ISSN 2313–5891 (Online)
ISSN 2304–974X (Print)

Ukrainian Food Journal

Volume 8, Issue 1
2019

Kyiv **Київ**

2019

Ukrainian Food Journal is an international scientific journal that publishes innovative papers of the experts in the fields of food science, engineering and technology, chemistry, economics and management.

Ukrainian Food Journal – міжнародне наукове періодичне видання для публікації результатів досліджень фахівців у галузі харчової науки, техніки та технології, хімії, економіки і управління.

Ukrainian Food Journal is abstracted and indexed by scientometric databases:

Ukrainian Food Journal індексується наукометричними базами:

Index Copernicus (2012)
EBSCO (2013)
Google Scholar (2013)
UlrichsWeb (2013)
CABI full text (2014)
Online Library of University of Southern Denmark (2014)
Directory of Research Journals Indexing (DRJI) (2014)
Directory of Open Access scholarly Resources (ROAD) (2014)
European Reference Index for the Humanities and the Social Sciences (ERIH PLUS) (2014)
Directory of Open Access Journals (DOAJ) (2015)
InfoBase Index (2015)
Chemical Abstracts Service Source Index (CASSI) (2016)
FSTA (Food Science and Technology Abstracts) (2018)
Emerging Sourses Citaton Index (2018)

Ukrainian Food Journal включено у перелік наукових фахових видань України з технічних наук, категорія А (Наказ Міністерства освіти і науки України № 358 від 15.03.2019)

Editorial office address:

National University
of Food Technologies
Volodymyrska str., 68
Ukraine, Kyiv 01601

Адреса редакції:

Національний університет
харчових технологій
вул. Володимирська, 68
Київ 01601

e-mail: **ufj_nuft@meta.ua**

Scientific Council of the National University of Food Technologies recommends the Journal for printing. Protocol № 8, 28.03.2019

Рекомендовано вченою радою Національного університету харчових технологій. Протокол № 8 від 28.03.2019

Ukrainian Food Journal publishes original research articles, short communications, scientific news in the related fields and also literature reviews.

Topics coverage:

Food engineering	Food nanotechnologies
Food chemistry	Food processing
Food microbiology	Economics and management in food industry
Physical property of food	Automation of food processes
Food quality and safety	Food packaging

Periodicity of the journal – 4 isues per year.

Reviewing a Manuscript for Publication. All scientific articles submitted for publication in "Ukrainian Food Journal" are double-blind peer-reviewed by at least two academics appointed by the Editors' Board: one from the Editorial Board and one, not affiliated to the Board and/or the Publisher.

Copyright. Authors submitting articles for publication are expected to provide an electronic statement confirming that their work is not an infringement of any existing copyright and will not indemnify the publisher against any breach of legislation and/or international standards in academic publishing. For the ease of dissemination all papers and other contributions become the legal copyright of the publisher unless agreed otherwise.

Academic ethics policy. The Editorial Board of "Ukrainian Food Journal" strictly follows all internationally acknowledged rules and regulations on academic publishing and academic ethics. For more details on this see: Miguel Roig (2003, 2006) "Avoiding plagiarism, self-plagiarism, and other questionable writing practices. A guide to ethical writing". The Editorial Board suggests all potential contributors of the journal, reviewers and readers to dully follow this guidance in order to avoid misconceptions.

For a **Full guide for All Authors** please visit our website:

http://ufj.ho.ua

International Editorial Board

Contents

Qualitative indicators of grain flakes of functional purpose

Svitlana Bazhay-Zhezherun, Galyna Simakhina, Ludmyla Bereza-Kindzerska, Nataliia Naumenko

National University of food Technologies, Kyiv, Ukraine

Keywords:

Activation
Wheat
Triticale
Oats
Flakes

Article history:

Received 03.09.2018
Received in revised form 19.10.2018
Accepted 28.03.2019

Corresponding author:

Svitlana Bazhay-Zhezherun
E-mail: Lananew_1@ukr.net

DOI:
10.24263/2304-974X-2019-8-1-3

Abstract

Introduction. Cereal raw materials are an important basis for the food industry. The research was carried out on the possibility of using a whole biologically activated grain of cereals to create functional food products.

Materials and methods. Compoundings of grain mixes of flakes and ready-made products on their basis is investigated. Protein was determined by Biuret method, starch content by polarimetric method. Fat was determined by the method of exhaustive extraction with chemically pure hexane. Vitamin E and substances with P-vitamin activity are determined colorimetrically. Vitamin C was carried out using a titrimetric method. The microbiological indices of the studied samples were determined by sowing them surface on agarized nutrient media.

Results and discussion. The influence of temperature regime and duration of cold conditioning of grain on its biological value is determined. With change of temperature regime to 12–18 ºC and the duration of conditioning of 24–30 h, the content in the grain of all water-soluble vitamins increases by 2–2,5 times, the amount of tocopherol grows by 5-7 times, the amount of substances with P-vitamin activity increases in 2,5 times.

The dependence of basic physical and technological parameters of grain flakes and organoleptic properties of finished products from their component composition is investigated. Increase in a mass fraction of oats to 50% leads to increased viscosity of porridge, due to an increase in the content of hemicellulose, decreases crumbility. Increasing the mass fraction of wheat grain to 35% leads to a more rigid structure of porridge, which is explained by the higher density of shell parts of wheat grain, compared with other components.

The degree of maintenance of the daily needs of the adult population in the macronutrients, at the expense of consumption of 100 grams of flakes, is: proteins – 18–22%, fats – 5–7%, carbohydrates – 13–16%, food fibers – 13,5%.

Taking into account the daily requirement of adult population in vitamins, 100 g of flakes mix allows you to meet the need for vitamin E by 67–76%; P by 17,4%.

The total number of colony-forming units of mesophilic aerobic and facultative-anaerobic microorganisms in fresh samples of mixes of flakes and after their storage does not exceed $2 \cdot 10^3$ per g product, mold mushrooms and pathogenic microorganisms are absent.

Conclusions. Biologically activated grain of cereal cultures of wheat, bare grain oats and triticale is a source of valuable nutrients, for creation of mixes of flakes of functional purpose.

Introduction

Cereal products are the main and irreplaceable components of food ration [1], they contain a number of essential substances necessary for providing of the normal functioning of man organism [2]. The leading place of food products on a grain basis in the feed puts the goal of the technologists to achieve a higher level of their functional significance [3]. Scientists investigate the possibility of application new non-traditional types of raw materials, processing methods for improving the quality of food products [4].

Wide distribution is acquired by flakes and groats of quick-cooking [5]. The interest of consumers in this type of products is explained by the possibility of reducing their cooking time and the best food and taste properties, in comparison with traditional groats [1]. The classic type of cereals for the production of flakes is an oat [6]. Lately for the production of flakes apply the row of other cereal cultures – rye, wheat, barley, and also corn, millet, buckwheat as well [5]. This allows you to expand the assortment of products, increase its food value.

One of the most useful and delicious types of cereal products are mueslis, basis of that are the flakes of a several cereals, complemented by fruit and vegetable components [7]. So fairly successful are products that provide a combination of cereal flakes with different original fillings, which is from 30% to 50% of the product [5].

The literature does not contain data on the possibility of production of flakes based on biologically activated grain of wheat, bare grain oats and triticale.

Literature review

By a base product that is basis for the production of flattening groats and flakes there are groats not ground up. Flakes produce from the groats of top grade at their additional cleaning, steaming-out and flattening [6].

Studies have found that regular consumption of whole grains and products on its basis contributes to reducing the risk of diseases of the cardiovascular [1] and digestive systems of the body, the development of diabetes [2].Such influence is caused by the presence in the whole-grain products of a number of biologically active components: vitamins, mineral compounds, food fibers [7] that can increase the immunity and adaptive capacity of a person, improve the activity of the gastrointestinal tract, organs and systems, and reduce the development of metabolic syndrome [3]. It is proved that valuable difficult complexes contained in whole grain products are more useful than the separate isolated components [4]. The peripheral parts of the grain, the shell and the aleurone layer, the germ contain much more valuable micronutrients than the starch endosperm, in particular vitamins and vitamin-like compounds, phenolic compounds, phytosterols, lignans, and dietary fibers [8] that are characterized by high biological activity [9]. It is also known that the proteins of the aleuronic layer and the germ have a higher nutritional value, compared to the grain endosperm proteins [10].

Foods on the basis of grain cereals are the important dietary sources of antioxidants [2]. The authors have investigated that the content of phenolic compounds, in particular routine, quercetin, phenolic acids in grain, not cleaned from shells, is significantly higher, antioxidant activity is also 2-4 times higher than that of grain cleaned from shells [11]. The total content of antioxidants in whole-wheat products exceeds 3,5–6 times the corresponding value in fruits and vegetables [12].

Clinical trials by Swiss physicians have shown that whole grains groats reduce the risk of oral and pharyngeal cancers, esophagus and larynx, unlike refined grain products that can contribute to this disease [13].

Scientists confirm the protective role of whole grains products that contain food fiber and the necessary mineral compounds, in particular magnesium, in relation to development of diabetes, especially in the elderly [3]. Clinical researches have shown that low concentration of magnesium in plasma of blood are associated with insulin resistance; it is proved that wholegrain products significantly reduce glycemia [14].

The Polish scientists have analyzed the assortment of the most popular cereal wheat breakfasts. It is marked that these products are made from the grain released from shells were characterized by low protein content (about 6,2%), subzero content squirrel (close 6,2%), low total fiber content (6,49%) and soluble dietary fibers [5].

Sprouting of grain and seeds is used to soften the core, increasing the nutritional value, reducing anti-alimentary substances and improving the functional composition of proteins [6]. The germination process should be short-lived and carried out at low temperatures, as it results in the degradation of β-glucans that significantly affect blood cholesterol and glucose, activity of the cardiovascular system, are antibacterial, antitumor, radioprotective properties [15]. So with the germination of oats grain for 72 h, the content of β-glucans is reduced by 40-45% [15].

Germination assists the increase of bioavailability of food compounds, by their partial hydrolysis, mineral substances of whole grain of Fe and Zn, mastering of that is complicated through the presence of natural inhibitors [16], and also to the decline of activity of present antialimentary substances, such as inhibitors of enzymes, hemagglutinins, etc. [17].

Scientists propose to receive flour of high nutritional value, in particular, with improved amino acid, mineral and fatty acid composition of grains germinated in solutions of sea salt [18].

In order to increase the nutritional value of products, biologically activated grain of wheat is recommended for use in bread technology [19]. Scientists from Belarus developed a number of methods for processing biologically activated grain, for the production of flour, groats and other products of high food value [20]. Technology of flakes is worked out from red rice, that envisages the use of whole grain soaked during a few days, that assists the increase of food value and comprehensibility of raw material [21].

The objectives of the work is the scientific and practical ground of the expediency of using a whole biologically activated grain of wheat, bare grain oats and triticale to create a mixture of flakes of high food value.

Task of researches are the following:

- to propose regimes for the preparation of grain of cereal cultures to increase its nutritional value;
- to ground expediency of application of the biologically activated grain-growing raw material for modeling and creating mixtures of flakes of functional purpose;
- to investigate the influence of the composition of the formula of a mixture of flakes on the basis of biologically activated grain of wheat, triticale, and bare grain oats on the basic physical and technological parameters of the product;
- to determine the organoleptic parameters and the nutritional value of grain flakes;
- to investigate the basic indexes of quality of flakes on the basis of biologically activated grain;
- to calculate the provision of day's norm in vitamins and basic substances due to the consumption of mixture of grain flakes;
- to define the indexes of microbiological stability of the product.

Materials and methods

Materials

During realization of experimental researches used grain of wheat, triticale and bare grain oats, respectively, sorts Mironivska 137, Molfar, Solomon [22], to the harvest of 2017; made standards of mixtures of flakes.

Methods of determination of nutrition value of flakes.

Preparation of investigated samples of flakes envisaged grain cleaning, washing and disinfection, hydrothermal treatment at 12–16 ºC in three cycles, each of which included the intensive moistening of grain for 4 hours, followed by evaporation for 4 to 6 hours, the total duration of 26–30 h. [23], rolling of grain, drying at a temperature of 40–45 ºC to a humidity of 12–14%; combination according to the formulation [6]. Humidity of the investigated samples was determined by drying to a constant mass for temperatures 105 °C [6]. Protein content was determined by Biuret method [24], which is based on the properties of proteins in an alkaline environment to form a blue-violet color with a biuret reagent whose intensity is determined on a spectrophotometer; starch – polarimetric method [25]. Fat was determined by the method of exhaustive extraction with chemically pure hexane [26]. Vitamins E and P were determined colorimetrically [27], determination of vitamin C was performed by titrimetric method. The method is based on extracting vitamin C from the test sample with an acid solution (chloride acid, metaphosphorus or a mixture of acetic and metaphosphoric) followed by titration visually or potentiometrically with sodium 2,6-dichlorophenolindophenolate solution [28].

Method of determination of microbiological indicators of flakes

The microbiological indexes of mixture of flakes determined in the prepared samples, humidity of that folded 11-12%. For this purpose, the samples studied were planted superficially on agarified nutrient media: meat-peptic agar (detection of mesophilic aerobic and facultative anaerobic microorganisms – MAFAnM), wort-agar (yeast and mushrooms). Cups with crops were incubated for 2 to 3 days at a temperature of 37 °C to establish a total amount m/o (MAFAnM). Crops on cups with an environment a wort-agar for the exposure of mushrooms and yeasts were incubated at 28 °C during a 5–7 days [29].

Results and discussion

Researches of influence of prescription composition of grain flakes on their indicators of quality

Scientists have shown that the consumption of grain products plays an important role in maintaining a normal body mass index, providing the body with energy, mineral compounds, vitamins, and lowering cholesterol levels in the blood [30]. Effective are biological methods of preparation of grain raw materials, which contribute to the increase of activity of native enzymes – soaking, sprouting, hydrothermal treatment at low temperatures, fermentation. Such treatment contributes to the highest natural degradation of anti-nutrients – phytate, inhibitors of digestive enzymes, increasing the bioavailability of macronutrients. The use of

biologically activated grain of cereals for the creation of products for health and functional purposes is a new progressive trend.

Traditional grain preparation includes hydrothermal treatment, in particular, cold conditioning, involves moisture of the grain and prolonged evaporation at a temperature of 30 to 40 °C. Hydrothermal treatment regimes include steam under pressure and grain tempering [31]. Such heating does not contribute to the synthesis of vitamins in the grain.

We have scientifically substantiated and proposed the use of significantly lower temperatures in the cold condition of grain – 12–18ºC. The process of treatment it is recommended to conduct in three cycles, each of which involves intensive humidification of grain for 4 hours, followed by evaporation for 4 to 6 hours, the total duration of 24–30 h, which contributes to increasing of humidity of grain to 30–35%. Such preparation causes activation of the enzyme complex. As a result of the intensification of enzymatic processes, increasing the bioavailability of carbohydrates and proteins, activating the synthesis of vitamins and vitamin-like substances [15]. Due to the activation of the enzyme complex, biological changes occur in the structure of the grain, it begins to sprout, is in the so-called "awakened state" [20]. By this treatment, the content of valuable micronutrients increases significantly, which is related to the internal biological processes in the plant organism.

Our previous investigations have shown that for this treatment, the content of all water-soluble vitamins increases by 2–2,5 times, the amount of tocopherol increases 5-7 times, depending on the culture and sort of grain; substantially increases the content of vitamin-like substances, in particular, substances with P-vitamin activity, inositol, in comparison with grain native [32].

Taking into account principles of health feed, the row of compounding of mixtures of flakes is worked out with the use of grain of wheat, bare grain oats and, triticale. Including our experimental data and applying the calculated method of food combinatorics, the percentage content of the formulation components of the mixture is calculated, which provides the highest amount of vitamins B, vitamins C and E, inositol in the finished product.

Prepared prototype finished products and studied their basic quality indicators (Table 1).

The influence of the amount of components of the mixture of flakes on the quality parameters of the finished product is investigated. It has been experimentally found that flakes obtained from the grain mixture, which include 30–45% oats, 20-30% wheat and 35-40% triticale are as friable, have a pleasant taste and a smell of cooked flakes. An increase in the mass fraction of oat to 50% leads to an increase in the viscosity of porridge, due to an increase in the content of hemicellulose, the scatteriness decreases, which is not expedient. Increasing the mass fraction of wheat grain up to 35% leads to a more rigid structure of porridge, which is explained by the higher density of the shell parts of wheat grain compared to the bare grain of oats and triticale, which is not appropriate.

It was investigated that the ratio of the main constituents of substances in developed grain flakes, which makes 12,2–12,7% (proteins): 3,8–4,4% (fats): 54,4–57% (carbohydrates) is more acceptable than in a number of cereals: manna, wheat, rice and the most popular types of cereal wheat breakfast [5, 33].

There was experimentally determined that the content of food fibers in developed grain flakes is 2.7 – 2.9%. It is researched that according to the water-holding ability, the data of the food fibers belong to the group of medium-water-binding, they have a positive influence on the processes of digestion, occupy a considerable volume in the intestine and increase its peristalsis [13]. The presence of food fibers, which are natural food sorbents, capable of adsorbing toxic substances, heavy metal salts, radionuclides, bile acids, and cholesterol, are an important factor in their functional action on the human body [34].

Table 1

Recipes of mixes of flakes and a characteristic of their nutritional value and organoleptic indicators

№	Recipe components			Nutritional value				Vitamin content, mg%.		Organoleptic indicators of the finished product
	Wheat	Oats bare grain	Triticale	Proteins	Fats	Carbo-hydrates	Energy value, kcal	E	P	
1	15	50	35	12,85	4,58	46,25	277,45	11,85	8,57	The porridge is viscous, not crumbly enough, the smell and taste is characteristic of this product
2	20	45	35	12,68	4,38	47,26	279,2	11,65	8,64	Porridge is a measure of crumbly, pleasant taste and a smell of cooked flakes
3	25	40	35	12,51	4,18	48,26	280,74	11,55	8,74	Porridge is a measure of crumbly, pleasant taste and a smell of cooked flakes
4	30	30	40	12,22	3,79	50,17	283,67	11,36	8,84	Porridge is a measure of crumbly, pleasant taste and a smell of cooked flakes
5	35	35	30	12,29	3,96	49,37	282,42	11,45	8,78	The porridge is hardened with slipping, the smell and taste is characteristic of this product

Determination of biological value and physical-technological indicators of quality of grain flakes

We have determined the content of vitamins in grain flakes. The developed product, due to the preliminary preparation of grain – biological activation, during which the grain sprouts, contains a significantly higher amount of antioxidants – vitamins C, E and substances with P vitamin activity, compared with traditional grain products. These data are consistent with studies by other scientists who have found a significant increase in the content of vitamins at grain germination [35, 36]. The flakes also contain vitamins of group B: thiamine – 0.48 to 0.62 mg%, riboflavin 0.25 to 0.32 mg%, pyridoxine 0.86 to 1.2 mg%, inositol 154 to 162 mg% nicotinic acid – 4.5–6.3 mg%.

The basic physical and technological parameters of quality of flakes mixes from biologically activated grain are determined (Table 2).

Table 2

Physical and technological indicators of grain flakes quality

№	Indicator	The ratio of the formulation components of the mixture flakes (wheat: oats: triticale),%.		
		20 : 45 : 35	**25 : 40 : 35**	**30 : 30 : 40**
1.	Humidity,%.	11,5	12,0	11,8
2.	Volumetric mass, g/l	420	426	438
3.	The middle particle size , mm	5,2	6,0	6,4
4.	The angle of the natural inclination, deg	64	62	65
5.	Angle of sliding on metal, deg	15	16	18
6.	Actual density, g / l	432	440	454
7.	Cohesiveness	1,1	1,2	1,4

It should be noted that the ratio of individual components of the mixture of flakes influences the physical and technological parameters [6]. All samples have a permissible value of humidity. The middle particle size of a mixture of flakes depends on the grain size of the raw material and is in the range acceptable for food products on grain basis [31]. The values of indexes of by volume mass and actual density indicate the high quality of the grain product. Optimum values of cohesiveness of grain mixtures characterize their ability to move freely when unloaded from containers and during transportation [6]. The obtained results correlate with the data, determined on the physical and technological parameters of corn flakes [37, 38].

Scientists note that whole grain products are essential for daily diets; they contain a unique set of nutrients that are prevention of cardiovascular disease and diabetes [37].

Using the norms of human physiological needs in the main nutrients and energy [39], the calculation of maintenance of the day's norm (DN) in vitamins and basic substances was made due to the consumption of a mixture of flakes from biologically activated grain. Quantitative indicators are given in tables 3,4.

Table 3

Providing daily need for vitamins per 100 g of a mixture of flakes

Vitamin	Content in a mixture of flakes, mg	Norms of consumption, mg		Maintenance DN,%.	
		Men	Women	Men	Women
E	11,5	17	15	67,6	76,6
P	8,7	50		17,4	17,4
C	4,2	70		6	6

Consequently, taking into account the day's requirement of adult population in vitamins, 100 g of mixture of flakes allows you to meet the need for vitamin E by 67–76% and vitamin P by 17,4% and vitamin C by 6%. The obtained results indicate that a mixture of flakes from biologically activated wheat, oats and triticale is a functional product [40]. These products are able to provide the body with vitamins antioxidants every day, which is important in antioxidant protection of the body [41].

Table 4

Nutritional and energy value of a mixture of flakes

Indicator		Mixture of flakes	Daily norms consumption		Ensuring day norm,%.	
			Men	Women	Men	Women
Proteins, g		12,4	67	55	18,5	22,5
Fats, g		4,03	68	56	5,92	7,19
Carbo-hydrates, g	Starch	52,7	392	320	13,4	16,4
	Food fibers	2,7	20		13,5	13,5
Energy value, kcal		327	2450	2000	13,34	16,35

It is established that the degree of ensuring of the day's needs of the adult population of the first group of labor intensity in the macronutrients, at the expense of consumption of 100 grams of cereal flakes is: proteins – 18–22%, fats – 5–7%, carbohydrates – 13–16%, food fibers – 13,5%. The presence of a significant amount of food fibers in the flakes is a positive factor, since these natural sorbents lower the level of cholesterol in the blood and the risk of development of tumors of the upper digestive and respiratory tract [13, 42, 43].

The total number of colony-forming units of mesophilic aerobic and facultative-anaerobic microorganisms (KFU MANFAnM) was determined in the finished mixture of flakes, as well as during storage of the product for 6 months. The results of microbiological studies are presented in Table 5.

As a result of the conducted research it was established that the microbiological seedability of mix flakes on the basis of biologically activated grain of wheat, triticale and oat does not exceed the values of permissible values of microbiological seedability [29], storage of mixtures of flakes during 6 months does not significantly impair their quality, these grain products are safe from the point of view of microbiological purity.

Table 5

Microbiological indicators of a mixture of flakes based on biologically activated grain

Sample	Microbiological indicators		
	MAFAnM, KFU / g, no more	Mold fung, **KFU / g, not more**	**Pathogenic microorganisms, including Salmonella in 25 g**
Cereals, normative value	$5 \cdot 10^3$	50	Not allowed
A mixture of flakes after drying	$3 \cdot 10^2$	Not found	Not found
A mixture of flakes after 6 months storage	$2 \cdot 10^3$	Not found	Not found

Conclusions

According to the results of analytical and experimental studies, the prescription composition of grain flakes on the basis of biologically activated grain of wheat, oats and triticale has been developed. This product has a functional purpose and is essential for a diet, since it enables you to satisfy your daily needs not only in the required macronutrients, but also in important vitamins and food fibers.

References

1. Anderson J.W. (2003), Whole grains protect against atherosclerotic cardiovascular disease, *Proc. Nutr. Soc.,* pp. 135–142.
2. Okarter N. (2010), Health benefits of whole grain phytochemicals, *Crit Rev. Food Sci Nutr.*, pp. 193–208.
3. Adom K.K., Sorrells R.H. (2003), Phytochemical Profiles and Antioxidant Activity of Wheat Varieties, *J. Agric. Food Chem*., pp. 7825–7834.
4. Liu R.H. (2004), Potential synergy of phytochemicals in cancer prevention: mechanism of action, *J. Nutr*., pp. 3479–3485.
5. Rzedzicki Z., Sykut-Domańska E., Popielewicz J. (2008), Quality of wheat breakfast cereals available on the polish market, *J. Food Nutr. Sci*, 58, pp. 307–312
6. Egorov G.A., Petrenko T.M. (2000), *Tekhnologiya muki i krupy*, Izdatel'skij kompleks MGUPP.
7. S. Senhofa, E. Straumite, M. Sabovics, D. Klava, R. Galoburda,T. Rakcejeva (2015), The effect of packaging type on quality of cereal muesli during storage, *Agronomy Research,* 13(4), P.1064–1073.
8. Jacobs D.R., Pereira M.A., Stumpf K., Pins J.J. (2002), Whole grain food intake elevates serum enterolactone, *Br. J. Nutr.,* pp. 111–116.
9. Liukkonen K.H. Katina K., Wilhelmsson A., Myllymäki O., Lampi A.M. (2003). Process-induced changes on bioactive compounds in whole grain rye, *Proc. Nutr. Soc.*, pp. 117–122.

10. Kazakov E. D., Karpilenko G.P. (2005), Biohimiya zerna i hleboproduktov GIORD, Sankt-Peterburg.
11. Kerienė I., Mankevičienė A., Bliznikas S., Jablonskytė-rašče D., Maikštėnienė S, Česnulevičienė R. (2015), Biologically active phenolic compounds in buckwheat, oats and winter spelt wheat, *Zemdirbyste-Agriculture*, 102(3), pp. 289–296.
12. Miller, H.E.; Rigelhof, F., Marquart, L., Prakash, A. Kanter, M. (2000), Antioxidant Content of Whole Grain Breakfast Cereals, Fruit and vegetables, *J. Am Coll Nutr*, pp. 312–319.
13. Levi F, Pasche C, Lucchini F, Chatenoud L, Jacobs DR & La Vecchia C (2000) Refined and whole grain cereals and the risk of oral, oesophageal and laryngeal cancer, *European Journal of Clinical Nutrition*, 54, pp. 487–489.
14. Meyer K.A., Kushi L.H., Jacobs D.R., Slavin J.E., Sellers T.A., (2000), Carbohydrates, dietary fiber, and incident type 2 diabetes in older women, *American Journal of Clinical Nutrition*, 71, pp.921–930.
15. Review A., Havrlentová M., Petruláková Z., Burgárová A., Gago F. (2011), Cereal β-glucans and their Significance for the Preparation of Functional Foods, *Czech J. Food Sci*, 29(1), pp. 1–14
16. Sandberg A. (2002), Bioavailability of minerals in legumes, *British Journal of Nutrition*, 88(3), pp. 281–285.
17. Delcour J., Hosney C. (2010), *Principles of Cereal Science and Technology*, Geneve.
18. Kravchenko M., Krivoruchko M. (2012), YAkist' boroshna iz zerna pshenici, proroshchenogo u rozchini mors'koï harchovoï soli, *Tovari i rinki*, 2 p.106–110.
19. Capalova, I. EH., Sotnikov O.M. (1999), Povyshenie biologicheskoj cennosti hleba putem bioaktivacii zerna pshenicy. Vliyanie prorashchivaniya na himicheskij sostav i kachestvo klejkoviny, *Hlebopechenie Rossii*, 6, pp. 26–27.
20. Sharshunov V.A., Urbanchyk E.N., Kasyanova L.A. (2012), Biotechnology techniques for a more efficient use of the cereal resources of Belarus, *Vesti National Academy of Sciences of Belarus,* 1., p.101–106.
21. Kanchana. A., Arun A. (2017), A Research On Reminiscence and Acclimation of Oryza Sativa Flakes. Snacks Among Adolescents, *Current Research in Nutrition and Food Science*, 5(3), pp. 330–337.
22. Donets M.M. (2007), *Nasinnytstvo z osnovamy selektsii*, Ahrarna osvita, Kyiv.
23. S. Bazhay-Zhezherun, T. Romanovska, M. Antoniuk (2016) Improving the nutritional value of grains by biological activation, *Ukrainian Food Journal*, 5(3), pp. 476–484.
24. Kononskyi O. I. (2002), *Orhanichna khimiia*, Vyshcha shkola, Kyiv.
25. Native starch – Determination of starch content – Ewers polarimetric method / International Standard IS0 10520:1997(E), International Organization for Standardization, Geneve, Switzerland.
26. Erickson M. (2005) Deep Frying. Chemistry, Nutrition, and Practical Applications, Urbana, Illinois.
27. Ermakov A.Y. (1987), Metodi byokhymycheskoho issledovonyia rasteniy, Lenynhrad.
28. Majidi M., Hazim Y-ALQubury (2016), Determination of Vitamin C(ascorbic acid) Contents in various fruit and vegetable by UV-spectrophotometry and titration methods, *Journal of Chemical and Pharmaceutical Sciences*, 9(4), pp. 2972-2974.
29. Food Standards Australia New Zealand (2016), ISBN: 978-0-642-34594-3.
30. Barton B.A., Eldridge A.L., Thompson D., Affenito S.G., Striegel-Moore R.H., Franko D.L., Albertson A.M., Crockett S.J. (2005), The relationship of breakfast and cereal consumption to nutrient intake and body mass index: The National Heart, Lung and Blood Institute Growth and Health Study, *J. Am. Diet. Assoc*., 105, p.1383–1389.

31. Egorova E.YU., Obrezkova M.V., Gur'yanov YU.G. (2011), *Zerno i zernoprodukty: zerno, muka, krupy. Tekhnologiya i ocenka kachestva*, BTI AltGTU, Bijsk.
32. Simakhina G.A., Bazhay-Zhezherun S.A., Mykoliv T.I., Bereza-Kindzerska L.V., Antoniuk M.M. (2017), The Use Of The Biologically Activated Grain Is In Technology Of Health Products, *East European Scientific Journal*, 4. p.147–153.
33. Taranenko L. (2004), Grechka potribna vsim, *Zerno i hlib*, 4, pp. 37.
34. Asp N.G. (2001), Development of dietary fiber methodology, *Advanced Dietary Fiber Technology Blackwell Science*, pp. 77–88.
35. Kim Y. S., Kim J.G., Lee Y.S., Kang I.J. (2005), Comparison of the chemical components of buckwheat seeds and sprouts, *J. Korean Soc. Food Sci. Nutr.,* 34, pp. 81–86.
36. Kim, Sun Ju and Sarker, Md. Zaidul Islam and Suzuki, Tatsuro and Mukasa, Yuji and Hashimoto (2008) Comparison of phenolic compositions between common and tartary buckwheat (Fagopyrum) sprouts, *Food Chemistry*, 110(4), pp. 814–820.
37. Milenko B. Košutić1, Lato L., Jelena S. (2017), Improving the nutritive characteristics of corn flakes enriched with functional components, *Hem. ind.,* 71(6), pp. 495–502.
38. Slavin, J.L., Jacobs D., Marquart L. (2001), Grain Processing and Nutrition, *Clinical Review Biotechnology*, 21, pp. 49–66.
39. Pavlotska L.F., Dudenko N.V., Dymytriievych L.R. (2015), *Osnovy fiziolohii, hihiieny kharchuvannia ta problemy bezpeky kharchovykh produktiv*, Universytetska knyha, Sumy.
40. Ukrainets A.I., Simakhina H.O. (2009), Tekhnolohiia ozdorovchykh kharchovykh produktiv, NUHT, Kyiv.
41. Biel W., Bobko K., Maciorowski R. (2009), Chemical composition and nutritive value of husked and naked oats grain, *Journal of* Cer*eal Science*, 49, pp. 413–418.
42. Tappy L. (2006), Effects of break fast cereals containing various amounts of beta-glucan fibres on plasma glucose and insulin responses in NIDDM subjects, *Diabetes Care,* 19, pp. 831–834.
43. Soukoulis Ch., Lebesi D., Tzia C. (2009), Enrichment of ice cream with dietary fibre: Effects on rheological properties, ice crystallisation and glass transition phenomena, *Food Chemistry*, 115, pp. 665–671.

Morphological characteristics of starch granules of Eastern and Central European potato varieties (Solanum Tuberosum)

Anastasia Zabolotets[1], Vladimir Litvyak[1], Aliaksei Yermakou[2], Gulnazym Ospankulova[3]

1 – Scientific and Practical Center of the National Academy of Sciences of Belarus on Food, Minsk, Republic of Belarus
2 – Belarusian National Technical University, Minsk, Republic of Belarus
3 – S. Seifullin Kazakh AgroTechnical University, Nur-Sultan, Republic of Kazakhstan

Keywords:

Starch
Native
Morphology
Grain
Potato

Article history:

Received 12.08.2018
Received in revised form 12.11.2018
Accepted 28.03.2019

Corresponding author:

Vladimir Litvyak
E-mail: besserk1974@mail.ru

DOI:
10.24263/2304-974X-2019-8-1-4

Abstract

Introduction. Studies have been conducted to determine the effect of the size of starch granules on the quality of starch obtained from Eastern and Central European potato varieties.

Materials and methods. The object of study is native potato starch, produced according on technical regulatory legal acts from 15 potato varieties Belarusian, of Russian, of Ukrainian and of German selection. The morphological structure of starch granules was evaluated on a LEO 1420 scanning electron microscope. The contrast of the images was achieved by the metallization of preparations made with gold in the EMITECH K 550X vacuum system.

Results and its discussion. Grains of native starch obtained from Eastern and Central European potato varieties vary significantly in both shape and size, which, to a large extent, apparently, determine the technological features of starch production. It is noticed that, depending on the selection of Belarusian, German, Russian, Ukrainian, the size of the grains of native potato starch of Eastern and Central European varieties of potatoes varies within: 5–90,26; 8,38–83,47; 5,3–88,7; 12,36–70 microns, respectively.

The highest starch content in grains obtained from Eastern and Central European potato varieties was found in the German selection variety *«Kormoran»* – 23%. Among the varieties under consideration, it is he who has the highest starchiness, but at the same time small starch granules (the average size of the granules is 24,0 microns). Lowest starch content in grains derived from Eastern and Central European potato varieties was the lowest in the Russian *«Krepysh»* variety, 11,1%, with an average granule size of 30,1 microns. On this basis, it can be assumed that with a larger average size of starch grains the starch content decreases, and with a smaller average size, the percentage of starch content increases.

According to our results, as well as well-known data, starch grains have an oval, spherical or irregular shape, their diameter varies between 0,001–0,2 mm. Starch grains are divided into simple and complex: simple grains are homogeneous formations; complex − a combination of smaller particles. The density of starch is on average 1,5 kg/m^3.

Based on the analysis of the characteristics of the structure of native starch, it can be assumed that the main structural characteristic of the structure of native starch, determining its properties, is starch grain (granule).

Conclusion. The morphological structure of native starch granules depends on the type of potato and can vary widely: from 5–7 microns to 80–90 microns, which affects the quality of the starch produced.

Introduction

The range of products of starch-treacle production is quite large and amounts to several hundred items [1–32]. The main raw materials for the production of starch and starch products are potatoes [5], corn [18–19, 22, 27], wheat [11, 15–16], rye [2], barley [12, 20], oats [4], rice [19, 21], buckwheat [2, 5], tapioca [23, 24], lentils [26], banana [25] etc.

Potato starch is widely used in the food industry, in cooking, in the production of certain types of sausages, to stabilize confectionery creams, and to produce artificial sago. Starch obtained from potatoes is also used for technical purposes in the textile, paper, printing industry, as well as in everyday life [1].

The use of starch is largely determined by its properties associated with the morphological structure. The peculiarities of starch morphology depend on the biochemical mechanisms of starch accumulation and are primarily determined by the grade of the raw material and its quality.

The size of starch granules has a significant effect on the quality of starch. Small granules swell worse and, accordingly, gelatinize more slowly, in addition, they are worse stored and inferior in taste to large ones.

In this regard, the purpose of this work was a comparative study of the morphological structure of native potato starch of Eastern and Central European varietal origin.

Materials and methods

Materials

The object of study is native potato starch, produced according on technical regulatory legal acts (TNLA) [33] from 15 potato varieties (*Solanum tuberosum* L.) [34–37], of which ten (*«Atlant»* (*«Атлант»*), *«Lazurit»* (*«Лазурит»*), *«Lasunok»* (*«Ласунок»*), *«Lileya»* (*«Лилея»*), *«Mag»* (*«Маг»*), *«Skarb»* (*«Скарб»*), *«Suzor'ye»* (*«Сузорье»*), *«Yavar»* (*«Явар»*), *«Vesnyanka»* (*«Веснянка»*)) Belarusian, thirteen (*«Lazar'»* (*«Лазарь»*), *«Divo»* (*«Диво»*), *«Vestnik»* (*«Вестник»*), *«Effect»* (*Эффект*), *«Bryanskiy delikatesc»* (*«Брянский деликатес»*), *«Favorit»* (*«Фаворит»*), *«Grand»* (*«Гранд»*), *«Vasilek»* (*«Василек»*), *«Velikan»* (*«Великан»*), *«Nakra»* (*«Накра»*), *«Kolobok»* (*«Колобок»*), *«Krepysh»* (*«Крепыш»*), *«Meteor»* (*«Метеор»*)) of Russian, two (*«Dzvin»* (*«Дзвин»*), *«Leleka»* (*«Лелека»*)) of Ukrainian and four (*«Albatros»*, *«Kormoran»*, *«Kranich»*, *«Sonata»*) of German selection.

Methods

Scanning Electron Microscopy (SEM)

Sample preparation. Metallization of native starch preparations was carried out with gold in the EMITECH K 550X vacuum unit.

Analysis. Scanning electron micrographs of native starch granules were obtained using a LEO 1420 scanning (raster) electron microscope (Germany) [38–41].

The principle of operation of the scanning electron microscope consists in scanning the sample surface with a focused electron beam and analyzing particles reflected from it and X-ray radiation resulting from the interaction of electrons with matter. In a scanning electron microscope, an electron beam (electron probe) is focused by electromagnetic lenses of a capacitor and a lens [6]. A special device – deflector deflects the electron beam (primary

electrons), which slides on the surface (raster). The secondary electrons (reflected from the surface) are perceived by the detector and are focused on the screen of the scanning electron microscope creating a three-dimensional image. The scanning electron microscope allows working in a wide range of magnifications from ×10 (which is equivalent to an increase in a strong hand-held lens) to ×1000000, which is ≈ 500 times the increase limit of the best optical microscopes. The scanning surface is necessarily sprayed with metal: platinum, gold, silver, aluminum [6].

Determination of starch content in potato tubers

The starchiness of potatoes is determined on the scales of the Steam [42].

Scales (Figure 1) consist of a long rocker, on which there are two parallel rulers with movable weights: a small one on the front ruler and a large one with a inside-in mobile ruler on the rear ruler. The back ruler is graded in divisions expressing the percentage of litter content (from 0 to 60%). It is used to determine the contamination of potatoes and weigh the samples for analysis. The front line is graded on the percentage of starch in the range from 10 to 30% with an accuracy of up to 0,1% and is used for weighing in water by moving a small weight along the rocker arm [42].

The content of starch in the test sample of potatoes is determined by the position of the small weight on a certain division of the rocker arm on reaching equilibrium. The definition itself is made when a large weight is shifted all the way to the left, and a mobile ticker on it is pushed to the right. The ruler ends with directional arrows, above which there are two mobile small weights used to balance the tare of the scales, as follows. Two baskets (upper and lower) are suspended for a short rocker. In a tank in which the lower basket is placed, water is poured at a temperature of 17,5°C. Both weights are pushed back and the mobile ruler is pushed to the left on the big weight until it stops. After this, the scales open and achieve the exact coincidence of the index arrows, first roughly: moving a large load on the short arm of the rocker arm, and then precisely: with small weights [42].

Due to the fact that the balance is calibrated at a temperature of 17,5°C, it is necessary to measure the temperature of the water in the tank with each determination and to make a temperature correction (Table 1).

Table 1

Corrections for water temperature in determining starchiness on potato scales

Water temperature when weighing, °C														
7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
	Add to scales										From indications of scales to take away			
0,27	0,26	0,25	0,23	0,20	0,17	0,15	0,12	0,09	0,06	0,02	0,02	0,08	0,10	0,12

Statistical Analysis

Statistical processing of the research results obtained using modern computer tools in accordance with generally accepted methods [3].

Results and discussion

Results

Potato starch granules

Scanning electron micrographs of grains of native starch obtained from Eastern and Central European potato varieties are shown in Figures 2 and 3. Figures 4, 5 and 6 show a grain size analysis of grains of native starch obtained from Eastern and Central European potato varieties (the distribution of starch granules according to their size).

The granulometric analysis of the grains of native starch was carried out on the basis of the results given in Table 2. Table 1 shows the average, minimum and maximum grain sizes of native starches obtained from Eastern and Central European potato varieties with the characteristics of the statistical processing of the studied sample.

Native starch grains obtained from Eastern and Central European potato varieties vary significantly in both shape and size, which, to a large extent, apparently, determine the technological features of starch production [4–6].

It was established that the average grain size of native starch obtained from Eastern and Central European potato varieties *«Atlant», «Lazurit»*, *«Lasunok»*, *«Lileya»*, *«Mag»*, *«Skarb»*, *«Suzor'ye»*, *«Yavar»*, *«Uladar», «Vesnyanka», «Albatros»*, *«Kormoran»*, *«Kranich»*, *«Sonata»*, *«Lazar'»*, *«Divo»*, *«Vestnik»*, *«Effect»*, *«Bryanskiy delikatesc»*, *«Favorit»*, *«Grand»*, *«Vasilek»*, *«Velikan»*, *«Nakra»*, *«Kolobok»*, *«Krepysh»*, *«Meteor»*, *«Dzvin»*, *«Leleka»* respectively, will be: 28,23; 23,89; 21,61; 26,03; 37,12; 25,54; 37,13; 26,02; 26,29; 32,85; 33,72; 23,96; 33,90; 28,38; 27,68; 30,35; 34,13; 27,52; 21,32; 12,9; 33,1; 32,6; 31,4; 51; 31,9; 30,1; 27,9; 33,47; 33,27 microns (tab. 1) [34]. At the same time, the minimum and maximum grain size of native starch obtained from Eastern and Central European potato varieties *«Atlant», «Lazurit»*, *«Lasunok»*, *«Lileya»*, *«Mag»*, *«Skarb»*, *«Suzor'ye»*, *«Yavar»*, *«Uladar», «Vesnyanka», «Albatros»*, *«Kormoran»*, *«Kranich»*, *«Sonata»*, *«Lazar'»*, *«Divo»*, *«Vestnik»*, *«Effect»*, *«Bryanskiy delikatesc»*, *«Favorit»*, *«Grand»*, *«Vasilek»*, *«Velikan»*, *«Nakra»*, *«Kolobok»*, *«Krepysh»*, *«Meteor»*, *«Dzvin»*, *«Leleka»* fluctuates within: 7,84–56,22; 7,92–66,81; 5–56,25; 7,91–59,46; 12,92–65,42; 6,62–64,12; 14,58–67,64; 9,12–59,41; 6,43–58,39; 7,9–90,26; 8,46–62,64; 8,38–58,82; 14,31–83,47; 10,97–62,64; 10,14–55,69; 15–56,58; 13,68–60,53; 9,47–58,94; 7–68,7; 5,3–38,4; 27,4–60,2; 11,4–88,7; 11,6–61,4; 15,1–80,8; 15,1–64,1; 10–61,1; 10,3–52,7; 12,36–70; 15,14–60,28 microns (tab. 2) [34].

It should be noted that the maximum average grain size of native starch obtained from Eastern and Central European potato varieties was noted for *«Nakra»* variety and was 51 microns. The minimum average grain size of native potato starch was found in variety *«Favorite»* – 12,9 microns. Both varieties of native potato starch belong to the varieties of Russian selection [34, 41].

Table 2

Morphological characteristics of native starch isolated from Eastern and Central European potato varieties

Options	Native starch extracted from Eastern and Central European potato varieties						
	Varieties of Belarusian breeding						
	«Atlant»	«Lazurit»	«Lasunok»	«Lileya»	«Mag»	«Skarb»	«Suzor'ye»
The average	28,23	23,89	21,61	26,03	37,12	25,54	37,13
Minimum	7,84	7,92	5,00	7,91	12,92	6,62	14,58
Maximum	56,22	66,81	56,25	59,46	65,42	64,12	67,64
Average starch content,%.	18,5	14,7	18,5	14,1	19	14,5	19,1
The distribution of granules in size	A				B	A	

Continuation of Table 2

Options	**Native starch extracted from Eastern and Central European potato varieties**						
	Varieties of Belarusian breeding			**German selection varieties**			
	«Yavar»	«Uladar»	«Vesnyanka»	«Albatros»	«Kormoran»	«Kranich»	«Sonata»
The average	26,02	26,29	32,85	33,72	23,96	33,90	28,38
Minimum	9,12	6,43	7,89	8,46	8,38	14,31	10,97
Maximum	59,41	58,39	90,26	62,64	58,82	83,47	62,64
Average starch content,%.	11,4	14,7	18,3	17,2	23	16,7	16,9
The distribution of granules in size	A			B	A	B	A

Continuation of Table 2

Options	Native starch extracted from Eastern and Central European potato varieties						
	Russian breeding varieties						
	«Lazar'»	«Divo»	«Vestnik»,	«Effect»	«Bryanskiy delikatesc»	«Favorit»	«Grand»
The average	27,68	30,35	34,13	27,52	21,32	12,9	33,1
Minimum	10,14	15,00	13,68	9,47	7	5,3	27,4
Maximum	55,69	56,58	60,53	58,94	68,7	38,4	60,2
Average starch content,%.	20,4	21	18,5	19,5	16,6	14,5	15,7
The distribution of granules in size	A	C	A	C	D	C	B

Continuation of Table 2

Options	Native starch extracted from Eastern and Central European potato varieties							
	Russian breeding varieties						Varieties of Ukrainian breeding	
	«Vasilek»	«Velikan»	«Nakra»	«Kolobok»	«Krepysh»	«Meteor»	«Dzvin»	«Leleka»
The average	32,6	31,4	51,0	31,9	30,1	27,9	33,47	33,27
Minimum	11,4	11,6	15,1	15,1	10,0	10,3	12,36	15,14
Maximum	88,7	61,4	80,8	64,1	61,1	52,7	70,00	60,28
Average starch content,%.	14,3	17,4	20,1	13,2	11,1	13,5	18	18,5
The distribution of granules in size	C				D	C	A	

A – Monomodal, B – Bimodal, C – Threemodal, D – Fourmodal

It is noticed that, depending on the selection of Belarusian, German, Russian, Ukrainian, the size of the grains of native potato starch of Eastern and Central European potato varieties ranges from: 5–90,26; 8,38–83,47; 5,3–88,7; 12,36–70 microns, respectively [34].

The highest starch content in grains obtained from different varieties of potatoes was found in the German selection variety *«Kormoran»* – 23%. This variety is of particular interest. Among the varieties under consideration, it is he who has the highest starchiness, but at the same time small starch granules (the average size of the granules is 24,0 microns) [34, 41].

The starch content in grains obtained from Eastern and Central European potato varieties was the lowest in the Russian *«Krepysh»* variety, 11,1%, with an average granule size of 30,1 microns [34, 41].

On this basis, it can be assumed that with a larger average size of starch grains the starch content decreases, and with a smaller average size, the percentage of starch content increases.

As can be seen from Figures 1 and 2, fifteen varieties of native starches obtained from Eastern and Central European potato varieties *(«Atlant», «Lazurit», «Lasunok», «Lileya», «Skarb», «Suzor'ye», «Yavar», «Uladar», «Vesnyanka», «Kormoran», «Sonata», «Lazar'», «Dzvin», «Leleka»*) the distribution of starch grains is monomodal in size (one fraction is clearly distinguished), four types of native potato starch (*«Mag», «Albatros», «Kranich», «Grand»*) have a bimodal (two-fractional) size distribution of starch grains, in eight varieties of native potato starch (*«Divo», «Effect», «Favorite», «Vasilek», «Velikan», «Nakra», «Kolobok», «Meteor»*), the distribution of starch grains is threemodal (three-fractional) in size, and in two varieties of native potato starch (*«Bryanskiy delikatesc»* and *«Krepysh»*), the distribution of starch granules is fourmodal (four-fractional) [34].

Amount of starch in potato tubers

It should be noted that the average starch content in grains obtained from Eastern and Central European potato varieties is as follows: *«Atlant»* – 18,5%, *«Lazurite»* – 17,7%, *«Lasunok»* – 18,5%, *«Lileya»* – 14,1%, *«Mag»* – 19%, *«Skarb»* – 14,5%, «*Suzor'ye*» – 19,1%, *«Yavar»* – 11,4%, *«Uladar»* – 14,7%, *«Vesnyanka»* – 18,3%, *«Albatros»* – 17,2%, *«Kormoran»* – 23%, *«Kranich»* – 16,7%, *«Sonata»* – 16,9%, *«Lazar'»* – 20,4%, *«Divo»* – 21,0%, *«Vestnik»* – 18,5%, *«Effect»* – 19,5%, *«Bryanskiy delikatesc»* – 16,6%, *«Favorite»* – 14,5%, *«Grand»* – 15,7%, *«Vasilek»* – 14,3%, *«Velikan»* – 17,4%, *«Nakra»* – 20,1%, *«Kolobok»* – 13,2%, *«Krepysh»* – 11,1%, *«Meteor»* – 13,5%, *«Dzvin»* – 18%, *«Leleka»* – 18,5% [34, 41].

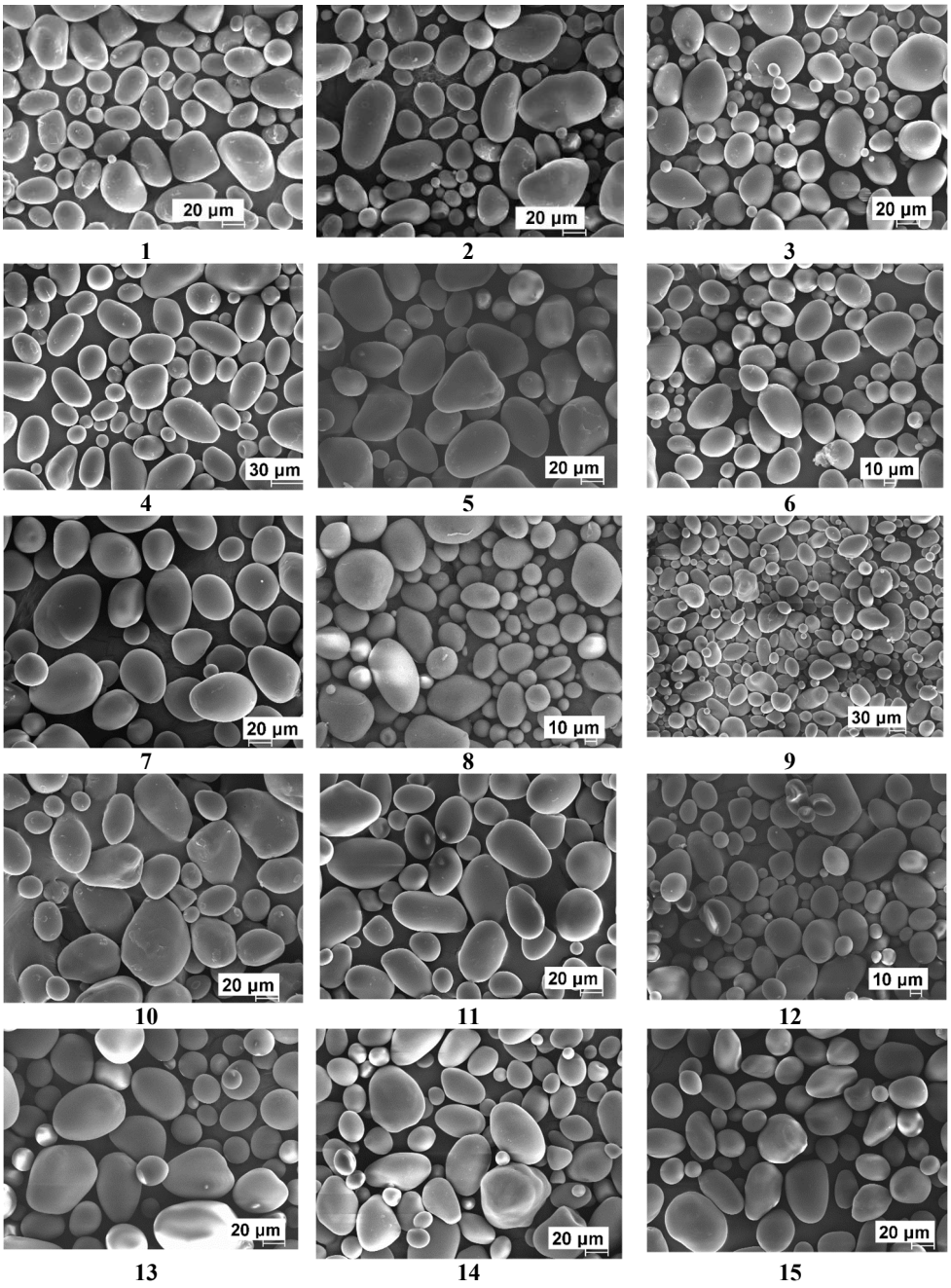


Figure 1. Scanning electron micrographs of native starch grains, isolated from Eastern and Central European potato varieties
1 – «Atlant», 2 – «Lazurit», 3 – «Lasunok», 4 – «Lileya», 5 – «Mag», 6 – «Skarb», 7 – «Suzor'ye», 8 – «Yavar», 9 – «Uladar», 10 – «Vesnyanka», 11 – «Albatros», 12 – «Kormoran», 13 – «Kranich», 14 – «Sonata», 15 – «Lazar'»

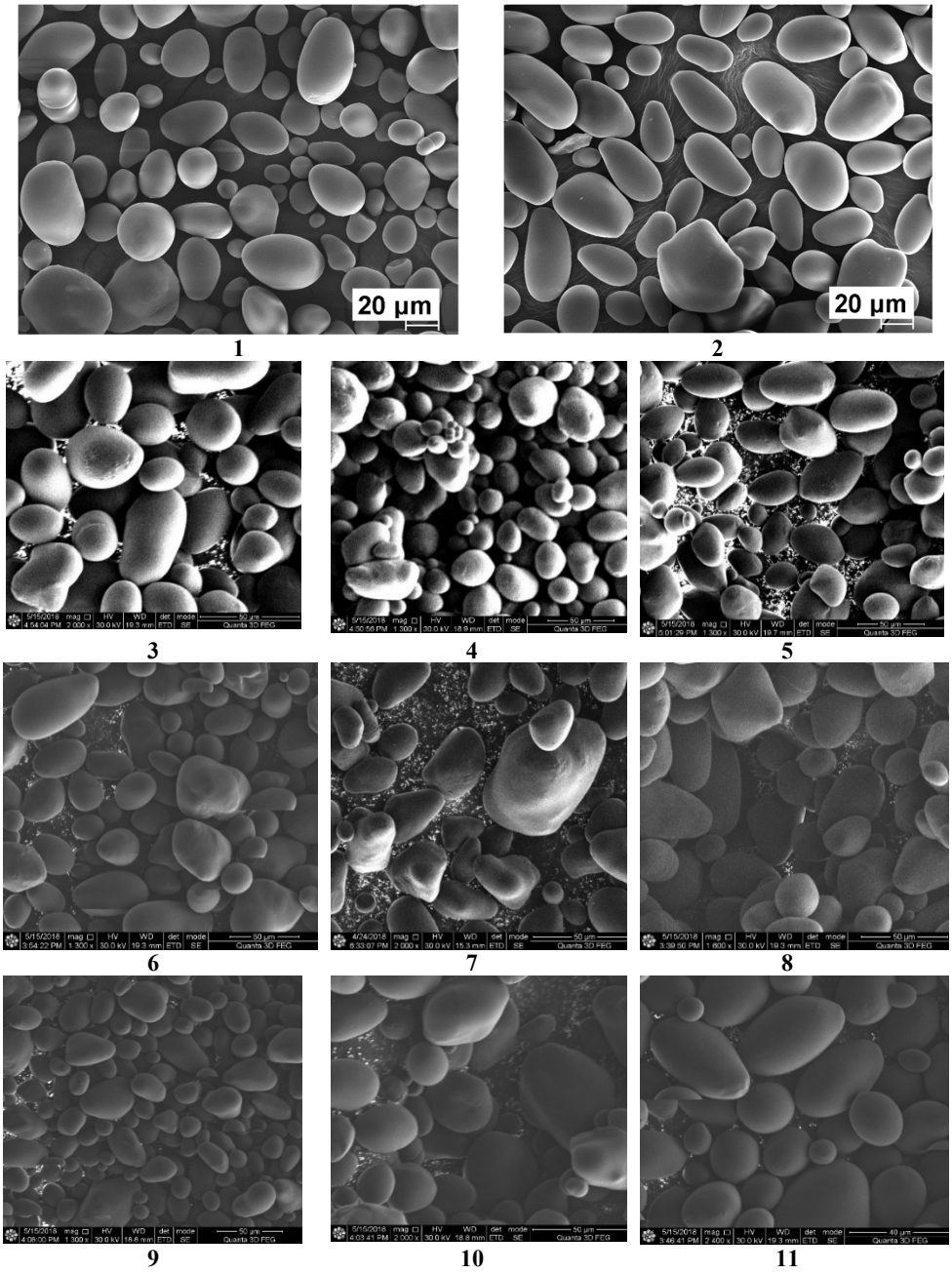


Figure 2. Scanning electron micrographs of native starch grains, isolated from Eastern and Central European potato varieties
1 – «Dzvin», 2 – «Leleka», 3 – «Bryanskiy delikatesc», 4 – «Favorit», 5 – «Grand», 6 – «Vasilek», 7 – «Velikan», 8 – «Kolobok», 9 – «Krepysh», 10 – «Meteor», 11 – «Nakra»

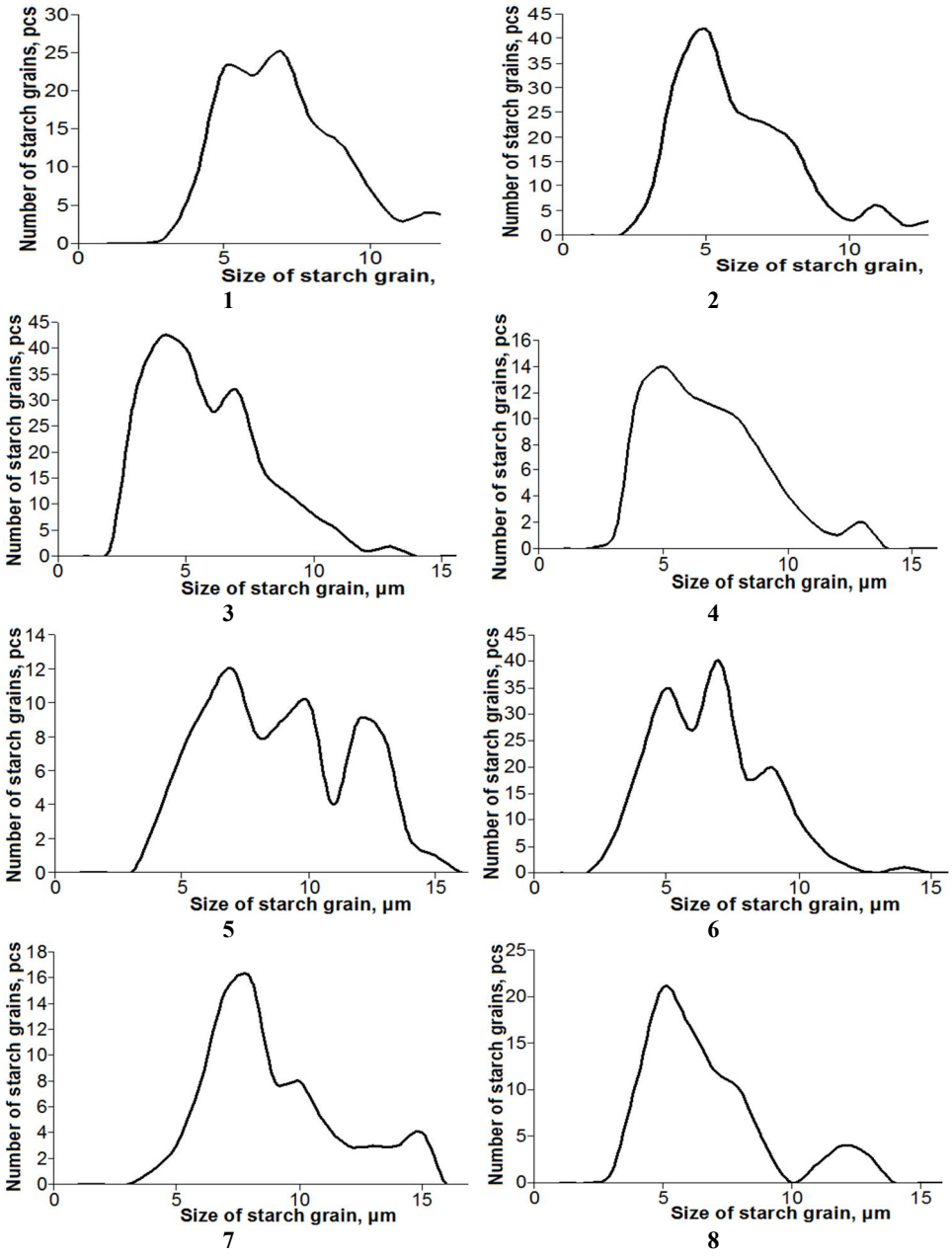
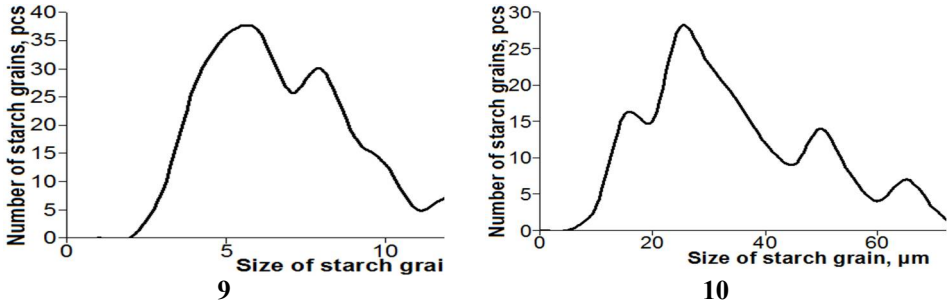
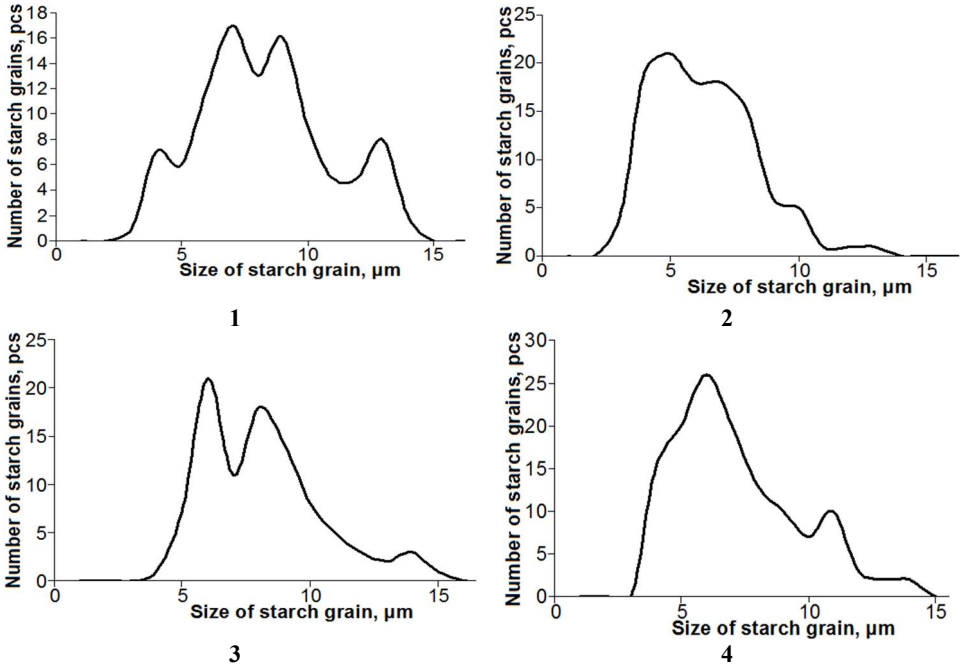


Figure 3. Granulometric analysis of grains of native starch, isolated from Eastern and Central European potato varieties:
1 – «Atlant», 2 – «Lazurit», 3 – «Lasunok», 4 – «Lileya», 5 – «Mag», 6 – «Skarb», 7 – «Suzor'ye», 8 – «Yavar»



9 10

Figure 3 (*Continuation*). Granulometric analysis of grains of native starch, isolated from Eastern and Central European potato varieties:
9 – «Uladar», 10 – Vesnyanka»



1 2

3 4

Figure 4. Granulometric analysis of grains of native starch, isolated from Eastern and Central European potato varieties:
1 – «Albatros», 2 – «Kormoran», 3 – «Kranich», 4 – «Sonata»

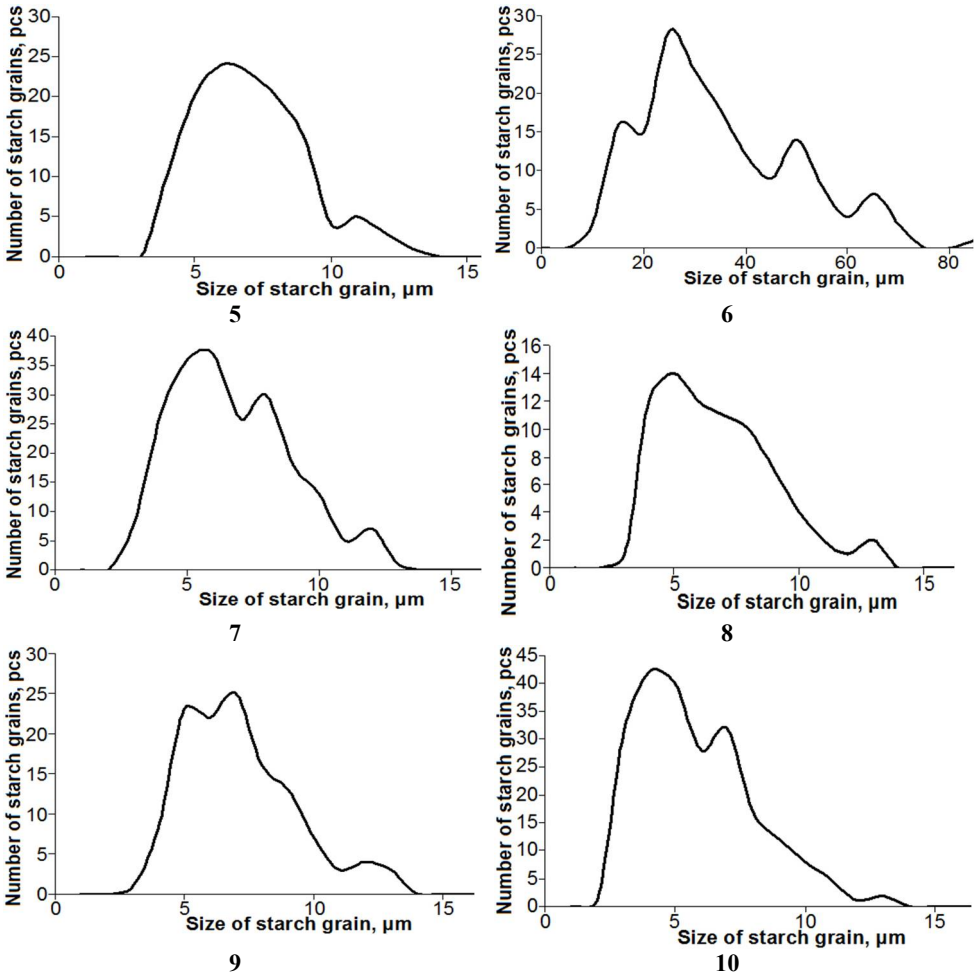


Figure 4 (*Continuation*). Granulometric analysis of grains of native starch, isolated from Eastern and Central European potato varieties:
5 – «Lazar'», 6 – «Bryanskiy delikatesc», 7 – «Favorit», 8 – «Grand», 9 – «Vasilek», 10 – «Velikan»

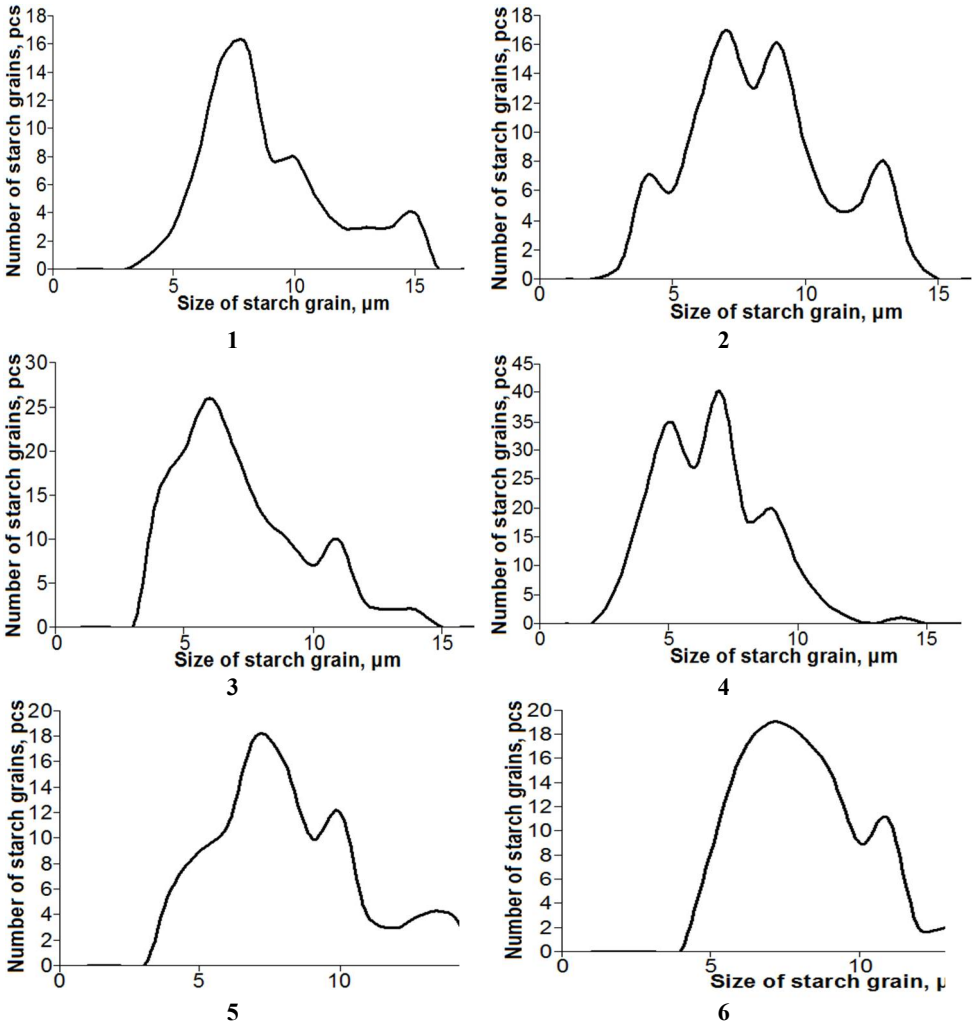


Figure 5. Granulometric analysis of grains of native starch, isolated from Eastern and Central European potato varieties
1 – «Kolobok», 2 – «Krepysh», 3 – «Meteor», 4 – «Nakra», 5 – «Dzvin», 6 – «Leleka»

Discussion of the results

Native starch is a natural polymer in which monomers (residues of α-D-glucopyranose) are bound by α-(1→4)- and α-(1→6)-glucoside bonds, forming amylose (linear polysaccharide) and amylopectin (branched polysaccharide buildings). Starch fractions (amylose and amylopectin) are compactly packed into starch grains (or granules) [1, 3, 32].

The source of starch-containing raw materials and the peculiarities of the structural organization of native starch largely determine the technological methods used for the fullest

and most gentle extraction of the seeds of native starch from the plant cell [2, 35]. To obtain native starch, it is required to prepare for processing vegetable starch-containing raw materials, destroy the plant cell, extract the native starch, wash it with clean water from associated impurities, dehydrate, dry, pack up and pack [2]. Thus, methods are known for processing potatoes for starch using a variety of technological schemes equipped with various types of processing equipment used for these purposes [34]. However, regardless of the hardware design, each of these methods includes the production stages that are common to all modern technologies for the production of potato starch: preparing potatoes for processing, grinding, extracting potato (cell) juice and pulp, cleaning starch, dehydrating it and drying [1, 8 – 10].

According to our results, as well as well-known data [1–2, 7–9], starch grains have an oval, spherical or irregular shape, their diameter varies between 0,001–0,2 mm. Starch grains are divided into simple and complex: simple grains are homogeneous formations; complex − a combination of smaller particles. The density of starch is on average 1,5 kg/m^3 [8].

Based on the analysis of the characteristics of the structure of native starch, it can be assumed that the main structural characteristic of the structure of native starch, determining its properties, is starch grain (granule) [1, 2, 34, 35]. Thus, the peculiarities of the size and shape of starch grains determine (determine) the manifestation of the following properties (characteristics) of starch [8]:

1. The amount of bound moisture (the larger the starch granule, the more bound moisture is in the starch and vice versa).
2. The temperature of gelatinization (the larger the starch granule, the lower the temperature of its gelatinization and vice versa).
3. The ratio of the starch fractions of the branched fraction of amylopectin and linear amylose (the formation of starch granules is due to the interaction of the linear sections of amylopectin with each other or with amylose).
4. Rheological characteristics of starch paste (viscosity of starch paste is due to the ratio of amylopectin and amylose starch fractions).

The temperature of gelatinization, the amount of bound moisture, the viscosity of starch paste, the ratio of starch fractions, the color of the iodine sample and other physicochemical properties determine (determine) the size and shape of the starch grains [37].

Conclusion

The morphological structure of native starch granules depends on the potato variety and can vary widely. The small granules of potato starch of the *«Lasunok»* and *«Skarb»* varieties are only 5–7 microns in size, while the large starch granules of the varieties *«Vasilek», «Nakra», «Kranich», «Vesnyanka»* reach 80–90 microns. The shape of the granules themselves is also varied: correct and irregular oval, irregular many-sided, rounded (mainly characteristic of small granules).

The experimental data presented suggest that the size and morphology of starch granules is related to the starch content in grains of different varieties of native potato starch. With a larger average size of starch grains, the starch content decreases, and with a smaller average size, the percentage of starch content increases.

References

1. Gliuk N.G., Zhushman A.I., Ladur T.A., Shtyrkova E.A. (1985), *Krakhmal i krakhmaloprodukty*, Agropromizdat, Moscow.

2. Andreev N.R. (2001), Osnovy proizvodstva nativnykh krakhmalov, *Pishchepromizdat*, Moscow.
3. Belitz H.-D., Grosch W., Schieberle P. (2009), *Food Chemistry. 4th revised and expanded edition*, Springer-Verlag, Leipzig.
4. Makella M.J., Laakso S. (2006), Studies on Oat Starch with a Celloscope: Granule Size and Distribution, *Starch/Starke*, 36, pp. 159–163.
5. Sylvia de Pater, Martien Caspers, Marijke Kottenhagen, Henk Meima, Renaldo Ter Stege, Nick de Vetten (2006), Manipulation of starch granule size distribution in potato tubers by modulation of plastid division, *Plant Biotechnology Journal*, 4, pp. 123–134.
6. Jane J-L., Kasemsuwan T., Leas S., Zobel H., Robyt J.F. (1994), Anthology of starch granule morphology by scanning electron microscopy, *Starch/Starke*, 46, pp. 121–129.
7. J.BeMiller, R. Whistler (2009), *Starch: chemistry and technology. 3th edition*, Academic Press. Burlington.
8. Pérez S., Bertoft E. (2010), The molecular structures of starch components and their contribution to the architecture of starch granules: a comprehensive review, *Starch/Starke*, 62, pp. 389–420.
9. Buleon A., Colonna P., Planchot V., Ball S. (1998), Starch granules: structure and biosynthesis, *Int. J. Biol. Macromol.*, 23, pp. 85–112.
10. A.C. Eliasson (2004), *Starch in food: structure, function and application*, Woodhead Publishing ltd, Cambridge.
11. Yonemoto P.G., Calori D.M.A., Franco C.M.L. (2007), Effect of granule size on the structural and physico- chemical characteristics of wheat starch, *Ciênc. Tecnol. Aliment.*, 27, pp. 761–771.
12. Takeda Y., Takeda C., Mizukami H., Hanashiro I. (1999), Structures of large, medium and small starch granules of barley grain, *Carbohyd. Polym.*, 38, pp. 109–114.
13. Swinkels J.J.M. (1985), Composition and Properties of commercial Native Starches, *Starch/Starke*, 37, pp. 1–5.
14. Zobel H.F. (1988), Molecules to Granules: A Comperehensive Starch Review, *Starch/Starke*, 40, pp. 44–50.
15. Dengate H., Meredith P. (1984), Variation in size distribution of starch granules from wheat grain, *J. Cereal. Sci.*, 2, pp. 83–90.
16. Stoddard F.L. (1999), Survey of starch particle-size distribution in wheat and related species, *Cereal. Chem.*, 76, pp. 145–9.
17. Lindebloom N, Chang P.R., Tyler R.T. (2004), Analytical, Biochemical and Hysiochemical Aspects of Starch Granule Size, with Emphasis on Small Granule Starches: A Review, *Starch/Starke*, 56, pp. 89–99.
18. Uarrota V.G., Amante E.R., Demiate I.M., Vieira F., Delgadillo I., Maraschin M. (2013), Physicochemical, thermal, and pasting properties of flours and starches of eight Brazilian maize landraces (*Zea mays* L.), *Food Hydrocolloid*, 30, pp. 614–624.
19. Hebeish A., El-Rafie M.H., El-Sisi F., Abdel Hafiz F., Abdel–Rahman A.A. (1994), Oxidation of maize and rice starches using potassium permanganate with various reductants, *Polym. Degrad. Stab.*, 43, pp. 363–372.
20. Halal S.L.M., Colussi R., Pinto V.Z., Bartz J., Radunz M., Carreño N.L.V., Dias, A.R.G., Zavareze E.R. (2015), Structure, morphology and functionality of acetylated and oxidised barley starches, *Food Chem*., 168, pp. 247–256.
21. Ribeiro L.S., Cordoba L.P., Colman T.A., Oliveira C.S., Andrade M.M.P., Schnitzler E. (2014), Influence of some sugars on the thermal, rheological and morphological properties of «pinhão» starch, *J.Therm. Anal. Calorim*., 117, pp. 935–942.
22. Malucelli L.C., Lacerda L.G., Carvalho Filho M.A.S., Fernández D.E.R., Demiate I.M., Oliveira C.S., Schnitzler, E. (2015), Porous waxy maize starch, *J. Therm. Anal. Calorim.*, 120, pp. 525–532.
23. Colman T.A.D., Demiate I.M., Schnitzler E. (2014), The effect of microwave radiation on some thermal, rheological and structural properties of cassava starch, *J. Therm. Anal. Calorim.*, 115, pp. 2245–2252.
24. Beninca C., Colman T.A.D., Lacerda L.G., Carvalho-Filho M.A.S., Demiate I.M., Schnitzler E. (2013), Thermal, rheological, and structural behaviors of natural and modified cassava starch granules, with hypochlorite solutions, *J. Therm. Anal. Calorim.,* 111, pp. 2217–2222.

25. Carmona-Garcia R., Sanchez-Rivera M.M., Mendez-Montealvo G., Garza-Montoya B., Bello-Perez L.A. (2009), Effect of the cross–linked reagent type on some morphological, physicochemical and functional characteristics of banana starch (*Musa paradisíaca*), *Carbohydr. Polym*., 76, pp. 117–122.
26. Joshi M., Aldred P., McKinight S., Panozzo J.F., Kasapis S., Adhikari R., Adhukari, B. (2013), Physicochemical and functional characteristics of lentil starch, *Carbohydr. Polym.*, 92, pp. 1484–1496.
27. Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1992), *Compendium of food additive specifications, vol. 1 and 2. FAO Food and Nutrition Paper 52/1,* Food and Agriculture Organization of the United Nations, Rome.
28. Galliard T. (1987), Starch: properties and potential, J. Wiley & Sons*, Chichester*.
29. Luallen T. Measuring starch in food (2004), *Starch in Food Structure, Function and Applications*, Polytechnic University of Valencia, Valensia, 1, pp. 185–207.
30. Passos M.L. (2010), *Innovations in food engineering: new techniques and products*, CRC Press, Boka Raton.
31. Copeland L., Blazek J., Salman H. et. al. (2009), Form and functionality of starch, *Food Hydrocoll*, 23, pp. 1527–1534.
32. Damodaran A.M., Parkin K.L., Fennema O.R. (2007), *Fennema's Food Chemistry. 4th edition,* CRC Press, Boka Raton.
33. Information Internet: Potato starch. Technical conditions, Available at: http://files.stroyinf.ru/Data/327/32700.pdf
34. Litvyak V.V., Butrim S.M., Moskva V.V., Kozlova L.N., (2010), Morfologicheskaya kharakteristika krakhmal'nykh granul kartofelya (Solanum tuberosum) razlichnykh sortov, *Vesci NAN Belarusi. Ser. biyal. navuk*, 3, p. 99–103.
35. Litvyak, V.V., Yurkshtovich N.K., Butrim S.M., Moskva V.V., (2013), *Atlas. Morfologiya krakhmala i krakhmaloproduktov*, Minsk: *Belaruskaya navuka*, p. 217.
36. Litvyak V.V., Ospankulova G.H., Shaimerdenova D.A., Yurkshtovich N.K., Butrim S.M., Roslyakov Yu.F., (2016), *Atlas*: *morfologiya polisakharidov*, TOO «EDIGE», Astana, p. 335.
37. Litvyak V.V., Roslyakov Yu.F., Butrim S.M., Kozlova L.N., (2013), *Krakhmal i krakhmaloprodukty: monografiya*, pod red. d-ra tehn. nauk, professora Yu.F. Roslyakova, FGBOUVPO «KubGTU», Krasnodar, p. 204.
38. Goldstein J., Yakovica H., (1978), *Prakticheskaya rastrovaya elektronnaya mikroskopiya*, M.: Mir, p. 656.
39. Goldstein J., Newbury D., Eclin P., Joy D., Fiori Ch., Lifshin F., (1984), *Prakticheskaya rastrovaya elektronnaya mikroskopiya i rentgenovskiy analiz*: *v 2-h knigah*, Mir, Moskva.
40. Goldstein J., Newbury D., Eclin P., Joy D., Fiori Ch., Lifshin F., (1984), *Rastrovaya elektronnaya mikroskopiya i rentgenovskiy microanaliz*: *v 2-h knigah: Kniga 2., Per. s angl.,* Mir, Moskva
41. Litvyak Vladimir (2018), Size and morphological features of native starch granules of different botanical origin, *Ukrainian Food Journal*, 7(4), pp. 563–576.
42. Information Internet: Potatoes. Determination of starchiness and density of potatoes, Available at: http://www.spec-kniga.ru/tehnohimicheski-kontrol/tekhnohimicheskij-kontrol-brodilnyh-proizvodstv/syre-brodilnyh-proizvodstv-kartofel.html

Extracts from physalis leaves (Physalis peruviana L.) for prospective application in medicine and cosmetics

Tanya Ivanova[1], Venelina Popova[1], Nadezhda Mazova[1], Albena Stoyanova[1], Stanka Damyanova[2]

1 – University of Food Technologies, Plovdiv, Bulgaria
2 – University of Russe, Branch Razgrad, Bulgaria

Keywords:

Physalis
Extracts
Polyphenols
Flavonoids
Triterpenes

Article history:

Received 06.09.2018
Received in revised form 28.10.2018
Accepted 28.03.2019

Corresponding author:

Venelina Popova
E-mail: vpopova2000@abv.bg

DOI:
10.24263/2304-974X-2019-8-1-5

Abstract

Introduction. The aim of research is to characterize the chemical composition of physalis leaves and the obtaining of extracts rich in bioactive compounds intended for medical and cosmetic applications.

Materials and methods. Extraction of dried physalis leaves was carried out under the following conditions: hydromodule – 1:10 (w/v), solvents – 95, 70, 50 and 30 vol.% ethanol, temperature – 20, 40and 60°C, and duration – 1, 3 and 5 h. The content of polyphenols, flavonoids and triterpenes in the leaves and in the obtained extracts was determined by HPLC.

Results and discussion. The analyzed physalis leaves from variety Plovdiv and from the bio-farm were with 8.32% and 8.79% moisture, respectively. The plant materials contained 9.62% and 10.58% tannins, respectively. Extract color varied by solvent concentration: yellow-orange (with 30% ethanol), yellow-brown (50% ethanol), green-brown (70% ethanol), and brown (95% ethanol). The experimental data and the derived equations showed that the two main factors – temperature and duration, had a strong influence on the content of extracted tannins. The optimal conditions of the process were: 5-hour extraction at a temperature of 60°C, with 30 and 50% ethanol for the leaves from Plovdiv genotype, and with 50 and 70% ethanol – for the bio-farm genotype. Twelve phenolic acids were identified in the leaves and extracts from Plovdiv genotype and 10 – in those from the bio-farm genotype. Rutin was the dominant flavonoid in the leaves and extracts from both genotypes. The major triterpene in the leaves and in the extracts was oleanolic acid, followed by betulin.

The extracts from physalis leaves are rich in bioactive substances (phenolic acids, flavonoids and triterpenes), and have the prospective for possible application in medicinal and cosmetic products.

Conclusions. This study provides for the first time data about the optimal conditions for the extraction of *Physalis peruviana* leaves, as well as information about the content of certain biologically active components in the leaves and in the obtained extracts. These are the first results reported about physalis genotypes grown in Bulgaria.

Introduction

The genus *Physalis* (family Solanaceae) comprises nearly 120 wild and cultivated species common to many countries around the world [1]. Among them, the most widely distributed and commercially important is *Physalis peruviana* L. [2], also known as Cape gooseberry, goldenberry, Inca berry, or simply physalis. The edible part of the plant is the fruit – ovoid in shape, cherry-sized, yellow-to-orange-fleshed and juicy. It is described as tomato-like in flavor and appearance, though the taste (sweet and sour) is much richer with a hint of tropical luxuriance [3]. The fruit is hidden in an inflated calyx or fruit basket, protecting it from insects, birds, diseases and harsh climate conditions [2, 3].

While physalis fruit, along with many other subtropical berries [4], has been long acknowledged as a source of valuable bioactive and nutritional substances, there is scarce data about the chemical composition of physalis leaves or leaf-derived bioactive products.

In a study by Ertürk et al. [5], the total phenolic and flavonoid content of an ethanol extract from physalis leaves were estimated to 1.368 mg GA/g and 0.635 mg QE/g, respectively. Wu et. al. [6] obtained ethanolic, aqueous and supercritical CO_2 extracts from physalis leaves. Total flavonoid and phenol contents were 37.39 mg/g and 18.57 mg/g, respectively, in the aqueous extract, and 94.97 mg/g and 85.81 mg/g – in the ethanolic extract. The presence of phytochemicals with different biological activities (alkaloids, saponins, tannins, steroids, terpenoids, and flavonoids) was detected in aqueous extracts from physalis leaves, although it was not quantified [7]. Cirigliano et al. [8] studied crude physalis extracts and the data indicated that the extract and its two major withanolides (withanolide E and 4-β-hydroxy withanolide E) could be used to develop baits to control the fruit fly *Ceratitis capitata*. An extract of physalis leaves showed antibiotic activity against Gram-positive bacteria from genus *Staphylococcus* [9].

In folk medicine, the juice of physalis leaves has been used in the treatment of worm and bowel complaints, while heated leaves are applied as a poultice [1, 10, 20].

Although physalis is not a popular crop in Bulgaria, an original local variety of *P. peruviana* named Plovdiv has been selected and officially recognized by the national authorities in 2006 [11].

The above-presented brief review on available data clearly identifies the lack of sufficient scientific evidence about the chemical composition of physalis leaves and about the obtaining of extracts rich in bioactive compounds intended for medical and cosmetic applications, which is set as the objective of current study.

Materials and methods

Plant Material

Leaves of two genotypes of cultivated phyisalis (*Physalis peruviana* L.) were investigated. The first genotype was the only local Bulgarian variety of physalis named Plovdiv and was grown in the region of Plovdiv city, located in Central South Bulgaria [11]. The second genotype was grown and provided by a certified bio-farm (Versol Ltd.), located in Lik village, North-West Bulgaria. Fresh leaves were picked in the period September-October, and then were air-dried in the shade. Dried leaves were isolated in plastic bags and stored at a temperature of 5-8°C until processing.

Chemicals

HPLC grade methanol and acetonitrile, as well as phenolic acid and flavonoid standards were purchased by Sigma (Sigma-Aldrich Chemie GmbH, Germany).

Chemical analyses

The moisture content of the leaves was determined by drying (at 105°C) to constant weight [12], and all data in the study were calculated on a DW basis. The content of tannins was determined by titration of hot water extract with potassium permanganate solution using indigo carmine as indicator [12]. The HPLC analysis of polyphenols, flavonoids and triterpenes in the plant material and in the extracts was according to Marchev et al. [13, 14].

Obtaining of extracts

Extraction was carried out in laboratory conditions, in a batch static mode, at a ratio of raw material to solvent = 1:10 (w/v). Four solvents were used for the extraction, representing different ethanol concentrations: 95, 70, 50 and 30 vol.%. The solvent, its concentrations and the hydromodule were chosen on the basis of authors' own published data. The influence of the technological factors – temperature and duration of extraction, was examined by mathematical modeling of the experiment (Table 1).

Table 1

Mathematical modeling of the experiment

Variant	Duration, (x_1), h	Temperature, (x_2), °C
1	20	1
2	20	3
3	20	5
4	20	7
5	40	1
6	40	3
7	40	5
8	40	7
9	60	1
10	60	3
11	60	5
12	60	7

Process effectiveness was evaluated in terms of the quantity of extracted tannins [15].

Statistical analysis

Based on experimental data, the equations of tannin extraction were derived, and their coefficients were verified for significance by Student's test and for adequacy – by Fisher's test. All experiments were for performed at least three times. Statistical significance was assessed by Student's-test or ANOVA. Differences between means were considered statistically significant if $p > 0.05$. The figures were created with MicroCal™ Origin 9.1 software.

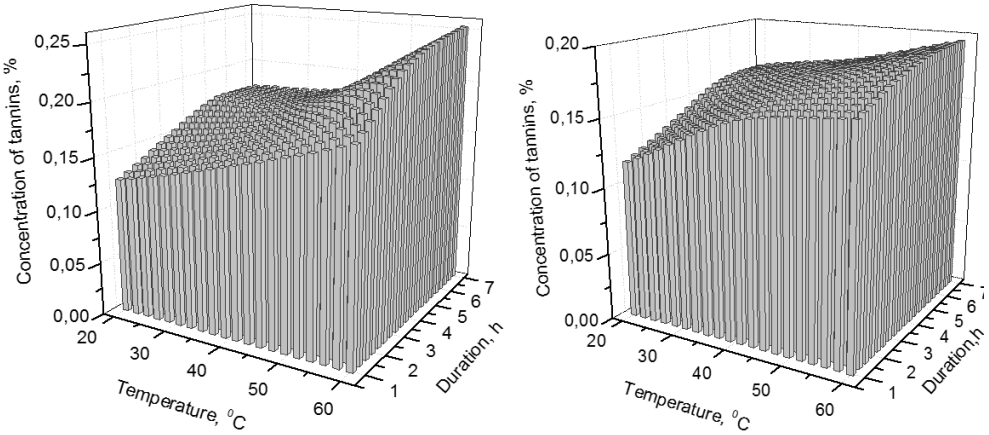
Results and discussion

Obtaining and characteristics of ethanol extracts from physalis leaves

The analyzed physalis leaves from variety Plovdiv and from the bio-farm were with 8.32% and 8.79% moisture level, respectively. The plant materials contained 9.62% and 10.58% tannins, respectively for the two genotypes.

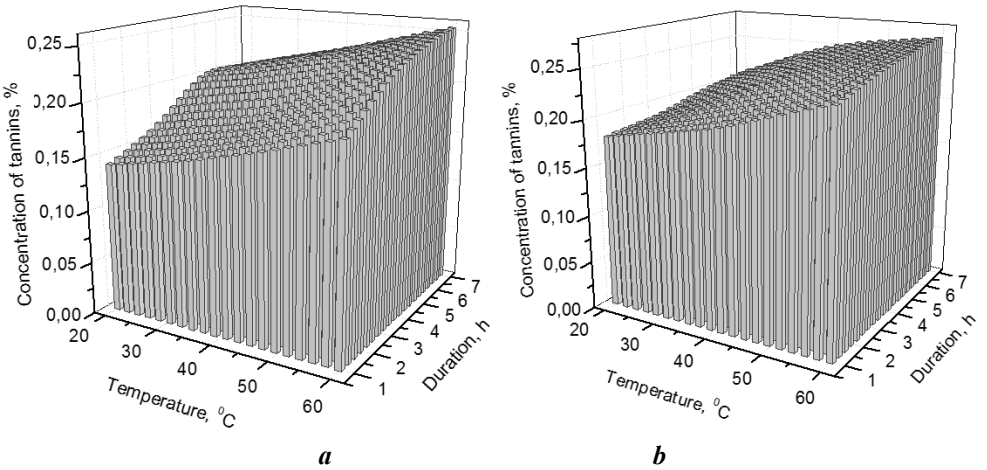
The obtained ethanol extracts from physalis leaves were liquids, and their color varied by solvent concentration: yellow-orange (with 30% ethanol), yellow-brown (50% ethanol), green-brown (70% ethanol), and brown (95% ethanol).

Figures 1– 4 present the results about the tannin content in the extracts from the two physalis genotypes, according to the scheme of the experiments that have been carried out. The results show that the elevation of temperature from 20 to 60 °C, and the extension of process duration from 1 to 5 h, both increase the content of extracted tannins, independent of ethanol concentration or the origin of the leaves. Extraction for 7 h resulted in an insignificant increase in the amount of extracted tannins.



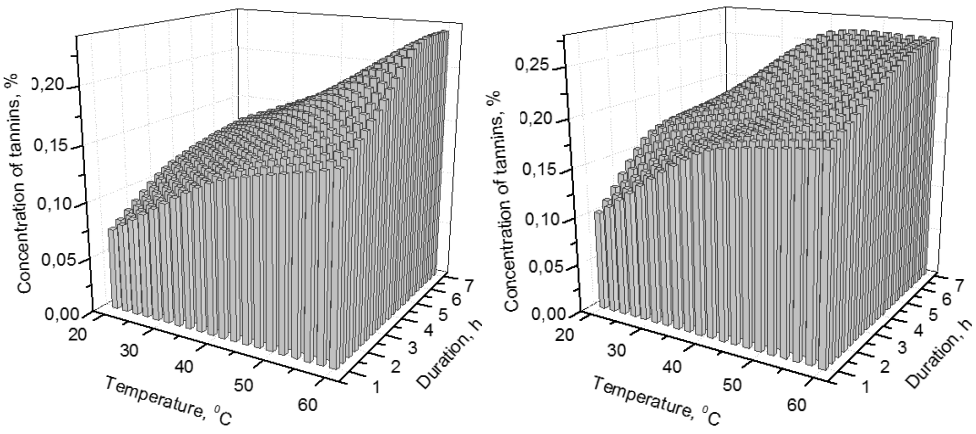
a *b*

Figure 1. Content of tannins (%) in extracts from physalis leaves with 30% ethanol: a – Plovdiv genotype; b – bio-farm genotype.



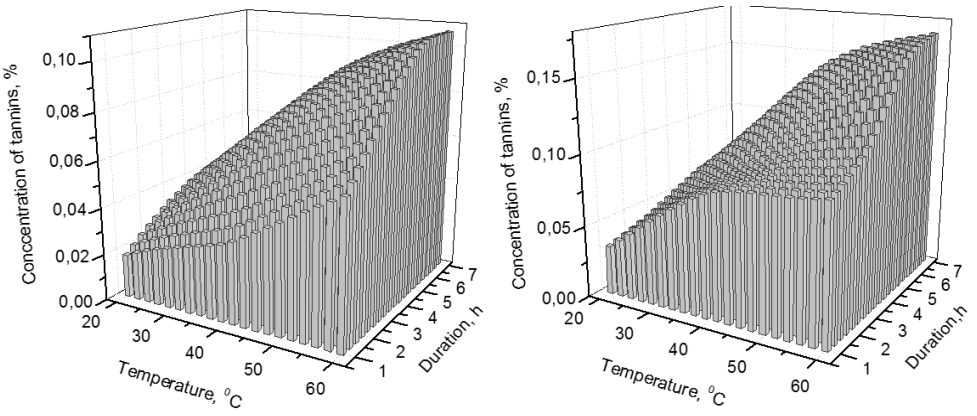
a *b*

Figure 2. Content of tannins (%) in extracts from physalis leaves with 50% ethanol: a – Plovdiv genotype; b – bio-farm genotype.



a *b*

Figure 3. Content of tannins (%) in extracts from physalis leaves with 70% ethanol: a – Plovdiv genotype; b – bio-farm genotype.



a *b*

Figure 4. Content of tannins (%) in extracts from physalis leaves with 95% ethanol: a – Plovdiv genotype; b – bio-farm genotype.

Reasonably, the two main factors of the extraction process – duration (x_1) and temperature (x_2), had a strong influence on the content of extracted tannins. The relation between tannin content (y,%.) and process parameters (x_1, h and x_2, °C) is confirmed by the obtained equations, which were verified as adequate and with significant coefficients:

Solvent	Equation
	Leaves from Plovdiv genotype
30% ethanol	$y=0.177 + 0.036x_1 + 0.023x_2 + 0.002x_1x_2 + 0.015x_1^2 - 0.006x_2^2$
50% ethanol	$y = 0.190 + 0.027x_1 + 0.028x_2 - 0.003x_1x_2 + 0.005x_1^2 + 0.002x_2^2$
70% ethanol	$y = 0.151 + 0.047x_1 + 0.026x_2 + 0.006x_1x_2 - 0.003x_1^2 - 0.001x_2^2$
95% ethanol	$y = 0.063 + 0.026x_1 + 0.019x_2 + 0.006x_1x_2 - 0.001x_1^2 - 0.005x_2^2$
	Leaves from the bio-farm genotype
30% ethanol	$y = 0.167 + 0.022x_1 + 0.014x_2 - 0.004x_1x_2 - 0.006x_1^2 - 0.002x_2^2$
50% ethanol	$y = 0.225 + 0.032x_1 + 0.013x_2 + 0.001x_1x_2 - 0.003x_1^2 - 0.006x_2^2$
70% ethanol	$y = 0.204 + 0.042x_1 + 0.029x_2 - 0.005x_1x_2 - 0.012x_1^2 - 0.006x_2^2$
95% ethanol	$y = 0.088 + 0.038x_1 + 0.025x_2 + 0.011x_1x_2 - 0.008x_1^2 + 0.007x_2^2$

The highest concentration of tannins in the extracts was obtained under the following conditions: 5-hour extraction at a temperature of 60°C, with 30 and 50% ethanol for the leaves from variety Plovdiv, and with 50 and 70% ethanol – for the leaves from the bio-farm. The lowest concentration of tannins was obtained with 95% ethanol, regardless of leaves' origin. The differences in tannin concentration in the extracts obtained with the four ethanol concentrations reflect the significant influence of the solvent, and can be explained with the different selectivity of water-ethanol mixtures used in the extraction of bioactive molecules. The same pattern of the influence of process temperature, duration and ethanol concentration on the content of tannins in liquid extracts has been reported in other studies on essential oil-bearing and medicinal plants, such as tobacco [15], mint [16], thyme [17], and rosemary [18].

Polyphenols in physalis leaves and extracts

The results about the content of phenolic acids and flavonoids in the leaves and extracts of *P. peruviana* are shown in Tables 2 and 3.

Data show that in the leaves and extracts from Plovdiv genotype were identified 12 phenolic acids, and the dominant was rosmarinic acid, followed by salicylic, sinapic and ferulic acids. In the leaves and extracts from the bio-farm genotype were identified 10 phenolic acids, among which dominated protocatechuic acid, followed by chlorogenic, sinapic and vanillic acids. In the leaves and extracts from both genotypes, rutin was the dominant flavonoid, which corresponded to the results obtained by Ertürk et. al. [5].

On a genotype basis, data about flavonoids showed higher levels of the flavon glycoside hesperetin in the leaves of variety Plovdiv, but lower – in the extracts, compared, respectively, to the leaves and extracts from the bio-farm genotype. The concentration of the flavon glycoside myricetin in the leaves and extracts from the bio-farm genotype were characteristically higher.

The content of salicylic, caffeic, p-coumaric, sinapic, ferulic and rosmarinic acids in the samples from Plovdiv genotype was significantly higher than the respective content in the leaves and extracts from the second genotype. Gallic acid, rosmarinic acid, luteolin, kaempherol, and apigenin were not identified in the leaves and extracts from the bio-farm genotype. Despite that, the contents of chlorogenic acid and myricetin were higher than those in the leaves and extracts from variety Plovdiv.

Table 2

Content of polyphenols in leaves and extracts from Plovdiv genotype (*P. peruviana*)

Compounds	Leaves, µg/g DW	Ethanol extracts, µg/mL			
		30%.	50%.	70%.	95%.
Gallic acid	-[a)]	53.91±0.51	16.75±0.15	-	-
Protocatechuic acid	142.77±1.39	-	39.39±0.38	7.33±0.07	2.34±0.02
Salicylic acid	1161.62±10.54	26.21±0.25	254.77±2.50	194.10±1.90	1.20±0.01
Chlorogenic acid	193.67±1.89	17.40±0.16	19.98±0.18	31.32±0.30	1.61±0.01
Vanillic acid	236.47±2.30	19.92±0.18	62.85±0.60	51.27±0.50	17.08±0.16
Caffeic acid	170.61±1.69	34.49±0.33	45.48±0.44	33.30±0.30	16.82±0.15
Syringic acid	63.05±0.62	16.36±0.15	18.21±0.17	9.74±0.09	1.57±0.01
p-Coumaric acid	665.00±6.62	119.56±1.10	136.63±1.34	125.92±1.21	58.40±0.51
Sinapic acid	776.05±7.74	183.59±1.78	203.10±2.00	159.07±1.54	65.01±0.61
Ferulic acid	722.23±7.20	113.62±1.10	120.33±1.19	97.83±0.93	54.59±0.50
Cinnamic acid	24.63±0.20	18.79±0.17	6.79±0.06	1.36±0.01	0.52±0.00
Rosmarinic acid	2316.41±22.11	322.03±3.18	508.56±5.00	529.27±5.20	238.49±2.30
Myricetin	17.94±0.17	5.12±0.04	5.70±0.05	4.99±0.04	-
Hesperetin	40.85±0.39	8.77±0.08	10.01±0.09	12.77±0.11	3.22±0.03
Quercetin	6.87±0.06	2.61±0.02	2.54±0.02	2.44±0.02	2.43±0.02
Luteolin	1.44±0.01	1.04±0.01	0.62±0.00	0.38±0.00	0.37±0.00
Kaempferol	3.62±0.03	1.98±0.18	1.48±0.01	1.57±1.50	1.40±0.01
Apigenin	31.09±0.30	tr[b)]	tr	tr	tr
Rutin	4996.37±48.50	738.54±7.25	999.09±9.91	1040.12±10.00	395.68±3.80
Hyperoside	226.96±2.20	-	34.41±0.31	36.11±0.32	15.02±0.14

[a)] not identified; [b)] trace amount

These results reveal the presence of various classes of bioactive polyphenols in the leaves and in the leaf extracts obtained from the two genotypes of physalis grown in Bulgaria. The observed differences in the profile and content distribution of polyphenols are attributed to the impact of plant genotype. The results agreed well with previous findings about polyphenol content in *Physalis* leaves, for example: total phenolic concentration of 30.9-129.2 mg/g [19] and 129 mg/g [26]; total flavonoid concentration of 37.39-226.19 mg/g [6] and 23.036 mg/g [26]. Studies on the determination of flavonoid and phenolic acid content in different *Physalis* species has been focused exclusively on the fruits, but current results reveal that leaves' potential of accumulating these secondary metabolites is even higher than that of the fruits [26]. Expectedly, the numerical results for the polyphenol content varied from data found in the scientific literature for other plant materials [22, 23, 24, 25]. The established differences in terms of leaf chemical chemical composition between the present investigation and the reported data may be due to the environmental conditions under which the plants were grown, as well as to the impact of species, origin and extraction technique.

Table 3

Content of polyphenols in leaves and extracts from bio-farm genotype (*P. peruviana*)

Compounds	Leaves, µg/g DW	Ethanol extracts, µg/mL			
		30%.	50%.	70%.	95%.
Gallic acid	-[a)]	-	-	-	-
Protocatechuic acid	933.22±9.28	-	-	-	-
Salicylic acid	-	47.94±0.45	49.45±0.45	67.64±0.65	52.26±0.51
Chlorogenic acid	324.33±3.20	28.65±0.26	21.29±0.20	25.83±0.24	17.64±0.16
Vanillic acid	248.56±2.41	3.68±0.03	68.62±0.65	67.55±0.36	40.74±0.38
Caffeic acid	44.90±0.40	13.01±0.11	31.60±0.30	37.75±0.36	25.16±0.22
Syringic acid	39.25±0.38	1.13±0.01	8.47±0.08	6.01±0.05	2.60±0.02
p-Coumaric acid	79.65±0.75	15.13±0.104	13.16±0.11	11.01±0.10	6.14±.05
Sinapic acid	282.59±2.79	39.06±0.37	181.69±1.78	13.18±0.11	5.55±0.04
Ferulic acid	108.89±1.00	21.71±0.20	27.22±0.25	45.32±0.43	15.26±0.14
Cinnamic acid	8.45±0.08	0.84±0.00	1.77±0.01	3.29±0.03	1.94±0.01
Rosmarinic acid	-	-	-	-	-
Myricetin	142.09±1.40	33.67±0.31	32.27±0.30	36.50±0.33	33.31±0.30
Hesperetin	32.58±0.30	49.42±0.47	24.52±0.21	14.31±0.12	15.65±0.14
Quercetin	27.17±0.25	16.32±0.15	-	-	-
Luteolin	-	-	-	-	-
Kaempferol	-	-	-	-	-
Apigenin	-	-	-	-	-
Rutin	2953.44±2.90	272.04±2.70	431.82±4.30	537.00±5.30	333.79±3.27
Hyperoside	209.11±0.19	37.28±0.18	28.77±0.09	50.53±0.33	28.48±0.09

[a)] not identified

Triterpenes in physalis leaves and extracts

The results about the content of triterpenes in the leaves and extracts of *P. peruviana* are shown in Tables 4 and 5.

Table 4

Content of triterpenes in leaves and extracts from Plovdiv genotype *(P. peruviana)*

Compounds	Leaves, µg/g DW	Ethanol extracts, µg/mL			
		30%.	50%.	70%.	95%.
Betulin	105.83±1.00	-[a)]	-	87.24±0.86	73.98±0.72
Betulinic acid	42.36±0.40	-	-	67.41±0.66	35.04±0.34
Oleanolic acid	264.90±2.60	46.98±0.45	70.01±0.69	86.97±0.85	31.10±0.30
Ursolic acid	-	30.77±0.29	60.73±0.60	58.69±0.57	8.18±0.07

[a)] not identified

Table 5

Content of triterpenes in leaves and extracts from bio-farm genotype *(P. peruviana)*

Compounds	Leaves, μg/g DW	Ethanol extracts, μg/mL			
		30%.	50%.	70%.	95%.
Betulin	- [a)]	122.21±1.20	124.36±1.21	201.91±19.97	394.44±38.00
Betulinic acid	-	-	-	-	-
Oleanolic acid	889.83±8.71	8.15±0.08	208.93±19.89	200.87±19.54	176.52±1.68
Ursolic acid	-	-	-	-	-

[a)] not identified

Three triterpenes were identified in the leaves of Plovdiv genotype, and the dominant were oleanolic acid (264.9 μg/mg) and betulin (105.83 μg/mg). In the second genotype, only oleanolic acid was identified, but in a significantly (nearly four-time) higher concentration (889.83 μg/mg). In total, four triterpenes were identified in the extracts obtained from the leaves of Plovdiv genotype, and there was a clear differentiation between the extracts (30 and 50% ethanol vs. 70 and 95% ethanol). In the extracts obtained with the lower solvent concentrations only oleanolic and ursolic acids were identified (total content 77.75 and 130.74 μg/mL, respectively). The extracts of the second sub-group were considerably richer in triterpenoids (with a total of 300.31 and 148.30 μg/mL, respectively), and the dominant were betulin, betulinic acid and oleanolic acid. In the extracts obtained from the leaves of the bio-farm genotype only two triterpenes were identified – oleanolic acid and betulin, but in higher concentrations (three-to-four times higher). Data from the study suggest that, in terms of triterpenoid content, the more suitable solvents would be 70 and 95% ethanol.

Our results clearly differentiate between the extracts on a triterpene basis, and the differences reflect the impact of plant genotype and solvent selectivity. With regard to data from previous research on triterpenoid concentration in physalis leaves and leaf extracts, it is even harder to make comparisons, than in the case of polyphenols. To the best of our knowledge the triterpenoid content of physalis leaves, and especially – of genotypes grown in Bulgaria, has not been determined elsewhere. Similar to the observations above, current results about the triterpene content in physalis leaves and ethanol leaf extracts characterize the species as distinctive form other plants, regarding literature data [24, 25]. The differences in the triterpene composition between the present investigation and the reported data may be attributed to environmental conditions, plant material origin and other influencing factors.

Conclusion

To the best of our knowledge, data achieved by this study provide for the first time the optimal conditions for the extraction procedure of *Physalis peruviana* L. leaves, as well as information about the content of certain biologically active components in the leaves and in the obtained extracts. These are the first results reported about physalis genotypes grown in

Bulgaria. The extracts contained phenolic acids, flavonoids and triterpenes, and have the prospective for possible application in different medicinal and cosmetic products, but additional investigations are undoubtedly required.

Acknowledgements. The authors acknowledge the support by the Science Fund of the University of Food Technologies, Plovdiv, Bulgaria (project 04/18-N). The authors are most thankful to Prof. Nikolay Panayotov (Agricultural University, Plovdiv) and to Mr. Miroslav Peshovsky (Versol bio-farm, Lik village) for providing the plant material.

References

1. Sharma N., Bano A., Dhaliwal H., Sharma V. (2015), Perspectives and possibilities of Indian species of genus *Physalis* (L.) – A comprehensive review, *Eur. J. Pharm. Med. Res.,* 2, pp. 326–353.
2. Puente L., Pinto–Munoz C., Castro E., Cortes M. (2011), *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review, *Food Res. Int.*, 44, pp. 1733–1740.
3. Ramadan M., El–Ghorab A., Ghanem K. (2015), Volatile compounds, antioxidants, and anticancer activities of cape gooseberry fruits (*Physalis peruviana* L.): an in vitro study, *J. Arab Soc. Med. Res.,* 10, pp. 56–64.
4. Kalugina I., Telegenko L., Kalugina Y., Kyselov S. (2017), The nutritional value of desserts with the addition of Gooseberry family raw materials from the Northern Black Sea Region, *Ukrainian Food Journal*, 6(3), pp. 459– 69.
5. Ertürk O., Colayvaz M., Can Z., Karaman Ü., Korkmaz K. (2017), Antioxidant, antimicrobial activities and phenolic and chemical contents of *Physalis peruviana* L. from Trabzon, Turkey, *Indian J. Pharm. Educ. Res.*, 51(3), pp. 213–216.
6. Wu S., Chang S., Lin D., Wang S., Hou F., Ng L. (2009), Supercritical carbon dioxide extract of *Physalis peruviana* induced cell cycle arrest and apoptosis in human lung cancer H661 cells, *Food Chem. Toxicol.*, 47, pp. 1132–1138.
7. Maobe M.A.G., Gatebe E., Gitu L., Rotich H. (2013), Preliminary phytochemical screening of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya, *Eur. J. Appl. Sci.*, 5(1), pp. 1–6.
8. Cirigliano A., Colamarino I., Mareggiani G., Bado S. (2008), Biological effects of *Physalis peruviana* L. (Solanaceae) crude extracts and its major withanolides on *Certitis capitate* Wiedemann (Diptera: Tephritidae), *Bol. San. Veg. Plagas*, 34, pp. 509–515.
9. Zhang Y., Deng G., Xu X., Wu S., Li S., Li H. (2013), Chemical components and bioactivities of cape gooseberry (*Physalis peruviana*), *Int. J. Food Nutr. Saf.*, 3(1), pp. 15–24.
10. Chopra R.N., Nayar S.L., Chopra I.C. (1986), *Glossary of Indian Medicinal Plants.* Council of Scientific and Industrial Research, New Delhi, India.
11. Panayotov N. (2009), Plovdiv – the first Bulgarian variety of physalis (*Physalis peruviana* L.*), Agr. Sci*., 1(1), pp. 9–12 (in Bulgarian).
12. *Russian Pharmacopoeia*, 11th edition (1990), Medicina, Moscow (in Russian).
13. Marchev A., Georgiev V., Badjakov I., Kondakova V., Nikolova M., Pavlov A. (2011), Triterpenes production by rhizogenic callus of *Salvia scabiosifolia* Lam. obtained via

Agrobacterium rhizogenes mediated genetic transformation, *Biotechnol. Biotechnol. Equip.*, 25, pp. 30–33.
14. Marchev A., Georgiev V., Ivanov I., Badjakov I., Pavlov A. (2011), Two–phase temporary immersion system for *Agrobacterium rhizogenes* genetic transformation of sage *(Salvia tomentosa* Mill.), *Biotechnol. Lett.*, 33, pp. 1873–1878.
15. Popova V. (2015), *Obtaining and analysis of aromatic extraction products for expanding the usability of tobacco (Nicotiana tabacum* L.), DSc Dissertation, University of Food Technologies, Plovdiv, Bulgaria (in Bulgarian).
16. Balinova-Cvetkova A., Stojanova A. (1999), Production of plant extracts for cosmetic applications. Part 2. Peppermint (*Mentha piperita* L.), *Pol. J. Cosmetol.*, 2(1), pp. 57–60.
17. Damianova S., Tasheva S., Stoyanova A., Damianov D. (2008), Investigation of extracts from thyme (*Thymus vulgaris* L.) for application in cosmetics, *J. Essent. Oil Bear. Pl.*, 11(5), pp. 443–450.
18. Damianova S., Tasheva S., Stoyanova A., Damianov D. (2010), Investigation of extracts from rosemary (*Rosmarinus officinalis* L.) for application in cosmetics, *J. Essent. Oil Bear. Pl.,* 13(1), pp. 1–11.
19. Wang S.Y., Lin H.S. (2000), Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage, *J. Agric. Food Chem.*, 48, pp. 140–146.
20. Gręboszk-Krawczyk, M. (2018), The Impact of Nostalgia on the Brand Equity in Economy with Post-Communist Background.Economics and Sociology, 11(2), pp. 216-228, DOI:10.14254/2071-789X.2018/11-2/15
21. Cuvelier M.E., Richard H., Berset C. (1996), Antioxidant and phenolic composition of pilot-plant and commercial extracts of sage and rosemary, *J. Am. Oil Chem. Soc.*, 73, pp. 645.
22. Baydar H., Özkan G., Erbaş S., Altındal D. (2009), Yield, chemical composition and antioxidant properties of extracts and essential oils of sage and rosemary depending on seasonal variations, *Acta Horticulturae*, 826, pp.383–389.
23. Pop A.-M., Tofană M., Socaci S., Nagy M., Fărcaş A., Borş M., Salanţă L., Feier D., Vârva L. (2014), Comparative study regarding the chemical composition of essential oils of some *Salvia* species, *Hop and Medicinal Plants*, 42(1-2), pp.79–91.
24. Razboršek M., Voneina D., Doleček V., Vončina E. (2007), Determination of major phenolic acids, phenolic diterpenes and triterpenes in rosemary (*Rosmarinus officinalis* L.) by gas chromatography and mass spectrometry, *Acta Chimica Slovenica*, 54, pp. 60–67.
25. Jäger S., Trojan H., Kopp T., Laszczyk M., Scheffler A. (2009), Pentacyclic triterpene distribution in various plants – rich sources for a new group of multipotent plant extracts, *Molecules*, 14, pp. 2016–2031.
26. Medina-Medrano J. R., Almaraz-Abarca N., González-Elizondo M. S., Uribe-Soto J. N., González-Valdez L. S., Herrera-Arrieta Y. (2015), Phenolic constituents and antioxidant properties of five wild species of *Physalis* (Solanaceae), *Botanical studies*, 56(1), pp. 24.

Structure activity relationship of tautomers of curcumin: a review

Sameera A. Rege, Megha Arya, Shamim A. Momin

Institute of Chemical Technology, Mumbai, India.

Keywords:

Curcumin
Methylene
Degradation
Nucleophilic
Radical

Article history:

Received 25.08.2018
Received in revised form 21.12.2018
Accepted 28.03.2019

Corresponding author:

Sameera A. Rege
E-mail: sameerarege@gmail.com

DOI:
10.24263/2304-974X-2019-8-1-6

Abstract

Introduction. The aim of this review is to focus on the unique chemistry of curcumin to explain its dissimilar behaviour in different mediums.

Materials and methods. The papers regarding structure-activity relationship studies of curcumin as antioxidant or pro-oxidant were examined. This review summarizes the achievements made in this field since 1980.

Results and discussion. Curcumin is the principal curcuminoid found in turmeric and is generally considered as the most active constituent of turmeric compared to other curcuminoids. Enormous research has been carried out to explain the beneficial activities of curcumin. It has been the centre of attraction for potential treatment of an array of diseases such as cancer, Alzheimer, diabetes, allergies, arthritis and other chronic illnesses. It also possesses antioxidant activity at lower concentration. In contrast, the pro-oxidant effect has also been reported.

The behaviour of curcumin to act as antioxidant or pro-oxidant depends on its structural form. Curcumin exists in two tautomeric forms, keto and enol. In keto form, curcumin exerts antioxidant activity. The enol form is prone to degradation. Hence, it is essential to maintain curcumin in keto form. In polar and acidic medium, curcumin exists in keto form whereas in non-polar and basic medium, it undergoes degradation. The mechanisms of degradation of curcumin under different mediums are discussed. Under basic conditions, nucleophilic attack of hydroxyl group is involved and under non-polar conditions, free radical mechanism is involved. Degradation under basic conditions leads to complete breaking of the molecule while under non-polar conditions, it proceeds via peroxide intermediate formation, clarifying the pro-oxidant effect of curcumin. In either of the cases, vanillin is the degradation product besides other degradation products.

The discussion is further extended for the other two curcuminoids, viz. demethoxycurcumin and bisdemethoxycurcumin as well. The antioxidant activity of curcumin is the highest whereas bisdemethoxycurcumin exhibits least antioxidant activity amongst curcuminoids. However, the rate of degradation of curcumin is also maximum amongst curcuminoids followed by demethoxycurcumin and bisdemethoxycurcumin. This reveals that the electron donating methoxy group influences the activity of curcuminoids.

Thus, structure of a constituent is responsible for its activity.

Conclusion. The importance of a particular medium to achieve the beneficial activities of curcumin is confirmed.

Introduction

Curcuminoids are the major polyphenolic compounds found in turmeric rhizome. The curcuminoids include curcumin (the main bioactive component), demethoxycurcumin and bisdemethoxycurcumin [1, 2, 3]. The unique structure of curcumin, which has the phenolic hydroxyl groups, heptadiene chain and diketone moiety [4, 5, 6], is responsible for all the therapeutic activities of curcumin such as anti-inflammatory, antitumor, anticancer, anti-HIV, antibacterial, antidiabetic [7, 8, 9, 10, 11, 12, 13]. It is used as an antioxidant [9], a wound healing agent [14, 15, 16] and to prevent Alzheimer disease [17, 18]. Recently, it has also been reported as an antidepressant agent [19]. However, the pro-oxidant effect of curcumin is also observed [20]. This review emphasizes the fundamental of the stabilization and degradation of curcumin with the help of existing theories [21, 22], explaining its dissimilar behaviour.

Materials and methods

Review is constructed on the basis of previously available research articles.

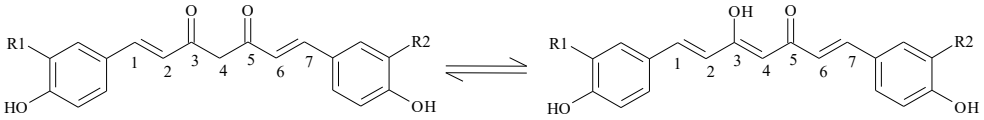
Result and discussion

Structure of keto and enol tautomers

The keto enol tautomerism in curcumin is because of the presence of carbonyl groups on the carbon number 3 and 5 in heptadiene ring [23, 24, 25, 26] (Figure 1). The stabilization of enol tautomer with respect to the keto tautomer is due to the conjugation of the carbonyl double bond with the enol double bond and a pi orbital system, i.e., phenyl group in conjugation with the conjugated C=C double bonds [27]. The enol tautomer is characterized by the formation of strong intramolecular hydrogen bonding compared to intermolecular hydrogen bonding which exists in the keto form [28]. The enolisation of curcumin brings about a fundamental change, i.e., the polar keto tautomer is converted to the non-polar enol tautomer. The dependency of the structure of curcumin on solvent has already been proved as it exhibits different λ_{max} in different solvents [29, 30]. Depending upon the polarity of the medium, curcumin exists in different proportion as a tautomeric mixture of keto and enol forms in the medium. Both the tautomers get solubilized in the medium through different forces. Polar-polar solubilization takes place by means of dipole-dipole forces, while non-polar–non-polar and polar–non-polar solubilization takes place by means of dispersion forces. Like prefers like. Hence, in the polar medium, the activity of keto form predominates while in the non-polar medium, the activity of enol form predominates [31].

Role of methylene group in curcumin

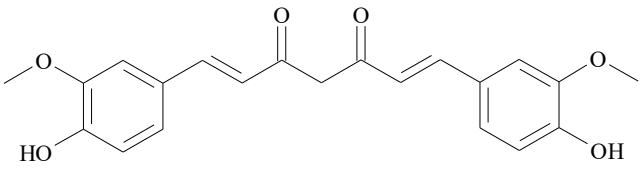
In the keto form of curcumin (1-i), the heptadienone linkage between the two methoxyphenol rings contains an active methylene group. Jovanovic et al. [32] proved that curcumin acts as an antioxidant only in the keto form by donating H-atom from the methylene group, i.e., $-CH_2$ group which is between two electron withdrawing carbonyl groups. The resulting carbon radical formed after abstraction of H-atom (3) is stabilized by resonance (4 and 5) (Figure 2). The active methylene group takes up the reaction site only in acidic or polar medium.



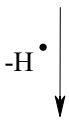
1 Keto form **2** Enol form

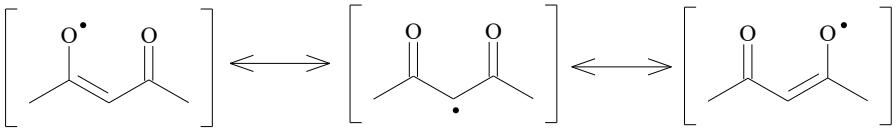
Figure 1. Tautomerism of curcuminoids

where,
(i) R1 = R2 = -OCH_3; Curcumin (Diferuloylmethane)
(ii) R1 = -OCH_3, R2 = -H; Demethoxycurcumin (*p*-Hydroxycinnamoyl feruloylmethane)
(iii) R1 = R2 = -H; Bisdemethoxycurcumin (*p,p'*-Dihydroxydicinnamoylmethane)



1-i





4 **3** **5**

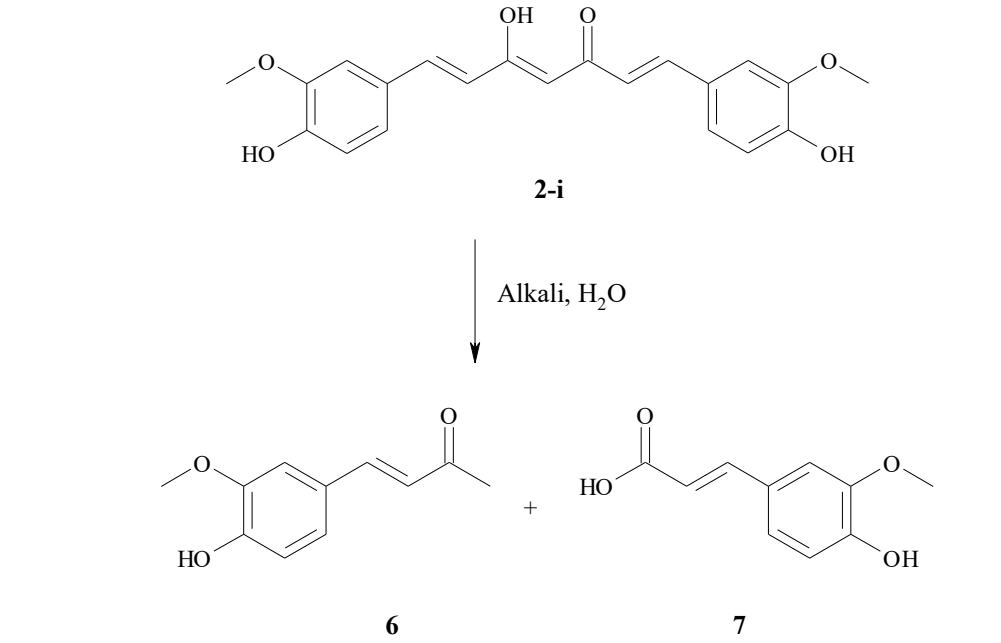
Figure 2. Donation of H-atom from active methylene group

In basic or non-polar medium, the phenolic moiety of curcumin primarily gets involved in the reaction and -CH_2 group involves in enolic form (2-i) [33]. Curcumin is prone to degradation in alkaline [34] and non-polar medium [31].

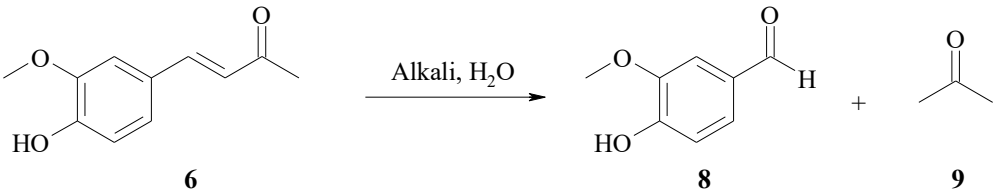
Degradation of curcumin

Various studies have been carried out to study the degradation of curcumin. Depending upon the medium, whether it is alkaline or non-polar, degradation of curcumin takes place through different pathways.

In basic medium, the degradation occurs by nucleophilic attack of basic -OH (hydroxyl) ion. Feruloylmethane (6) and ferulic acid (7) are formed by the alkaline hydrolysis of curcumin. The feruloylmethane further undergoes hydrolysis to form vanillin (8) and acetone (9) [35] (Figure 3). Thus, the degradation pathway under basic medium involves breaking of heptadienone moiety, leading to disappearance of active methylene group that mainly imparts antioxidant activity to curcumin. Hence, curcumin cannot act as an antioxidant.



(i) Formation of primary products

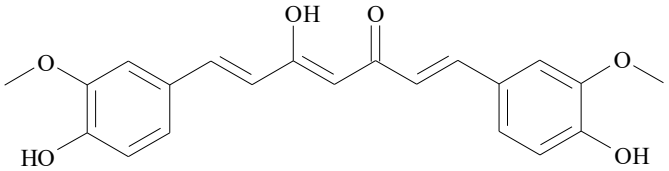


(ii) Formation of secondary products

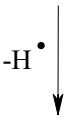
Figure 3. Degradation of curcumin under alkaline medium

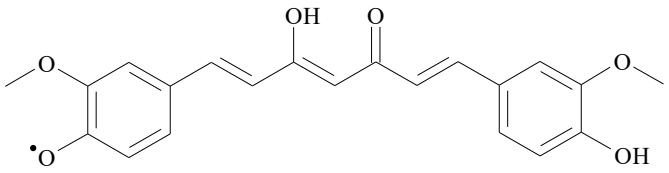
In non-polar medium, Masuda et al. [36] postulated the degradation of curcumin through radical formation to form vanillin and ferulic acid [37, 38, 39]. The enol form of curcumin degrades to form oxy radical I (10) initially, which further gets converted to carbon radical I (11) and carbon radical II (12). Because of conjugation, carbon radical II is more stable than carbon radical I. Both the radicals independently react with molecular oxygen to produce two types of peroxy radicals (13, 16), which get cyclized at adjacent positions. The

cyclic intermediates (14, 17) get decomposed to form stable products through an abstraction of the hydrogen atom from hydrogen atom donor compounds. The enol form of trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal (15-i) and vanillin are formed by the carbon radical I [40] whereas ferulic acid is formed by the carbon radical II. The enol form of trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal can tautomerize to keto form (15-ii) (Figure 4). Thus, the degradation pathway under non-polar medium involves autoxidation of curcumin. Hence, curcumin acts as a pro-oxidant.

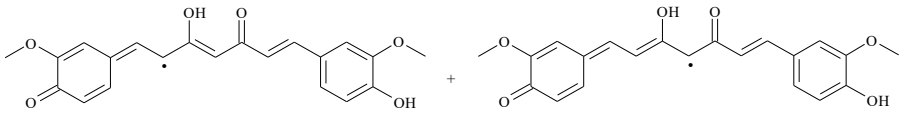


2-i





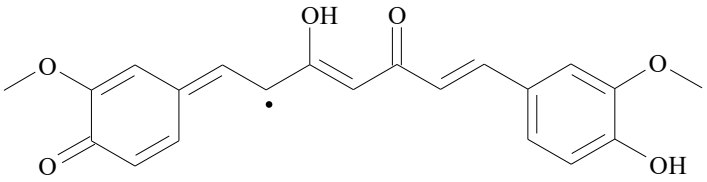
10
Oxy radical I (Highly unstable)



11
Carbon radical I (Minor)

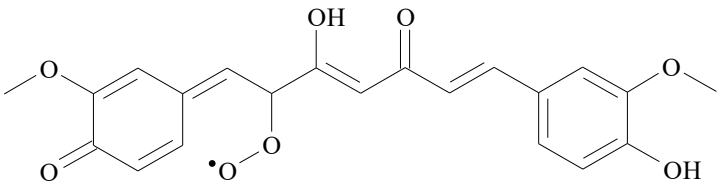
12
Carbon radical II (Major)

(i) Radicals generated by curcumin



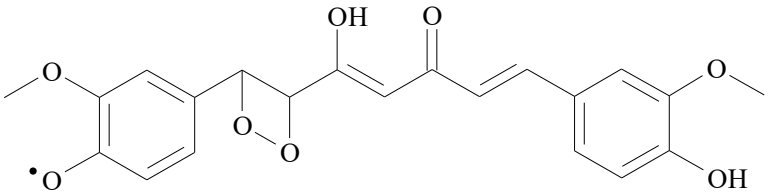
11

O_2



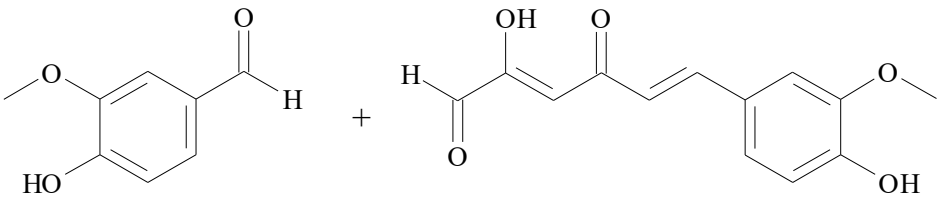
13

Cyclization

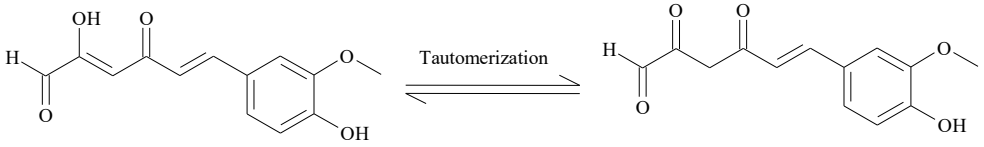


14

$+ H^{\bullet}$



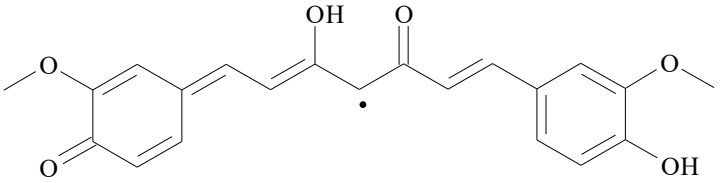
8 + **15-i**



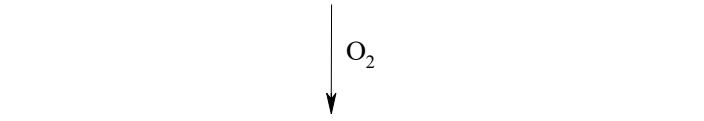
15-i
enol form

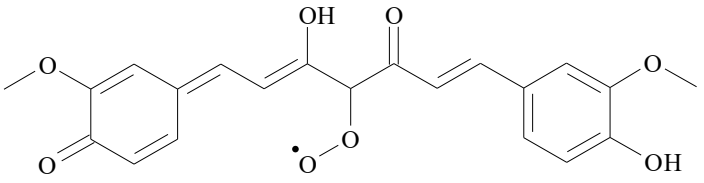
15-ii
keto form

(ii) Reaction of radical I with O_2

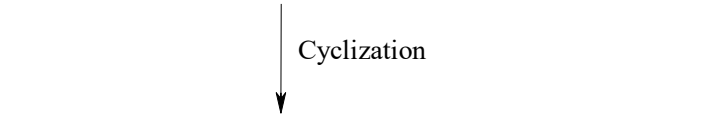


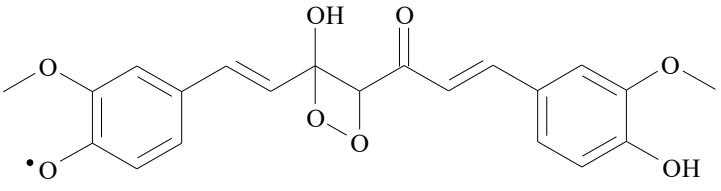
12



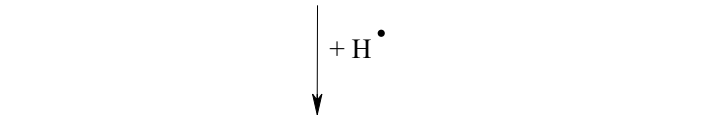


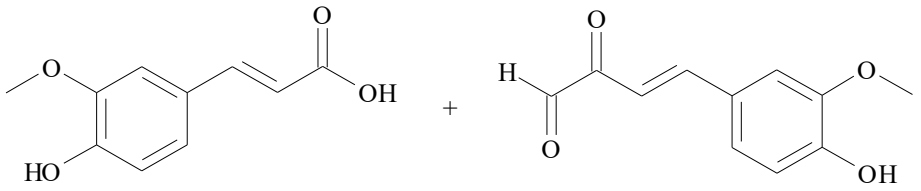
16





17





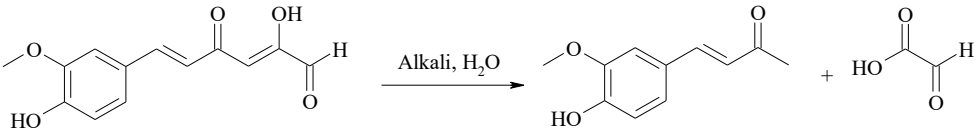
7 **18**

(iii) Reaction of radical II with O_2

Figure 4. Degradation of curcumin under non-polar medium

Degradation of trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal

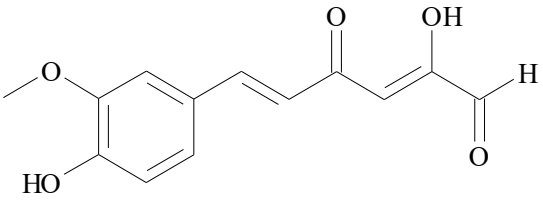
It can be envisaged [40] from the studies that similar to curcumin, the enol tautomer of trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal (15-i) may undergo degradation under alkaline medium to form feruloylmethane (Figure 5), which gets converted to vanillin and acetone on hydrolysis as mentioned previously.



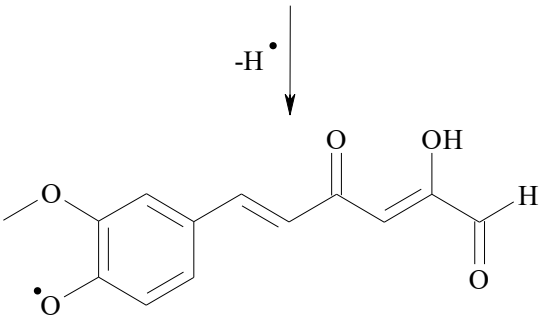
15-i **6** **19**

Figure 5. Degradation of Trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal under alkaline medium

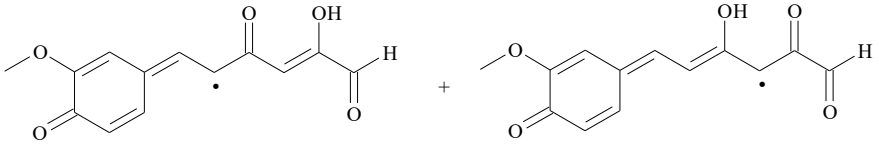
In non-polar medium, the enol tautomer of trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal undergoes degradation to form oxy radical II (20), which gets converted to carbon radical III (21) and carbon radical IV (22). Like carbon radical II, carbon radical IV is more stable than carbon radical III because of conjugation. The radicals so formed combine with molecular oxygen forming two types of peroxy radicals (23, 26), which get cyclized at adjacent positions (24, 27). As discussed previously, the stable products from these cyclic intermediates are obtained by simultaneous abstraction of the hydrogen atom from hydrogen atom donor compound and decomposition (Figure 6). Carbon radical III forms vanillin while carbon radical IV forms ferulic acid.



15-i



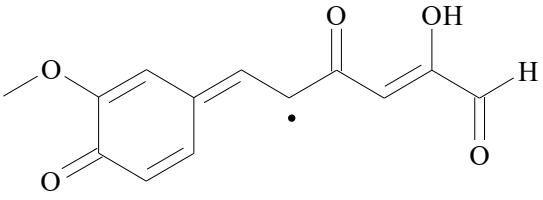
20
Oxy radical II (highly unstable)



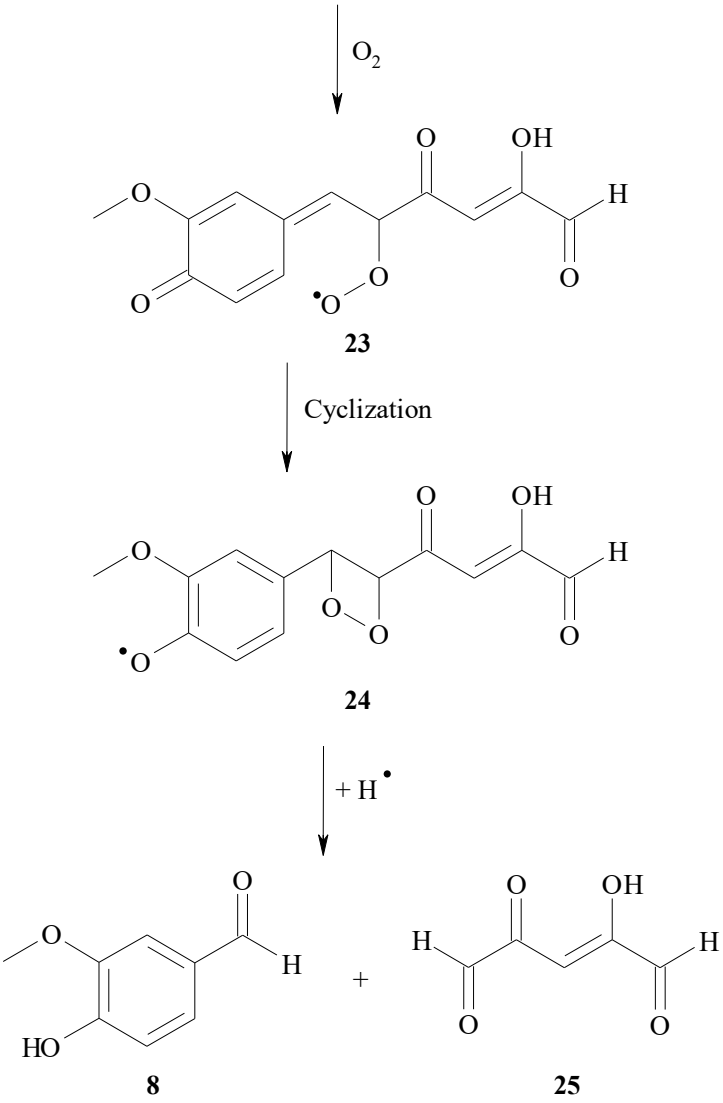
21
Carbon radical III (Minor)

22
Carbon radical IV (Major)

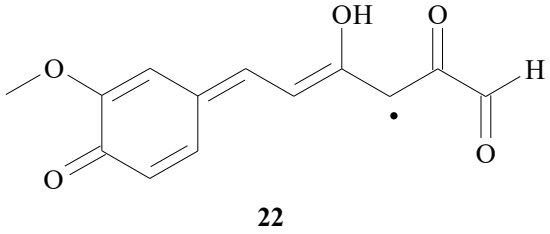
(i) Radicals generated by Trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal

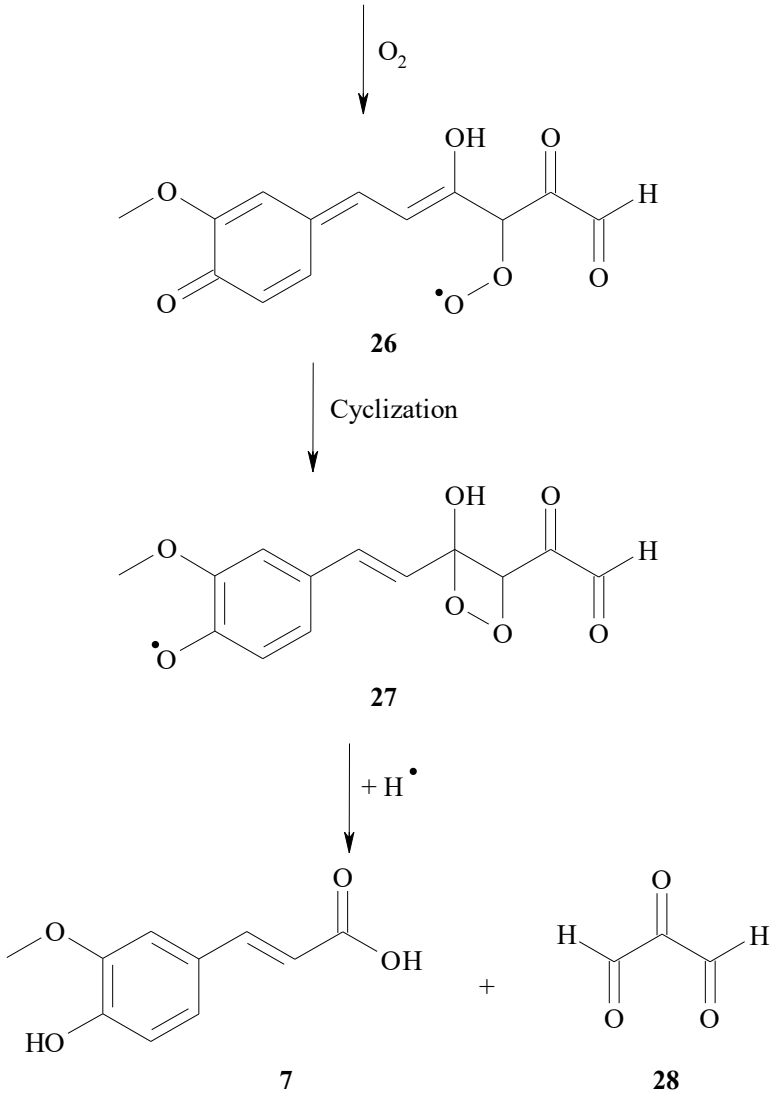


21



(ii) Reaction of radical III with O_2





(iii) Reaction of radical IV with O_2

Figure 6. Degradation of Trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal under non-polar medium

Inference about degradation products

The aforementioned mechanisms indicated that vanillin is the degradation product of curcumin in alkaline as well as non-polar medium along with other products in the respective mediums. Consequently, these mechanisms support the formation of more amount of vanillin during the degradation of curcumin.

The overall discussion is represented in Figure 7.

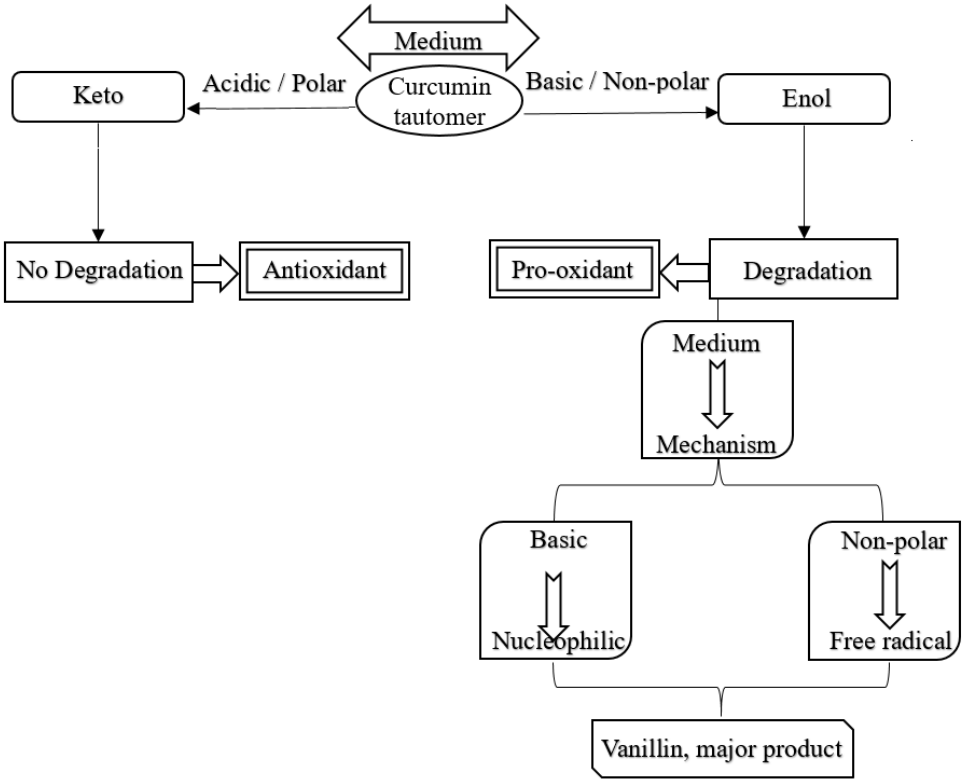


Figure 7. The activity of tautomers of curcumin in different mediums

Structure activity relationship of demethoxycurcumin and bisdemethoxy-curcumin and comparison with curcumin

General introduction about demethoxycurcumin and bisdemethoxycurcumin. Similar to curcumin, the other two curcuminoids (i.e., demethoxycurcumin and bisdemethoxycurcumin) contain phenolic hydroxyl groups, heptadiene chain and diketone moiety (Figure 1), which account for the various therapeutic activities of demethoxycurcumin and bisdemethoxycurcumin such as antioxidant, anti-inflammatory, anticancer [41, 42, 43]. Both are useful for the prevention of Alzheimer disease [44]. Since phenolic groups and heptadienone moiety are responsible for the general chemistry of curcumin as notified earlier, the theories postulated for curcumin hold good for other two curcuminoids.

Comparison of antioxidant activity of curcumin with demethoxycurcumin and bisdemethoxycurcumin. Although all the three curcuminoids exhibit identical activities, their reactivity differs. Jayaprakasha et al. [41] evaluated that the antioxidant potential of curcumin is the highest followed by demethoxycurcumin and bisdemethoxycurcumin. This finding is valid only when curcuminoids do not undergo degradation, by employing acidic or

polar medium. On the contrary, under basic or non-polar medium, bisdemethoxycurcumin is less susceptible to degradation than demethoxycurcumin, which is still less susceptible to degradation than curcumin [45]. Besides pH or nature of the medium, the structure of the phenolic compounds has an impact on the stability of the phenolic compounds [46]. The methoxy group plays an important role in determining the activity of curcuminoids.

Nevertheless, in curcuminoids the antioxidant activity is mainly due to the active methylene group; the presence of electron donating methoxy group at *ortho* to phenolic hydroxyl group also contributes to the antioxidant activity of the molecule, by increasing the electron density on the hydroxyl group by means of an inductive effect [47]. Amongst three curcuminoids, the existence of two methoxy groups in curcumin ensures the maximum antioxidant activity. By the virtue of the occurrence of one methoxy group in demethoxycurcumin, it has better antioxidant activity than bisdemethoxycurcumin, which is devoid of methoxy group [48].

The electron donating group favours enol tautomer [49]. Hence, owing to two methoxy groups, the equilibrium shifting towards formation of enol tautomer is maximum in case of curcumin and minimum in case of bisdemethoxycurcumin among three curcuminoids. Correspondingly, bisdemethoxycurcumin is less prone to degradation than demethoxycurcumin, which in turn is less prone to degradation than curcumin.

Accordingly, in acidic or polar solvent, the rate of antioxidant activity of curcuminoids and in basic or non-polar solvent, the rate of degradation of curcuminoids is same, i.e., curcumin > demethoxycurcumin > bisdemethoxycurcumin.

Conclusion

Curcumin is a specially gifted molecule provided by Mother-Nature to protect humans from chronic health problems. By looking at its chemical structure, we can presume that its chemistry is also very simple. However, with increasing scientific research, curcumin appears to be a more complex, unique and difficult structure to comprehend. It is a symmetrical molecule found abundance in turmeric with relatively high stability in its natural form. In view of the degradation study of curcumin, this review attempts to justify the formation of different degradation products under various reaction mediums. The heptadienone moiety in curcuminoids needs to be protected from degradation to avail its beneficial effects. Since the mechanisms are elucidated on the basis of reported literature, further studies need to be carried out regarding degradation of curcuminoids, which will account for the formation of unknown degradation products.

References

1. Jayaprakasha G.K., Jena B.S., Negi P.S., Sakariah K.K. (2002), Evaluation of antioxidant activities and antimutagenicity of turmeric oil: A byproduct from curcumin production, *Zeitschrift für Naturforschung C,* 57(9–10), pp. 828–835.
2. Ravindranath V., Satyanarayana M.N. (1980), An unsymmetrical diarylheptanoid from Curcuma longa, *Phytochemistry,* 19(9), pp. 2031–2032.
3. Limtrakul P., Anuchapreeda S., Buddhasukh, D. (2004), Modulation of human multidrug–resistance MDR–1 gene by natural curcuminoids, *BioMed Central Cancer,* 4(13), pp. 1–6.
4. Ruby A.J., Kuttan G., Babu K.D., Rajasekharan K.N., Kuttan R. (1995), Anti–tumor and antioxidant activity of natural curcuminoids, *Cancer Letters,* 94(1), pp. 79–83.

5. Kunchandy E., Rao M.N.A. (1990), Oxygen radical scavenging activity of curcumin, *International Journal of Pharmaceutics,* 58(3), pp. 237–240.
6. Sokmen M., Khan M.A. (2016), The antioxidant activity of some curcuminoids and chalcones, *Inflammopharmacology,* 24(2–3), pp. 81–86.
7. Niranjan A., Prakash D. (2008), Chemical constituents and biological activities of turmeric (Curcuma longa L.) – A review, *Journal of Food Science and Technology,* 45(2), pp. 109–116.
8. Jain S.K., Rains J., Jones, K. (2006), Effect of curcumin on protein glycosylation, lipid peroxidation, and oxygen radical generation in human red blood cells exposed to high glucose levels, *Free Radical Biology and Medicine,* 41(1), pp. 92–96.
9. Stano J., Grancai D., Neubert K., Kresanek, J. (2000), Curcumin as a potent antioxidant, *Ceská a Slovenská Farmacie,* 49(4), pp. 168–170.
10. Cole G.M., Teter B., Frautschy S.A. (2007), Neuroprotective effects of curcumin, *Advances in Experimental Medicine and Biology,* 595, pp. 197–212.
11. Thangapazham R.L., Sharma A., Maheshwari R.K. (2007), Beneficial role of curcumin in skin diseases, *Advances in Experimental Medicine and Biology,* 595, pp. 343–357.
12. Sun X., Liu Y., Li C., Wang X., Zhu R., Liu C., Liu H., Wang L., Ma R., Fu M., Zhang D., Li Y. (2017), Recent advances of curcumin in the prevention and treatment of renal fibrosis, *BioMed Research International,* 2017, pp. 1–9.
13. Qin S., Huang L., Gong J., Shen S., Huang J., Ren H., Hu H. (2017), Efficacy and safety of turmeric and curcumin in lowering blood lipid levels in patients with cardiovascular risk factors: a meta–analysis of randomized controlled trials, *Nutrition Journal,* 16(68), pp. 1–10.
14. Kant V., Gopal A., Pathak N.N., Kumar P., Tandan S.K., Kumar D. (2014), Antioxidant and anti–inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin–induced diabetic rats, *International Immunopharmacology,* 20(2), pp. 322–330.
15. Govindarajan V.S. (1990), Turmeric – Chemistry, technology, and quality, *Critical Reviews in Food Science and Nutrition,* 12(3), pp. 199–301.
16. Ammon H.P., Wahl M.A. (1991), Pharmacology of Curcuma longa, *Planta Medica,* 57(1), pp. 1–7.
17. Ono K., Hasegawa K., Naiki H., Yamada M. (2004), Curcumin has potent anti–amyloidogenic effects for Alzheimer's beta–amyloid fibrils in vitro, *Journal of Neuroscience Research,* 75(6), pp. 742–750.
18. Ruidas B., Chaudhury S.S. (2017), Role of curcumin in treatment of Alzheimer disease, *International Journal of Neurorehabilitation,* 4(3), pp. 1–7.
19. Panahi Y., Badeli R., Karami G.R., Sahebkar A. (2015), Investigation of the efficacy of adjunctive therapy with bioavailability–boosted curcuminoids in major depressive disorder, *Phytotherapy Research,* 29(1), pp. 17–21.
20. Semwal A.D., Sharma G.K., Arya S.S. (1997), Antioxygenic activity of turmeric (Curcuma longa) in sunflower oil and ghee, *Journal of Food Science and Technology,* 34(1), pp. 67–69.
21. Muhammad A., Shahab–Uddin A., Khan U., Hannan A., Mohiuddin E., Asif M. (2010), Curcuma longa and curcumin: A review article, *Romanian Journal of Biology – Plant Biology,* 55(2), pp. 65–70.
22. Suresh D., Gurudutt K.N., Srinivasan K. (2009), Degradation of bioactive spice compound: curcumin during domestic cooking, *European Food Research and Technology,* 228(5), pp. 807–812.
23. (2004), *Curcumin, Chemical and Technical Assessment,* 61st JECFA. pp. 1–8. Available at: ftp://ftp.fao.org/es/esn/jecfa/cta/CTA_61_Curcumin.pdf.

24. Kawano S., Inohana Y., Hashi Y., Lin J.M. (2013), Analysis of keto–enol tautomers of curcumin by liquid chromatography/mass spectrometry, *Chinese Chemical Letters,* 24(8), pp. 685–687.
25. Payton F., Sandusky P., Alworth W.L. (2007), NMR study of the solution structure of curcumin, *Journal of Natural Products,* 70(2), pp. 143–146.
26. Rege S.A., Momin S.A., Wadekar S.D., Bhowmick D.N., Pratap A.P. (2014), Effect of demethoxycurcumin and bisdemethoxycurcumin on antioxidant activity of curcumin in refined sunflower oil, *Journal of Food Processing and Preservation,* 38(1), pp. 296–303.
27. Kalsi P.S. (2006), Chapter 2: Delocalized Chemical Bonding, *Organic Reactions and Their Mechanisms (Second Edition)*, New Delhi, India: New Age International (P) Limited, pp. 37–80.
28. Skyes, P. (2013), Chapter 10: Carbanions and Their Reactions, *A Guidebook to Mechanism in Organic Chemistry (Sixth Edition)*, New Delhi, India: Pearson Education, Ltd., pp. 270–298.
29. Chignell C.F., Bilskj P., Reszka K.J., Motten A.G., Sik R.H., Dahl T.A. (1994), Spectral and photochemical properties of curcumin, *Photochemistry and Photobiology,* 59(3), pp. 295–302.
30. Khopde S.M., Priyadarsini K.I., Palit D.K., Mukherjee T. (2000), Effect of solvent on the excited–state photophysical properties of curcumin, *Photochemistry and Photobiology,* 72(5), pp. 625–631.
31. Rege S.A., Momin S.A., Bhowmick D.N., Pratap A.P. (2012), Stabilization of emulsion and butter like products containing essential fatty acids using kalonji seeds extract and curcuminoids, *Journal of Oleo Science,* 61(1), pp. 11–16.
32. Jovanovic S.V., Steenken S., Boone C.W., Simic M.G. (1999), H–atom transfer is a preferred antioxidant mechanism of curcumin, *Journal of American Chemical Society,* 121(41), pp. 9677–9681.
33. Jagannathan R., Abraham P.M., Poddar P. (2012), Temperature–dependent spectroscopic evidences of curcumin in aqueous medium: A mechanistic study of its solubility and stability, *The Journal of Physical Chemistry B,* 116(50), pp. 14533–14540.
34. Khurana A., Ho C.T. (1988), High performance liquid chromatographic analysis of curcuminoids and their photo–oxidative decomposition compounds in Curcuma longa L., *Journal of Liquid Chromatography,* 11(11), pp. 2295–2304.
35. Tonnesen H.H., Karlsen J. (1985), Studies of curcumin and curcuminoids: V. Alkaline degradation of curcumin, *Zeitschrift für Lebensmittel–Untersuchung und –Forschung,* 180(2), pp. 132–134.
36. Masuda T., Hidaka K., Shinohara A., Maekawa T., Takeda Y., Yamaguchi H. (1999), Chemical studies on antioxidant mechanism of curcuminoid: Analysis of radical reaction products from curcumin, *Journal of Agricultural and Food Chemistry,* 47(1), pp. 71–77.
37. Sandur S.K., Pandey M.K., Sung B., Ahn K.S., Murakami A., Sethi G., Limtrakul P., Badmaev V., Aggarwal B.B. (2007), Curcumin, demethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti–inflammatory and anti–proliferative responses through a ROS–independent mechanism, *Carcinogenesis,* 28(8), pp. 1765–1773.
38. Zhu J., Sanidad K.Z., Sukamtoh E., Zhang G. (2017), Potential roles of chemical degradation in the biological activities of curcumin, *Food & Function,* 8(3), pp. 907–914.
39. Schneider C., Gordon O.N., Edwards R.L., Luis P.B. (2015), Degradation of curcumin: From mechanism to biological implications, *Journal of Agricultural and Food Chemistry,* 63(35), pp. 7606–7614.
40. Wang Y.J., Pan M.H., Cheng A.L., Lin L.I., Ho Y.S., Hsieh C.Y., Lin J.K. (1997), Stability of curcumin in buffer solutions and characterization of its degradation products, *Journal of Pharmaceutical and Biomedical Analysis,* 15(12), pp. 1867–1876.

41. Jayaprakasha G.K., Rao L.J., Sakariah K.K. (2006), Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin, *Food Chemistry,* 98(4), pp. 720–724.
42. Ramsewak R.S., DeWitt D.L., Nair M.G. (2000), Cytotoxicity, antioxidant and anti–inflammatory activities of curcumins I–III from Curcuma longa, *Phytomedicine,* 7(4), pp. 303–308.
43. Zhang L.J., Wu C.F., Meng X.L., Yuan D., Cai X.D., Wang Q.L., Yang J.Y. (2008), Comparison of inhibitory potency of three different curcuminoid pigments on nitric oxide and tumor necrosis factor production of rat primary microglia induced by lipopolysaccharide, *Neuroscience Letters,* 447(1), pp. 48–53.
44. Park S.Y., Kim D.S. (2002), Discovery of natural products from Curcuma longa that protect cells from beta–amyloid insult: a drug discovery effort against Alzheimer's disease, *Journal of Natural Products,* 65(9), pp. 1227–1231.
45. Price L.C., Buescher R.W. (1997), Kinetics of alkaline degradation of the food pigments curcumin and curcuminoids, *Journal of Food Science,* 62(2), pp. 267–269.
46. Friedman M., Jurgens H.S. (2000), Effect of pH on the stability of plant phenolic compounds, *Journal of Agricultural and Food Chemistry,* 48(6), pp. 2101–2110.
47. Khan M.A., Shahidi F. (2001), Effects of natural and synthetic antioxidants on the oxidative stability of borage and evening primose triacylglycerols, *Food Chemistry,* 75(4), pp. 431–437.
48. Somparn P., Phisalaphong C., Nakornchai S., Unchern S., Morales N.P. (2007), Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives, *Biological and Pharmaceutical Bulletin,* 30(1), pp. 74–78.
49. Antonov L., Deneva V., Simeonov S., Kurteva V., Crochet A., Fromm K.M., Shivachev B., Nikolova R., Savarese M., Adamo C. (2015), Controlled tautomeric switching in azonaphthols tuned by substituents on the phenyl ring, *ChemPhysChem.* 16(3), pp. 649–657.

Characteristics of proteolytic processes during the isolation of natural casein phosphopeptides

Volodymyr Yukalo, Olha Krupa, Liudmyla Storozh

Ternopil Ivan Puluj National Technical University, Ternopil, Ukraine

Keywords:

Casein
Phosphopeptides
Proteolysis

Article history:

Received 01.08.2018
Received in revised form 30.11.2018
Accepted 28.03.2019

Corresponding author:

Olha Krupa
E-mail: cmakota@ukr.net

DOI:
10.24263/2304-974X-2019-8-1-7

Abstract

Introduction. The aim of this work is studying of the proteolytic processes features in the production of natural phosphopeptides of the milk casein complex.

Materials and methods. Casein substrate was isolated by isoelectric sedimentation from skimmed milk. Proteolysis was performed using pancreatin. The degree of proteolysis was determined spectrophotometrically by the absorption of low molecular weight products of proteolysis at λ = 280 nm. The yield of phosphopeptides was determined gravimetrically after precipitation with ethanol in the presence of calcium ions. Electrophoresis of phosphoproteins of the substrate and products of proteolysis was carried out in an alkaline system of homogeneous polyacrylamide gel in the presence of urea.

Results and discussion. Natural phosphopeptides were obtained by proteolysis of the phosphoproteins of the casein complex with pancreatin (E:S = 1:100) under physiological conditions (37° C, pH 7.9). At different stages of proteolysis, the yield of phosphopeptides and peptides soluble in 10% trichloroacetic acid was determined. Degree of proteolysis has increased monotonically throughout the studied period. The yield of phosphopeptides reaches its maximum in the 90th minute of proteolysis and then decreases continuously. The yield of phosphopeptides is lower than it was used proteolytic preparations of microbial origin. The results obtained by gel filtration and electrophoresis in a polyacrylamide gel indicate that a decrease in the yield of phosphopheptides after the 90th minute of proteolysis may be caused by the phosphopeptide's molecular weight decrease. Most of phosphopeptides obtained in the 90th minute of proteolysis have a molecular weight up to 2000 Da, which is characteristic for the already known biologically active phosphopeptides.

Conclusions. During the proteolysis of casein with pancreatin in physiological conditions, the total yield of proteolysis products increases monotonously. The yield of phosphopeptides has maximum. Gel filtration and electrophoresis data indicate that this is due to a decrease in the molecular mass.

Introduction

The proteins of milk casein complex are precursors of a large number of various biologically active peptides with difference action on various physiological systems of the organism [1, 2]. Among them, one of the most common and important is phosphopeptides [3]. Phosphopeptides are formed in the course of a normal digestion of a casein proteins complex in the intestine following the action of proteolytic enzymes of the pancreas. It is believed that their main function is the ability to bind ions of macroelements (calcium, magnesium, ferrum), as well as trace elements (zinc, nickel, cobalt, selenium) and to promote their adsorption in the gastrointestinal tract. Herewith casein phosphopeptides are able to form complexes with metal ions, resistant to the action of proteolytic enzymes [4, 5].

For the first time, the biological effect of phosphopeptides was described in 1950 [6]. It was an independent of vitamin D calcification of bones in children with rickets. In further studies, the positive effect of casein phosphopeptides on the absorption of calcium, zinc and iron ions has been established [2, 3]. The mechanism of action of phosphopeptides in adsorption of metal ions has not been finally established. This may be the formation with the participation of phosphopeptides of calcium-selective channels in the cytoplasmic membrane. Phosphopeptides can bind ions of calcium and transport by endocytosis. Also, the biological effect of phosphopeptides is associated with the prevention of teeth caries [7]. It has been established that phosphopeptides can participate in the restoration of the mineral composition of teeth, inhibit the adhesion of cariogenic bacteria to the surface of teeth, and contribute to the accumulation of bioavailable calcium ions.

Due to the biological effect of casein phosphopeptides, they are of considerable interest as ingredients for functional products [8]. For the production of phosphopeptides, the proteolysis of casein substrates is carried out. Proteases of animal, plant and microbiological origin are used as proteolytic enzyme [3, 9]. As a result, a large number of phosphopeptide preparations were obtained, which differ in their primary structure, molecular weight and properties. These differences may be reflected in the biological activity of phosphopeptides [4]. Obviously, the greatest value can have phosphopeptides, formed in conditions that reproduce the conditions of caseins proteolysis in the gastrointestinal tract. We have previously justified the choice of proteolytic enzyme and casein substrate, established their proportion to obtain natural phosphopeptides [10]. Taking into account the above mentioned information, the purpose of the work is to characterize proteolytic processes in the production of natural casein phosphopeptides.

Materials and methods

To obtain total casein, fresh cow's milk was used. Proteolysis of casein substrate was carried out with pancreatin, produced by PJSC "Tekhnoloh" (Ukraine). All electrophoretic buffers and gels were prepared using reagents of the company "Reanal" (Hungary).

Obtaining of total preparation of milk casein complex proteins

The preparation of total casein was obtained from skimmed milk by precipitation at pH 4.6. The separation of lipids from casein was carried out by centrifugation (4000 g, 10 minutes) in two steps at two different temperatures: 30 and 4° C, respectively. This allows more completely separation of milk lipids. The sedimentation of total casein at an isoelectric point was carried out with an addition of chloride acid solution (1 mol/dm^3). The resulting

precipitate was washed with distilled water and dissolved with sodium hydroxide (1 mol/dm^3). In this case, the pH value should not exceed 7.9 [10]. At higher values of pH the dephosphorylation of caseins may occur. Precipitation and dissolution procedure was repeated twice. The third precipitation was carried out with acetic acid at a pH of 4.0. The resulting total casein sediment has been incubated in acetic acid solution (pH 4.0) for 5 hours at a temperature of 4° C to inactivate the natural proteases of milk [11].

Proteolysis of total casein

Proteolysis of 9% total casein was carried out with pancreatin at physiological values of temperature and pH (37° C, pH 7.9). The ratio of "enzyme: substrate" was 1:100 [12].

Determination of casein substrate concentration and products of its proteolysis

The concentration of proteins in preparations of total casein and products of its proteolysis in hydrolysates was determined spectrophotometrically during absorption at the 280 nm by spectrophotometer СФ-46. In this case, the commonly accepted absorption coefficient ($D_{1cm}^{1\%}$) for total casein was 8.2 [13].

Gel filtration

Gel filtration of total casein and products of its proteolysis was carried out on columns (2 × 35 cm) from the liquid chromatography kit of the company "Reanal" (Hungary) filled with Sephadex G-25 (fine) of the company "Pharmacia" (Sweden). Preparation of sephadexes and a chromatographic column for gel filtration was carried out in accordance with the methodological recommendations of the "Pharmacia" company [14]. The samples were dissolved in a chromatographic buffer (0.005 Tris HCl, pH 7.9, 6 M urea) and centrifuged to remove insoluble fragments by T-24 centrifuge (15000 g, 20 minutes). 5 ml of sample was taken from the obtained supernatant and applicated on sephadex, equilibrated with a chromatography buffer. Gel filtration was carried out at an elution rate of 25 ml/h, selecting 5 ml of eluate. For obtaining of chromatograms the optical density at 280 nm was measured by a spectrophotometer СФ-46.

Electrophoresis

The analysis of total casein and hydrolysates was carried out in a device of the Stadier type on vertical plates of a homogeneous polyacrylamide gel in an alkaline electrophoretic system (pH 7.9), which included 4.5 M urea [15]. Electrophoretic buffers and plates of polyacrylamide gel were prepared using reagents of "Reanal" company (Hungary). Polyacrylamide gel was obtained by mixing solutions according to Table 1.

Also the electrode buffer and the buffer for dissolution of samples were prepared, according to table 2.

Electrophoregrams were fixed with 7% acetic acid and stained with 0.5% amidoblack 10B [16,17].

Statistical analysis

The statistical analysis of the obtained results and the graphical representation of the experimental data were carried out using the Microsoft Excel 2007 program. The accuracy of the obtained results was provided by triple repetition of the experiments. The reliability of the obtained results was taken at $p < 0,05$.

Table 1

Composition of polyacrylamide gel

Solutions	Ration in polyacrylamide gel (volume)	Component	Amount
Gel	1 part	Acrylamide	13,5 g
		N,N'- methylenebisacrylamide	0,75 g
		Water	up to 100 ml
Buffer for gel (pH 7.9)	2 part	Tris(hydroxymethyl) aminomethane	0,609 g
		Ethylenediaminetetraacetic acid (disodium salt)	0,2 mg
		Veronal	1,1 mg
		2- mercaptoethanol	1,0 ml
		Urea	54,0 g
		Water	up to 100 ml
Catalyst and initiator solution	1 part	Ammonium persulfate	selected experimentally
		N,N,N',N'-tetramethyl-ethylenediamine	0,05 ml
		Water	up to 10 ml

Table 2

Composition of electrode buffer and sample buffer

Buffer	Component	Amount
Electrode buffer (pH 7.9)	Tris(hydroxymethyl) aminomethane	6,09 g
	Ethylenediaminetetraacetic acid (disodium salt)	2,0 g
	Veronal	11,0 g
	Water	up to 1000 ml
Buffer for dissolution of samples (pH 7.9)	Tris(hydroxymethyl) aminomethane	30,5 mg
	Ethylenediaminetetraacetic acid (disodium salt)	10,0 mg
	Veronal	55 mg
	2- mercaptoethanol	0,25 ml
	Urea	13,5 g
	Water	up to 50 ml

Results and discussion

Determination of the total yield of proteolysis products and phosphopeptides

When conducting proteolysis of total casein with pancreatin every 30 minutes, starting from the 30th from its beginning and up to the 180th minute inclusive, the samples were taken to determine the intensity of the process and the release of phosphopeptides. Soluble proteolysis products obtained after sedimentation uncleaved phosphoproteins with 10% trichloroacetic acid were diluted fortyfold with 5% acetic acid. For the obtained solutions,

the optical density was measured by a spectrophotometer СФ-46 at 280 nm. The results of proteolysis are shown in Figure 1 (1). Each point in the graph is the average value of three definitions. As can be seen from the graph, the degree of proteolysis monotonically increases throughout the period (up to the 180th minute). The slowdown in the intensity of proteolysis occurs after the 90th minute. Adamson and Reynolds have got similar results, obtained with enzyme preparations Novo trypsin and Pancreatin 4 NF [18].

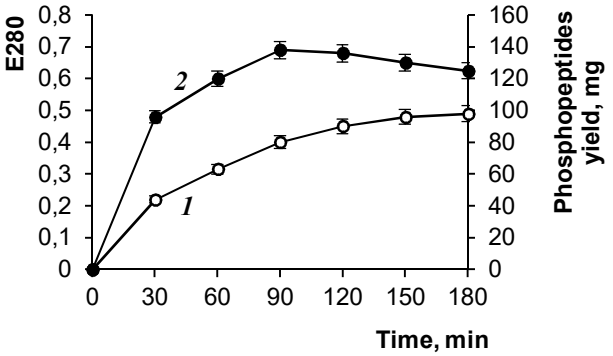


Figure 1. Proteolysis products (1) and phosphopeptides yield (2) in the process of proteolysis of the phosphoprotein substrate with pancreatin

Simultaneously, every 30 minutes of proteolysis samples have been taken for the extraction of phosphopeptides from the hydrolyzate. The basis was the methodology described earlier [19]. Herewith, the pH of the sampled hydrolyzate was adjusted to pH 4.6 with chloride acid solution of concentration 0.1 mol/dm^3. The unsolvated phosphoprotein and the large polypeptides sediment were separated by centrifugation at a centrifuge ОПН-8 (5000 rpm, 15 minutes). Then, 1 cm^3 of 10% $CaCl_2$ and 10 cm^3 of ethanol were added to 9 cm^3 of supernatant. The precipitate of phosphopeptides was centrifuged, washed with ethanol, dried to a constant weight and weighted. The results are shown in Figure 1 (2). The yield of phosphopeptides, obtained in physiological conditions, is lower than with the application of microbial proteolytic enzymes [9] and it approaches to the yield with trypsin using [19]. The analysis of the phosphopeptides extraction shows that after reaching a maximum (in the 90th minute), the value of the yield decreases. This occurs on the background of a further increase in a degree of proteolysis and may be due to a change in the phosphopeptides' molecular weight in the process of proteolysis [9, 18]. In this regard, to characterize the products obtained at various stages of proteolysis, we carried out gel filtration and electrophoresis.

Gel filtration and electrophoresis of proteolysis's products

Gel filtration on Sephadex G-25 allows estimating the molecular and weight distribution of proteolysis products in the range from 1000 to 5000 Da [14]. For gel filtration samples have been taken at different stages of proteolysis. The results of gel filtration are shown in Figure 2 and 3. For comparison, in each case, the chromatogram of the phosphoprotein substrate is shown in parallel. According to literature data, the molecular masses of the already known biologically active casein phosphopeptides do not exceed 2000 Da [8]. They are eluated in the 15th and subsequent chromatographic fractions, sampled during gel filtration. In the 90th minute (Figure 2), the greater part of proteolysis products, including phosphopeptides, have molecular weight up to 2000 Da. During further proteolysis (the 90th-180th minutes), the amount of low molecular weight peptides increases. At the same time, as can be seen from Figure 1 (2), the total amount of phosphopeptides up to the 90th minute does not change, and then begins to decrease.

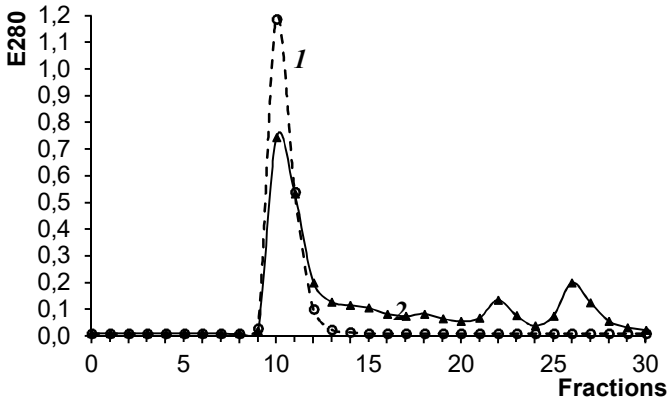


Figure 2. Chromatogram of the phosphoprotein substrate (1) and its hydrolyzate (2) obtained in the 60th minute of proteolysis with pancreatin

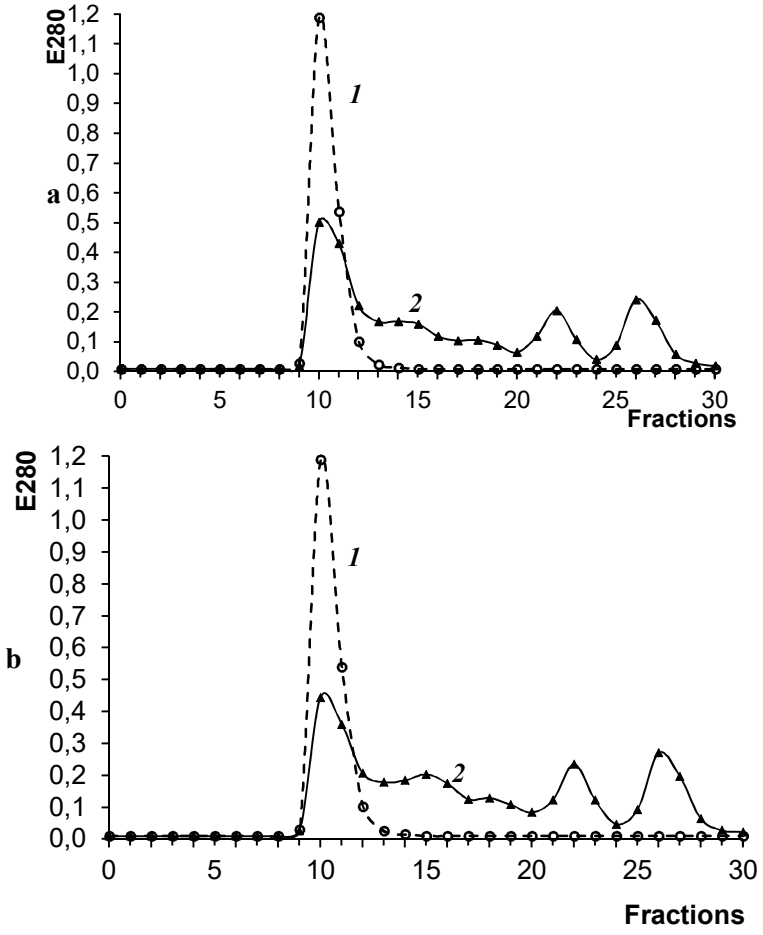


Figure 3. Chromatogram of the phosphoprotein substrate (a.1 and b.1) and its hydrolyzate, obtained in the 90th (a.2) and 120th minutes (b.2) of proteolysis with pancreatin

The samples for gel filtration were selected according to the scheme (Figure 4):

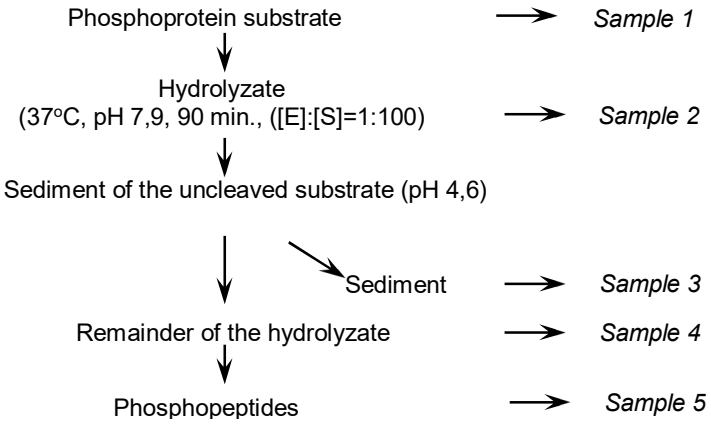


Figure 4. Scheme of sample selection for gel filtration.

The Figure 5 shows the chromatograms of the hydrolyzate obtained in the 90th minute under physiological conditions (37° C, pH 7.9), as well as the uncleaved substrate, sedimened at pH 4,6, the residue of the hydrolysate and the isolated phosphopeptides.

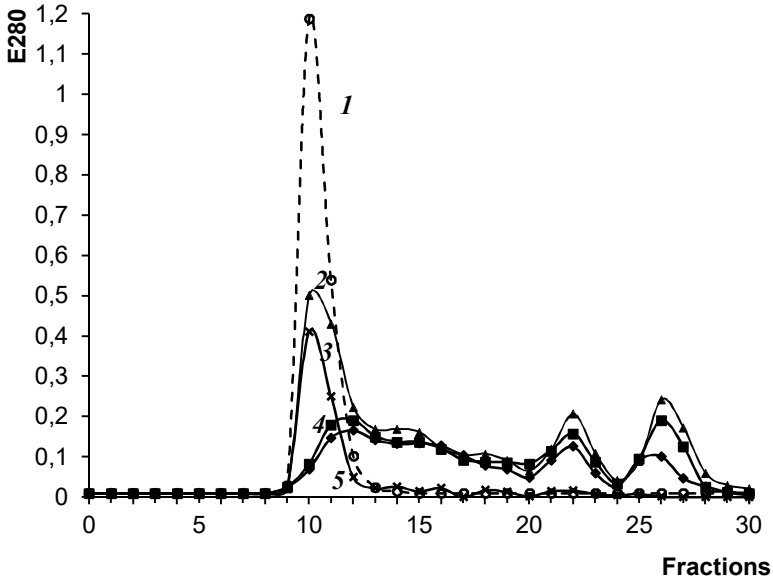


Figure 5. Chromatogram of phosphoprotein substrate (1), and products of its proteolysis, obtained in the 90th minute of proteolysis: 2 - hydrolyzate; 3 - uncleaved substrate; 4 - remainder of the hydrolyzate; 5-phosphopeptides

According to the gel filtration data at pH 4.6, polypeptides with a molecular weight greater than 5000 Da are sedimented from hydrolyzate (Figure 5). Scientific investigations confirm that under such conditions non-hydrolyzed proteins, as well as polypeptides with a large molecular weight are isolated [1, 13]. The chromatogram of the hydrolyzate residue (Figure 5 (4)) has a similar profile to the chromatogram of phosphopeptides (Figure 5 (5)). Moreover, among the remainder of the hydrolyzate, phosphopeptides present a larger part. This fact is consistent with published dates [18-20].

In addition to gel filtration, electrophoresis was used to characterize the hydrolyzate (Figure 6). The obtained results indicate that after the 15th minute, the main substrate phosphoproteins (α_{S1}-CN-8P, α_{S2}-CN-XP, β-CN-5P) are cleaved to form low molecular weight and separate macromolecular products that are retained in a polyacrylamide gel. In the 90th minute, high molecular peptide fractions are absent (Figure 6). Similar results with a large number of various low molecular weight phosphopeptides are described in other scientific researchs [21, 22].

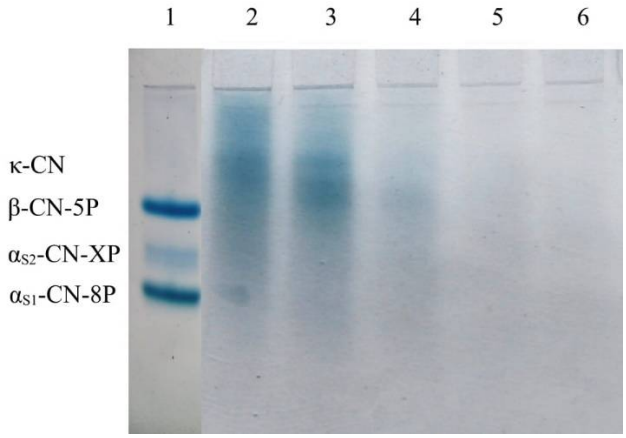


Figure 6. Electrophoregram of phosphoprotein substrate (1) and products of its proteolysis obtained in the 15th (2); 30th (3); 60th (4); 90th (5th); 120th (6) minutes

Conclusions

During the casein substrate proteolysis with pancreatin (E:S ratio 1:100) under physiological conditions (37° C, pH 7.9), the total yield of peptides that are soluble in 10% trichloroacetic acid and phosphopeptides does not coincide. The total amount of proteolyctic products monotonously increases throughout the whole period of proteolysis. The yield of phosphopeptides reaches a maximum in the 90th minute of proteolysis with a further decrease. The results of gel filtration and electrophoresis indicate that this fact is associated with a decrease in the molecular weight of phosphopeptides.

References

1. Fox P.F., Uniacke-Lowe T., McSweeney P.L.H., O'Mahony J.A. (2015), *Dairy Chemistry and Biochemistry (Second Edition)*, Springer, New York.

2. Iukalo A.V., Storozh L.A., Yukalo V.H. (2012), Proteiny kazeinovoho kompleksu moloka koriv (Bos taurus) yak poperednyky biolohichno aktyvnykh peptydiv, *Biotekhnolohiia,* 5(4), C. 21-33.
3. McSweeney P.L.H., O'Mahony J.A. (2016), *Advanced Dairy Chemistry. Proteins: Applied Aspects (Fouth Edition),* Springer, NewYork.
4. Park Y.W. (2009), *Bioactive components in milk and dairy products,* Wiley-Blackwell, Ames.
5. Park Y.W., Haenlein G.F.W. (2013), *Milk and Dairy Products in Human Nutrition: Production, Composition and Health,* Wiley-Blackwell, Ames.
6. Mellander O. (1950), The physiological importance of the casein phosphopeptide calcium salts II. Peroral calcium dosage of infants, *Acta Societatis Medicorum Upsaliensis,* 55(5-6), pp. 247-255.
7. Bullappa D., Puranik M.P., Uma S.R. (2015), Casein phosphopeptide-amorphous calcium phosphate: a review, *International Journal of Dental and Health Sciences,* 2(1), pp. 116-125.
8. Pinto G., Caira S., Cuollo M., Lilla S., Chianese L., Addeo F. (2012), Bioactive Casein Phosphopeptides in Dairy Products as Nutraceuticals for Functional Foods. In : *Milk Proteins*, In Tech, Croatia.
9. FitzGerald, R.J. (1998), Potential uses of caseinophosphopeptides. *International Dairy Journal,* 8, pp. 451-457.
10. Yukalo V.H., Storozh L.A., Shtokalo M.O. (2014), Vyznachennia umov otrymannia pryrodnykh bioaktyvnykh kazeinovykh fosfopeptydiv, *Scientific Messenger LNUVMBT named after S.Z. Gzhytskyj*, 16(3), pp. 192-200.
11. Yukalo V.G. (2005), Obtaining of casein protein complex fractions from cow milk, *Nutracos,* 5, pp. 17-19.
12. Yukalo V., Storozh L. (2013), The isolation of phosphopeptides from total casein and its fractions, *Food chemistry and technology*, 47(2), pp. 32-40.
13. Farrell H.M., Jimenez-Flores R., Bleck G.T., Brown E.M., Butler J.E., Creamer L.K., Hicks C.L., Hollar C.M., Ng-Kwai-Hang K.F., Swaisgood H.E. (2004), Nomenclature of the proteins of cows' milk – sixth revision, *Journal of Dairy Science,* 87 (6), pp. 1641-1674.
14. Pharmacia Fine Cemicals, (1985), *Gel Filtration. Theory and practice*, Uppsala, Sweden.
15. Yukalo A., Yukalo V., Shynkaryk M. (2009), Electrophoretic separation of the milk protein. *Proceeding of the International Conference on Bio and Food Electrotechnologies*, *22-23 October 2009*, Compiegne: France, pp. 227-231.
16. Osterman L.A. (1981), *Metody issledovaniya belkov i nukleinovykh kislot: Elektroforez i ultratsentrifugirovaniye (prakticheskoye posobiye)*, M.: Nauka.
17. Baerle A., Dimova O., Zadorojnai L., Tatarov P., Zenkovich A. (2014), Electrophoresis of oil-containing edible microcapsules with proteinpolyuronic shells, *Ukrainian Food Journal*, 3(2), pp.211-219
18. Adamson N.J., Reynolds E.C. (1997), Relationship between degree of casein hydrolysis and phosphopeptide release, *Journal of Dairy Research*, 64(4), pp. 505-514.
19. Adamson N.J., Reynolds E.C. (1995), Characterization of tryptic casein phosphopeptides prepared under industrially relevant conditions, *Biotechnology and Bioengineering*, 45(3), pp. 196-204.
20. Su R., Qi W., He Z., Yuan S., Zhang Y. (2007), Pancreatic hydrolysis of bovine casein: Identification and release kinetics of phosphopeptides, *Food Chemistry,* 104, pp. 276-286.
21. Ellegard, K.H. C. Gammelgard-Larsen, E.S. Sorensen, S. Fedosov (1999) Process Scale Chromatographic Isolation, Characterization and Identification of Tryptic Bioactive Casein Phosphopeptides, *International Dairy Journal*, 9(9), pp. 639-652.
22. Naqvi M.A., Irani K.A., Katanishooshtari M., Rousseau D. (2016) Disorder in Milk Proteins: Formation, Structure, Function, Isolation and Applications of Casein Phosphopeptides, *Current Protein and Peptide Science,* 17(4), pp. 368-379.

Surface morphology of soybean, pea, whey protein isolates, and their dried gels

Maksym Polumbryk[1], Olena Shestel[2], Olha Yatsenko[1], Nataliia Yuschenko[1], Ulyana Kuzmyk[1]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – Cherkassy State Technological University, Cherkassy, Ukraine

Keywords:

Morphology
Proteins
Whey
Soybean
Pea

Article history:

Received 12.08.2018
Received in revised form 26.11.2018
Accepted 28.03.2019

Corresponding author:

Maksym Polumbryk
E-mail:
polumbr@nuft.edu.ua

DOI: 10.24263/2304-974X-2019-8-1-8

Abstract

Introduction. The hydrocolloids of plant and animal origin are widely used in food technologies, especially in the meat and dairy products manufacturing. The aim of this study was to investigate the surface morphology of some proteins isolates of plant origin and hydrated gels made from them.

Materials and methods. The protein isolates of soybean, pea and whey as well as κ-carrageenan, and guar gum were used in this study. We prepared gels of the isolates with low fats milk at the ratio 1:6 and 1:8. Structural changes of soybean, pea, and whey protein as well as their hydrates were observed by use of scanning electron microscope JSM-6700F.

Results and discussion. The presence of coarse particles was observed in the samples of soybean and pea isolates, whereas sample of whey isolates had spherical shaped particles only. We found that, pea and soybean isolates did not differ significantly in their sample size, which was approximately 40 μm. The low hydration level of the isolates at a ratio of 1:5 lead to smooth surface formation with the large number of holes. The remains of the large globules can be identified in the sample of hydrated pea isolate as the edges of dried spheres, which sizes are similar to those of initial isolates. Increased hydration level up to 1:8 resulted in the crosslinking between the proteins macromolecules and lead to carcass formation. Polysaccharides incorporation into the mixture of stabilizers resulted in the surface formation of gels which morphological features were similar to those of protein isolates of pea, soybean, and whey. However, this was further resulted in the gel structure formation with the minor crosslinking

Conclusions. Increased amount of milk in the gels resulted in the development of 3D structure formation, as expected. Pea isolate was characterized by the great ability to the crosslinking between macromolecules of proteins.

Introduction

Several dairy products, such as yogurt, curd, and butter are widely present in a human diet. Modern trends in the substitution of raw materials of animal origin lead to thorough examination of structural features of the plant materials. Several beans, primarily soybean and pea are widely used as a source of protein since their chemical composition and technological properties are similar to the animal proteins [1, 2]. Moreover, consumption of these proteins is beneficial for human health due to a high amount of essential amino acids [1].

Introduction of these proteins into the formulations of dairy products has resulted in the reduction of fats content without any adverse effects in the texture of the final products [3]. Usually protein isolates are used in a preliminary hydrated form, so that it is necessary to evaluate effective water: protein ratio in each certain case. It should be noted, that insufficient amount of water may have negative effect on the technological properties, causing increased viscosity, unpleasant aftertaste, and low yield of final product [4]. It should be noted, that too much water resulted in the complicated retention of food carcass. Mixture or composition of several protein isolates are used in order to obtain food products, particularly meat and dairy with desired properties. This approach was an effective in the sausages production made from meat emulsion and in the curd manufacturing too [5]. Unfortunately, it is necessary to find effective ratio of hydrocolloids in the each certain case. Thus, structural changes of protein isolates during hydration are the subject of careful study, which may reveal the mechanisms of hydrocolloids transformations, and will help to simplify the search of their ratio in compositions.

Scanning electron microscopy is an important technique which has been widely used in the careful screening of the surface of food materials [6-8]. This method has found to be useful in the investigations of surface morphology of fermented sausages at different level of starch, fats and egg white in the formulation [9], and analysis of texture of frankfurter sausages with the modified fats profile [10]. The authors have suggested that sensory properties of the final products depend on the structural features of the product surface [10]. The more uniformly distributed holes on the surface the better quality of the final product. Application of SEM in the analysis of dairy products gives an opportunity to quantify differences of the surface parameters at the different duration of technological process [11]. Also, this method was useful in the analysis of fats distribution in the dairy products, such as butter, curd, milk, and other. It should be noted, that usually frozen samples of dairy product have examined by cryo scanning electron microscopy [12]. This technique is an extremely useful in the investigations of distribution of casein particles, fats, and oils as well as their form, shape, and transformations during technological treatment or storage [12]. Nevertheless, SEM method has used in the quantitative examination of the texture of dairy products too [8].

Purpose. The purpose of this study is a surface examination of several hydrated gels of protein isolates at different milk: protein ratio.

Materials and methods

Materials and samples preparation

The protein isolates of soybean, pea and whey as well as κ-carrageenan, and guar gum were purchased from Roeper (Germany). All the isolates were of high purity (≥ 95%.) grade.

Low fats milk (0.05%.) was purchased from a local supermarket "Auchan". We prepared gels of the isolates with low fats milk at the ratio 1:6 and 1:8. We have previously reported that the lower isolate: milk ratio was resulted in extremely high viscosity of the gel so that there were no possibilities of utilization of these gels for the food manufacturing purposes [3]. Moreover, we prepared the gel sample of the pea isolate, which was swelled during 24 h. The samples were retrained during 22-24 h. prior to heat treatment in order to achieve a maximal hydration of the samples. Further, the gels of protein isolates hydrated by skimmed milk at the ratio 1:6 and 1:8 were exposed to thermal treatment at 82 ± 2 °C during 5 and 10 minutes, respectively. This was done order to accelerate development of the protein network. Petri`s dish was layered by protein hydrates and dried at room temperature for 24 h followed by rinsing in chloroform for 40 min in order to remove particles of fats from the samples surfaces, and, finally, the samples were sustained in ethanol due to necessity of water elimination from the surfaces.

We have previously reported that the application of hydrocolloids composition based on the isolate of whey proteins resulted in the increased stabilization of butter paste [13]. The samples of gel of composition with low fats milk at the ratio 1:6 and 1:8 were prepared. The following components have used in the composition: isolate of milk proteins, isolate of whey proteins, guar gum, and κ-carrageenan at the ratio 10: 3: 0.3: 0.05.

Scanning electron microscopy set-up

Structural changes of soybean, pea, and whey protein as well as their hydrates were observed by use of scanning electron microscope JSM-6700F (JEOL, Japan. The microstructure of protein isolates was observed at ambient temperature. Samples were covered with platinum layer with the width of 10 nm prior to the experiments. The microphotographs were obtained at the accelerating voltage which was 10 kV and probe current 0,65 nA. Dried samples of the protein hydrates (0.2×0.2 sm, and 0.5–1 mm in depth) were fixed with the steel holder followed by operating under vacuum at the temperature 20 ± 0.1 °C.

Results and discussion

In the first stage, we tried to assess morphological parameters of initial isolates. The presence of coarse particles was observed in the samples of soybean (figure 1b) and pea isolates (figure 1 c), whereas sample of whey isolates had spherical shaped particles only (figure 1a). We found that, pea and soybean isolates didn`t differ significantly in their sample size. The particles with the mean diameter of 18-21 μm preferably were presented in these isolates and minor amount of fraction with the average size 40 μm was detected in these samples. In contrast, the sample of whey isolated consisted mainly large globules with the average size of about 40 μm with inclusion other particles, which cannot be separated into the certain fractions. It should be noted that, prolonged shooting of the samples resulted in the wrinkled globules formation which was observed due to the low thermal resistance of these samples.

Previously κ-carrageenan and guar gum were used as a minor components of mixture of stabilizers [13]. The structural features of κ-carrageenan are different to those of protein isolates. We didn`t detect presence of globules on the surface in the sample of this hydrocolloid (figure 1d). The presence of coarse particles and ellipsoid-like particles with the diameters 110–120 μm and 60–70 μm was observed on the surface of the sample of κ-carrageenan

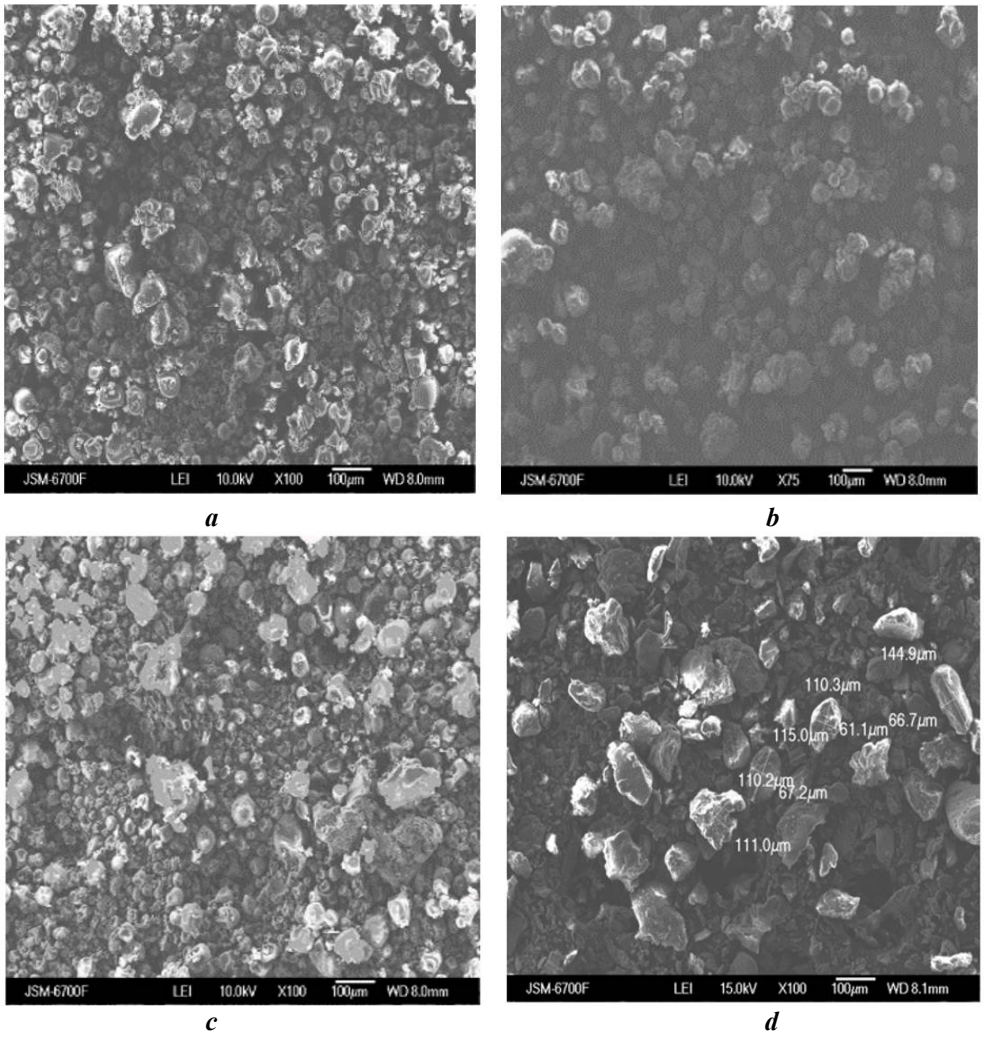


Figure 1. Electron microphotographs of whey protein isolate (a), soybean isolate (b), pea isolate (c), and κ-carrageenan (d)

The low hydration level of the isolates at a ratio of 1:5 lead to smooth surface formation with the large number of holes (figure 2). These holes were filled with water before drying. In our opinion, insufficient hydration of proteins resulted in the quternary structure disruption, while interconnections between protein macromolecules didn`t observed. Insight into figure 2D revealed that remains of the large globules can be identified in the sample of hydrated pea isolate as the edges of dried spheres, which sizes are similar to those of initial isolates. In our opinion the presence of fracturings on the surface of pea gels was most likely associated with incoplete hydration of the sample.Also, small globules, which were less than 1 μm were, still presented on the surface of other isolates. The presence of κ-carrageenan on the surface of dried gels wasn`t observed.

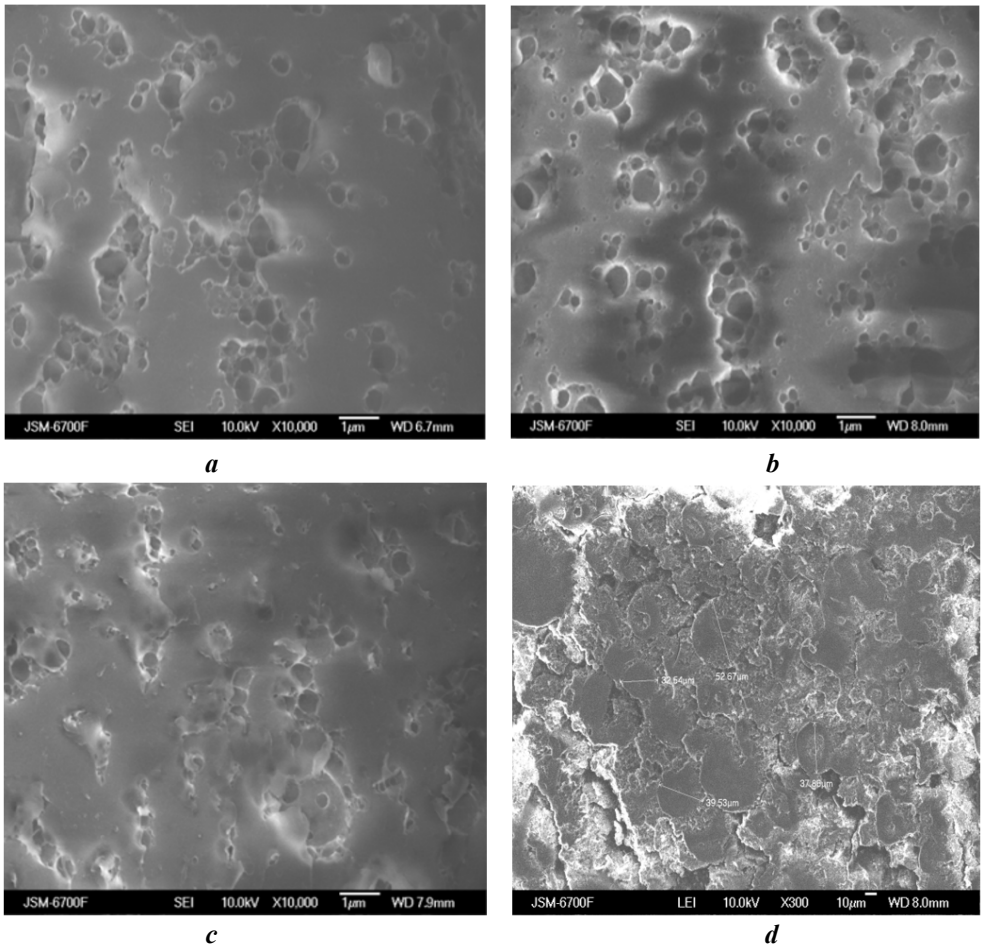


Figure 2. SEM images of dried gels of samples of whey protein isolate (a), spybean isolate (b), preliminary swelled pea isolate (c), and pea isolate without swelling (d) at protein: milk ratio 1:5

Further hydration of protein isolates was resulted in the surface formation without any globules. Rise of milk amount in the isolate: milk ratio up to 1:6 increases hydration of proteins. As it can be seen from the figure 3 all the samples of dried gels are characterized by smooth texture with large number of small holes. In contrast to the previous set of dried gels, preliminary hydration of isolates at the 1:6 isolate: milk ratio has negligible effect on the surface morphology of the gels.

We observed decreased number of pores and their diameter in the sample with 1:8 hydration ratio in compare to sample with 1:8 hydration ratio. By other words, increased hydration was resulted in the less porous structure formation. Similar findings were observed by Rovira et al. in the dried samples of goat chees [8]. A greater number of pores was found in the sample which was preliminary swelled during 24 h. This sample was characterized by the higher development of the proteins crosslinking than sample without preliminary swelling.

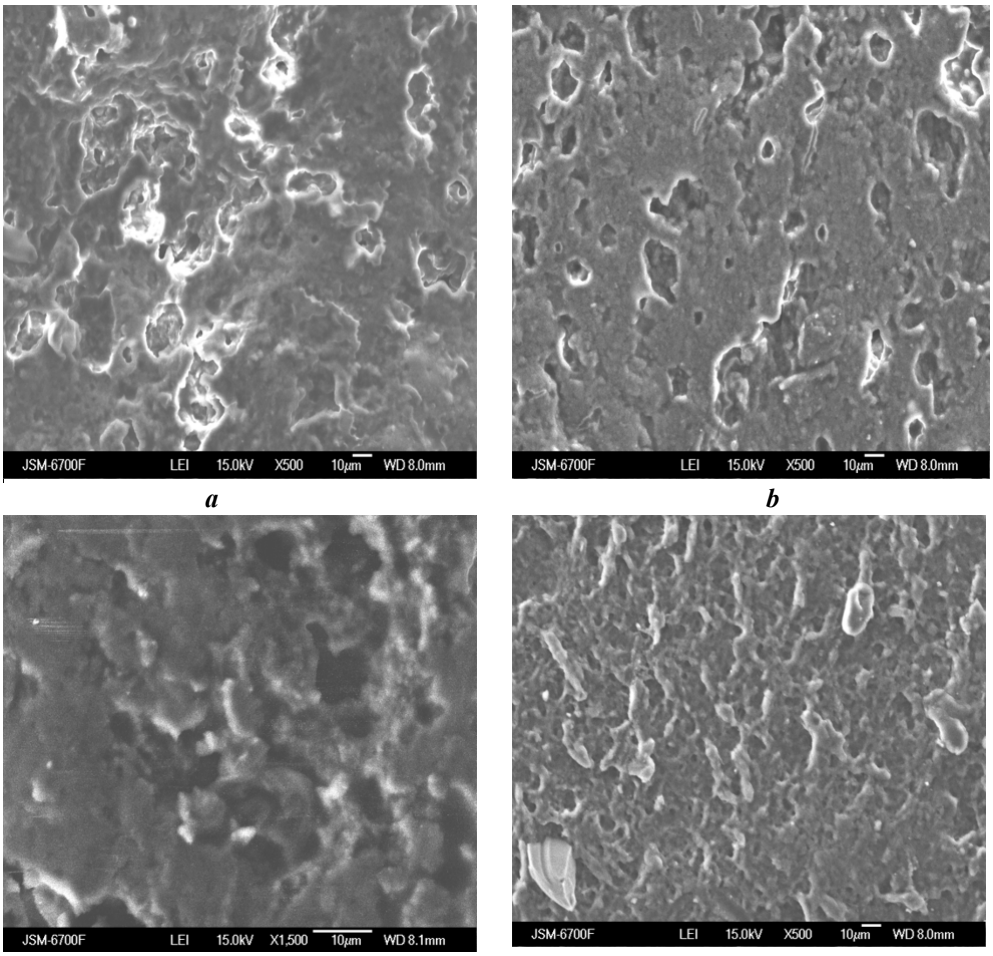


Figure 3. Electron microphotographs of dried gels of whey protein isolate (a), spybean isolate (b), preliminary swelled pea isolate (c), and pea isolate without swelling (d) at protein: milk ratio 1:6

Further hydration of these isolates at the ratio 1:8 was resulted in the crosslinking between different residues of protein macromolecules and three-dimenional net structure formation (fig. 4). This was particularly true in the sample of pea isolate (fig.4c), which surface consisted numerous holes and free spaces. Liu and coauth. found that the development of free dimensional structure of soybean proteins in their gels strongly dependent on the processing type [14]. Microwave heating was resulted in more viscoelsatic structure of soybean gels than the gels obtained by conventional method [14].

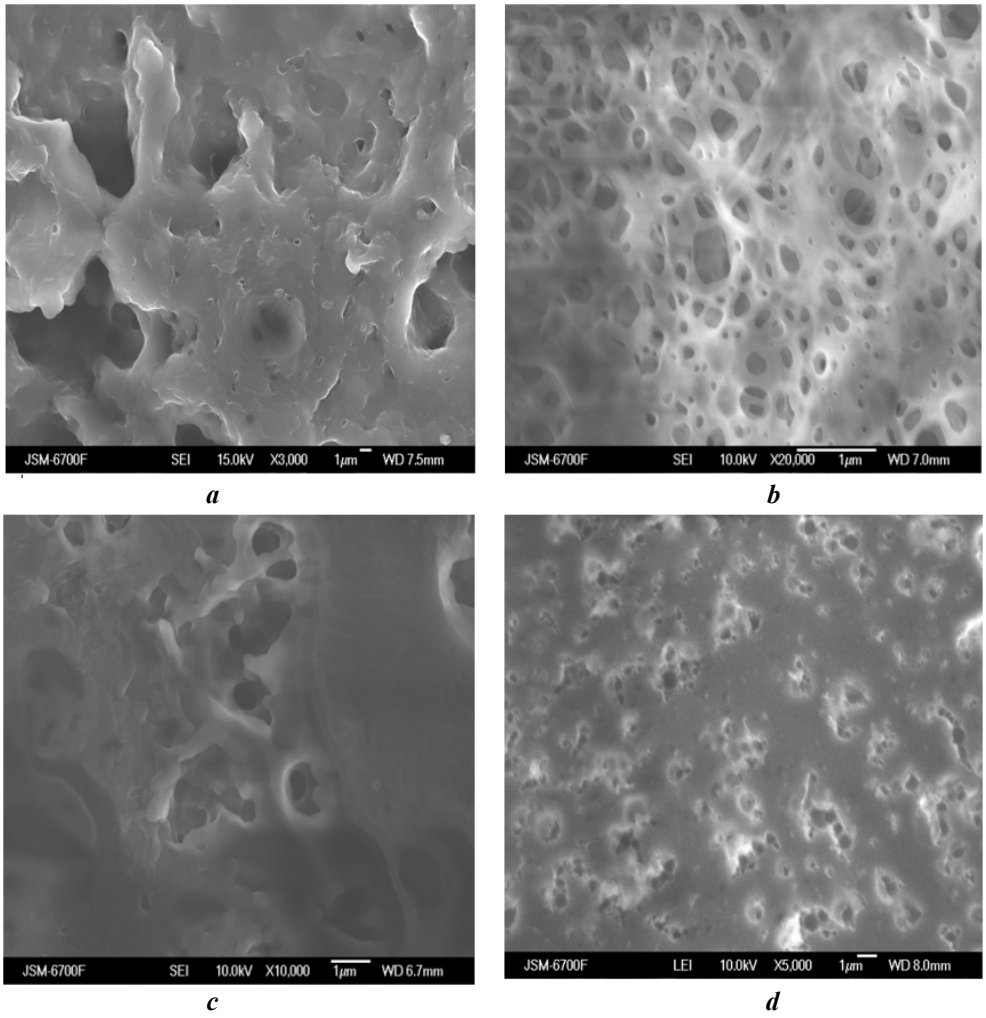


Figure 4. SEM images of hydrated samples of whey protein isolate (a), soybean isolate (b), and pea isolate (c), and pea isolate without swelling (d) at protein: milk ratio 1:8

We didn`t studied with higher isolate: milk ratio due to both full hydration of proteins and adverse effect on the texture of the gels caused by high amount of water.

Discussion

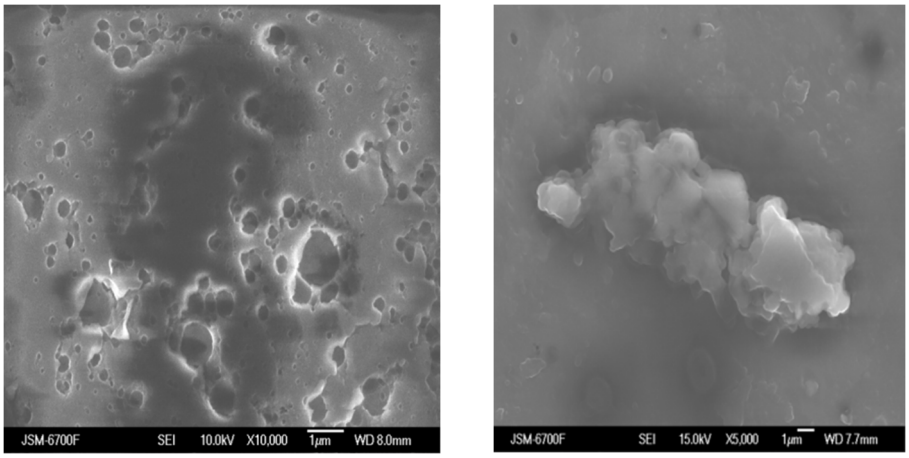
Utilization of protein isolates in the formulations of cheese and curd has lead to decrease of total cost of final products and has a positive efeect on the overall quality of the final product. Usually these isolates have used in the preliminary hydrated form, which resulted in the necessity of evaluation of the most effective water: isolate ratio. In this study scanning electron microsocopy was used for this purpose. Also, we used milk as water substitute in the gels formation. It has several advantages, especially presence of proteins which structural features are similar to proteins isolates. We prepared three series of gels of protein isolates

with a isolate:milk ratio 1:5, 1:6, and 1:8. The samples were dried at ambient temperature for 24 h and treated with chloroform and ethylalcohol in order to eleminate water and fats from the samples surfaces.

We observed fractures on the surface of gel made from not swelled pea isloate and globules remains on the surfaces of the other samples of gels prepared from isolate protein and milk in the ratio of 1:5. In our opinion these findings suggest that the milk amount isn`t enough for the full hydration of proteins. Probably, the more prolonged drying will cause similar findings in the other samples with low hydration level. It should be noted, that "air side" surface was rough whereas the surface which was contacted with the flat glass was smooth. Similar observations were indicated by Kowalczyk et al. during observation of pea gels [15]. Whey have suggested that rougness of the surface which has contacted with air related to the presence of small (5-20 μm) particles of pea isolates [15]. From figure 2, it can be seen that the presence of globules of the isolates didn`t detected on the surface of the samples. All the images of dehydrated protin fels given in figure 2 obtained from smooth side.

Increased hydration level up to 1:8 resulted in the crosslinking between the proteins macromolecules and carcass formation (figure 4). The samples didn`t differ by their structural features. It is known that the development of microstructure starts from rearrangement of casein micelles of whey proteins. As it has observed by Rovira et al., increased production time of goat cheese resulted in the continous network of proteins formation due to binding of weakly hydrated aggregates of the proteins [8]. Also, formation of protein carcass has observed by Lin et al, in the samples of tofu made from soybean milk [16]. SEM images of dehydrated tofu samples has characterized by large number of the small pores between protein macromolecules, which cuased three dimensional honeycomb-like structure formation [16].

The gels produced from milk and protein isolates are extremely important in the production of batter pastes. Several polysaccharides usually added into the composition in order to improve their technological properties. Utilization of κ-carrageenan in these products make it possible to decrease amount of composition of stabilizers, while guar gum incorporation may resulted in the improvement of final product during freezing and thawing processes. We have found that the incorporation of κ-carrageenan has resulted in the formation of highly crosslinked gels which cannot be used in the batter pastes. We prepared samples of gels made from low fat milk and composition of hydrocolloids based on milk proteins and whey isolate proteins at the composition and milk ratio 1:6 and 1:8. As it can be seen from figure 5a, incorporation of polysaccharides into the mixture of stabilizers resulted in the surface formation of gels which morphological features were similar to those of protein isolates of pea, soybean, and whey (figure 3). However, the sample of gel, which was hydrated by low fat milk, had the structure with the minor crosslinking (figure 5). In our opinion, crosslinking between molecules of proteins is a key factor of gels structure in determining of possibility of the gel application in the butter pastes. The strong crosslinking, which was observed in the sample of pea isolate, was pointed that it would not to be used in these products manufacturing without significant changes of technological processes and amount of ingredients in the formulations, while the isolates of soybean and whey are the better choice for this purpose. Therefore utilization of the composition of the stabilizers allows decreasing the development of crosslinking between and decrease amount of added hydrocolloids.



a *b*

Figure 5. SEM images of hydrated samples of hydrocolloid composition at protein: milk ratio 1:6 (a) and 1:8 (b)

Conclusions

This study proved that SEM is a promising technique which helps to choose components in the composition of stabilizer dependently on the product type. It was found that the increased amount of milk resulted in the development of 3D structure formation, as expected. Pea isolate was characterized by the great ability to the crosslinking between macromolecules of proteins. Therefore it cannot be used in the butter pastes, but may be especially useful in the cheese manufacturing. This study is limited by a low number of studied proteins isolates. Further study on larger number of proteins isolates is necessary to investigate role of hydration level on the 3D- structure development.

References

1. Han B.-Z.; Rombouts F. M.; Nout M.R. (2004), Amino acid profiles of sufu, a Chinese fermented soybean food, *J. Food Compos. Anal.*, 37, pp. 689–698.
2. Xiong T., Ye X., Su Y., Chen X., Sun H. et al. (2018), Identification and quantification of proteins at adsorption layer of emulsion stabilized by pea protein isolates, *Coll. Surf. B Biointerfaces.*, 171, pp. 1–9, DOI: 10.1016/j.colsurfb.2018.05.068.
3. Guo Y., Zhang X., Hao W., Xie Y., Chen L. et. al. (2018), Nano-bacterial cellulose/soy protein isolate complex gel as fat substitutes in ice cream model, *Carbohydr Polym.*, 198, pp. 620–630, Doi: 10.1016/j.carbpol.2018.06.078.
4. Kochubei-Lytvynenko O., Yatsenko O., Yushchenko N., Kuzmyk U. (2018), Investigation of functional-technological properties of soya protein, *EUREKA: Life Sciences*., №. 5, pp. 39–43, Doi: 10.21303/2504-5695.2018.00716.
5. Kochubei-Lytvynenko O., Yatsenko O., Yushchenko N., Kuzmyk U. (2018), A stabilizing system for butter pastes based on the dry concentrates of milk protein.

East.-Europ. J. Enterprise Technol., 5(11), pp. 30–36, DOI: 10.15587/1729-4061.2018.143105.

6. Niu H., Xia X., Wang C., Kong B., Liu Q. (2018), Thermal stability and gel quality of myofibrillar protein as affected by soy protein isolates subjected to an acidic pH and mild heating, *Food Chem.*, 242, pp. 188–195, DOI: 10.1016/j.foodchem.2017.09.055.
7. Chen J. (2007), Surface texture of foods: perception and characterization, *Crit. Rev. Food Sci. Nutr.* 47, pp. 583–98. doi: 10.1080/10408390600919031.
8. Rovira S., Lopez M.B., Ferrandini E., Laencina J. (2011), Microstructure quantification by scanning electron microscopy and image analysis of goat cheese curd, *J. Dairy Sci.* 94 , pp.1091–1097, DOI: 10.3168/jds.2010-3996.
9. Mohamed H.M., Emara M.M., Nouman T.M. (2011), Effect of cooking temperatures on characteristics and microstructure of camel meat emulsion sausages, *J. Sci. Food Agric.*, 96, p. 2990–2997. doi: 10.1002/jsfa.7468.
10. Caballo J., Fernandez P., Barreto, G.,et.al. (1996), Morphology and texture bologna sausage as related to content of fat, starch and egg white, *J. Food Sci*, 61(3), pp. 652–655, DOI: https://doi.org/10.1111/j.1365-2621.1996.tb13179.x
11. Liu, W., Lanier, T. (2015), Combined use of variable pressure scanning electron microscopy and confocal laser scanning microscopy best reveal microstructure of comminuted meat gels, *LWT – Food Sci. Technol.*, 62, pp. 1027–1033., DOI: 10.1016/j.lwt.2015.02.001.
12. Ong L., Dagastine R R., Kentish S.E., Gras S.L. (2011), Microstructure of milk gel and cheese curd observed using cryo scanning electron microscopy and confocal microscopy, *LWT – Food Sci. Technol.*, 44, pp. 1291–1302, DOI: 10.1016/j.lwt.2010.12.026.
13. Kochubei-Lytvynenko O., V.M. Pasichnyi, Yatsenko O., Yushchenko N., Kuzmyk U. (2018), Prospects of usage of pea protein isolate in buttery pastes technology, *Sci. Lett. NUFT.*, 24(6), pp. 152–159, DOI: 10.24263/2225-2924-2018-24-6-19.
14. Liu H.H., Kuo M.I. (2011) Effect of microwave heating on the viscoelastic property and microstructure of soy protein isolate gel, *J. Texture Stud.*, 42, p. 1–9.
15. Kowalczyk D., Gustaw W., Zieba E., Lisiecki S. et. al. (2016), Microstructure and functional properties of sorbitol-plasticized pea protein isolate emulsion films: Effect of lipid type and concentration, *Food Hydrocoll.*, 60(4), pp. 353–363, DOI: 10.1016/j.foodhyd.2016.04.006.
16. Lin H.F., Lu C.P., Hsieh J.F., Kuo M.I. (2016), Effect of ultrasonic treatment on the rheological property and microstructure of tofu made from different soybean cultivars, *Innovative Food Sci. Emerging Technol.*, 37, pp. 98–105, DOI: 10.1016/j.ifset.2016.08.013.

Effect of hydrolytic enzymes pretreatment on the oil extraction from pumpkin seeds

Tamara Nosenko, Ganna Vovk, Tamara Koroluk

National University of food Technologies, Kyiv, Ukraine

Keywords:

Pumpkin
Seeds
Oil
Protease
Cellulase
Pressing

Article history:

Received 23.09.2018
Received in revised form 01.11.2018
Accepted 28.03.2019

Corresponding author:

Tamara Nosenko
E-mail:
tamara_nosenko@ukr.net

DOI: 10.24263/2304-974X-2019-8-1-9

Abstract

Introduction. The aim of the present study was to evaluate the effect of enzyme mixture on the cell integrity, oil yield of cold-pressing and dynamics of solvent extraction of pumpkin seeds oil.

Materials and Methods. Protolad, Alkaline, acid proteases and Cellulad (ENZIME, Ukraine) were used for pumpkin seeds pretreatment. The cells integrity was evaluated by the method of immediate "shaking". The cold pressing was carried out on the laboratory screw press. The solvent oil extraction rate was estimated using a Soxhlet extractor as oil quantity extracted after one extraction cycle.

Results and discussion. It was detected that main increase of pumpkin seed cells integrity destruction comparing with a control sample had been happened after 2-hour of enzyme pretreatment. Further incubation of ground seeds with enzymes did not lead to significant increase of "open" cells content in the mixture. It was shown that using of different kind of proteolitic enzymes for pumpkin seeds pretreatment resulted in increase of destroyed cells quantity from 3 to 10.4%. Using of proteases and cellulase mixture for pumpkin seeds pretreatment had resulted in further increase of level of pumpkin seed cells "revealing" by 10%. The oil yield of cold pressed pumpkin oil after enzyme pretreatment with protease (70%.) and cellulase (30%) mixture was increased from 62.3 (control sample) to 70.0%. from total oil content of seeds. The rate of solvent extraction of oil from pumpkin seeds had increased after enzyme pretreatment, that means 25.4 and 17.7% of oil were extracted after 80 min extraction from mass of enzyme pretreated and control seeds, respectively. There was no difference of peroxide content between enzyme pretreated sample and control.

Conclusions. Using of proteases and cellulases mixture for pumpkin seeds pretreatment had resulted in increase of destroyed cells quantity, following by increase of cold pressed pumpkin oil as well as rate of solvent extraction of oil from pumpkin seeds.

Introduction

Pressing and solvent extraction are the two methods for oil obtaining from oil seeds. The solvent extraction is more effective for oil yield, that gives possibility to recover almost total oil content. The disadvantages of this method are process safety and environmental issues associated with solvent extraction process. The pressing is more environment friendly but does not provide the whole oil extraction. Thus it is important to develop the effective and environment friendly technology of oil recovering from oil materials.

In this context, different enzymatic technologies were proposed for oil extraction from raw materials. Firstly, the enzyme assisted aqua extraction was used to obtain oil and protein from oil seeds. In order to increase the efficiency of water extraction of oils and proteins from oilseeds, it was proposed to use hydrolytic enzymes to destroy cell walls, as well as protein frames surrounding oleosomes [1-10]. The oil yield had varied from about 60 to 90%. depending from proteases and cellulases used and kind of oil material. The weakness of enzyme assisted aqua extraction is the necessarity to separate of oil-in-water emulsion and low oil yield. Enzyme assisted technology was proposed to obtain the partly hydrolyzated protein from sunflower meal [11,12].

In parallel, enzyme assisted technologies were used for oil seeds pretreatment before their processing. The cellulase, hemicellulase, pectinase and proteases are usually used for this purpose. It was shown that press oil yield had increased from 72 to 90-93%. after pretreatment of rape seeds by carbohydrases and proteases [13–16]. The increase of press soy bean oil yield after enzyme pretreatment was shown in [17–19].

The effect of different enzyme preparations (Viscozyme L, Kemzyme, and Feedzyme) on the oil yield, physicochemical and antioxidant properties of cold pressed flaxseed oil were assessed in [20]. The oil yield from enzyme-treated cold pressed flaxseeds, although lower than Soxhlet extracted oil yield, was considerably higher when compared with the control Most of the physicochemical parameters such as refractive index, density, iodine number, free fatty acid contents, saponification value, color and fatty acid profile did not differ significantly between the control and enzyme pretreated oil. At the same time the peroxide value, *para*-anisidine value, conjugated dienes, triens and induction period (Rancimat method) of oil from enzyme-treated flaxseeds were superior compared with the control. The effects of enzyme-assisted cold-pressing (EACP) on the oil yield and physicochemical properties of hemp seed oil were also investigated using five enzyme preparations [21]. The oil yield from enzyme-treated hempseeds were found to be significantly higher than that determined for the control. There were no significant variations observed for the values of iodine number, refractive index, density, unsaponifiable matter and fatty acid composition between the enzyme-extracted and control hempseed oils. The values of saponification value, free fatty acids, iodine value and peroxide value were slightly varied between the oils tested. A relatively higher content of tocopherols (4.8–14.1%) as well as improved Rancimat profiles were observed in the enzyme extracted oils compared to the control.

To the best of our knowledge, there are no reports about pumpkin seeds oil recovering using EACP. The aim of the present study was to evaluate the effect of proteases and cellulases on the cell integrity, oil yield of the EACP and dynamics of solvent extraction of pumpkin seeds oil.

Materials and methods

Materials

Pumpkin (*Cucurbita pepo*) seeds were purchased from a local market (Kyiv, Ukraine). Neutral protease from *Bacillus subtilis* (Protolad, 70 FIP-U/g, optimum pH 6.5-7.0, ENZIME, Ukraine), alkaline protease from *Bacillus licheniformis* (Alkalase, 2.4 AU/g, optimum pH 8.5-9.0, ENZIM, Ukraine) and acid protease (ENZIM, Ukraine) were used for hydrolysis of cell proteins. Cellulad (ENZIM, Ukraine) − a complex enzyme preparation of cellulases for the hydrolysis of non-starch polysaccharides, obtained by directed fermentation of the breeding strain *Tr. reeseii*. In addition to the main activity of cellulase, the preparation contains significant amounts of hemicellulase and xylanase. All chemicals used for experiments were at least analytical grade.

Enzyme treatment and pressing

Clean seeds were ground using a coffee grinder and then subjected to incubation with 0.6%. (by seed weight) of the enzyme preparations over a period of 2 h at (51±3) °C and 50%. moisture level. The hydrolyzed sample was dried at 100 °C in a drying oven to inactivate the enzymes and to readjust the moisture to the desired level (6–7%) prior to pressing. Pressing of the hydrolyzed and dried seed sample for oil extraction was done in a laboratory screw press at (60±5) °C. A control sample of pumpkin seeds was also processed under the same set of conditions, except for the enzyme adding during treatment.

Evaluation of the cells integrity by the method of immediate "shaking"

10 g of ground pumpkin seeds were placed in a flask, 200 cm^3 of petroleum ether was added and the contents of the flask were shaken for 3 seconds (exactly). Then the flask was left for 10 seconds (exactly), after which the ether solution of oil was quickly and carefully transferred to another flask. The obtained solution was filtered to a weighed flask. The filter was washed by several portions of the ether. The ether from the weighed flask was evaporated and the resulting oil was dried to a constant weight. Simultaneously another 10 g of ground pumpkin seeds were taken to determine the oil content by the extraction in the Soxhlet apparatus. The mass fraction of oil extracted from the instantaneous culling and expressed as a percentage of its total content corresponds to the number of "exposed" cells.

The number of destroyed cells was determined as a percentage of oil extracted by the immediate "shaking" from the total oil content in the ground pumpkin seeds:

$$X = \frac{a_1 \cdot 100}{a_2},$$

where a_1 - oil extracted by the immediate "shaking",%; a_2 - the total oil content of ground pumpkin seeds, determined by the extraction in the Soxhlet apparatus,%.

Measurement of dynamics of oil solvent extraction

The enzyme pretreatment of pumpkin seeds was carried out as described in 2.2. The pretreated and control seeds were taken to filtering paper shells, weighted and placed in a Soxhlet extractor. The extractor was fitted with a condenser and a 0.5 L round bottomed flask. The extraction of oil was done in a water bath for 80 min, using about 350 mL *n*-

hexane. The duration of extraction cycle was about 10 min. After every extraction cycle the three filtering paper shells were withdrawn from extractor, hexane was evaporated, dried and weighted. The level of oil extraction was calculated using the following equation:

$$O = \frac{(m_0 - m_n)}{m_0} \cdot 100,$$

where m_0 - the initial mass of filtering paper shell, m_1 - the mass of filtering paper shell after every extraction cycle, n - the number of extraction cycle.

Determinations of peroxide values

Determinations of peroxide values of the enzyme extracted and control pumpkin seed oils were made following AOCS official methods [24].

Statistical analysis

All the experiments were conducted in triplicate and statistical analysis of the data was performed using the statistical software Statistica [25]. A probability value at $p< 0.05$ was considered statistically significant. Data are presented as mean values±standard deviation calculated from triplicate measurings.

Results and discussion

Influence of enzyme pretreatment of pumpkin seeds on the cell integrity

To establish the appropriate conditions for seeds pretreatment firstly we have study the influence of treatment duration by proteases on the cell integrity. It was detected, that after first hour of treatment about 5.5%. increase of destroyed cells were observed comparing with a control sample (Fig. 1) and next hour of treatment have resulted in next 9.6%. of destroyed cells content increase. Further incubation of ground seeds with enzymes did not lead to significant increase of "open" cells content in the mixture. It was decided to use two-hour enzyme treatment for the next study. At the same time enzyme pretreatment duration of hemp seeds was up to 6 hours in study of Latif S. and Anwar F. [21].

The data obtained have demonstrated that using of different kind of proteolitic enzymes for pumpkin seeds pretreatment resulted in increase of destroyed cells quantity from 3 to 10.4%. (Fig. 2). The acid proteases were the most effective enzymes in "revealing" of pumpkin seeds cells, showing the highest increase of destroyed cells content in oil material.

Obviously, that proteins are important substances for the integrity of pumpkin seeds cells, which play a significant role in the building of the intracellular membranes, including that surrounding the oleosomes and in the adhesion of cell membrane to cell walls. Enzyme pretreatment facilitates the breakdown of the protein network surrounding the lipid bodies and also supports the conversion of the complex seed lipoprotein molecules into simple lipid and protein molecules, thereafter enhancing both the oil availability and extractability [22, 23].

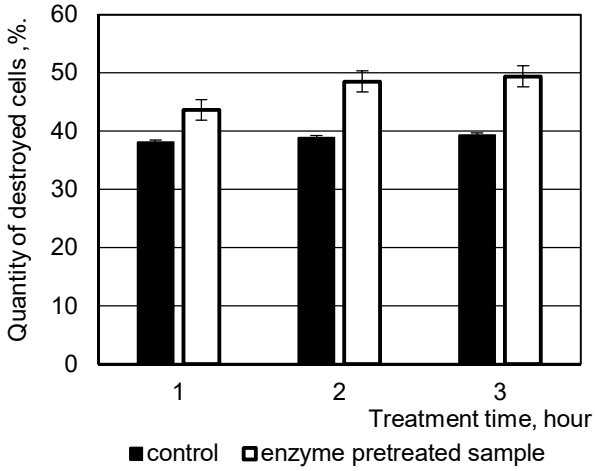


Figure 1. The influence of proteases treatment duration on the quantity of destroyed cells in control and enzyme pretreated ground pumpkin seeds

The accessibility of cell oil depends from the integrity of cell walls, that are building from cellulose. It was supposing, that adding of cell-wall-degrading enzymes to the enzyme cocktail for seeds pretreatment has to enhance the enzymes effect on the cell structure and improve the accessibility of oil.

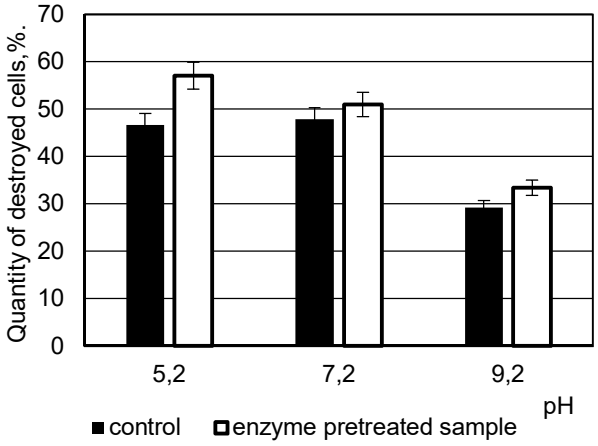


Figure 2. The influence of different proteases on the quantity of destroyed cells in control and enzyme pretreated ground pumpkin seeds

The data obtained have shown that using of protease and cellulase for pumpkin seeds pretreatment had resulted in some increase of destroyed cells in ground pumpkin seeds mixture (Fig 3). The maximum level of "revealing" of pumpkin seeds cells had increased to about 61%. under influence of mixture of 30% neutral protease and 70% cellulase that is 10%. higher than after treatment by this protease itself. The growing of content of destroyed cells was about 3%. under using of acid protease and cellulase mixture (Fig 3, b).

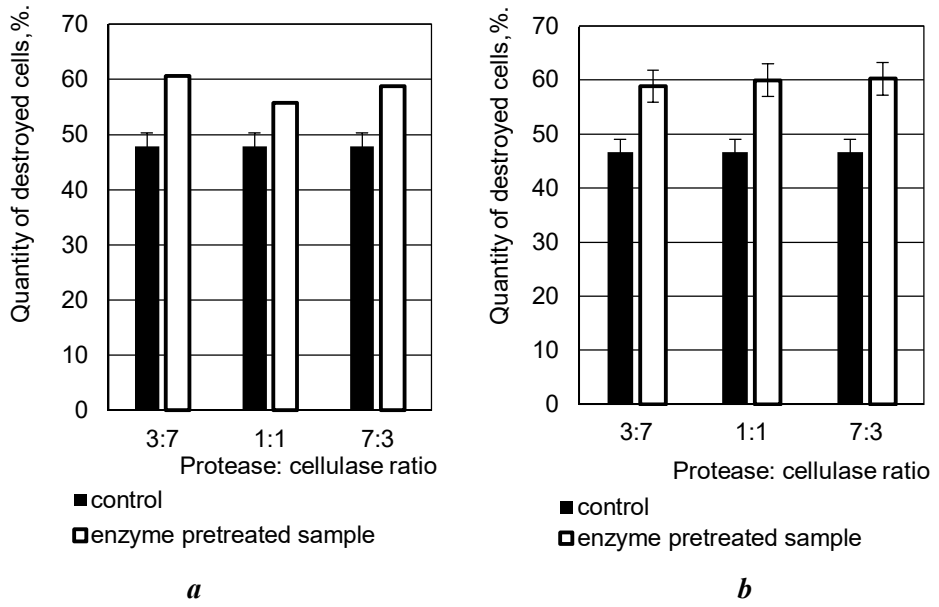


Figure 3. The quantity of destroyed cells in control and enzyme pretreated ground pumpkin seeds on dependence the proteases kind and protease: cellulase ratio (a - neutral protease and cellulase mixture, b - acid protease and cellulase mixture)

Influence of enzyme pretreatment of pumpkin seeds on oil recovery

The cold pressing of dried pumpkin seeds (seeds moisture was 7.0±0.1) were carried at (60±5) °C. The oil yield of cold pressed pumpkin oil after enzyme pretreatment of seeds with neutral protease (70%.) and cellulase (30%.) mixture was increased from 62.3 (control sample) to 70.0%. from total oil content, which was 42.4%. Our data are in accordance with the results obtained for hemp seeds with the enzyme of complex activity (mainly a-amylase, b-glucanase, cellulase complex, hemicellulase complex, protease and xylanase activities) [21] as well as with results obtained for flax seeds [20] and rape seeds [13] cold pressing after enzyme pretreatment.

Commonly, the enzyme pretreatment of oil seeds are using as a technique to increase the press oil yield, mainly cold pressed oil, as heat pressing and solvent extraction of oil seeds give a high recovering of oil on their own. But in our study we have researched the dynamics of oil solvent extraction from oil material under effect of enzyme pretreatment.

The rate of solvent extraction of oil from pumpkin seeds was influenced by enzyme mixture pretreatment also (Fig. 4). Enhancement of oil extraction as result of enzyme mixture pretreatment was observed from the beginning of the extraction process. After first 10 min of extraction about 6.6%. of oil were extracted from control seed samples, whereas 10.3%. from enzyme pretreated seeds, respectively. The difference between two samples was 7.7%. after 80 min extraction, that is 25.4 and 17.7%. of oil were extracted from mass of enzyme pretreated and control seeds, respectively. These were about 60 and 40%. of total oil content for enzyme pretreated and control seeds, respectively.

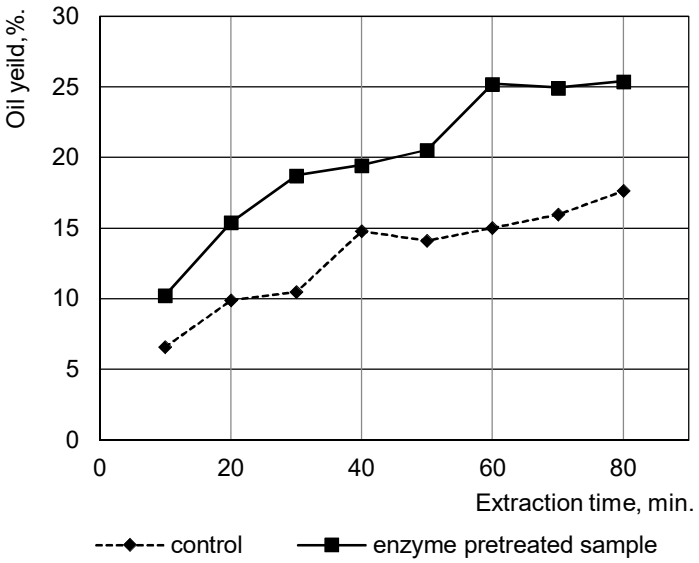


Figure 4. Dynamics of solvent extraction of oil from control and enzyme pretreated pumpkin seeds

It was possible to suggest that enzyme treatment at 40 °C and 50%. moisture level of oil seeds for 2 hours could result in increase of oil quality and probably its biological value. To elucidate this phenomena the main chemical parameters of oil samples had been evaluated. There were not any differences in acidity of oil, obtained from the pretreated seeds and from control seeds, the acid value of pumpkin oil was in the range from 2 to 2.4 mg KOH/g. The similar results were obtained concerning the measuring of oil oxidation level. It had not been demonstrated significant ($p<0.05$) difference of oil peroxide values, they were 2.47 and 2.44 mmol 1/2 O/kg for control and enzyme pretreated pumpkin seed oils, respectively. These results are corresponded to the data obtained for cold presss oils from enzyme pretreated hemp seeds [21], flax seeds [20] and rape seeds [13].

There were no reliable diversity in the composition of fatty acids between the studied samples of oils [26]. The ratio between the individual representatives of sterols in the studied samples of pumpkin oil almost did not differ. The analysis of the tocopherol homologies and their total content in the oil samples shows that tocopherol content in the oil obtained from the seeds after processing with hydrolytic enzymes was 68% higher than in the control [26]. Thus, these results mean that enzyme pretreatment of pumpkin seeds did not decrease the oil quality and improve the biological value of oil.

Conclusions

The results of the present study have showed that the enzymes added during the pretreatment of pumpkin seeds resulted in considerably higher content of destroyed cells in the mixture of ground pumpkin seeds. The mixture of 30%. neutral protease and 70%. cellulase was the most effective, increasing the content of destroyed cells in enzyme pretreated seeds by 10%. comparing with a control. The increase of cold pressed pumpkin

seed oils from enzyme pretreated seeds was observed as result of enzyme influence on the cells integrity. The enzyme pretreatment of pumpkin seeds had accelerated solvent extraction of oil comparing with untreated seeds. The enzyme pretreatment of pumpkin seeds had not adversely affect the quality of the oil. This suggests the use of proteases and cellulases mixture in preparing for the extraction of oil from pumpkin seeds.

Acknowledgement. The research was carried out under financial support of the NAS of Ukraine within the framework of the scientific research project № 0117U001245 "Unconventional vegetable oil technologies with the applying of enzyme preparation".

References

1. Towa L. T., Kapchie V. N., Hauck C. et al. (2010), Enzyme assisted aqueous extraction of oil from isolated oleosomes of soybean flour, *J. Am. Oil Chem. Soc.* 87(3), pp. 347–354.
2. Lamsal B. P., Murphy P. A., Johnson L. A. (2006), Flaking and extrusion as mechanical treatments for enzyme-assisted aqueous extraction of oil from soybeans, *J. Am. Oil Chem. Soc.,* 83(11),pp. 973–979.
3. Moura J. M., Campbell K. A., Mahfuz A. et al. (2008), Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification, *J. Am Oil. Chem. Soc.,* 85(10), pp. 985–995.
4. Moura J. M., Johnson L. A. (2009), Two-stage countercurrent enzyme-assisted aqueous extraction processing of oil and protein from soybeans, *J. Am. Oil Chem. Soc.,* 86(3), pp. 283–289.
5. Moura J. M., Almeida N. M., Johnson L. A. (2009), Scale-up of enzyme-assisted aqueous extraction processing of soybeans, *J. Am. Oil Chem. Soc.,* 86(8), pp. 809–815.
6. Moura J. M., Almeida N. M., Jung S. et al. (2010), Flaking as a pretreatment for enzyme-assisted aqueous extraction processing of soybeans, *J. Am. Oil Chem. Soc.,* 87(12), pp. 1507-1515.
7. Freitas P. S., Couri L., Janklonka F. et al. (1997), The combined application of extrusion and enzymatic technology for extraction of soybean oil, *Lipid / Fett,* 99(9), pp. 333–337.
8. Latif S., Anwar F. (2011), Aqueous enzymatic sesame oil and protein extraction, *Food Chem.,* 125(2), pp. 679-684.
9. Moura J. M., Maurer D., Jung S. et al. (2011), Integrated Countercurrent Two-Stage Extraction and Cream Demulsification in Enzyme-Assisted Aqueous Extraction of Soybeans, *J. Am. Oil Chem. Soc.*, pp. 1045-1051.
10. Moura J. M., Maurer D., Jung S. et al. (2011), Pilot-plant proof-of-concept for countercurrent two-stage enzyme-assisted aqueous extraction processing of soybeans, *J. Am. Oil Chem. Soc.*, 88(10), pp. 1649-1658.
11. Nosenko T., Kubaychuk O., Vovkodav N., Cherstva A. (2014), Influence of partial hydrolysis on the protein extraction from Sunflower meal, *Ukrainian Journal of Food Science*, 2(2), pp. 244-252.
12. Nosenko T., Mank V., Zhukova Y., Cherstva A. (2016), Composition and properties of partially hydrolyzed sunflower protein isolates, *Ukrainian Food Journal,* 5(3), pp. 451-461.
13. Sosulski K, Sosulski F. W. (1990), Enzyme pre-treatment to enhance oil extractability in canola. In: Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology (edited by F.Shahidi), pp. 277-289. New York NY: Van Nostrand Reinhold Publishers.
14. Sosulski K., Sosulski F. W. (1993), Enzyme-aided vs. two-stage processing of canola: technology, product quality and cost evaluation, *J. Am. Oil Chem. Soc.,* 70, pp. 825-829.
15. Sosulski K. Sosulski F. W., Coxworth E. (1998), Carbohydrase hydrolysis of canola to enhance oil extraction with hexane, *J. Am. Oil Chem. Soc.,* 65, pp. 357–361.

16. Cherstva A., Lastovetska A, Nosenko T. (2016), Using of enzymes to extract of rapeseed oil by pressing, *Ukrainian Journal of Food Science*, 4(1), pp. 85-92.
17. Rosenthal A., Pyle D. L., Niranjan K. (1996), Aqueous and enzymatic processes for edible oil extraction, *Enzyme Microbial Technology,* 19, pp. 402–420.
18. Smith D. D., Agrawal Y. C., Sarkar B. C., Singh B. N. P., (1993), Enzymatic hydrolysis pretreatment for mechanical expelling of soybeans, *J. Am. Oil Chem. Soc.,* 70, pp. 885-890.
19. Bargale P. C., Sosulski K., Sosulski F. W. (2000), Enzymatic hydrolysis of soybean for solvent and mechanical oil extraction, *J. Food Process. Engineer*., 23, pp. 321-327.
20. Anwar F., Zreen Z., Sultana B., Jamil A. (2013), Enzyme-aided cold pressing of flaxseed (Linum usitatissimum L.): Enhancement in yield, quality and phenolics of the oil, *Grasas y aceites,*64, pp. 463-471.
21. Latif S., Anwar F. (2009), Physicochemical studies of hemp (*Cannabis sativa*) seed oil using enzyme-assisted cold-pressing, *Eur. J. Lipid Sci. Technol*., 111, pp. 1042–1048.
22. Murphy D. J. (1993), Structure, function and biogenesis of storage lipid bodies and oleosins in plants, *Prog Lipid Res.*, 32, pp. 247–280
23. Tzen J. T. C., Huang A. H. C. (1992), Surface structure and properties of plant seed oil bodies, *J. Biol. Chem.*, 117, pp. 327–335.
24. AOCS: Official and Recommended Practices of the American Oil Chemists Society. 5th Edn. AOCS Press, Champaign, IL (USA) 1997.
25. Tamara Nosenko, Oksana Kubaychuk, Natalya Vovkodav, Alyona Cherstva (2014), Influence of partial hydrolysis on the protein extraction from Sunflower meal, *Ukrainian Journal of Food Science*, 2(2), pp. 244-252.
26. Nosenko T. T., Vovk H. O., Koroliuk T. A., Holubets O. V. (2018), Vplyv poperednoi fermentatyvnoi obrobky nasinnia na sklad presovoi harbuzovoi olii, *Naukovi pratsi NUHT*, 24(5), pp. 244-251.

Substantiation of rational modes of semi-finished milk-plant stuffings freezing

Tetiana Yudina[1], Victoriia Gnitsevych[1], Iryna Nazarenko[2]

1 – Kyiv National University of Trade and Economics, Kyiv, Ukraine
2 – Donetsk National University of Economics and Trade named after Mykhailo Tugan-Baranovsky, Kryvyy Rih, Ukraine

Keywords:

Stuffing
Semi-finished
Milk
Freezing
Storage

Article history:

Received 12.09.2018
Received in revised form 13.11.2018
Accepted 28.03.2019

Corresponding author:

Tetiana Yudina
E-mail: Yudina2902@gmail.com

DOI:
10.24263/2304-974X-2019-8-1-10

Abstract

Introduction. The rational regimes of freezing of semi-finished milk-plant stuffings based on buttermilk concentrate were substantiated and experimentally confirmed.

Materials and methods. Milk-carrot, milk-pumpkin, milk-zucchini stuffings, as well as cottage cheese control sample stuffing, were studied. The research was carried out on a high-resolution spectrometer Tesla BS 567A with a working frequency of 100 MHz on protons by the Kuntz method.

Discussion of the results of study. It was established that at the temperature of 20 °C in the nuclear magnetic resonance spectrum of the semi-finished milk-plant stuffing an intense water signal is recorded, which significantly decreases with intensity while sample cooling to a temperature of -25 °C. The presence of a nuclear magnetic resonance signal indicates that non-freezing water does not disappear completely when cooling the sample to a temperature of -25 ° C, which implies the possibility of biochemical reactions proceesing in the stuffing. It is determined that developed semi-finished stuffings require a deeper overcooling than a control sample, since in designed samples of stuffings water binding with carbohydrates is processing.

It was investigated that at -25 °C the control sample contains 0,21 g of H_2O per gram of dry matter, when the semi-finished milk-carrot stuffing contains 0,40 g of H_2O per gram of dry matter, milk-pumpkin stuffing – 0,39 g of H_2O per g of dry matter, milk-zucchini stuffing – 0,37 g of H_2O per gram of dry matter at the same temperature. At -30 °C the control sample contains 0,20 g of H_2O per gram of dry matter, when in the semi-finished milk-carrot stuffing the content of H_2O reaches 0,32 g per gram of dry matter, in the milk-pumpkin stuffing – 0.25 g per gram of dry matter, milk-zucchini stuffing – 0,24 g per gram of dry matter at the same temperature. It is proved that the smallest amount of non-freezing water in the stuffings is kept at a temperature of -25 ... -30 °C and amounts 0,26 ... 0,40 g of H_20 per 1 g of dry matter, which allows to recommend a given range of temperatures for their rapid freezing.

Conclusions. The temperature of milk-plant stuffings freezing is substantiated (-25…-30 °C). The possibility of frozen stuffings storage at a temperature of -18...-19 °C, which is the standard temperature of industrial freezing chambers, is proved.

Introduction

A promising direction in creating qualitatively new foods is the combination of dairy and plant raw materials [1].

The technology of fundamentally new foods in the form of multifunctional semi-finished products on the basis of protein-carbohydrate dairy and plant raw materials is developed [2]. The new approach to the use of uninvolved natural properties of dairy and plant raw materials gives the possibility to maximize their functional properties, increasing the economic efficiency of technologies by reducing the use of nutritional additives, as well as increasing the nutritional and biological value of finished products [1].

Semi-finished milk-plant stuffings can be used for the production of a wide range of restaurant foods (sweet dishes, flour dishes, pastry, e.t.c.) [3].

It is advisable to store the developed semi-finished milk-plant dtuffings in frozen form [2]. The advantages of the use of frozen semi-finished milk-plant stuffings in the foods production at restaurant establishments are the possibility of using quick-frozen semi-finished products in the fast food system, reducing of labor and producing costs, the flexibility of the technological process, the long storage time of semi-finished milk-plant stuffings, significant expansion of the range of culinary products in restaurants and possibility to transport frozen semi-finished products at long distances [4, 22].

Literature review

To extend the storage time of foods it is expedient to use a freezing process, in particular quick freezing [22]. Quick freezing is one of the methods of preservation, which guarantees long-term storage of raw material properties due to the action of low temperatures on the development of microflora [5]. The decrease in temperature is accompanied by a slowing down of reactions related with the activity of enzymes and microorganisms [5, 22].

The effect of low temperatures on food causes changes in their consistency and structure, affects the quality of products after defrosting [5–7]. The degree of these changes depends on the speed and temperature of freezing, the duration and conditions of storage, the method of defrosting and composition of products [6,7].

The study of the influence of the freezing-defrosting process on the structure of milk-protein foods could be found in the works of domestic and foreign scientists [8–18].

It is known [8] that a large number of small crystals is formed in the process of milk-protein products quick freezing. In general, the negative influence on the quality of the milk-protein product is made by the pressure of the formed crystals of ice on the protein complexes, which leads to their breaks, cuts and loss of the native structure [9]. Besides, an increase in the concentration of dissolved chemicals occuring during crystallization creates the conditions for the rearrangement of protein micelles, changes in the structure of the product, which leads to an intense moisture distribution [8]. At the same time, the difference in the quality of milk-protein semi-finished foods based on the cottage cheese, frozen in various ways, almost disappears after several months of storage at -20 °C due to the migratory recrystallization – the growth of large crystals after melting of small ones [9]. The mover of this process is considered to be the temperature difference in the middle of the product and on its surface, as well as the difference in pressure on the surface of large and small crystals [8].

The technology of cottage cheese, which envisages the enrichment by the microparticulates of serum proteins, is developed. The storage time of the developed product exceeds the storage time of cottage cheese by 30% [9].

A method to increase the storage time of cottage cheese using different fermentation, protein coagulation and serum removal techniques is known [10]. The method of improvement of the refrigeration reservation technology of cottage cheese is developed. The method justifies the choice of optimal parameters of cottage cheese microwave defrosting, but requires experimental confirmation [11].

In the work [12] the changes of organoleptic parameters of protein bars during storage were investigated. The bars were stored at the temperature 22 °C, 32 °C or 42 °C for 42 days. It was established that the bars made using milk-protein concentrate have a higher softness regardless of the storage temperature. It is determined that the change in the surface color of the bars based on the milk-protein concentrate is minimal when stored at 22 °C, but increased at 32 °C and 42 °C [12].

The rheological properties and solubility of milk-protein concentrate during storage are investigated [13,14]. It is established that the final complex module and the tension decrease exponentially with an increase in the storage time of the milk protein concentrate. The increase in the storage temperatures intensify this effect [13]. The solubility also decreases exponentially over time, and serum proteins remain soluble, unlike caseins that become lactosilated [14].

In the work [15] the effect of low temperatures on the structure of the milk-protein concentrate was investigated and it was determined that the quick freezing of the studied foods at -20...-30 °C with subsequent storage at that temperature is rational. It is determined that the rational storage period of milk-protein concentrate, taking into account the change of color-parametric characteristics in the storage process, is 30 days from the moment of manufacturing.

It is proved that the making of protein-plant mixtures before freezing contributes to the losses reducing beyond the regulatory of milk-protein base (cottage cheese) due to changes in the state of the free-bound moisture and its leakage after defrosting [16, 17]. It is confirmed that rice and wheat extruders in combination with cottage cheese have the ability to change the state of the free-bound moisture – to prevent active synergistic phenomena after defrosting [18].

In the technology of semi-finished milk-plant stuffings the use of carrot, pumpkin, and zucchini purees is proposed to provide compatibility with the milk protein base (buttermilk concentrate) at the organoleptic level, economic expediency (use of local raw materials of the regions) and perform the technological function of wet-binding, due to high content of pectin substances in vegetable purees [2,3].

In connection with the foregoing, the study of the effect of plant raw materials on the state of water during freezing-defrosting of semi-finished milk-plant stuffings and the definition of rational modes of their freezing is an urgent task.

The purpose of the work is to study the influence of plant raw materials on the state of water during freezing-defrosting of semi-finished milk-plant stuffings and the definition of rational regimes of their freezing.

To reach the goal, the following tasks were solved:

– to investigate the influence of plant raw materials on the processes of phase transition and the state of water during freezing-defrosting of semi-finished milk-plant stuffings;
– to substantiate the freezing temperature of milk-plant stuffings;
– to substantiate the temperature of further storage of frozen milk-plant stuffings.

Materials and methods

Researched materials

As materials were defined: semi-finished milk-plant stuffings (milk-carrot stuffing, milk-pumpkin stuffing, milk-zucchini stuffing) and a control sample (cottage cheese stuffing).

Cottage cheese stuffing was obtained by adding flour and melange prepared in advance to the wiped cottage cheese and by mixing them.

To obtain milk-plant stuffings, milk-protein concentrate based on buttermilk is wiped, mixed with prepared melange, wheat flour, sugar (salt), carrots, pumpkin or zucchini purees, then the mixture is stirred for 5·60 s, syringed in a cellophane shell and freezed until reaching the temperature of 3 ºC in the center of the loaf.

The prescription components are taken in the following ratios, weight%:

1. For milk-carrot stuffing: milk-protein concentrate based on buttermilk – 68,0%, carrot puree – 16,0%, melange – 6,0%, wheat flour – 6,0%, sugar – 4,0%;
2. For milk-pumpkin stuffing: milk-protein concentrate based on buttermilk – 68,5%, pumpkin puree – 15,0%, melange – 6,0%, wheat flour – 6,5%, sugar – 4,0%;
3. For milk-zucchini stuffing: milk-protein concentrate based on buttermilk – 71,0%, zucchini puree – 15,0%, melange – 6,0%, wheat flour – 7,0%, salt – 1,0%.

Description of methods

The research was carried out on a high-resolution spectrometer Tesla BS 567A with a working frequency of 100 MHz on protons by the Kuntz method [19].

Results processing

For statistical probability, all experiments under laboratory conditions were performed in a fivefold repetition. The results of experimental studies were subjected to statistical processing by the least squares method to determine the error of the obtained data. In the series of each experiment, the average value of the indicator and the dispersion were calculated:

$$\bar{y} = \frac{\sum_{y=1}^{n} y_i}{n}, S_i^2 = \frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{n-1},$$

where $\bar{y}$ – average value of the indicator; y_i – value of the indicator in each experiment session; n – number of parallel experiment sessions.

In order to calculate the reliability of the obtained results, the Student's criterion was used. To check the differences between the two meanings, the formula was used:

$$t = \frac{\overline{X}_a - \overline{X}_b}{\sqrt{x_a^2 + x_b^2}},$$

where t – the Student's criterion;

$\overline{X_a}$, $\overline{X_b}$ – the average of the A and B sample;

x_a, x_b – the error of the arithmetic mean of A and B sample.

Results and discussion

Results of study of nuclear magnetic resonance spectrum of semi-finished milk-carrot stuffing

Typical spectrum of nuclear magnetic resonance of semi-finished milk-carrot stuffing at temperatures of + 20 °C and -25 °C are shown in Figure 1. The signals of the water and the standard – tetramethylsilane (TMS) – are recorded in the nuclear magnetic resonance spectrum.

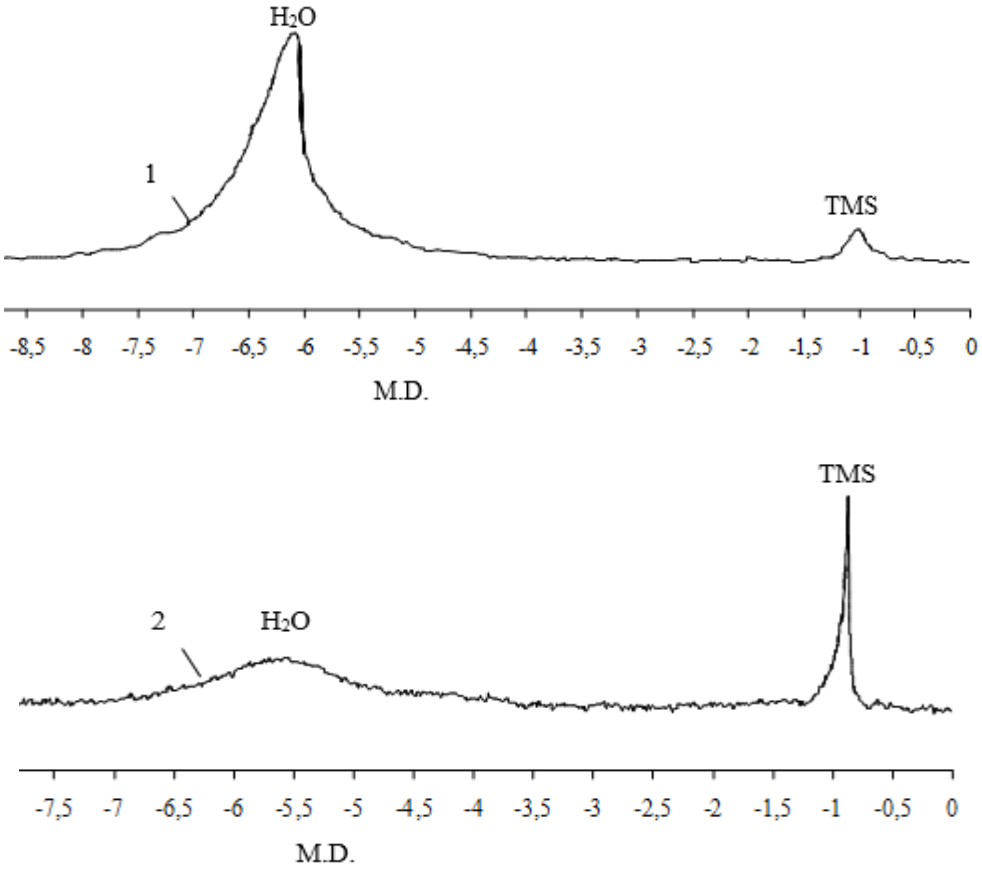


Figure 1. Spectrum of $^{-1}$H- nuclear magnetic resonance of semi-finished milk-carrot stuffing based on buttermilk concentrate:
1 - at the temperature of 20 °C, 2 - at the temperature of -25 ° C

Analysis of data given at Figure 1 shows that at a temperature of 20 °C in the nuclear magnetic resonance spectrum of semi-finished milk-carrot stuffing (Figure 1) an intensive water signal is recorded, which decreases significantly with intensity while sample cooling to a temperature of -25 °C. The presence of a nuclear magnetic resonance signal indicates

that non-freezing water does not disappear completely when cooling the sample to a temperature of -25 °C, which implies the possibility of biochemical reactions procceding in the stuffing.

Taking into account that the intensity of the nuclear magnetic resonance signal, that is the area under the corresponding resonance line, is the measure of the substance amount, the effect of freezing temperature on the amount of non-freezing water in semi-finished milk-carrot stuffing was investigated in the next step.

Study of the freezing temperature effect on the amount of non-freezing water in the control sample

The Figure 2 shows the results of the study of the freezing temperature effect on the amount of non-freezing water in the control sample.

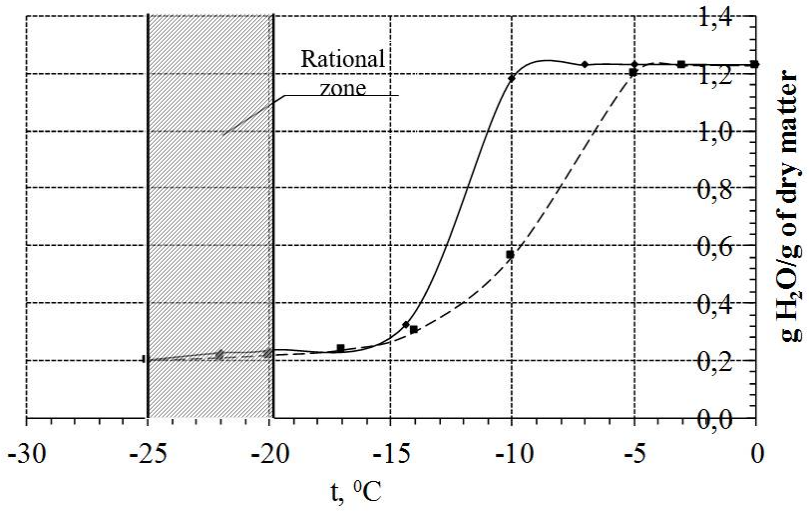


Figure 2. The influence of freezing temperature on the amount of non-freezing water in the control sample (——— – cooling, - - - - - – heating)

In the temperature range from 0 °C to -9 °C while cooling the intensity of the water signal in the spectrum of nuclear magnetic resonance does not change (Figure 2), that is, all water in the sample is recorded as non-freezing. Crystallization of ice in the control sample while its cooling is observed in the temperature range -9 ... -15 °C, which is recorded at a sharp drop in the intensity of the water nuclear magnetic resonance signal. The amount of non-freezing water in the control sample decreases from 1,24 g of H_2O per gram of dry matter to 0,28 g of H_2O per gram of dry matter.

A slight decrease in the amount of non-freezing water (up to 0,20 g of H_2O per gram of dry matter) is observed with a decrease in temperature to -17 ... -18 °C, with further decrease in temperature the amount of non-freezing water does not change. At -25 °C the amount of non-freezing water in the control sample amounts 0,20 g of H_2O per gram of dry substance.

The amount of non-freezing water in the experimental sample at a temperature below 0 °C does not coincide at the cooling and heating stages (Figure 2), which is explained by the overcooling of the liquid in the sample at a temperature decrease. Water systems are characterized by a very high propensity to overcooling [20, 21]. Conversely, while heating a

frozen water solution, the melting occurs at the «liquid – solid» equilibrium point. Therefore, in the cooling-heating cycle in water systems the fraction of non-freezing water is sensitive to the temperature hysteresis, which is proved by the data of Figure 2.

Results of the study of the freezing temperature effect on the amount of non-freezing water in semi-finished milk-plant stuffings

At the next stage, the effect of freezing temperature on the amount of non-freezing water in semi-finished milk-carrot (Figure 3), milk-pumpkin (Figure 4), milk-zucchini (Figure 5) stuffings was investigated.

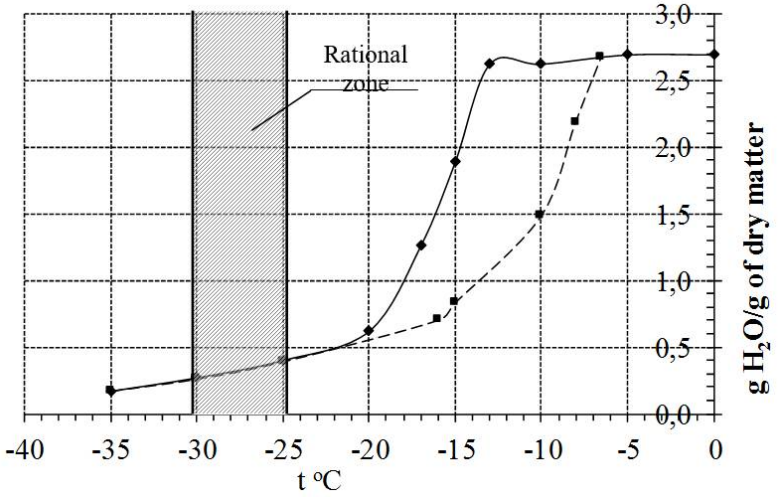


Figure 3. The influence of freezing temperature on the amount of non-freezing water in the semi-finished milk-carrot stuffing (——— – cooling, - - - - - – heating)

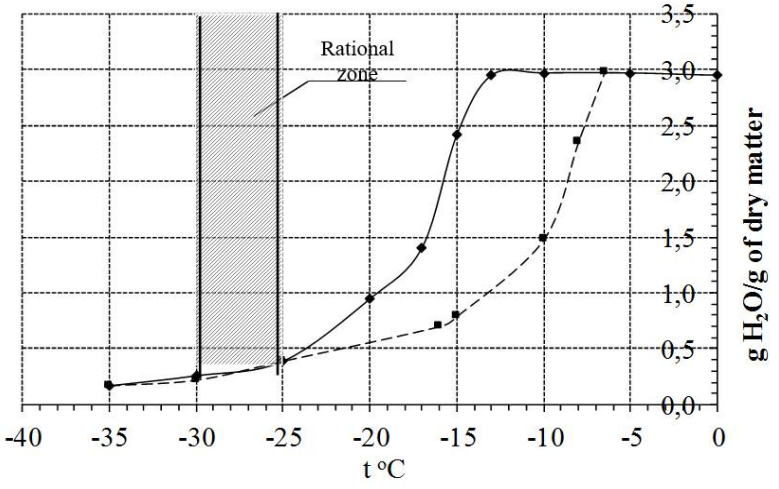


Figure 4. The influence of freezing temperature on the amount of non-freezing water in the semi-finished milk-pumpkin stuffing (——— – cooling, - - - - - – heating)

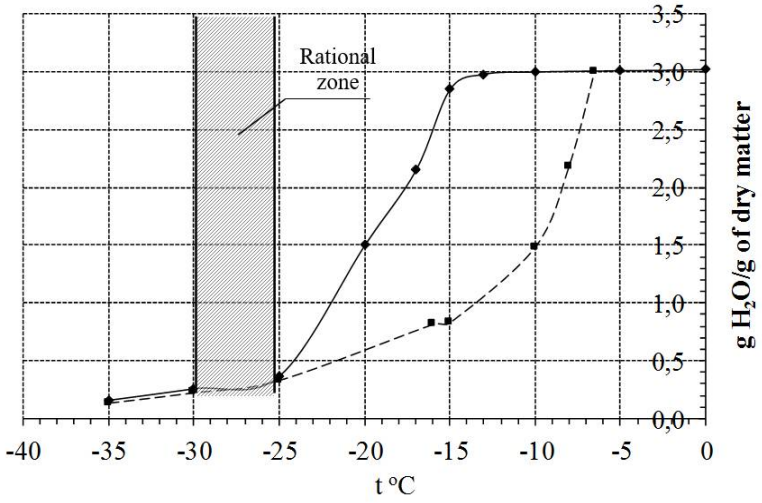


Figure 5. The influence of freezing temperature on the amount of non-freezing water in the semi-finished milk-zucchini stuffing (——— – cooling, - - - - - – heating)

Analysis of Figures 3–5 shows that the crystallization of ice with a decrease in temperature begins in the overcooled state of the samples.

Substantiation of the freezing temperature of semi-finished milk-plant stuffings

It is determined that the developed semi-finished milk-plant stuffings require a deeper overcooling than a control sample. This is related to the presence of vegetable purees in the stuffing, containing a large amount of carbohydrates, which bind water and stabilize its condition in the composition of stuffings. Such impact of vegetable purees creates more favorable conditions for the long-term quality storage of the frozen stuffings.

The comparison of the non-freezing water presence, registered by the method of nuclear magnetic resonance in protons at the temperature of -25 °C, indicates an increase in this water fraction amount in all investigated semi-finished milk-plant stuffings comparing with this amount in the control sample. For example, at -25 °C the control sample contains 0,21 g of H_2O per gram of dry matter, when for the semi-finished milk-carrot stuffing the amount of H_2O per gram of dry matter is 0,40 g, for milk-pumpkin stuffing – 0,39 g, for milk-zucchini stuffing – 0,37 g at the same temperature. At the temperature of -30 °C the control sample contains 0,20 g of H_2O per gram of dry matter, and the semi-finished milk-carrot stuffing – 0,32 g of H_2O per gram of dry matter, milk-pumpkin stuffing – 0,25 g of H_2O per gram of dry matter, milk-zucchini stuffing – 0,24 g of H_2O per gram of dry matter. This fact further confirms the conclusion that in the samples of semi-finished milk-plant stuffings the water is bound by carbohydrates (sugars).

The conducted studies (Figures 3-5) show that the smallest amount of non-freezing water in samples of semi-finished milk- plant stuffings is kept at a temperature of -25...-30 °C and amounts 0,26...0,40 g of H_20 per 1 g dry matter or 8,1...11,1%. of its amount in stuffing samples at a temperature of 0 °C. It is established that during further freezing, the content of non-freezing water is almost not reduced, which allows to recommend an indicated temperature range for the freezing of semi-finished stuffings.

Substantiation of the further storage temperature of frozen milk-plant stuffings

It is determined that further storage of semi-finished milk-plant stuffings at a temperature of -18...-19 °C (which is a normative temperature of the majority of industrial freezing chambers used in the food industry and recommended for storage of frozen foods and semi-finished products) contributes to a slight increase in the content of non-freezing water in them. If we take into account that at the temperature of -25...-30 °C the amount of non-freezing water in semi-finished stuffings is the smallest and makes 8,1...11,1%. of its amount at 0 °C, then at the temperature of -18...-19 °C, which is recommended as the storage temperature of semi-finished stuffings, the non-freezing water will amount 22,2...23,8%. Since the increase in the amount of non-freezing water in semi-finished milk-plant stuffings is insignificant, the temperature of -18...-19 °C can be recommended for the further storage of frozen stuffings.

Conclusions

1. Presence of carbohydrates in carrot, pumpkin or zucchini purees increases the content of bound water in stuffings and creates more favorable conditions for the long-term storage of frozen semi-finished milk-plant stuffings.
2. The smallest amount of non-freezing water in semi-finished milk-plant stuffings is kept at a temperature of -25...-30 °C and is equal to 0,26...0,40 g of H_20 per 1 g of dry matter, which allows to recommend this temperature range for quick freezing of stuffings.
3. Frozen semi-finished milk-plant stuffings should be kept at a temperature of -18...-19 °C during the storage.
4. To determine the rational storage terms of semi-finished milk-plant stuffings, changes in their microbiological parameters, organoleptic properties and acidity during storage should be investigated, which is the prospect of further research.

References

1. Lipatov N.N., Sazhinov S.Iu., Bashkirov O.I. (2001), Sovokupnoe kachestvo tekhnologicheskikh protsessov molochnoi promyshlennosti i kolichestvennye kriterii ego otsenki, *Khranenie i pererabotka selkhozsyria*, 4, pp. 33–34.
2. Dejnychenko G., Gnitsevych V., Yudina T., Nazarenko I., Vasylieva O. (2016), Determination of process raw processing options in technology pektynovmisnoyi milk-vegetable stuffing, *Eastern Eurppean Jornal of Enterprise technologies. Technology and equipment of food production,* 5, 11 (83), pp. 25-31. doi: 10.15587/1729-4061.2016.81209
3. Yudina T.I., Nazarenko I.A.. Nykyforov R.P. (2015), Doslidzhennia yakosti molochno-roslynnykh farshiv na osnovi kontsentratu zi skolotyn, *Skhidno-Evropeiskyi zhurnal peredovykh tekhnologii,* 3, 10 (75), pp. 10-14. doi: 10.15587/1729-4061.2015.43407
4. Marianna Lysenko (2014), The Problem of Ensuring the Economic Security of Dairy Industry in Ukraine, *Economics & Sociology*, 7(2), pp. 160–-171, DOI: 10.14254/2071-789X.2014/7-2/13
5. Zhou Y.-G., Li D., Wang L.-J., Li Y., Yang B.-N., Bhandari B., Chen X.D., Mao Z.-H. (2009), Effect of water content on thermal behaviors of common buckwheat flour and starch, *Journal of Food Engineering*, 93 (2), pp. 242-248.

6. *Kolmakova N. (2003), Pektin i ego primenennie v razlichnykh pishchevykh proizvodstvakh,* Pishchevaia promyshlennost*, 6, pp. 60-62.*
7. *Dubinina A.A., Odarchenko A.M., Odarchenko D.M., Zinchenko O.V. (2003), Fazovi perekhody i skluvannia doslidzheno v ovochevykh i fruktovykh pastakh metodom dyferentsialnoi skanuiuchoi kalorymetrii,* Kharchova i pererobna promyslovist*, 2, pp. 16-17.*
8. Pod red. Dzh. A. Evans (2010), *Zamorozhennye pishchevye produkty. Nauchnye osnovy i tekhnologiia*, Proffesiia, Sankt-Peterburg.
9. Korotkov E.G., Melnikova E.I., Kuznetsova I.V., Stanislavskaia E.B. (2016), Issledovanie form sviazi vlagi v tvoroge s mikropartikuliatom syvorotochnykh belkov, *Molochnaia promyshlennost*, 8, pp. 31–32.
10. Zobkova Z.S., Zenina D.V., Fursova T.P., Gavrilova A.D., Shelaginova I.R. (2016), Biotekhnologicheskie i mikrobiologicheskie aspekty proizvodstva tvoroga s uvelichennym srokom godnosti, *Molochnaia promyshlennost*, 4, pp. 49–50.
11. Fridenberg G.V., Lukashova T.A. (2014), Sovershenstvovanie tekhnologii kholodilnogo rezervirovaniia tvoroga, *Molochnaia promyshlennost*, 6, pp. 59–62.
12. Banach J. C., Clark S., Lamsal B.P. (2014), Texture and other changes during storage in model high-protein nutrition bars formulated with modified milk protein concentrates, *LWT – Food Science and Technology,* 56 (1), pp. 77–86.
13. Hunter R.J., Hemar Y., Pinder D.N., Anema S.G. (2011), Effect of storage time and temperature of milk protein concentrate (MPC85) on the renneting properties of skim milk fortified with MPC85, *Food Chemistry,* 125 (3), pp. 944–952.
14. Anema S.G., Pinder D.N., Hunter R.J., Hemar Y. (2006), Effects of storage temperature on the solubility of milk protein concentrate (MPC85), *Food Hydrocolloids*, 20 (2-3), pp. 386–393.
15. Gnitsevych V., Yudina T., Deinychenko L., Nykyforov R., Nazarenko I.(2018), Survey of characteristics of dairy-protein concentrates in the low-temperature storage process, *Eastern European Jornal of Enterprise technologies. Technology and equipment of food production*, 1, 11 (91), pp. 16-21, DOI: 10.15587/1729-4061.2018.120749
16. Ivanov S.V., Hrek O.V., Krasulia O.O. (2014), Vyznachennia stanu volohy metodom ICh-spektroskopii v bilkovo-roslynnykh sumishakh, *Naukovi pratsi NUKhT*, 20 (5), pp. 185-192.
17. Hrek O.V., Tymchuk A.V. (2011), Krioskopichna temperatura sumishei na osnovi syru kyslomolochnoho, *Prodovolcha industriia APK*, 3, pp. 20-23.
18. Onopriichuk O.O., Petryna A.B., Hrek O.V. (2007), Doslidzhennia form zviazkiv krokhmalmistkoi syrovyny z volohoiu molochnoi osnovy, *Naukovyi visnyk Lvivskoi natsionalnoi akademii veterynarnoi medytsyny im. S.Z. Gzhytskoho*, 2 (33), pp. 172–175.
19. Kuntz I.D., Brassfield T.S., Law W.D., Purcell B.V. (1972), Hydratation of macromolecules, *Science,* 163 (3873), pp. 1329-1331.
20. Wolfe J., Bryant G., Koster K. (2002), What is 'unfreezable water', how unfreezable is it and how much is there?, *Cryoletters,* 23, pp. 157-166.
21. McClatchey K.D. (2002), Clinical Laboratory Medicine, *Philadelphia : Lippincott Williams&Wilkins*, pp. 381–424.
22. Labutina N.V. (2004), *Povyshenie effektivnosti tekhnologii khlebobulochnykh izdelii iz zamorozhennykh polufabrikatov s ispolzovaniem rzhanoi muki*, Moscow.

Application of surface-active substances produced by Nocardia vaccinii IMB B-7405 for the treatment of vegetables

Tetiana Pirog[1,2], Kristina Beregova[1], Bogdana Geichenko[1], Viktor Stabnikov[1]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukraine

Keywords:

Vegetables
Biosurfactant
Nocardia vaccinii IMV B-7405
Shelf life

Article history:

Received 24.09.2018
Received in revised form 27.12.2018
Accepted 28.03.2019

Corresponding author:

Viktor Stabnikov
E-mail:
vstabnikov1@gmail.com

DOI: 10.24263/2304-974X-2019-8-1-11

Abstract

Introduction. Application of surface-active substances (SAS) produced by *Nocardia vaccinii* IMV B-7405 for shelf live extension of vegetables was studied.

Materials and methods. Organic vegetables such as tomato, cucumbers, and squashes were washed with the solution of SAS produced by *N. vaccinii* IMV B-7405 with concentrations of 0.25 or 0.5 g/L. Microbiological analysis was done before the beginning of the vegetables storage. Evaluation of vegetable quality was conducted by viewing during time of the storage.

Results and discussion. The results of our research showed the efficiency of the application of biosurfactant produced by *Nocardia vaccinii* IMV B-7405 using industrial wastes for shelf life extension of vegetables. Results of visible observations as well as microbiological analysis showed that the treatment of vegetables with SAS solutions at the concentrations of 0.25 and 0.5 g/L was more effective than washing them with tap water. The total number of heterotrophic bacteria and fungi in the samples decreased after treatment of vegetables with SAS of *N. vaccinii* IMV B-7405 by 16–34 and 3–14 times, respectively, meanwhile the washing of vegetables with tap water decreased total microbial number only by 2–2.5 times. It was shown that vegetables washed with water spoiled faster than those treated with SAS solution. The advantages of application of this biosurfactant for vegetables post-harvest treatment are that (1) it can be used at the lower by 2–6 times concentration in comparison with other reported in literature microbial SAS, and (2) it can be produced using industrial wastes that will reduce the cost of its production.

Conclusion. Biosurfactant produced by *Nocardia vaccinii* IMV B-7405 can be used for the treatment of vegetables to extent their shelf life.

Introduction

Shelf life of fresh vegetables is a matter of great importance. According to statistics of the Food and Agriculture Organization of the United Nations (FAOSTAT), the developed countries lose only 5% of the harvest due to the more perfect technologies of vegetables storage, meanwhile in the other countries the losses of harvest can be above 20% [1, 2]. For example, in China the losses of 30 million tons of annual garden-stuffs economic damages can be estimated as 30 billion yuan [1-3]. Ukraine is one of the leading world producers of vegetables. However, the total post-harvest losses were assessed as 800–964 thousand tons in 2017 [4], which consist about US$30–37 million. To extend shelf life of vegetables they can be treated using physical methods such as hot-water immersion, low temperature storage, modified atmosphere packaging technology, the treatment with gamma radiation or by pulsed electric field and chemical methods such as application of acidifying agents, chemical reductants, inhibitors of enzymes activities, sequestrants [5-8]. The treatment of the vegetables using physical methods can cause their mechanical damages such as bruises, abrasions and cuts which make them more sensitive to desiccation and shriveling, increase in respiration rate and ethylene emission, speed up the wilting and enzymatic browning. The high temperatures can cause yellow discoloration of green vegetables, and the use of modified atmosphere packing stipulates determination of optimal gas composition for every certain product [8]. Application of the physical methods for vegetables treatment also requires special equipment.

Application of synthetic chemical substances for surface treatment of vegetables is not appreciated by consumers because of the health concerns. For example, application of such widely used as a post-harvest dip of crops synthetic antioxidants as ethoxyquin and diphenylamine are under regulatory restrictions [7]. So, the global trend is to decrease the use of the synthetic chemical substance for prolongation of the vegetables shell life. To do it, the development of alternative safe methods is needed. The antimicrobial and antiadhesion efficiency of nontoxic biodegradable microbial surface-active substances (SAS), for example, sophrolipids and rhamnolipids, to treat the agricultural products was shown [9–13]. However, this treatment is costly. To diminish the cost of the post-harvest treatment of vegetables with microbial polysaccharides the strain producing surface active substances that are by one order of magnitude more active than known substances was selected and studied. The strain of hydrocarbon-oxidizing bacteria *Nocardia vaccinii* IMV B-7405 isolated from the oil-polluted soil was able to synthesize substances, which had surface activity and emulsion properties [13]. It was shown that this biosurfactants had also antimicrobial and antiadhesive properties [14, 15] and was active against phytopathogenic bacteria of the geniuses *Pseudomonas* and *Xanthomonas* [16].

The aim of the present research was testing of the surface active substances produced by *N. vaccinii* IMV B-7405 for the vegetables treatment to extent their shelf life.

Materials and methods

Microorganism

The strain *Nocardia vaccinii* IMV B-7405 was isolated from the oil-polluted soil [13] and was deposited in the Collection of Microorganisms of Institute of Microbiology and Virology NAS of Ukraine. The biosurfactant produced by *N. vaccinii* IMV B-7405 was a set of neutral, glyco- and aminolipids. Neutral lipids contained mycolic and n-alkanoic acids, glycolipids contained trehalose diacelates and trehalose mycolates [14].

Cultivation of *N. vaccinii* IMV B-7405

Bacterial strain *N. vaccinii* IMV B-7405 was cultivated in the liquid mineral medium with the following composition, g/L: $NaNO_3$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; $CaCl \cdot 2H_2O$, 0.1; KH_2PO_4, 0.1; $FeSO_4 \cdot 7H_2O$, 0.001, yeasts extract, 0.5 vol%, distilled water 1 L. Crude glycerol which is waste from biodiesel production plant, Poltava region, Ukraine, was used as a source of carbon in the concentration of 2 vol.%. Inoculum was prepared by cultivation of the strain *N. vaccinii* IMB B-7405 in the medium described above with 0.5 vol.%. of crude glycerol. The cultural liquid with the concentration of bacterial cells 10^4–10^5 cells/mL was taken from the exponential growth phase and was added to the medium for the strain cultivation, 10 vol.%. Cultivation of *N. vaccinii* IMV B-7405 was conducted in the flasks with the volume of 750 mL in 100 mL of medium under shaking at 320 rpm at 30 ºC during 120 hours.

Production of surface-active substances

The cultural liquid after cultivation was centrifuged at 5000×g for 45 minutes (laboratory centrifuge LP–8, Kiev, Ukraine). The Folch solution (chloroform and methanol in volume ratio 2:1) was used for extraction of surface-active substances. The supernatant was placed in the funnel, then Folch solution was added (ratio 1:1), and the mixture was shaken for 5 min and left for phase separation. The lower fraction (organic extract 1) was removed, and the extraction procedure was repeated twice to obtain organic extracts 2 and 3. All extracts were combined and evaporated on the rotor evaporator ER-1M2 (Russia) at the temperature 50 ºC and pressure 0.4 atm to the constant weight. The dry SAS was dissolved in water and solution of biosurfactant with its concentration of 0.25 - 0.5 g/L was used in the research.

Vegetables

Organic vegetables such as tomato red (Admiral variety) and green (Malachite casket variety), cucumbers (Conni F1 variety), squashes (Airoil variety) were grown in the open ground without treatment with pesticides in Gvozdev, Kyiv Oblast, Ukraine, GPS 50°14'53.5"N 30°28'41.3"E). The harvested vegetables were ripe, without visible damages and infections

Vegetable washing

To study the influence of the vegetable treatment with SAS of *N. vaccinii* IMV B-7405 on the storage duration, picked up vegetables were divided into three groups with 10 – 30 pieces in each. Vegetables of the first group were not treated at all, vegetables of the second group were washed with tap water, and vegetables of the third group were washed with the solutions of SAS with concentrations of 0.25 or 0.5 g/L. Vegetables were placed in the glass cylinder, 250 mL of tap water or the SAS solution was added, treatment lasted for 5 min, and after that vegetables were taken off and placed on the plates at the room temperature for observation [8]. Microbiological analysis was done before the beginning of the vegetables storage.

Microbiological analysis

Some vegetables from each group were taken with sterile pincers, then they were placed into sterile porcelain jar and were pestling. Homogenized mixture, 10 g, were placed into flask with 90 mL of sterile tap water and shaken. The quantity of microbial cells (colony-forming units, CFU) was determined by the plate diluting method. The quantity of heterotrophic bacteria was determined by their growth on the beef-extract agar-agar (BEAA) at 37 ºC for 24 hours, and the quantity of the fungi was determined by their growth on the Wort Agar for microbiology at 30 ºC for 48 hours.

Evaluation of vegetable quality

Evaluation of vegetable quality was conducted by viewing during time of the storage. The experiment was finished when the signs of deterioration (usually on the seventh day) such as decay, changes of color and texture, the presence of the cracks and wrinkling were evident on all vegetables.

Statistical analysis

All experiments were done in triplicates, the number of the parallel determinations varied from 3 to 5. Statistical analysis was done using computer program Statistix 10.0 for Windows version 11.5. The average means (M) and standard deviations (SD) were calculated for the experimental results.

Results and discussion

From the different vegetables, which are cultivated in Ukraine, the cucumbers, the squashes, and the tomatoes were chosen for our experiments by the following reasons: (1) according to the data of FAOSTAT Ukraine occupies sixth place in the world on the cucumbers production going ahead USA and Spain and is within the top twenty exporting countries [17]; Ukraine is a leader in Europe in the production of tomatoes, and the part of tomato's production contributes 20%. of overall fruit-and-vegetable production of the country [18]. In addition, tomatoes unlike vegetable marrows and cucumbers belong to the soft garden-stuffs, so at the longtime transportation or storage they are quickly infected by microorganisms and spoiled.

In the studies of the influence of microbial SAS on the extension of the storage time of fruit-and-vegetables products high enough concentration of preparations, 1 – 3 g/L, were used [2, 8, 18–21]. Our previous studies showed that SAS synthesized by *N.vaccinii* IMV B-7405 had high antimicrobial activities [16]: the minimum inhibiting concentration (MIC) against phytopathogenic bacteria *Pectobacterium carotovorum* UKM B-1095, *Pseudomonas syringae* pv. *atrofaciens* UKM B-1015 and *Xanthomonas campestris* pv. *campestris* UKM B-1049 were 14−52 μg/mL, meanwhile MIC of SAS reported in the literature were higher. So, MIC of SAS synthetized by *Candida bombicola* ATCC 22214 against 18 phytopathogenic fungi (*Alternaria tomatophilia*, *Alternaria solani*, *Alternaria alternata*, *Aspergillus niger*, *Aureobasidium pullulans*, *Bacillus cinerea*, *Chaetomium globosum*, *Fusarium asiaticum*, *Fusarium austroamericana*, *Fusarium cerealis*, *Fusarium graminearum*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Penicillium digitatum*, *Penicillium funiculosum*, *Phytophtera infestans*, *Phytopthora capsici, Ustilago maydis*) and 7 phytopathogenic bacteria (*Acidovorax carotovorum*, *Erwinia amylovora*, *Pseudomonas*

cichorii, *Pseudomonas syringae*, *Pectobacterium carotovorum*, *Ralstonia solanacearum, Xanthomonas campestris*) was in the range 2.5–10 mg/mL [23]. Therefore, in our present study the lower concentration of SAS produced by *N.vaccinii* IMV B-7405 (0.25–0.5 g/L) and used in experiments was comparable with the concentrations reported in the literature (1-3 g/L).

Visual supervision of the cucumbers and the squashes shown that the samples treated with SAS did not have the signs of spoilage after 7 days of storage in comparison with ones washed with water (Figure 1).

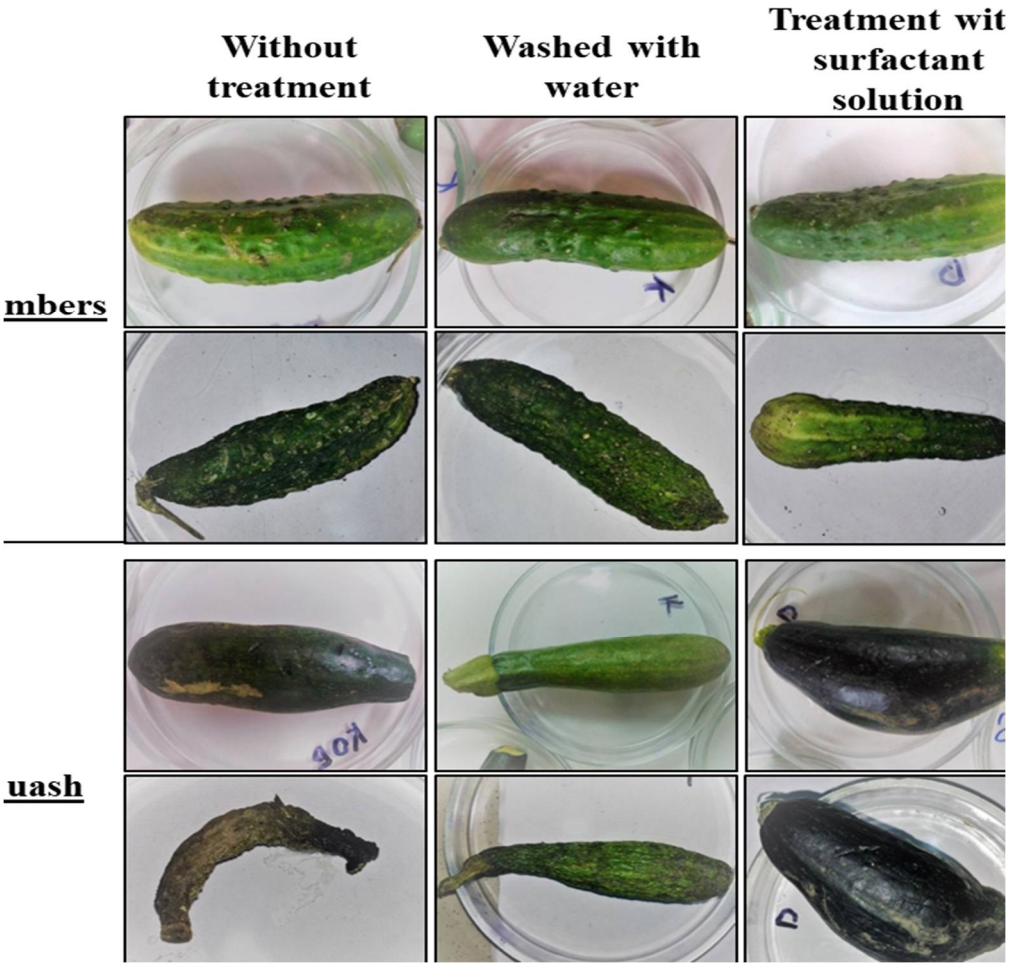


Figure 1. Effect of the treatment of the cucumbers and squashes with SAS produced by *N. vaccinii* IMV B-7405 (0.5 g/L) on their storage.

The spoilage of the non-treated and washed with water tomatoes was observed on the third day of their storage; meanwhile tomatoes treated with SAS did not show the signs of spoilage even on the seventh day (Figure 2).

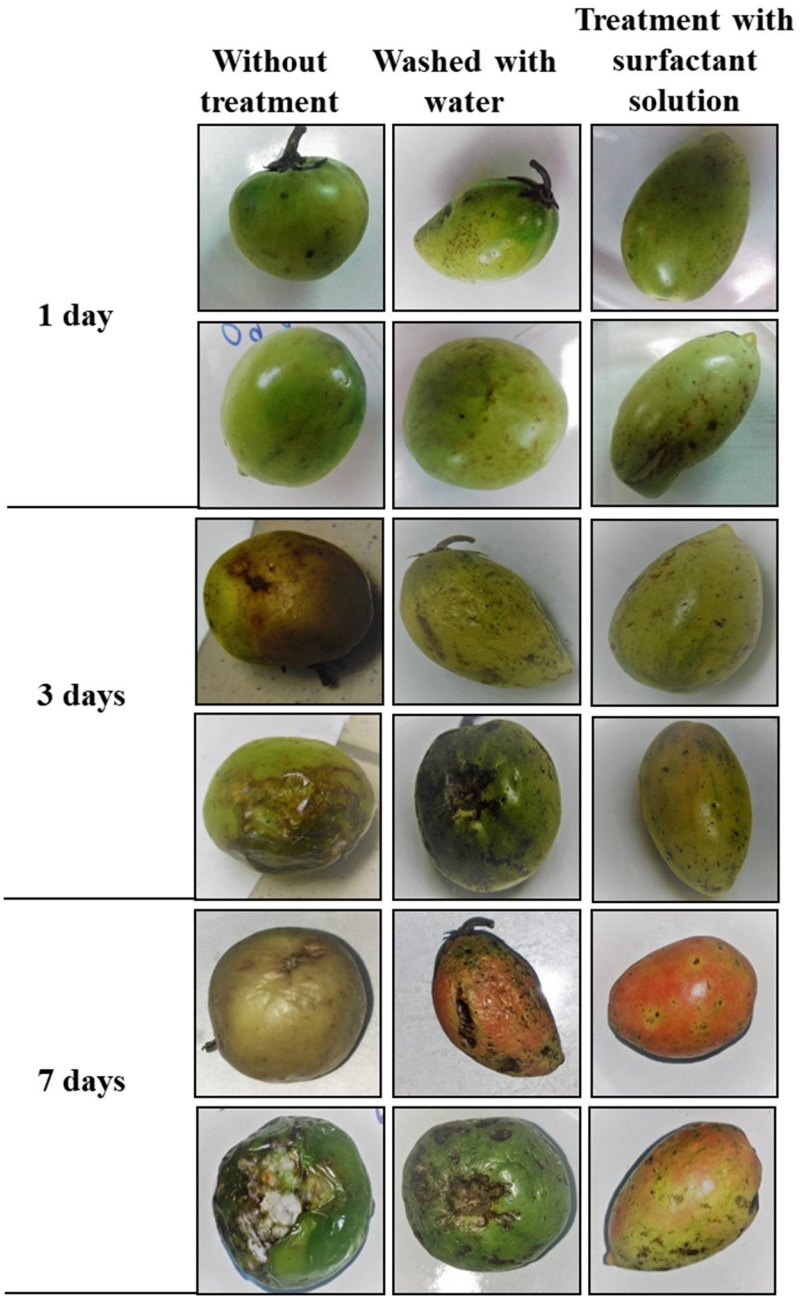


Figure 2. Effect of the treatment of green tomatoes with SAS produced by *N. vaccinii* IMV B-7405 (0.5 g/L) on their storage.

The results of microbiological analysis of the surface of the green tomatoes without any treatment, after washing with water and after the treatment with SAS are shown in the Figure 3.

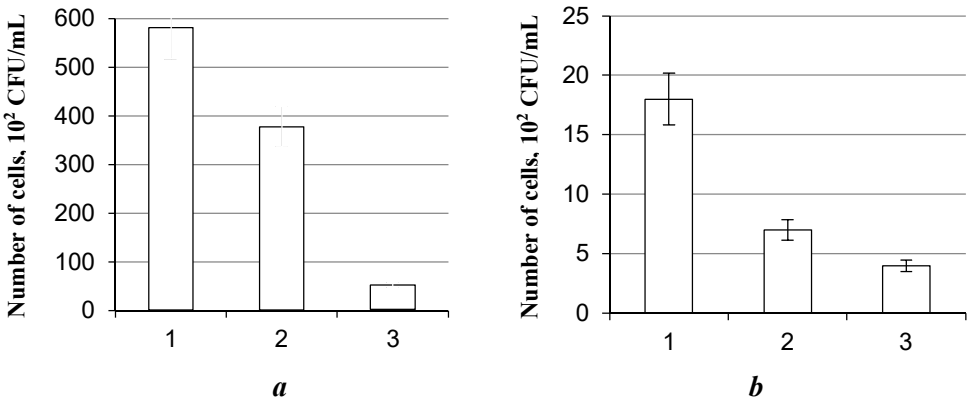


Figure 3. Total number of heterotrophic bacteria (A) and fungi (B) on the surface of green tomatoes depending on the method of their treatment:
1 – without treatment; 2 – treatment with water;
3 – treatment with surfactant solutions, 0.5 g/L.

It was shown that after washing the quantity of the bacterial and fungi cells on the surface of green tomatoes was diminished by 1.6 and 2.4 times, meanwhile after treatment with the SAS solution it was diminished by 10.8 and 2.8 times, respectively, in comparison with the non-treated green tomatoes (Figure 3, A,B).

Red tomatoes were treated with the solutions of SAS of 0.5% and 0.25%. It was shown, that in the case when the treatment of the red tomatoes was done with the solutions of SAS at the concentrations of 0.25 or 0.5 g/L the quantity of bacterial cells diminished by 6 and 14 times, and the concentrations of fungi diminished by 1.5 and 7 timed, respectively, in comparison with the tomatoes washed with water (Figure 4, A, B).

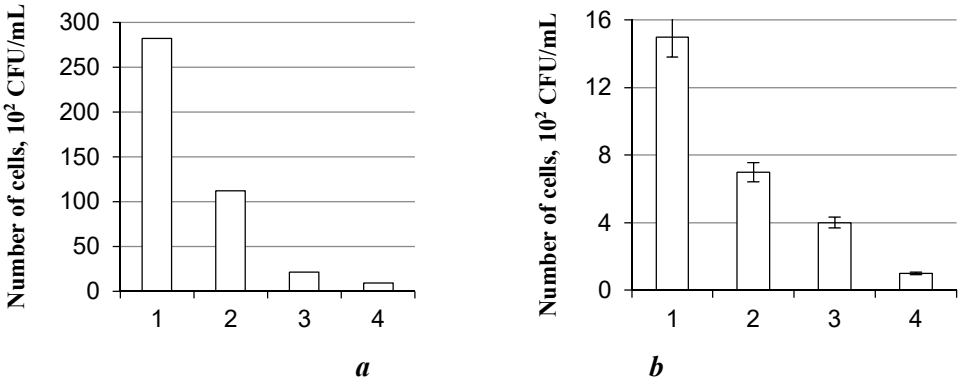


Figure 4. Total number of heterotrophic bacteria (A) and fungi (B) on the surface of red tomatoes depending on the method of their treatment: 1, without treatment; 2, treatment with water; 3, treatment with surfactant solutions, 0.25 g/L; 4, treatment with surfactant solutions, 0.5 g/L.

According to the visual observation, external appearance of tomatoes treated with the SAS solutions with concentration 0.25 or 0.5% on the seventh day was almost the same (Figure 5). Taking into account the economic consideration, dosage of SAS 0.25 g/L was recommended for the treatment of vegetables for extension of their shelf life, notwithstanding that concentration of SAS 0.5 g /L was more effective.

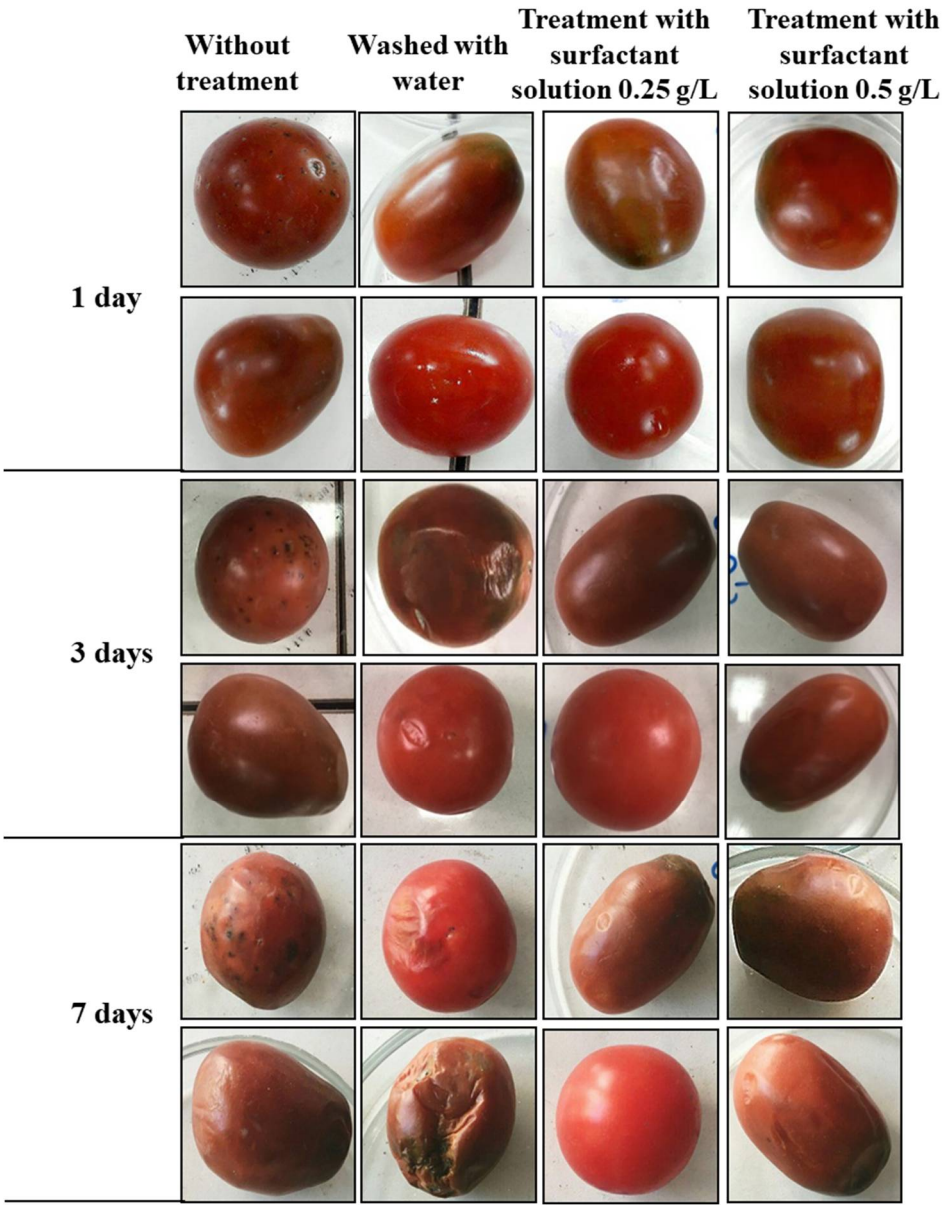


Figure 5. Effect of the treatment of the red tomatoes with different concentrations of SAS produced by *N. vaccinii* IMV B-7405 on their storage

Currently, there are known only some studies of applications of microbial surfactants for fruit and vegetables treatment, and the industrial production of these surfactants (rhamno- and sophorolipids) is conducted by only few companies, the most of which work in the USA [24]. American companies AGAE Technologies LLC, Jeneil Biosurfactant Co. LLC, Paradigm Biomedical Inc., Rhamnolipid Companies Inc. produce rhamnolipids to be used in pharmaceuticals industry, agriculture, for bioremediation of polluted environment, and to increase of oil production. Japanese companies Saraya Co. Ltd. and Allied Carbon Solutions (ACS) LTD Japan produce sophorolipids, which are used in the manufacturing of cleaning and hygiene products, as well as in agriculture.

Low yield of biosynthesized SAS and their high production cost are the major restrictions for their wide use. One of the ways to diminish the cost of biosurfactant production is the use of the waste materials as substrates for SAS biosynthesis [25], for example, wastes from fat-and-oil, sugar, and milk industries, agriculture and forestry, production of biodiesel, and used (overdone) oils. The most suitable substances for the biosynthesis of the microbial surfactants are oil-containing wastes, which unlike lignin- and cellulose-containing wastes, milk whey, and technical glycerin, do not need preliminary treatment and purification.

It was shown in our previous studies that the wastes from the biodiesel production and used sunflower oil can serve as the substrates for cultivation of *N.vaccinii* IMB B-7405 and synthesis of biosurfactant [14, 26]. Additionally, this biosurfactant can be used for the vegetables treatment in the concentrations much lower than ones that are reported in the literature. So, sophorolipids produced by *Wickerhamiella domercqiae* Y2a CGMCC3798 were used to treat citruses, peaches and apricots in the concentrations of 1–3 g/L [2]. It was shown that the strongest hyphae growth spread of *Aspergillus niger, Aspergillus flavus* and *Penicillium* (agent of fruits putrefaction) was suppressed at concentration of the sophorolipids fruit preservative of 3 g/L [2]. Inhibiting effect of the treatment of *Eugenia uniflora* (Surinam cherry, pitanga) with rhamnolipids solution produced by *Pseudomonas aeruginosa* LBI in the concentration of 1 g/L was shown [7]. It is known that biosurfactants can be used at the lower concentrations but only in combination with other components, for example, with synthetic SAS or with microorganisms, which are antagonists of phytopathogens. So, Yan with coauthors showed the synergetic effect of rhamnolipids of *Pseudomonas aeruginosa* (0.5 g/L) and suspension of the yeasts *Rhodotorula glutinis* (1×10^8 cells/mL) in suppressing *Alternaria alternata* on cherry tomato [19]. A simultaneous application of *R. glutinis* and rhamnolipids to treat the tomato surface to control post-harvest diseases was more effective than application of yeasts and biosurfactants alone. Application of 0.25% solution of sophorolipids, synthesized by *Candida bombicola* ATCC 22214, in combination with synthetic SAS polyethylene glycol was effective against *Erwinia chrysanthemi* ATCC 11663 and *Xanthomonas campestris* ATCC 13951 on the surface of chikoos, tomatoes, cucumbers, and citruses [22].

Conclusions

The results of our research showed the efficiency of the application of biosurfactant produced by *Nocardia vaccinii* IMV B-7405 for shelf life extension of vegetables. The advantages of application of this biosurfactant for vegetables post-harvest treatment are that (1) it can be used at the lower by 2-6 times concentration in comparison with other reported in literature microbial SAS, and (2) it can be produced using industrial wastes that will reduce the cost of its production.

So, use of biosurfactant synthesized by *N.vaccinii* IMB B-7405, which will be produced using industrial wastes, can resolve such important problems as utilization of the toxic industrial wastes, decrease of the cost of biodiesel production, and extend the shelf life of vegetables during their storage and transportation.

References

1. Kader A.A. (2005), Increasing food availability by reducing postharvest losses of fresh produce. V International Postharvest Symposium; 2005 June 30; Verona, Italy; 2005, pp. 2169–2176.
2. Chen J., Yuan B. (2012), Sophorolipid fruit preservative and use thereof in fruit preservation, CN 101886047 B.
3. Sharma R.R., Singh D., Singh R. (2009), Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. *Biological Control*, 50(3), pp. 205–221, doi.org/10.1016/j.biocontrol.2009.05.001.
4. *Ukrainian club of agrarian business*. 06.11.2018, Available at: http://ucab.ua/ua/doing_agribusiness/agrarni_rinki/ovochi.
5. Khadka R.B., Marasini M., Rawal R., Gautam D.M., Acedo A.L.Jr. (2017), Effects of variety and postharvest handling practices on microbial population at different stages of the value chain of fresh tomato (*Solanum lycopersicum*) in Western Terai of Nepal. *BioMed Research International*, Article ID 7148076, doi: 10.1155/2017/7148076.
6. Galani J.H.Y., Patel J.S., Patel N.J., Talati J.G. (2017), Storage of fruits and vegetables in refrigerator increases their phenolic acids but decreases the total phenolics, anthocyanins and vitamin C with subsequent loss of their antioxidant capacity. *Antioxidants* (Basel), 6, e59, doi: 10.3390/antiox6030059.
7. Anbudhasan P., Surendraraj A., Karkuzhali S., Sathishkumaran P. (2014), Natural antioxidants and its benefits, *International Journal of Food and Nutritional Sciences*, 3(6), pp. 225-232.
8. Dilarri G., da Silva V.L., Pecora H.B., Montagnolli R.N., Corso C.R., Biddia E.D. (2016), Electrolytic treatment and biosurfactants applied to the conservation of *Eugenia uniflora* fruit. *Food Science and Technology*, 36(3), doi.org/10.1590/1678-457X.00516.
9. de Oliveira M.R., Magri A., Baldo C., Camilios-Neto D., Minucelli T., Celligoi M.A.P. (2015), Review: Sophorolipids a promising biosurfactant and its applications, *International Journal of Advanced Biotechnology and Research*, 6(2), pp. 161–174.
10. Sinumvayo J.P., Ishimwe N. (2015), Agriculture and food applications of rhamnolipids and its production by *Pseudomonas aeruginosa. Journal of Chemical Engineering and Process Technology*, 6, doi.org/10.4172/2157-7048.1000223.
11. Kaur H.P., Prasad B., Kaur S. (2015), A review on application of biosurfactants produced from unconventional inexpensive wastes in food and agriculture industry, *World Journal of Pharmaceutical Research*, 4(8), pp. 827–842.
12. Varnier A.L., Sanchez L., Vatsa P., Boudesocque L., Garcia-Brugger A., Rabenoelina F., et al. (2009), Bacterial rhamnolipids are novel MAMPs conferring resistance to *Botrytis cinerea* in grapevine. *Plant Cell and Environments*, 32(2), pp. 178–193, doi: 10.1111/j.1365-3040.2008.01911.x.
13. Pirog T.P., Shevchuk T.A., Voloshina I.N., Grechirchak N.N. (2005), Use of claydite-immobilized oiloxidizing microbial cells for purification of water from oil. *Applied Biochemistry and Microbiology*, 41(1), pp. 51–55, doi.org/10.1007/s10438-005-0010-z.
14. Pirog T., Sofilkanych A., Konon A., Shevchuk T., Ivanov S. (2013), Intensification of surfactants' synthesis by *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* K-8 on fried oil and glycerol containing

medium. *Food and Bioproducts Processing*, 91(2), pp. 149−157, doi.org/10.1016/j.fbp.2013.01.001.
15. Pirog T.P., Konon A.D., Beregovaya K.A., Shulyakova M.A. (2014), Antiadhesive properties of the surfactants of *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 and *Nocardia vaccinii* IMB B-7405. *Microbiology*, 83(6), pp. 732–739, doi.org/10.1134/S0026261714060150.
16. Pirog T.P., Konon A.D., Sofilkanich A.P., Iutinskaia G.A. (2013), Effect of surface-active substances of *Acinetobacter calcoaceticus* IMV B-7241, *Rhodococcus erythropolis* IMV Ac-5017, and *Nocardia vaccinii* K-8 on phytopathogenic bacteria. *Applied Biochemistry and Microbiology*, 49(4), pp. 360–367, doi.org/10.1134/S000368381304011X.
17. *Food and Agricultural commodities production*. Commodities by country/ FAOSTAT. Food and Agriculture Organization of the United Nations. Rome, 2013, Available at: http://faostat.fao.org/site/339/default.aspx.
18. Shatkovsky A.P. (2018), Tomato. Situation and opportunity for the development of open field vegetable production, Available at: http://agroua.net/plant/catalog/cg-8/c-28/info/cag-408/.
19. Yan F., Xu S., Chen Y., Zheng X. (2014), Effect of rhamnolipids on *Rhodotorula glutinis* biocontrol of *Alternaria alternata* infection in cherry tomato fruit. *Postharvest Biology and Technology*, 97, pp. 32–35, https://doi.org/10.1016/j.postharvbio.2014.05.017.
20. Karthik R., Gobalakrishnan S., Hussain A.J., Muthezhilan R. 2013. Efficacy of Bacteriocin from *Lactobacillus* Sp. (AMET 1506) as biopreservative for seafood's under different storage temperature conditions, *Journal of Modern Biotechnology*, 2, pp. 59–65.
21. Perneel M., D'hondt L., De Maeyer K., Adiobo A., Rabaey K., Höfte M. (2008), Phenazines and biosurfactants interact in the biological control of soil-borne diseases caused by *Pythium* spp., *Environmental Microbiology*, 10(3), pp. 778–788, doi:10.1111/j.1462-2920.2007.01501.x.
22. Dengle-Pulate R., Joshi J., Bhagwat S., Prabhune A. (2015), Application of sophorolipids synthesized using lauryl alcohol as a germicide and fruit-vegetable wash, *World Journal of Pharmaceutical Research*, 3, pp. 1630–1643.
23. Mnif I., Ghribi D. (2016), Glycolipid biosurfactants: main properties and potential applications in agriculture and food industry, *Journal of the Science and Food Agriculture,* 96, pp. 4310–4320, doi: 10.1002/jsfa.7759.
24. Randhawa K.K.S., Rahman P.K. (2014), Rhamnolipid biosurfactants − past, present, and future scenario of global market. *Frontiers in Microbiology*, 5, Article number 454, doi.org/10.3389/fmicb.2014.00454.
25. Santos D.K., Rufino R.D., Luna J.M., Santos V.A., Sarubbo L.A. (2016), Biosurfactants: multifunctional biomolecules of the 21st century. *International Journal of Molecular Sciences*, 17(3), 401. doi: 10.3390/ijms17030401.
26. Pirog T., Shulyakova M., Sofilkanych A., Shevchuk T., Mashchenko O. (2015), Biosurfactant synthesis by *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinia* IMV B-7405 on byproduct of biodiesel production, *Food and Bioproducts Processing*, 93, pp. 11–18, doi.org/10.1016/j.fbp.2013.09.003.

Influence of nanoparticles on the solventogenesis of bacteria *Clostridium beijerinckii* IMV B-7806, *Clostridium acetobutylicum* IMV B-7807

Serhii Skrotskyi, Serhiy Voychuk, Liudmyla Khomenko, Olga Vasyliuk, Valentyn Pidgorskyi

D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Science of Ukraine, Kyiv, Ukraine

Keywords:

Bacteria
Clostridium
Biobutanol
Nanoparticles

Article history:

Received 10.10.2018
Received in revised form 19.12.2018
Accepted 28.03.2019

Corresponding author:

Serhii Skrotskyi
E-mail:
skro.science@gmail.com

DOI: 10.24263/2304-974X-2019-8-1-12

Abstract

Introduction. Given the fact that nanoparticles (NPs) have long been used by the nature, the problem of use of alternative human-designed NPs for achievement of desired biological or medical effects remains relevant for today. In the field of biotechnology NPs can appear as catalysts of biochemical processes, as well as protectors (cryo-, osmo-, etc.), sorbents of toxic metabolites, conductors and mediators, signaling molecules, etc.

Materials and methods. The influence of nanoparticles of metals (iron oxides, cerium, silver, gold and gadolinium) on the biosynthesis of butanol by the strains of acetone-butyl bacteria *Clostridium beijerinckii* IMV B-7806 and *C. acetobutylicum* IMV B-7807 was studied.

Results and discussion. Synthesis of main products of ABE-fermentation (acetone, butanol and ethanol) was affected in presence of NPs in the culture medium. It was shown that for the strain *Clostridium beijerinckii* IMV B-7806, all studied NPs suppressed the synthesis of butanol compared to control. In case of presence of silver oxide NPs during butanol synthesis by strain *C. acetobutylicum* IMV B-7807 there was observed a tendency ($p \leq 0.16$) for increase of butanol yield from 9.0 ± 0.6 g/L in control up to 11.1 ± 1.8 g/L and 11.1 ± 1.1 g/L in the presence of 0.1 mkM and 10 mkM NPs in medium respectively. The nature of changes depended on type of NPs and their concentration. The optimal concentrations of the studied NPs were estimated. Also assumptions on possible mechanisms of the NPs` effect on the ABE fermentation process were formulated. The regulatory potential of the NPs for the coordination of the ABE-fermentation processes and synthesis of fatty acids has been studied in order to increase the yield of the target product.

Conclusion. The effect of the NPs on the synthesis of organic solvents by acetone-butyl bacteria is strain-specific and determined by the growth properties of bacteria and by the functioning of specific enzyme systems as well.

Introduction

More and more attention has been paid to the biological synthesis of chemicals, which expands the capabilities of industrial microbiology by reducing production costs, and this respectively allows to get high profits. Therefore there exist constantly increasing interest in obtaining bioobutanol, bioethanol, and other biofuels through microbial conversion processes of sugars and various starch-containing substances [1–4].

The main problem in obtaining biofuels is the toxicity of end products for microorganisms themselves This significantly limits the potential of microbial synthesis [3]. Today, different approaches are used to solve this problem and to intensify the process of ABE fermentation by using more stable and overactive strains of bacterial producers, obtained by methods of genetic engineering, as well as by optimizing production through using more advanced systems of accumulation and discharge of end products, etc [3, 4]. One of the most promising areas of modern science is the study of properties of nanoparticles (NPs) and nanomaterials (NMs). NPs and NMs can not only influence certain individual biological processes, but simultaneously change both the biological properties of the organism (microorganism) and also properties of the external environment, and thereby create new conditions of existence [5–8]. As a result of this living organisms acquire new features - resistance to pressure of stress factors, intensification of physiological and biochemical functions, and so on. The unique properties of NPs are increasingly used in various spheres of life - medicine, technology, biology, agro-industrial complex and other areas [6-11]. Nanoparticles (NPs) are part of the nature itself, since many of them are natural compounds and perform certain biological functions in cells. This indicates the promise of the use of alternate human-designed NPs to obtain the desired effect [7]. There are more and more applications of nanoparticles in biology and medicine: direct use of them in “as is” form, for example as antimicrobial agents [8]; as selective indicator molecules for some pathogenic microorganisms [9]; sorbents for immobilization of enzymes [10], as delivering agents for diagnostic and therapeutic substances [11, 12]. For medical use nowadays there already exist biodegradable nanoparticles synthesized from albumin [13], polyalkylcyanoacrylate [14], polylactategluclate [15], and solid lipids [16]. However, in recent years nanoparticles based on metal oxides are gaining more and more popularity [13,17,18]. In the field of biotechnology, NPs can act as catalysts of biochemical processes, as well as various protectors (cryo-, osmo-, etc.), sorbents of toxic metabolites, conductors and mediators, signaling molecules, etc.

Currently, an attention is only beginning to be focused on the study of the influence of nanoparticles on microorganisms of various genera and species. Progress in research related to this problem is complicated by the extremely small number of publications, the lack of a broad methodological base, the need for developing new control methods, etc [1, 2, 6, 9, 17, 18]. The structure of NPs is largely determined by the method of their production. By the spatial structure 3 main classes of NPs [5] are known: three-dimensional particles obtained by explosion of conductors, plasma synthesis, the restoration of thin films, etc .; two-dimensional objects - films obtained by methods of molecular and ionic stratification, etc .; one-dimensional objects - nanowiskers, nanotubes, nanofibers, which are obtained by the method of molecular stratification, the introduction of substances into cylindrical micropores, etc [8, 12, 13]. Also, there are nanocomposites - materials obtained by introducing NPs to any matrix. Nanoparticles of metals have different shapes and in most cases are crystallic, although some of them can be amorphous.

In this paper, we have focused on the study of the effect of NPs of metal oxides on the synthesis of butanol, which is the final product of acetone-butyl fermentation. This topic was

almost not studied, despite heightened interest of scientists around the world in the synthesis of biofuels. There is only small number of publications on this subject [18].

The aim of research was to analyze the potential use of metal nanoparticles for intensification of ABE fermentation processes of the acetone-butyl bacteria to increase the synthesis of butanol.

Materials and methods

Bacteria *Clostridium beijerinckii* strain IMV B-7806 and *C. acetobutylicum* strain IMV B-7807 that are deposited in the Depository of the D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine were used in the study.

Nanoparticles. Magnetic nanoparticles were prepared by the method [10] and were kindly given for the study by Dr. Pud O. A. from the Institute of Bioorganic Chemistry of NAS of Ukraine. Other nanoparticles were kindly presented by Dr. Zholobak N.M. from the D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine.

Electronic microscopy. Determination of the size of nanoparticles and their general morphology was carried out by electron microscopy. For this purpose, 5 mkl suspensions of nanoparticles were dropped on a surface of a carbon coated copper grids and dried at room temperature. After that, the nanoparticles were analyzed using a transmission electron microscope JEM-1400 (Jeol, Japan) at an accelerating voltage of 80 kV and an instrumental magnification of x50,000 - x100,000.

Analysis of nanoparticles sizes. The size of nanoparticles was determined from the digital images obtained by electron microscopy. Images analysis was done with a help of the image analysis software ImageJ version 1.50 (National Institutes of Health, USA).

Study of the influence of nanoparticles on the synthesis of butanol. Suspension of bacterial cells in the active phase of growth was obtained by cultivation on liquid thioglycolic nutrient media (Himedia) of the following composition (g/L): tryptone -15.0; yeast extract - 5,0; glucose -50.0; sodium chloride -2,5; L-cysteine - 0.5; sodium thioglycolate - 0,5; sodium rezazurin - 0,5; agar-agar - 0.75. After sterilization (1.1 atm for 15 minutes), the medium was cooled to 25 ° C and poured into test tubes. After that in order to provide anaerobic conditions of cultivation 1.5 ml of sterile vaseline oil was added in each tube. For investigation prepared nutrient media were sowed with 5% of the inoculum using a 18–24 h bacterial culture of the corresponding *Clostridium* strain.

Disperse nanoparticle systems were added to the sterile medium at a concentrations of 10.0; 1.0; 0.1 ηM. Subsequently, the contents of the test tube were thoroughly mixed with vortex (Mancor) for 30 seconds and inoculum (5%) was added. As a control we used non-inoculated tubes containing same medium with or without addition of nanoparticles. Cultivation of cells was carried out under anaerobic conditions at a temperature of 37 ° C. The content of butanol and other ABE-fermentation products was determined at the end point after 72 hours of cultivation.

The determination of butanol content was carried out on a gas-liquid chromatography. The quantitative determination of short-chain fatty acids was studied by gas chromatography - mass spectrometry (GC-MS) with use of GC-MS instrument Agilent

6890N / 5973inert (Agilent Technologies, USA), HP-INNOWax capillary column (30m × 0.25mm × 0.25μm) (J & W Scientific, USA). The separation was carried out with a temperature gradient of 20 ° C / min from 40 to 250 °C, using helium as gas carrier, a flow rate through a column of 1 ml / min and volume of injection - 0.2 μl. As an internal standard, isovalerylate acid was used. The identification of short-chain fatty fatty acids was performed using libraries of NIST02 mass spectra and standard short chain fatty acid solutions (Sigma-Aldrich, USA).

Statistical analysis was performed with the help of Microsoft Excel and STATISTICA version 10 (StatSoft, Inc. 2011) software packages. The significance of difference between the mean values was determined with *t*-test and was considered reliable at $p \leq 0.05$. A two-way ANOVA was used to evaluate the influence of different factors on the studied parameters.

Results and discussion

Much attention is paid to the study of the interaction of microorganisms with metal ions, due to their key role in various biotechnological and natural processes. Analysis of the nanoparticles that we used in the study with electron microscopy showed that the smallest size had gold nanoparticles that ranged from 4 nm to 13 nm (Table 1, Figure 1).

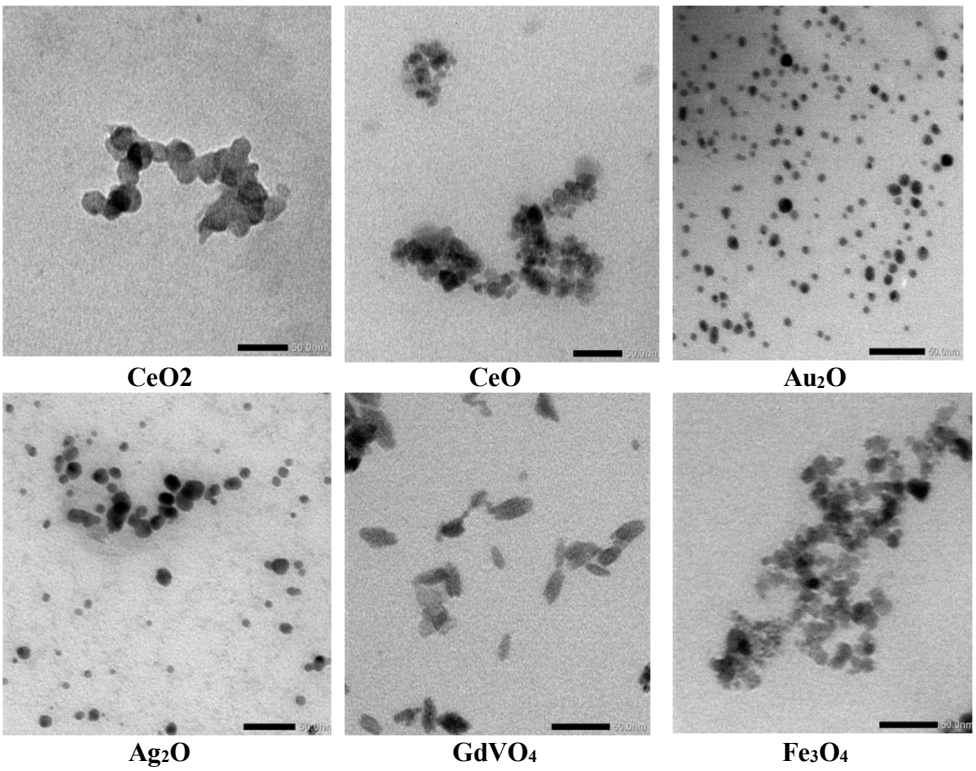


Figure 1. Electron microscopy of the nanoparticles that were used in the study. Bar is 50 nm. Magnification ×80 000

Table 1

Nanoparticles sizes (in *nm*)

Nanoparticle	Mean ± Sd	Maximum	Minimum
CeO_2	22.6±1.5	24.6	20.4
CeO	13.7±5.6	22.3	7.4
Ag_2O	13.2±5.3	22.1	5.6
Au_2O	7.6±3.1	13.9	4.0
$GdVO_4$	26.6±6.2	35.3	18.5
Fe_3O_4	12.3±5.2	20.8	5.1

The addition of nanoparticles (NPs) to the nutritional medium affected the synthesis of butanol which respectively altered the growth rates of bacteria and largely depended from NPs toxicity level. In particular for the strain *Clostridium beijerinckii* IMV B-7806 all investigated NPs inhibited the synthesis of butanol compared to control (tab.2). The effect of NPs had strain-specific and/or species-specific character. Also for the strain *C. beijerinckii* IMV B-7806 there was observed a general decrease in the formation of butanol in the presence of all the studied NPs. Significant inhibition of the final product synthesis was detected in the presence of iron and cerium oxides. Other NPs also have shown a tendency to suppress the butanol synthesis in this strain. The intensity of effect depended on the concentration of NPs in the culture medium. With the increase in NPs concentration, the effect of butanol synthesis inhibition was more expressed and approached to statistically significant. The opposite trend was observed for the butanol yield obtained during cultivation in presence of different NPs for the other strain - *C. acetobutylicum* IMV B-7807 (tab. 2). The presence of iron and cerium nanoparticles in the medium in general produced stimulating effect on the synthesis of butanol in this strain.

Table 2

Synthesis of butanol in *Clostridium* strains under the influence of nanoparticles

Nanoparticle	**Butanol, g/L**		***p***
	Mean	**Sd**	
***C. beijerinckii* B-7806**			
Control	10.4	1.8	-
Fe_3O_4	8.3	0.8	**0.020**
$GdVO_4$	9.3	0.9	0.193
CeO	8.1	0.8	**0.011**
CeO_2	8.9	1.1	0.094
Ag_2O	8.7	1.3	0.052
Au_2O	9.2	0.5	0.158
***C. acetobutylicum* B-7807**			
Control	9.0	0.6	-
Fe_3O_4	9.4	0.8	0.644
$GdVO_4$	8.8	1.2	0.803
CeO	8.5	0.9	0.582
CeO_2	9.4	1.2	0.670
Ag_2O	10.4	1.6	0.155
Au_2O	9.2	1.4	0.817
***C. acetobutylicum* B-7807**			
Control	9.0	0.6	-

However, the effective use of cerium oxide (CeO2) and iron oxide resulted in a slight stimulative effect compared to control. An increase in butanol synthesis was observed up to a maximum level of 9.4 g / l.

The effect of nanoparticles depended on their concentration, and increase of NPs level in medium led to both stimulating and depressing effects on the synthesis of organic solvents by studied acetone-butyl bacterial strains.

The stimulation of the butanol synthesis with iron nanoparticles produced insignificant influence on increasing of nanoparticles concentration in the culture medium, and presence of cerium oxide nanoparticles resulted in rapid increase in the butanol yield at NPs concentrations of 1 ηM and 10 ηM.Effect of gold nanoparticles in the *C. acetobutylicum* strain IMV B-7807 occurred only at a concentration of 0.1 ηM in which the yield of the synthesized butanol was 10.3 g/L, compared with 9 g/L in the control (Table 2). The maximum yield of butanol (almost 11.1 g/L) was fixed in presence of 0.1 ηM and 10.0 ηM of silver oxide NPs in the medium. In case of use of these NPs in concentration of 1.0 ηM the amount of synthesized butanol reduced to 8.9 g/L, indicating an inhibitory effect.

For the strain *C. acetobutylicum* IMV B–7807 the yield of butanol during cultivation in presence of various NPs in concentrations 0.1-10 ηM remained unchanged. In case of silver oxide NPs presence there was observed a tendency ($p \leq 0.16$) for increase of butanol yield in compare with control level 9.0 ± 0.6 g/L up to 11.1 ± 1.8 g/L and 11.1 ± 1.1 g/L in presence of NPs in concentrations 0.1 and 10 ηM respectively.

Observed effect of iron oxide NPs indicates that synthesis of organic solvents in acetone-butyl bacteria is determined not only by growth parameters but also by functioning of enzyme systems themselves independently from bacterial growth process.

The nanoparticles of iron oxide and cerium used in the study were able to increase the synthesis of butanol by strain *C. acetobutylicum* IMV B-7807 on average in 1.5 times. And for strain *C. beijerinckii* IMV B-7806, the butanol synthesis rates remained unchanged in presence of nanoparticles and in control experiments. Obtained result indicates the existence of certain strain characteristics, which are probably related to absence of some special enzyme systems. Exactly this would allow to metabolize existing organic matter with this strain and synthesize ABE-fermentation products at the sufficient level. The primary and probably the most important factor is the presence of a set of certain enzymes or enzyme systems, that is the basis of specific features of one or another strain. The presence of a complete set of enzyme systems is a key factor that allows the full use of the metabolic potential of bacterial cells, while the presence of NPs is a secondary factor that can be either a mediator, or a catalyst for these processes, or perform other functions whose characteristics require separate scientific researches.

The fermentation time in strain *C. beijerinckii* IMV B-7806 decreases almost twice in the presence of iron oxide, and does not change in the presence of other NPs. In the strain *C. acetobutylicum* IMV B-7807 the total fermentation time on the contrary increases almost twice in the presence of iron, cerium and gold NPs. No significant correlation dependencies have been detected between formation of the final product andstart time of gas formation or the fermentation duration time.

Therefore, the effect of NPs was shown to be strain-specific. Efficacy of NPs action is concentration-dependent. Observed decrease of the butanol yield in the strain *C. beijerinckii* B-7806 and its insignificant induction in the presence of silver oxide in strain *C.acetobutylicum* IMV B-7807 have no dependence on the basic parameters of fermentation (start time of gas formation, duration of fermentation), and therefore is probably determined by other factors.

Results analysis

The possibility of widespread introduction of nanoparticles in various aspects of human life points to the high potential of these structures, in particular in the field of biotechnological production of liquid fuels, mainly through stimulation of fermentation processes. The study of the influence of these nanoparticles on the biosynthesis of butanol with clostridia was carried out for the first time. Despite the lack of literature data on the interaction of these nanoparticles with clostridia, our data suggest the prospective and feasibility of use of some nanoparticles to enhance the release of target products of ABE-fermentation. However, it is obvious that for other biotechnological processes, the use of these nanoparticles can also be effective.

Kim and co-authors used nanoparticles to increase the ethanol yield by *Clostridium ljungdahlii.* The fermentation of *C. ljungdahlii* with nanosized silicon dioxide more effectively increased the mass transfer. The concentrations of dissolved CO, CO2 and H2 increased by 272.9%, 200.2% and 156.1% respectively. Production of ethanol and acetic acid increased by 166.1% and 29.1%, correspondingly, as well [1].

Biomass, ethanol and acetic acid yield increased by 227.6%, 213.5% and 59.6%, respectively, in the presence of CoFe2O4 SiO2-CH3 nanoparticles [2].

While there are studies on the antimicrobial effects of nanoparticles on bacteria of the genus *Clostridium* [6].

The results obtained by us indicate the perspectivity and necessity of use of certain nanoparticles to increase the yield of the target product in ABE-fermentation, but it is obvious that use of NPs can be also effective for other biotechnological processes. Taking into account our previous studies and the results obtained at this stage, we noted that in order to establish the potential for the influence of the NPs on the synthesis of organic solvents in the ABE-fermentation, the effect of these NPs on the metabolic or physiological parameters of bacterial growth can be taken as the basis.In most cases it was established that the most effective in increasing the synthesis of butanol were NPs that effectively influenced (stimulated) growth processes and showed a positive influence on the economic and metabolic coefficients [18]. An example of such NP was the oxides of iron and cerium. However, the ability of silver oxide (at low concentrations) to stimulate the synthesis of butanol by cells of acetonobutyl bacteria of the strain *Clostridium acetobutylicum* IMV B-7807 indicates that, in addition to physiological and biochemical parameters, the nature of the stimulating and suppressing effects of the NPs should also be taken into account. Those NPs which are able to exhibit bacteriostatic effects (inhibiting growth processes) but at the same time do not inhibit the biochemical pathways for synthesis of target products may be more suitable for use in the biotechnological production of butanol. Such assumption requires additional experimental verification [1, 2, 18].

Conclusion

The NPs can directly or indirectly participate in certain enzymatic reactions and act as catalysts, mediators, etc. Role of NPs which they play in ABE fermentation process requires further investigations, which would allow to derive a theory explaining the biological effect of the NPs in ABE fermentation process. At current stage due to the lack of special studies, we can only confirm the presence of biological effect itself. However, the obtained result, namely – an increase of biobutanol yield in the presence of NPs of iron oxide and cerium, as well as its relative repeatability for different strains of acetone-butyl bacteria, allows the

recommendation of these NPs for further study at pilot plants with purpose of introduction into the biotechnological process. This would allow not only to receive organic solvents (the main products of ABE-fermentation - acetone, butanol and ethanol), but also to use the waste biomass for extraction of lipid fraction with further transformation into biodiesel.

Acknowledgements. Research was carried out within the project (DR No 0115U004278) of the targeted complex multidisciplinary program of scientific researches of Ukraine "Development of scientific principles of rational use of natural resources and sustainable development. Scientific basis for the rational use of natural resources and waste management problems".

References

1. Kim Y.K., Park S.E., Lee H., Yun J.Y. (2014), Enhancement of bioethanol production in syngas fermentation with *Clostridium ljungdahlii* using nanoparticles, *Bioresour. Technol*., 159, pp. 446-450, DOI:10.1016/j.biortech.2014.03.046
2. Kim Y.K., Lee H. (2016), Use of magnetic nanoparticles to enhance bioethanol production in syngas fermentation, *Bioresource Technology*, 204, pp. 139–144, DOI: 10.1016/j.biortech.2016.01.001
3. Jones D.T., Woods D. (1986), Acetone-butanol fermentation revisted, *Microbiology Reviews,* 50, pp. 484–524.
4. Papoutsakis E.T. (2008), Engineering solventogenic clostridia, *J. Current opinion in biotechnology,* 19, pp. 420–429
5. Andrusishina I. (2011), Nanochastitsy metallov: sposoby polucheniia, fiziko-khimicheskie svoistva, metody issledovaniia i otsenka toksichnosti, Suchasni problemi toksikologiï, 3, pp. 5–14.
6. Lee W.T., Wu Y.N., Chen Y.H., Wu S.R., Shih T.M., Li T.J., Yang L.X., Yeh C.S., Tsai P.J., Shieh D.B. (2017), Octahedron iron oxide nanocrystals prohibited *Clostridium difficile* spore germination and attenuated local and systemic inflammation, *Scientific Reports,* 7(1), 8124, DOI: 10.1038/s41598-017-08387-y.4.
7. Zinchenko A. (2014), Nanochastitsy kak transportery diagnosticheskikh i terapevticheskikh substants. Mikrobnye biotekhnologii: fundamentalnye i prikladnye aspekty: sbornik nauchnykh trudov, Belaruskaia navuka, Minsk, 6. pp. 36–50
8. Antonovskaia L.I., Skorb E.V., Sviridov D.V., Beliasova N.A. (2008), Antimikrobnaia aktivnost plenochnykh dioksid-titanovykh fotokatalizatorov po otnosheniiu k grampolozhitelnym i gramotritsatelnym bakteriiam, *Doklady NAN Belarusi*., 52(2), pp. 83–87
9. Poleshchuk N.N, Astashonok A.N, Rubanik L.V., Zhavnerko G.K., (2012), Nanotekhnologicheskie podkhody k izucheniiu strukturnoi organizatsii i detektsii Chlamydia trachomatis, *Zdravookhranenie*, 11. pp. 33–38.
10. Beresnev A. I.,. Kvach S. V, Zinchenko A. I. (2014), Immobilizatsiia bakterialnykh nukleozidfosforilaz na magnitnye nanochastitsy, *Mikrobnye biotekhnologii: fundamentalnye i prikladnye aspekty. Sb. Nauchnykh trudov,* 6. pp. 26–36.
11. Parveen S., Misra R., Sahoo S. K. (2012), Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging, *Nanomedicine,* 8(2), pp. 147–166, DOI:10.1016/j.nano.2011.05.016

12. Marty J.J., Oppenheim R.C., Speiser P. (1978), Nanoparticles – a new colloidal drug delivery system, *Pharm. Acta Helv.,* 53, pp.17–23.
13. Couvreur P., Kante B., Roland M., Guiot P., Bauduin P., Speiser P. (1979), Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties, *J. Pharm. Pharmacol,* 31(5), pp. 331–332.
14. Gurny R., Peppas N.A., Harrington D.D., Banker G.S. (1981), Development of Biodegradable and Injectable Latices for Controlled Release of Potent *Drugs, Drug Development and Industrial Pharmacy*, 7(1), pp. 1–25. DOI: 10.3109/03639048109055684.
15. Siekmann B, Westesen K., (1992), Submicron-sized parenteral carrier systems based on solid lipids, *Pharm Pharmacol Lett*., 1, pp. 123–126.
16. Rao C. N. R., Ramakrishna Matte H. S. S., Voggu Rakesh and Govindaraj A. (2012), Recent progress in the synthesis of inorganic nanoparticles, *Dalton Trans.,* 41, pp. 5089–5120.
17. Mongkolkajit J., Pullsirisombat J., Prichanont S., Tantayakom V., Prasitchoke P., Phisalaphong M. (2010), 2 γ-Alumina Doped Alginate Gel for Cell Immobilization in Fermentation Processes, *AJChE*, 10(1). pp. 1–7.
18. Durán-Padilla V.R., Davila-Vazquez G., Chávez-Vela N.A., Tinoco-Valencia J.R., Jáuregui-Rincón J. (2014), Iron effect on the fermentative metabolism of Clostridium acetobutylicum ATCC 824 using cheese whey as substrate, Biofuel Research Journal, 4, pp. 129–133, DOI: 10.18331/BRJ2015.1.4.5

Influence of operating pressure on concentration polarization layer resistance in revers osmosis

Serhii Huliienko, Oksana Leshchenko

National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine

Keywords:

Osmosis
Membrane
Resistance
Polarization
Flux
Pressure

Article history:

Received 21.09.2018
Received in revised form 27.10.2018
Accepted 28.03.2019

Corresponding author:

Serhii Huliienko
E-mail: gunder@i.ua

DOI:
10.24263/2304-974X-2019-8-1-13

Abstract

Introduction. The experimental examination of hypothesis about linear dependence of concentration polarization resistance from pressure was carried out and the influence of hydrodynamic condition on this resistance is determined.

Materials and methods. The research was carried out with using of commercially available membrane modules TFC-75 type. The measurements of productivity were carried out with using of deionized water (total dissolved solids 5–15 mg/dm^3) and also NaCl solutions. The volumetric technique was used for flux measurements. The concentration was measured by conductometric technique.

Results and discussion. The membrane resistance during reverse osmosis of deionized water did not change with applied pressure in experimental conditions and was equal R_m=7,549·10^{13} m^{-1}.

The concentration polarization layer resistance (R_{cp}) increased from 0.65–1.29·10^{13} m^{-1} to 1.46–1.83·10^{13} m^{-1} with applied pressure increasing from 0.2 MPa to 0.6 MPa and from 0.65–1.46 m^{-1} to 1.29–1.83·10^{13} m^{-1} with increasing of feed concentration from 100 mg/dm^3 to 600 mg/dm^3. This increasing of R_{cp} value with pressure was linear which is in agreement with previously reported data for the ultrafiltration process. Moreover, in considered range of applied pressure, the exponential dependence of index of concentration polarization from applied pressure could be approximated by a linear equation with correlation coefficient 0.93. Therefore, assumption about linear dependence of concentration polarization layer resistance from pressure is reasonable and could be extended to reverse osmosis process for mentioned above conditions.

The increasing of concentration polarization layer resistance with increasing of applied pressure is determinated by higher values of transmembrane fluxes and lower values of mass transfer coefficient at higher values of applied pressure in the considered system. These results are in agreement with film theory of concentration polarization.

Conclusions. The exanimated hypothesis is validated for reverse osmosis in considered range of applied pressure. The correlation between concentration polarization layer resistance and index of concentration polarization was defined.

Introduction

The concentration polarization phenomenon and the scaling formation on membrane surface are the main problems in using of pressure-driven membrane processes in the food industry. In particular, during drinking water production with using of microfiltration and ultrafiltration the water flux decrease almost twice (from 102 dm^3/h until 50 dm^3/h) due to those effects [1], moreover, flux drop is observed also in case of membranes with antifouling modification [2] and membrane systems with pretreatment [3]. During pomegranate juice ultrafiltration the resistance of cake layer was 35.6–56.9% of the total resistance of mass transfer across the membrane which causes the dramatic drop of permeate flux [3], similar results was obtained for ultrafiltration of bergamot and kiwifruit juices [5]. During nanofiltration and reverse osmosis of milk whey, the rapid flux drop due to adsorption fouling and following flux decreasing due to concentration polarization and cake layer formation was observed [6]. In case of milk whey ultrafiltration flux drop for 67.5% because of those phenomena was observed over 20 hours of work [7] and during skimmed milk microfiltration the cake layer resistance was two times higher than membrane resistance [8]. Significant decreasing of permeate flux was also defined for microfiltration such products as corn distillery [9], beer [10] and wine [11].

The comprehensive review of mentioned phenomena was carried out by Shirazi and al. [12]. In particular, in that work, it was pointed out that decreasing in permeate flux is determinated by series resistances of mass transfer across the membrane which include membrane resistance, concertation polarization resistance, cake later resistance and pore blocking resistance. However, pore blocking resistance is not significant for most of the pressure-driven membrane process therefore in others works, for example, Luo and al [13] for investigation of nanofiltration processes and Macedo and al. [14] for analysis of ultrafiltrations, only the first three resistances were taken into account.

The methods for determination of membrane resistance and cake layer resistance are represented in works [12–14], moreover, Sioutopoulos and Karabelas [15] have determinated the dependences of fouling layer resistance from working parameters of ultrafiltration process in particular from applied pressure. But methods for determination of concentration polarization layer resistance are almost not described in the literature.

In most cases, the researches of concentration polarization have theoretical character and looked toward for development and assess the adequacy of mathematical models of that phenomena. For example, Geraldes and Afonso [16] have proposed the model based of extended Nernst-Planck equation, which allows to predict the parameters of concentration polarization (index of concentration polarization) for the case of nanofiltration and reverse osmosis of diluted multicomponent salt solutions. Song and Liu [17] have developed the model based on general salt balance and shear stress. Cavaco Morao and al. [18] have used for simulation the method of computational fluid dynamics (CFD). Kim and Hoek [19] have carried out the comparison of several analytical and numerical models for prediction of concentration polarization and have defined their satisfied accuracy in the range of operating parameters change which correspond to the real condition of reverse osmosis process operation. But in these works, the value of concentration polarization as such are not considered.

Shirazi and al. [12] have pointed out that for taking into account the concentration polarization layer resistance it is possible to use of mediate methods as it has been done in earlier works [20–22], in particular, Song [22] has pointed out, that influence of concentration polarization could be taken into account by decreasing of driving force. he most comprehensive study of the problem of concentration polarization layer resistance has been

carried out by Macedo and al [14]. They declare that under constant solute concentration, feed flow velocity and temperature, concentration polarization layer resistance depend from applied pressure, in particular, the assumption about the linear dependence between these values. But this hypothesis has been verified only for ultrafiltration and for one kind of feed solution – ovine milk whey. The values of the proportional coefficient in work [14] have been determinated from experiments and their magnitudes have varied more than three times under different condition of the ultrafiltration process. Moreover, the physical meaning of the proportional coefficient has not been disclosed. Therefore, at present time the possibility of direct taking into account of concentration polarization resistance during calculation of membrane equipment is overstructured. Simultaneously Macedo and al. [14] have proposed the technique of data analysis of productivity of pressure-driven membrane processes, which allows to determinate the fouling layer resistance. In a case of availability of reliable dependence for concentration polarization layer resistance, this technique would allow to determinate in working conditions the necessity of regeneration of membranes. For this reason, the determination of this dependence is a topic of great practical significance.

The purpose of present work is the examination of hypothesis about linear dependence of concentration polarization layer from applied pressure in case of reverse osmosis of salt solutions and determination of applied pressure and also hydrodynamic conditions on concentration polarization resistance value.

Materials and methods

Materials

The experiments were carried out with using of commercially available reverse osmosis membrane modules Dow Filmtec TW30-1812-50 (made in USA). The deionized water (reverse osmosis permeate with total dissolved solids (TDS) 5-15 ppm which correspond to the concentration of Sodium Chloride (NaCl) 5–15 mg/dm^3) and also NaCl solutions were used as feed solutions. The deionized water was used for measurements of membrane resistance and salt solution for measurements of concentration polarization layer resistance. The considered type of membrane modules is designed for tap water advanced treatment [23], for this reason for determination of concentration polarization layer resistance the solution which simulated TDS of tap water were used. According to [24] the overall mineralization in waters of Dnipro and Desna is 268–270 mg/dm^3, and TDS parameter of tap water in European countries (Spain, Italy, and France) according to [25–26] is in range 70–820 ppm, in most cases this parameter was in range 100–600 ppm. Therefore, the NaCl solutions with concentrations of 100, 200, 400 and 600 mg/dm^3 were used in experiments.

Description of experimental set-up

The existing experimental set-up (Figure 1) was used for carrying out experimental research. The set-up provides the measurements of permeate and retentate flow rate by volumetric technique through the use of tanks 5 and 6 with accuracy of ±2 ml and ±10 ml correspondingly (it was carried out the direct measurements of volume and time), and also it allows to measure the overall mineralization using portable TDS-meter with accuracy of 1 mg/dm^3. The applied pressure was measured by manometer 9, the temperature was controlled by Chromel-Copel thermocouples and software module IndexTem with the accuracy of 0.2 °C (it did not represented on the scheme).

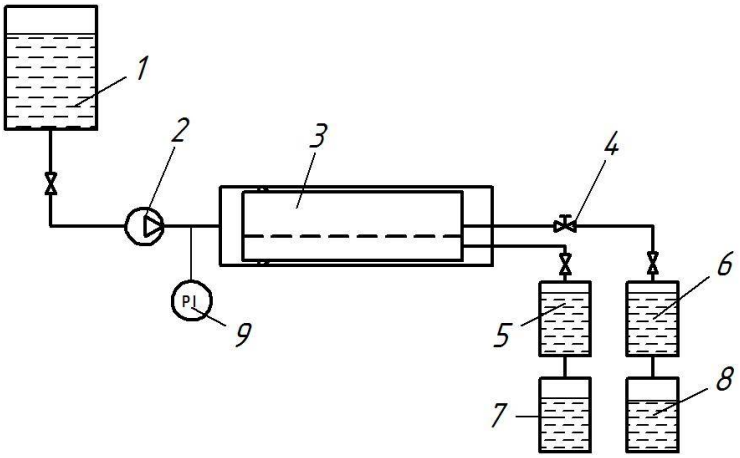


Figure 1. Scheme of experimental set-up:
1 – feed solution tank, 2 – pump, 3 – membrane unit, 4 – regulation valve, 5 – measuring tank for permeate, 6 – measuring tank for retentate, 7 – permeate collecting tank, 8 – retentate collecting tank, 9 – manometer.

Procedure of carrying out the experiments and main measurements

The feed solution was pumped with a predetermined pressure by pump 2 from tank 1 to plug flow membrane unit 3 in which the studied membrane module Dow Filmtec TW30-812-50 was inserted. During the membrane separation process, the permeate (desalinated solution) and retentate (concentrated) solution were generated which flowed to measurement tanks 5 and 6 and gone on to collecting tanks 7 and 8 correspondingly. The applied pressure was regulated by the needle valve on the retentate line 4 and was controlled by manometer 9. The measurements of permeate and retentate were carried out by volumetric technique, according to it the volumes of solutions which simultaneously collected in measuring tanks 5 and 7 during the determined time interval (120 s) were determined.

Processing the results of the research

The feed solution flow rate was determ

inated from well-known material balance equations. The mass balance for streams is following [27]:

$$L_f = L_p + L_r \tag{1}$$

where L_f is feed solution flow rate, m^3/s; L_p is permeate flow rate, m^3/s; L_r is retentate flow rate m^3/s.

The mass balance for the solute is following [27]:

$$L_f = \frac{x_p L_p + x_r L_r}{x_f}, \tag{2}$$

where x_f is solute concentration in feed solution, mg/dm^3; x_p is solute concentration in permeate, mg/dm^3; x_r is solute concentration in retentate, mg/dm^3.

The mean values of determinated from equation (4) and (5) was used for further calculations. If the difference between that values was more than 5% the results obtained in such experiment were discarded as mistaken.

The transmembrane flux was determinated from the relationship [28]:

$$J = \frac{L_p}{F}, \tag{3}$$

where F is membrane surface area, m^2; for the membrane module under consideration F=0.46 m^2 (directly measured value).

The value of Reynolds number was used for analysis of hydrodynamic conditions in module [27]:

$$\mathrm{Re} = \frac{w_e \cdot d_e \cdot \rho}{\mu}. \tag{4}$$

Since in membrane module the flow rate change with channel length due to penetration of a part of the feed solution through the membrane in present research the mean value calculated from the permeate and retentate flow rate was used as a determining velocity, m/s [27]:

$$w_e = \frac{L_f + L_r}{2 \cdot S}, \tag{5}$$

where S is membrane channel cross section, m^2. For the membrane module under consideration S= $3.675 \cdot 10^{-4}$ m^2 (directly measured value).

The equivalent diameter was used as determining linear dimension. For spiral wound membrane modules it can be represented in a form [28]:

$$d_e = 2 \cdot \delta, \tag{6}$$

where δ is spacer net width, m.

Determination of concentration polarization layer resistance

The calculations of resistance were carried out based on transmembrane flux value which according to [12–14] can be described by equation, $m^3/(m^2 \cdot s)$:

$$J = \frac{\Delta p - \Delta \pi}{\mu \cdot \left(R_m + R_{cp} + R_f \right)}, \tag{7}$$

where Δp is applied pressure (driving force), Pa; $\Delta\pi$ is osmotic pressure of feed solution, Pa; μ is coefficient of dynamic viscosity of feed solution, Pa·s; R_m is membrane resistance, m^{-1}; R_{cp} is concentration polarization layer resistance, m^{-1}; R_f is fouling resistance.

In new membrane modules fouling is absent consequently in this case the value of R_f will be equal to zero. Moreover, when deionized water is used as testing solution due to absence (or negligible amount) of solute the solution osmotic pressure $\Delta\pi$ would tend to zero and concentration polarization phenomena would not appear. Therefore, the membrane resistance can be defined from relationship [12]:

$$R_m = \frac{\Delta p}{\mu \cdot J}. \tag{8}$$

For thin-film composite membrane the flux drop due to compaction of membrane structure according to [29] is observed under applied pressures of 0.5–1.45 MPa. Although in current study in particular cases the applied pressure was in mentioned above range, the duration of continuous operation was no longer than 180–300 s, therefore it may assume that

impact of compaction to membrane resistance was negligible. When this assumption is correct the R_m will be constant under any applied pressure [12].

For the case of salt solution separation thorough new membrane, the total membrane resistance would be the sum of R_m and R_{cp} values. Whereas the R_m is known, the concentration polarization resistance could be obtained from measurements results from dependency, found from the transformations of equations (7) and (8):

$$R_{cp} = \frac{\Delta p - \Delta \pi}{\mu \cdot J} - R_m \,. \tag{9}$$

Thus, for the solution with known values of osmotic pressure and dynamic viscosity the concentration polarization layer resistance could be determinated experimentally. The values of $\Delta\pi$ and μ depends on sort of substances (chemical composition of solution) and also they are functions of temperature and pressure. For monocomponent solutions, these values could be determinated from reference literature [30–31].

Results and discussion

The total resistance to mass transfer through the membrane was determinated on the results of the current study in the range of applied pressure of Δp=0.2..0.6 for deionized water and NaCl solutions with concentration 100, 200, 400 and 600 mg/dm^3 (Figure 2). The step of applied pressure variation was 0.1 MPa. In the case of deionized water, the deviation of total resistance values was less than 5% from the mean value which does not exceed of measurement error limit. Thus, the result shown that membrane resistance is constant and independent from applied pressure and the proposed assumption is confirmed. The mean value was R_m=7.549·10^{13} m^{-1} and this magnitude was used in further calculations.

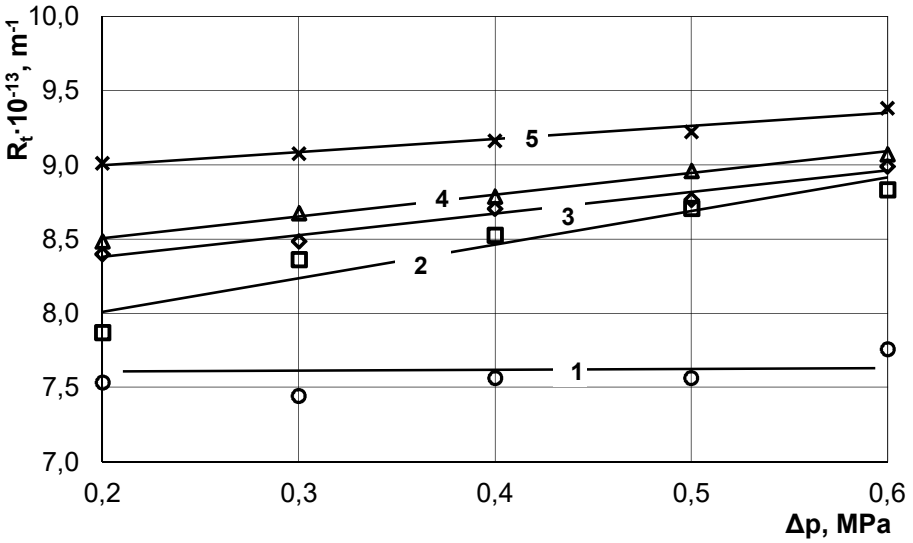


Figure 2. The dependence of total resistance to mass transfer thorough the membrane from applied pressure:
1 – Deionized water; 2 –NaCl solution, 100 mg/dm^3; 3 – NaCl solution, 200 мг/дм^3; 4 –NaCl solution, 400 mg/dm^3; 5 –NaCl solution, 600 mg/dm^3

During NaCl solutions separation using the membranes of consideration the total resistance increased with increasing of feed concentration, and applied pressure. Since the new membrane modules were used in experiments and NaCl concentration was lower than saturation limit on two orders of magnitude [30], the fouling resistance could be considered as absent, therefore total resistance increasing was determinated by concentration polarization. The concentration polarization layer resistance calculated using equation (9) is shown on Figure 3.

Thus, the equation (9) allow to determinate concentration polarization during reverse osmosis of salt solution. In order to validation of obtained results reliability, it is necessary to analyze them on the agreement with received theoretical foundations.

The increasing of concentration polarization resistance with feed concentration increasing is excepted and determinated by increasing in a corresponded number of times of concentration in boundary layer near membrane which reduce diffusion mass transfer toward membrane and increase driving for on reverse diffusion flow [12].

The increasing on concentration polarization layer resistance with applied pressure increasing is in agreement with results obtained in work [14]. In the considered range of applied pressure, this dependence could be approximated by a linear equation. For validation of such results on the agreement with existing received theoretical foundations about the influence of applied pressure on parameters of concentration polarization should be considered.

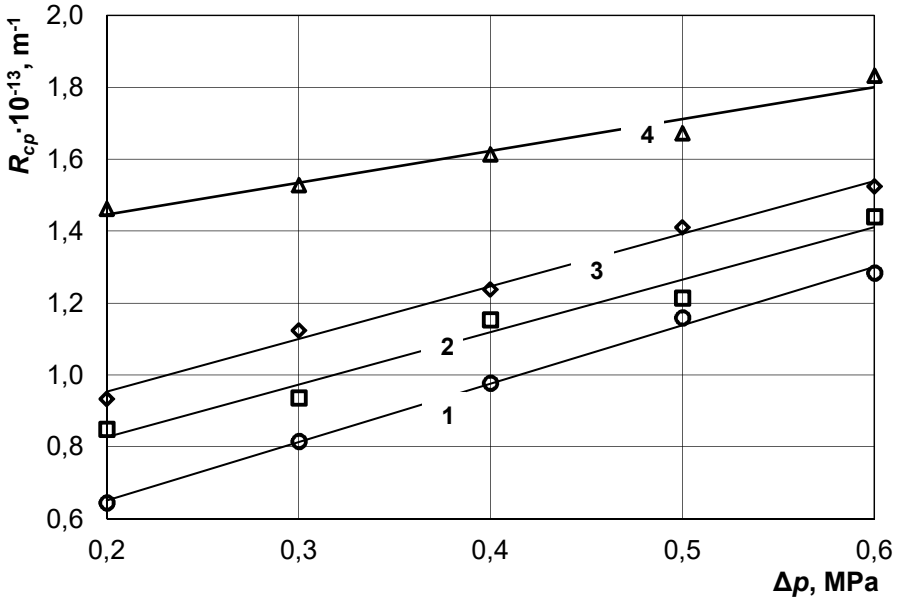


Figure 3. Dependence of concentration polarization layer resistance from applied pressure:
1 – NaCl solution, 100 mg/dm^3; 2 – NaCl solution, 200 mg/dm^3;
3 – NaCl solution, 400 mg/dm^3; 4 – NaCl solution, 600 mg/dm^3

According to film theory of concentration polarization [14, 28] for evaluation of concentration polarization influence the value of the index of concentration polarization is used:

$$f = \frac{c_m}{c_b} = \exp\left(\frac{J}{k}\right), \tag{10}$$

where c_m is solute concentration in boundary layer near membrane surface; c_b is solute concentration in bulk of feed solution; k is mass transfer coefficient.

In a case of nonideal selectivity equation (10) should be rewritten in the form [28]:

$$f = \frac{c_m}{c_b} = \frac{\exp\left(\frac{J}{k}\right)}{\phi - (\phi - 1)\cdot\exp\left(\frac{J}{k}\right)}. \tag{11}$$

At that the rejection coefficient (selectivity) is determinated as [28]:

$$\phi = 1 - \frac{c_p}{c_f}, \tag{12}$$

where c_p is solute concentration in permeate, kg/m^3; c_f is solute concentration in feed solution, kg/m^3.

As mentioned in [12, 28] the index of concentration polarization value and correspondingly concentration in the boundary layer, increase with transmembrane flux increasing and mass transfer coefficient decreasing. Increasing of applied pressure determinate increasing of transmembrane flux (Figure 4) which govern the increasing of the index of concentration polarization that correlated with increasing of resistance in this layer (Figure 5).

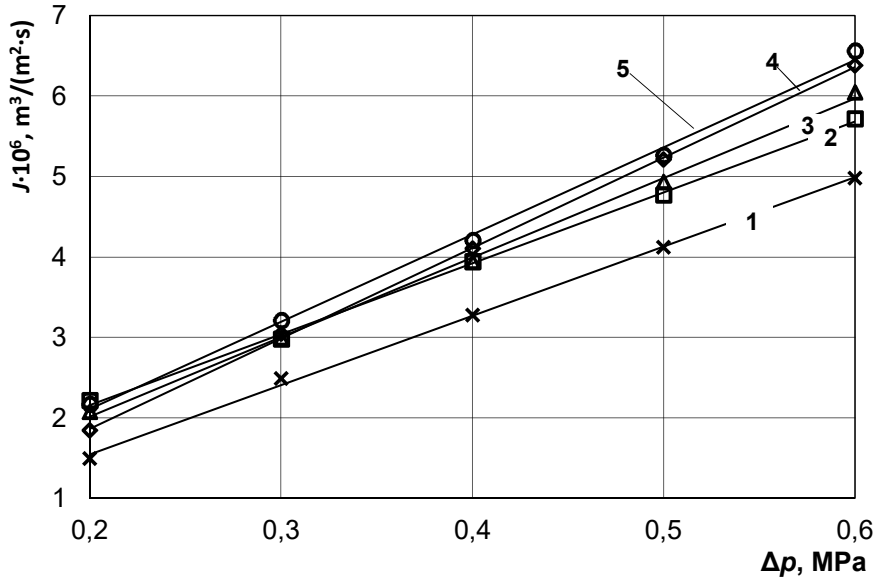


Figure 4. Dependence of transmembrane flux from applied pressure:
1 – Deionized water; 2 –NaCl solution, 100 mg/dm^3; 3 – NaCl solution, 200 мг/дм^3; 4 –NaCl solution, 400 mg/dm^3; 5 –NaCl solution, 600 mg/dm^3

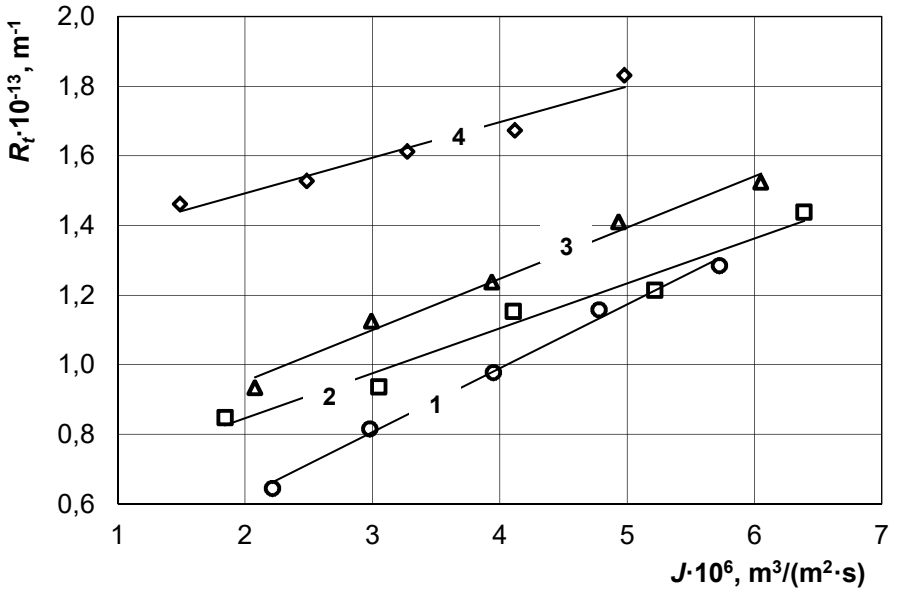


Figure 5. Dependence of concentration polarization resistance to mass transfer thorough the membrane from transmembrane flux:
1 – NaCl solution, 100 mg/dm^3; 2 – NaCl solution, 200 mg/dm^3;
3 – NaCl solution, 400 mg/dm^3; 4 – NaCl solution, 600 mg/dm^3

It should be noticed that experiments were carried out under an ambient temperature which varied from 10°C to 17°C. This fact determinated observed deviation from decreasing of transmembrane flux with feed concentration increasing due to different values of dynamic viscosity of the solvent.

The mass transfer coefficient value depends on many factors, in particular, from hydrodynamic conditions in a membrane module and physical properties of a feed solution. Generally this parameter is calculated using dimensionless equations according to which increasing of liquid flow velocity (increasing of Reynolds number value) determinate increasing of mass transfer coefficient [12, 28]. The dependence of Reynolds number from applied pressure is shown on Figure 6.

In experimental research processing the applied pressure was regulated by variation of hydrodynamic resistance of retentate flow and under such conditions, higher values of applied pressure correspond to higher Reynolds number values. Furthermore, concentration polarization resistance decreases with Reynolds number increasing (Figure 7). Correspondingly increasing of applied pressure in the involved system lead to decreasing of mass transfer coefficient decreasing which determinate increasing of the index of concentration polarization and in this case concentration polarization resistance also should increase. Therefore, the obtained experimental results are in generally in qualitative agreement with film theory of concentration polarization.

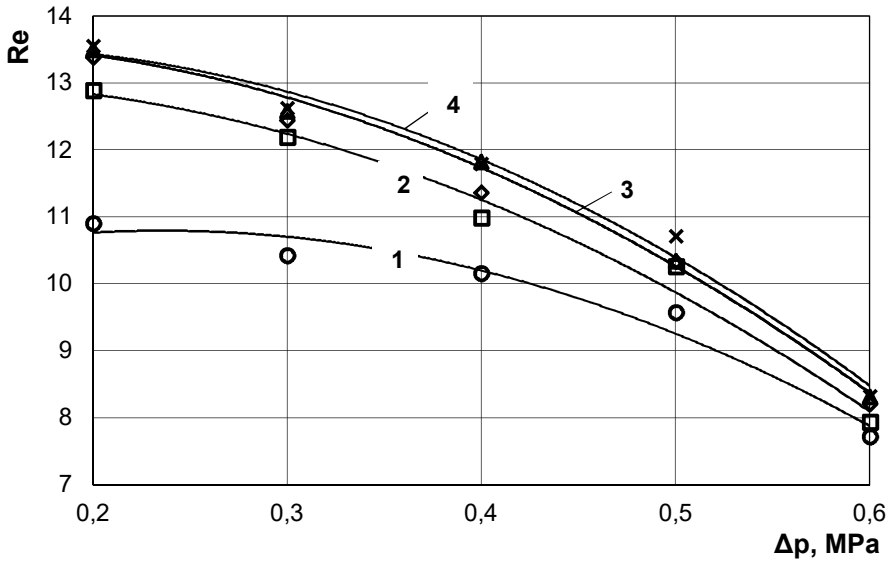


Figure 6. Dependance of Reynolds number from applied pressure:
1 – NaCl solution, 100 mg/dm^3; 2 – NaCl solution, 200 mg/dm^3;
3 – NaCl solution, 400 mg/dm^3; 4 – NaCl solution, 600 mg/dm^3

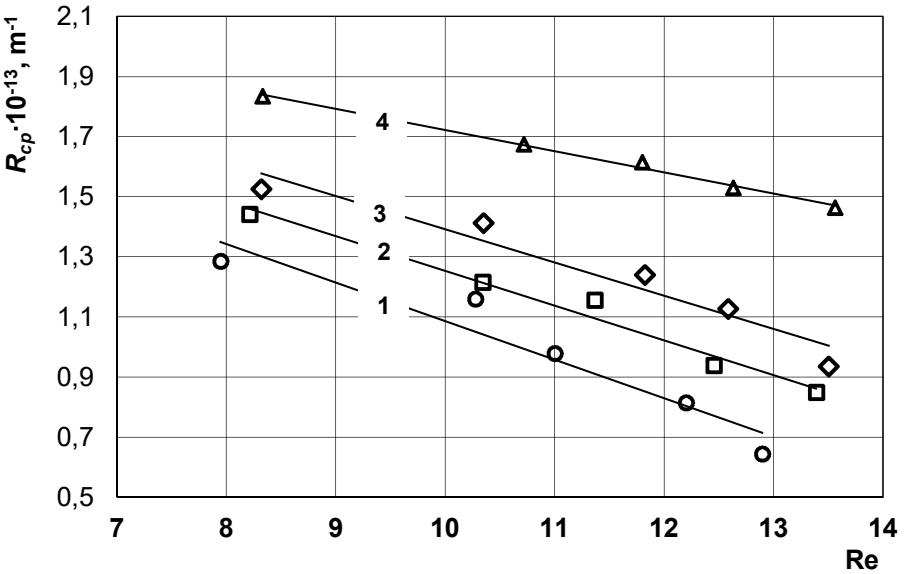


Figure 7. Dependence of concentration polarization layer resistance to mass transfer thorough the membrane from Reynolds number:
1 – NaCl solution, 100 mg/dm^3; 2 – NaCl solution, 200 mg/dm^3;
3 – NaCl solution, 400 mg/dm^3; 4 – NaCl solution, 600 mg/dm^3

It should be noticed that the dependence of concentration polarization layer resistance from Reynolds number could be approximated by a linear equation. The different values of Reynolds number under the same values of applied pressure are explained by different values of transmembrane flux for different feed solution concentration which determinated differences in retentate flux and correspondingly changes in determinated velocity calculated from equation (5).

In order to more reliable validation of defined results agreements with film theory, it should compare the variations of concentration polarization layer directly with the index of concentration polarization value. At that, since the experiments were carried out in range of Reynolds number [7.9, 13.5] which corresponds to the laminar regime of flow, the mass transfer coefficient values were estimated using the dimensionless equation for laminar flow in channel [28]:

$$\mathrm{Sh} = \frac{k \cdot d_e}{D} = 1{,}85 \cdot \left(\mathrm{Re} \cdot \mathrm{Sc} \cdot \frac{d_e}{L} \right)^{0{,}33}, \tag{13}$$

where D is diffusivity, m^2/s; $Sc = \nu/D$ is Schmidt number; L is channel length, m; ν is coefficient of kinematic viscosity, m^2/s.

The length of considered channel was $L = 0.26$ m (directly measured value), the value of coefficient of kinematic viscosity was determinated using reference literature [30–31] and diffusivity value were calculated using the Wilke–Chang equation [27]:

$$D = 5{,}06 \cdot 10^{-11} \frac{T_s}{\mu_l \cdot V_s} \tag{14}$$

where T_s is solvent absolute temperature, K; μ_l is solvent coefficient of dynamic viscosity, mPa·s; V_s is solute molar volume, cm^3/mole.

The values of the index of concentration polarization were calculated using equation (11), all parameters were determinated from the results of measurements. The dependence of concentration polarization index from applied pressure is shown on Figure 8.

The values of the index of concentration polarization according to equation (11) increase exponentially but in regarding range of applied pressure change this dependence could be approximated by a linear equation with sufficient accuracy for engineering calculation. In particular, for NaCl concentration of 200 mg/dm^3, the sample correlation coefficient is 0.97. Therefore, the assumption about linear dependance of concentration polarization layer resistance from applied pressure in several ranges of this parameter presented in work [14] is reasonable and could be used not only for a case of ultrafiltration and also for reverse osmosis.

It should be noticed that concentration polarization layer resistance increase in 1,25–2 times when applied pressure increase from 0.2 to 0.6 MPa (Figure 3). The index of concentration polarization in this case increase in 1.9–2.15 times (Figure 8). Moreover, the relationship between concentration polarization layer resistance and index of concentration polarization could be approximated by a linear equation with correlation coefficients more than 0.93 (Figure 9).

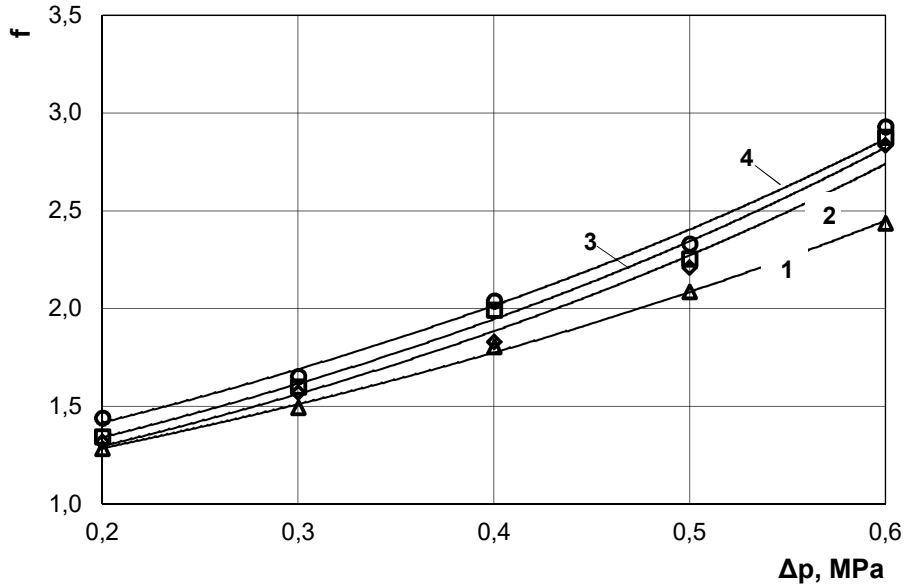


Figure 8. Dependence of index of concentration polarization from applied pressure
1 – NaCl solution, 100 mg/dm^3; 2 – NaCl solution, 200 mg/dm^3;
3 – NaCl solution, 400 mg/dm^3; 4 – NaCl solution, 600 mg/dm^3

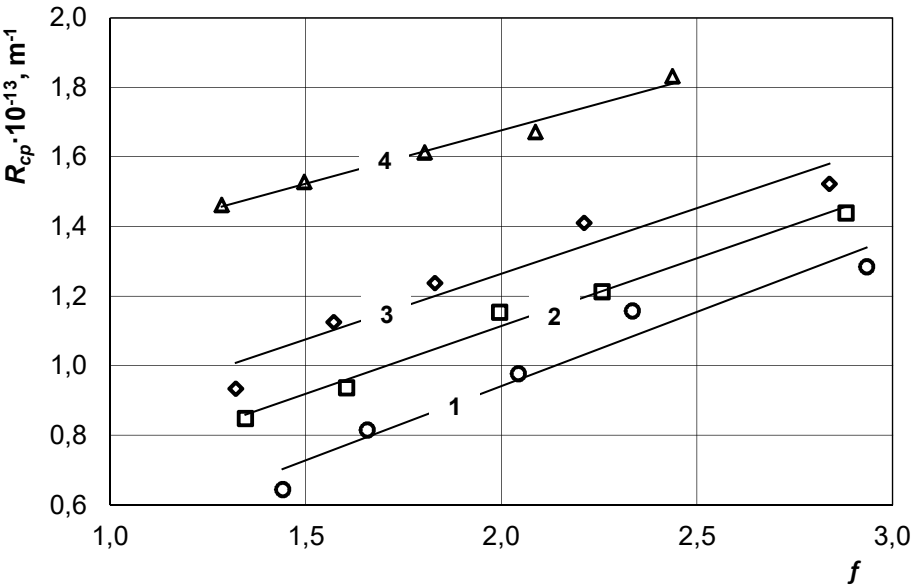


Figure 9. Dependence of concentration polarization layer resistance to mass transfer thorough the membrane
1 – NaCl solution, 100 mg/dm^3; 2 – NaCl solution, 200 mg/dm^3;
3 – NaCl solution, 400 mg/dm^3; 4 – NaCl solution, 600 mg/dm^3

Consequently, such good qualitative agreement of concentration polarization layer resistance calculated from equation (9) with well-known theoretical fundamentals confirms the possibility of using the before-mentioned technique in serial experiments. The results obtained from pilot experiments are not enough for the establishment of quantitative relationships among concentration polarization layer resistance and operating parameters including applied pressure. Also, this data are not enough for the determination of proportional coefficient which used in work [14]. Thus, the further investigation is necessary which would allow obtaining the reliable calculation dependencies in form of functions of operating parameters of pressure driven membrane separation processes (applied pressure, feed solution properties, hydrodynamic condition in modules etc.)

Conclusion

1. The hypothesis about linear dependence of concentration polarization layer resistance from applied pressure which used for analysis of milk whey ultrafiltration process is confirmed for the case of salt solutions reverse osmosis in the range of applied pressure 0.2–0.6 MPa.
2. In the considered range of applied pressure, the influence of membrane compaction is negligible and it could be considered that applied pressure does not affect the membrane resistance value for the considered type of membrane.
3. The obtained qualitative dependences of concentration polarization layer dependences from applied pressure, transmembrane flux and Reynolds number are in agreement with film theory. Moreover, the correlation between concentration polarization layer resistance and index of concentration polarization which allows assuming about the possibility to obtain of reliable calculation dependence of concentration polarization layer resistance from operating parameters of pressure driven membrane separation processes.

References

1. Chang H., Liang H., Qu F., Liu B., Yu H., Du X., Li G., Snyder S. (2017), Hydraulic backwashing for low-pressure membranes in drinking water treatment: A review, *Journal of Membrane Science*, 540, pp. 262–380.
2. Du J., Peldszus S., Huck P., Feng X. (2015), Modification of membrane surfaces via microswelling for fouling control in drinking water treatment, *Journal of Membrane Science*, 475, pp. 488–495.
3. Stoquart C., Servais P., Bérubé P., Barbeau B. (2012), Hybrid Membrane Processes using activated carbon treatment for drinking water: A review, *Journal of Membrane Science*, 411–412, pp. 1–12.
4. Onsekizoglu P. (2013), Production of high quality clarified pomegranate juice concentrate by membrane processes, *Journal of Membrane Science*, 442, pp. 264–271.
5. Ruby-Figueroa R., Saavedra J., Bahamonde N., Cassano A. (2017), Permeate flux prediction in the ultrafiltration of fruit juices by ARIMA models, *Journal of Membrane Science*, 524, pp. 108–116.
6. Kyrychuk I., Myronchuk V., Zmiievskii Y., Holiachuk S. (2015), Two-stage whey treatment by nanofiltration and reverse osmosis, *Ukrainian Food Journal*, 4 (4), pp. 638–647.
7. Chamberland J., Beaulieu-Carbonneau, Lessard M.-H., Labrie S., Bazinet L., Doyen A., Pouliot Y. (2017), Effect of membrane material chemistry and properties on biofouling susceptibility during milk and cheese whey ultrafiltration, *Journal of Membrane Science*, 542, pp. 208–216.
8. Jimenez-Lopez A.J.E., Leconte N., Garnier-Lambrouin F., Bouchoux A., Rousseau F., Gésan-Guiziou G. (2011), Ionic strength dependence of skimmed milk microfiltration: Relations between

filtration performance, deposit layer characteristics and colloidal properties of casein micelles, *Journal of Membrane Science*, 369, pp. 404–413.
9. Zmievskii Y., Dziazko Y., Myronchuk V., Rozhdestvenska L., Vilenskii A., Kornienko L. (2016), Fouling of polymer and organic-inorganic membranes during filtrarion of corn distillery, *Ukrainian Food Journal*, 5 (4), pp. 739–747.
10. van der Sman R.G.M., Vollebregt H.M., Mepschen A., Noordman T.R. (2012), Review of hypotheses for fouling during beer clarification using membranes, *Journal of Membrane Science*, 396, pp. 22–31.
11. El Rayess Y., Albasi C., Bacchi P., Taillandi P., Mietton-Peuch M., Devatine A. (2011), Cross-flow microfiltration of wine: Effect of colloids on critical fouling conditions, *Journal of Membrane Science*, 385–386, pp. 177–186.
12. Shirazi S., Lin C.-J., Chen D. (2010), Inorganic fouling of pressure-driven membrane processes – A critical review, *Desalination*, 250, pp. 236-248.
13. Luo J., Ding L., Su Y., Wei Sh., Wan Y. (2010), Concentration polarization in concentrated saline solution during desalination of iron dextran by nanofiltration, *Journal of Membrane Science*, 363, pp. 170–179.
14. Macedo A., Duarte E., Pinho M. (2011), The role of concentration polarization in ultrafiltration of ovine cheese whey, *Journal of Membrane Science*, 381, pp. 34–40.
15. Sioutopoulos D., Karabelas A. (2015), The effect of permeation flux on the specific resistance of polysaccharide fouling layers developing during dead-end ultrafiltration, *Journal of Membrane Science*, 473, pp. 292–301.
16. Geraldes V., Afonso M. D. (2007), Prediction of the concentration polarization in the nanofiltration/reverse osmosis of dilute multi-ionic solutions, *Journal of Membrane Science*, 300, pp. 20–27.
17. Song L., Liu C. (2012), A total salt balance model for concentration polarization in crossflow reverse osmosis channels with shear flow, *Journal of Membrane Science*, 401–402, pp. 313–322.
18. Cavaco Morao A., Brites Alves A., Geraldes V. (2008), Concentration polarization in a reverse osmosis/nanofiltration plate-and-frame membrane module, *Journal of Membrane Science*, 325, pp. 580–591.
19. Kim S., Hoek E. (2005), Modeling concentration polarization in reverse osmosis processes, *Desalination*, 186, , pp. 111–128.
20. van Den Berg G.B., Smolders C.A. (1990), Flux Decline in ultrafiltration process, *Desalination*, 77, pp. 101–133.
21. Wijmans J.G., Nakao S., Smolders C.A. (1984), Flux limitation in ultrafiltration: osmotic pressure model and gel layer model, *Journal of Membrane Science*, 20, pp. 115–124.
22. Song L. (1998), Flux decline in crossflow microfiltration and ultrafiltration: mechanisms and modeling of membrane fouling, *Journal of Membrane Science*, 139, pp. 183–200.
23. Weinman S., Fierce E., Husson S. (2019), Nanopatterning commercial nanofiltration and reverse osmosis membranes, *Separation and Purification Technology*, 209, pp. 646–657.
24. Kravchenko M.V. (2015), Fizyko-khimichnyi analiz pryrodnoi pytnoi vody riznykh dzherel vodopostachannia, *Ekolohichna bezpeka ta pryrodokorystuvannia*, 3(19), pp. 52–60.
25. Cidu R., Frau F., Tore P. (2011), Drinking water quality: Comparing inorganic components in botled water and Italian tap water, *Journal of Food Composition and Analysis*, 24, pp. 184-193.
26. Platikanov S., Hernández A., González S., Cortinac J.L., Tauler R., Devesa R. (2017), Predicting consumer preferences for mineral composition of bottled and tap water, *Talanta*, 162, pp. 1–9.
27. Thibodeaux L., Mackay D. (2011), *Handbook of Chemical Mass Transport in the Environment*, CRC Press, New York.
28. Mulder M. (2000), *Basic principles of membrane technology*, Kluwer academic publishers, Dordrecht.
29. Aghajani M., Wang M., Cox L., Killigore J., Greenbeng A., Ding Y. (2018), Influence of support-layer deformation on the intrinsic resistance of thin film composite membranes, Journal of Membrane Science, 567, pp. 49–57.
30. Nikolskij B. (2013). *Spravochnik himika tom 3. Himicheskoe ravnovesie i kinetika. svojstva.* [S.l.]: Book On Demand Ltd.
31. Yaws C. (1999), *Chemical properties handbook: physical, thermodynamic, environmental, transport, safety and heals related properties for organic and inorganic chevicals*, McGraw-Hill, New York.

Method of thermometric determination of thermophysical characteristics of thermolabs materials

Olga Mazurenko, Liliia Kharchenko, Dmytro Kolomiets, Oleksandr Mazurenko

National University of food Technologies, Kyiv, Ukraine

Keywords:

Thermometric
Heat transfer
Metrology
Determination
Thermolabs

Article history:

Received 12.07.2018
Received in revised form 21.12.2018
Accepted 28.03.2019

Corresponding author:

Dmytro Kolomiets
E-mail:
kdp1210@i.ua

DOI: 10.24263/2304-974X-2019-8-1-14

Abstract

Introduction. The analytical researches of methods and devices of determination of thermophysical properties of complicated structure and chemical composition of products of processing industry are carried out in order to increase the accuracy of measurements.

Materials and methods. The basis of the studied devices is thermoelectric converters of temperature and heat flow in the form of "auxiliary wall". To improve the accuracy of the measurements of the thermophysical characteristics of materials, the graph-analytical method of calibration of the devices was used.

Results and discussion. To study the thermophysical characteristics of thermolabial materials, the most suitable thermometric means of their complex definition, which allow to conduct research in the presence or absence of material phase transformations of its constituents. The calorimetric measuring instruments are based on the measurement of the temperature of the heat fluxes that penetrate the sample under different thermal conditions.

Thermal, electric and other transmission processes in thermometric materials determine the instability of the transformation function and form an instrumental error. On the basis of the calculation and graphic analysis of the thermal and capacitive resistance of the "device-sampler" system, a fundamentally new method has been developed, according to which the thermophysical characteristics of the material and the metrological characteristics of the device are determined in a complex and simultaneously based on the results of the experiment with samples of only the test material.

Conclusion. Simultaneous obtaining information on the values of the thermophysical characteristics of the material and the metrological characteristics of the device can improve the accuracy of the determination of the thermophysical characteristics of products.

Introduction

The intensity of heat exchange processes occurring in substances (materials), is quantified by changing their thermophysical characteristics (hereinafter TPC), in particular the thermal conductivity λ_x, mass c_x and volumetric $(c\rho)_x$ heat capacity, temperature diffusivity $a_x = \lambda_x / (c\rho)_x$ and thermal activity $\beta_x = \sqrt{\lambda_x \cdot (c\rho)_x}$. These TPC refer to one of the defining parameters of materials in their manufacturing, storage, application and operation in various industries. Therefore, when calculating both natural thermal processes and processes of heat treatment of materials [1, 2], it is necessary to take into account the TPC of those materials, using their known values given in reference literature, for example [3–6].

The accuracy of determination of TPC of materials depends on many factors, ranging from the perfection of methods and devices for determining TPC, primary and secondary means of measurement, and ending with equipment that provides the creation and maintenance of the desired thermal mode in the sample of the research material [7]. In many cases, special methods and devices [7–16] are used to determine the TPC of materials, which can be divided into three groups: determination of thermal conductivity; mass or volumetric heat capacity; and complex determination of TPC of materials – this is when λ_x, c_x or $(c\rho)_x$, as well as a_x and β_x of the material sample, and if necessary, the dependence of these characteristics on temperature are determined simultaneously during one experiment.

We should note that regardless of which of the selected groups includes the selected method and device for measuring the TPC of material, in the process of research it is necessary to take measures to determine the metrological characteristics (hereinafter-MC) of the device, in particular the sensitivity of the primary converters, the resistances of the heat transfer device to the sample of the research material, as well as the amount of heat required to change the temperature of the device, and others.

At the same time, one of the main MC of the device that determines the accuracy of the experimental determination of TPC of materials is ballast thermal R_b, and capacitive P_b resistances. Their value depends on the corresponding resistances of the electrical insulation of the temperature converters (hereinafter TC) and the heat flow converters (hereinafter HFC) of the TPC of device, the resistance of the contact of the sample with the elements of the device, the degree of uniformity of the temperature distribution and the heat flow density on the working surfaces of the device and the sample of the research material, etc. In the general case, to determine these ballast resistances, a series of special experiments with calibration of the device is carried out.

To determine the thermolabile materials TPC, in particular food products, in the temperature range, where phase transformations occur in the sample of the research material, it is advisable to use devices for complex determination of TFC of materials, which allow implementing a combination of stationary and transient modes. In the temperature range at which phase transformations do not occur in the sample of the test product, it is convenient to carry out the determination of TPC of the material in the regular mode of the second kind (quasi-stationary thermal mode) [7].

Given that the errors in determining MC of device to a large extent generally determine the reliability of the measurement results of the TPC of material, the aim of the study is to develop a method for the integrated determination of the MC of device and TPC of thermolabile materials – raw materials, intermediate and finished industrial products, in particular, in the processing industry.

Materials and methods

One of the most common devices for determining the thermal conductivity of flat samples of materials in Ukraine [11, 13] consists (Figure 1) from a down-flow condenser *1* and an electric heater *2* on the working surfaces of which flat primary heat flow converters *3* and temperature converters *4* are located and between which a sample of the research material *5* is placed.

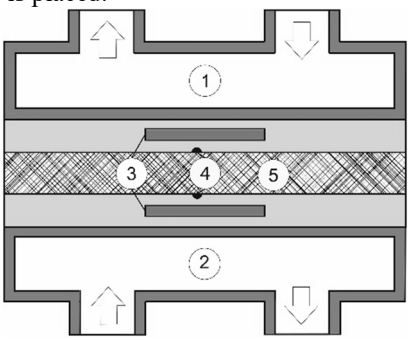


Figure 1. Device for determining the thermal conductivity of materials

The thermal conductivity of the sample of the research material is calculated from the measurement results in the stationary thermal mode of the temperature difference Δt on the surfaces of a flat sample with thickness h and the heat flow density q, which passes through the sample, by the formula:

$$\lambda_x = \frac{h}{\Delta t / q - R_b}, \quad (1)$$

where R_b is an additional (ballast) thermal resistance, the value of which is determined during the calibration of the device.

Similar in structure devices for determining the thermal conductivity of materials are widely used in Germany [14], the United States [15] and other countries.

When determining the heat capacity of materials, so-called conductive microcalorimeters are often used [16]. In these devices, to measure the amount of heat Q_m, supplied or withdrawn from the device and the sample of the research material with mass m_x (volume V_x), which causes a change in the average temperature of the sample by the value $\delta\bar{t}$, the batteries of their thermocouple elements are used. The measuring cell of such devices, where the sample of the research material is located, have the form of a cylindrical thermometric shell or a flat thermoelectric HFC. According to the measurement results, the mass c_x and volumetric $(c\rho)_x$ heat capacity of the material are calculated according to the formulas:

$$c_x = \frac{Q_m - Q_b}{m_x \cdot \delta\bar{t}}, \quad (2)$$

$$(c\rho)_x = \frac{Q_m - Q_b}{V_x \cdot \delta\bar{t}}. \quad (3)$$

where Q_b - the amount of heat consumed to change the temperature of the device by δt. Its value is determined by the calibration of the TPC device.

To date, to determine the heat capacity the methods of heat analysis during linear and step-by-step scanning of the heat capacity by the temperature of the material sample have become widespread [17–21].

Methods for determining the TPC of materials are reflected in the standards of Ukraine, that are fully consistent with the standards of Germany, USA and Japan [22-25].

The devices for complex determination of TPC by the basic structure, in fact, do not differ from the device shown in Figure 1. At the same time, it should be noted that the heat block of these devices can form various heat exchangers [7, 12]. Except for the electric heater and the flow heat exchanger shown in Figure 1, the heat block can be formed from two liquid

flow heat exchangers; a combination of flow heat exchanger and electric heater; a flow heat exchanger or an electric heater and a device that has a "developed" surface for convective heat withdrawal from the sample of the research material, etc. The application of one or another pair of heat exchangers in a TPC device for complex determination of TPC is mainly determined by the type of thermal modes and the temperature range of the study of TPC of materials [7].

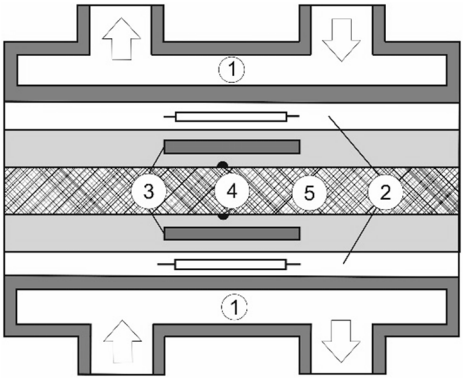


Figure 2. Device for determination of TPC of materials with combined heat block

Under such conditions, for the complex determination of TPC, in particular food products, it is advisable to use a device with a combined heat block (Figure 2). Such a unit consists of two flow heat exchangers *1* and two thin, electric heaters *2* with evenly distributed density of the heat release over the surface, which are located between the heat exchangers and the plate of the measuring cell of the device with the HFC *3* and TC *4* mounted therein. Both flow heat exchangers are supplied with a heat-transfer material of the same temperature that varies linearly over time. In this case, electric heaters are used to create a time-stable difference in the density of the heat flow on the surfaces of the test product *5*.

The thermal conductivity λ_x and the volumetric heat capacity $(c\rho)_x$ of the test sample of the product are calculated from the equations obtained as a result of solving the task of unsteady thermal conductivity with bilateral heating of the plate in the regular mode of the second kind [7]. Here, in contrast to the stationary thermal mode, where the temperature of the sample of the research material does not change, when it is heated (cooled) in the quasi–stationary thermal mode of the flat sample of the research material, the heat flow density on the "working" surfaces of the sample although does not change in time ($q_1(\tau)=const$, $q_2(\tau)=const$, $q_1(\tau)–q_2(\tau)=\Delta q=const$), but differ in value, that is $q_1 \neq q_2$. At that the temperature on the "working" surfaces of the sample changes linearly: $t_1(\tau)–t_2(\tau)=\Delta t=const \neq 0$.

Taking into account the ballast thermal R_b and capacitive P_b resistances of TPC device, the TPC of research material is calculated by the formulas:

$$\lambda_x = \frac{h}{2\cdot(t_1-t_2)/(q_1+q_2)-R_b} = \frac{h}{(\Delta t/\overline{q})-R_b}, \tag{4}$$

$$(c\rho)_x = \frac{(q_1-q_2)/(\delta\overline{t}/\Delta\tau)-P_b}{h} = \frac{(\Delta q/u_t)-P_b}{h}, \tag{5}$$

$$a_x = \frac{\lambda_x}{(c\rho)_x} = h\cdot\left(\frac{\Delta t}{\overline{q}}-R_b\right)^{-1}\cdot\left(\frac{\Delta q}{u_t}--P_b\right)^{-1}, \tag{6}$$

$$\beta_x = \sqrt{\lambda_x\cdot(c\rho)_x} = \sqrt{\frac{(\Delta q/u_t)-P_b}{(\Delta t/\overline{q})-R_b}} \tag{7}$$

where $u_t = \delta\bar{t} / \Delta\tau = 0,5 \cdot [(t_1 + t_2)_{\tau i} - (t_1 + t_2)_{\tau 0}] / (\tau_i - \tau_0)$ – the rate of change of the average temperature $\delta\bar{t}$ of the sample of research material in the time interval $\Delta\tau=\tau_i-\tau_0$ when heating (cooling) of the sample in the quasi-static thermal mode from the average temperature $\bar{t}_{\tau_0} = (t_1 + t_2)_{\tau_0} / 2$, at the moment of time τ_0, to the average temperature $\bar{t}_{\tau_i} = (t_1 + t_2)_{\tau_i} / 2$.

Results and discussion

From the results of the analysis of formulas (1)–(7) it follows that each of the considered methods of experimental determination of thermal conductivity, volumetric or mass heat capacity, as well as the complex determination of TPC of materials, in fact, is reduced to obtaining an equation or a system of equations of the following form:

$$R_m = R_x + R_b, \quad (8)$$

$$P_m = P_x + P_b \ , \quad (9)$$

where $R_x=h/\lambda_x$ and $P=h\cdot(c\rho)_x$ – respectively, the thermal and volumetric resistances of the sample of the research material; R_b and P_b – respectively, the ballast thermal and ballast capacitive resistances of the device R_m and P_m – respectively, the thermal and volumetric resistances of the "device-sample" system, measured during the experiment on the study of the TPC of the material.

The value of thermal and volumetric resistances of the system "device-sample" (R_m, P_m) is calculated by measuring the values obtained in the course of the experiment on the study of the TPC of the material.

The accuracy of the calculation results of R_m and P_m depends on the perfection of the system of maintaining the desired thermal mode in the device (material sample), the device itself, the primary and secondary measuring equipment, as well as the accuracy of determining the operating coefficients K_q and K_t (inverse sensitivity) of the primary TC and HFC of the device. Therefore, it is natural that the accuracy of the results of the calculations of the value of these resistances directly determines the reliability of the results of determining the TFC of the material.

As it follows from the results of the analysis of the structure of devices, as well as equations (1)–(7) for calculation of TPC of materials, thermal and volumetric resistances of the "device-sample" system are calculated based on the measurement of temperature (t_1, t_2) and heat flow density (q_1, q_2) on the "working" surfaces of a flat sample of the research material. But in practice, this is somewhat different, since the temperature and heat flow density on the surfaces of the material sample also have to be calculated. Thus, when thermoelectric TC and HFC are used to determine the temperature and density of the heat flow in the TPC device, which generate EMF, respectively, e_{t1}, e_{t2} and e_{q1}, e_{q1}, the calculation equation R_m and R_m when heating (cooling) the sample in the quasi-stationary thermal mode have the following form:

$$R_m = \frac{\Delta t}{\Sigma q / 2} = \frac{t_1 - t_2}{(q_1 + q_2) / 2} = \frac{K_t \cdot (e_{t1} - e_{e2})}{K_q \cdot (e_{q1} + e_{q2}) / 2} \ , \quad (10)$$

$$P_m = \frac{\Delta q}{u_t} = \frac{q_1 - q_2}{[(t_1 + t_2)|_{\tau_i} - (t_1 + t_2)|_{\tau_0}] / [2 \cdot (\tau_i - \tau_0)]} = \frac{K_q \cdot (e_{q1} - e_{q2})}{K_t [(e_{t1} + e_{t2})|_{\tau_i} - (e_{t1} + e_{t2})|_{\tau_0}] / [2 \cdot (\tau_i - \tau_0)]} \quad (11)$$

The operating coefficients K_t and K_q, respectively, TC and TPC or their inverse values – sensitivity, which are used to calculate the temperature t and the heat flow density q on the surfaces of the sample of the research material (differences $\Delta t=t_1-t_2$, $\Delta q=q_1-q_2$, and the average of the values $\bar{t}=(t_1+t_1)/2=\Sigma t/2$, $\bar{q}=(q_1+q_1)/2=\Sigma q/2$) also refer to the MC of the device.

The accuracy of their determination in a series of special experiments with calibration of the device, as well as ballast resistances, significantly affects the reliability of the results of the experimental determination of TPC of materials.

Currently, among the existing methods for determining the MC of TPC devices one of the most common techniques of which is associated with the use of working calibration standards [7-12].

In our opinion, this is due to the fact that the method of determining the MC of device using working calibration standards does not differ from the method of determining the TPC of materials. For example, according to the results of two experiments (further indicated as, respectively, 1 and 2) with samples of standard (s) material, which has thermal conductivity λ_s and volumetric heat capacity $(c\rho)_s$, at a thickness of samples $h_1\neq h_2$, that is, with samples that differ in thermal $R_{s1}\neq R_{s2}$ and volumetric $P_{s1}\neq P_{s2}$ resistances, we obtain systems composed of equations of the form (7) and (8), respectively :

$$\begin{cases}\left(\dfrac{\Delta t}{\bar{e}_q}\right)_1=\dfrac{K_q}{\lambda_s}\cdot h_1+K_q\cdot R_b=K_q\cdot R_{s1}+K_q\cdot R_b\\ \left(\dfrac{\Delta t}{\bar{e}_q}\right)_2=\dfrac{K_q}{\lambda_s}\cdot h_2+K_q\cdot R_b=K_q\cdot R_{s2}+K_q\cdot R_b\end{cases}. \tag{12}$$

$$\begin{cases}\left(\dfrac{\Delta e_q}{u_t}\right)_1=\dfrac{(c\rho)_s}{K_q}\cdot h_1+\dfrac{P_b}{K_q}=\dfrac{P_{s1}}{K_q}+\dfrac{P_b}{K_q}\\ \left(\dfrac{\Delta e_q}{u_t}\right)_2=\dfrac{(c\rho)_s}{K_q}\cdot h_2+\dfrac{P_b}{K_q}=\dfrac{P_{s2}}{K_q}+\dfrac{P_b}{K_q}\end{cases}, \tag{13}$$

As a result of solution of each of these systems of equations with respect to K_q we have:

$$K_q=\lambda_s\cdot\frac{\left(\dfrac{\Delta t}{\bar{e}_q}\right)_1-\left(\dfrac{\Delta t}{\bar{e}_q}\right)_2}{h_1-h_2}=\frac{\left(\dfrac{\Delta t}{\bar{e}_q}\right)_1-\left(\dfrac{\Delta t}{\bar{e}_q}\right)_2}{R_{s1}-R_{s2}}, \tag{14}$$

$$K_q=(c\rho)_s\cdot\frac{h_1-h_2}{\left(\dfrac{\Delta e_q}{u_t}\right)_1-\left(\dfrac{\Delta e_q}{u_t}\right)_2}=\frac{P_{s1}-P_{s2}}{\left(\dfrac{\Delta e_q}{u_t}\right)_1-\left(\dfrac{\Delta e_q}{u_t}\right)_2}. \tag{15}$$

In this case, the formulas for calculating of ballast thermal and volumetric resistances of the TPC device have the following form:

$$R_b = \left(\frac{\Delta t}{e_q}\right)_1 \cdot K_q^{-1} - R_{s1} = \left(\frac{\Delta t}{e_q}\right)_2 \cdot K_q^{-1} - R_{s2}, \quad (16)$$

$$P_b = K_q \cdot \left(\frac{\Delta e_q}{u_t}\right)_1 - P_{s1} = K_q \cdot \left(\frac{\Delta e_q}{u_t}\right)_2 - P_{s2} \ . \quad (17)$$

From the results of the analysis of the systems of equations (12) and (13) it follows that in the case of experiments with samples of the research material, which differ in thickness $h_1 \neq h_2$, that is, the thermal $R_{x1} \neq R_{x2}$ and volumetric $P_{x1} \neq P_{x2}$ resistances, there is no need to determine the ballast resistances of the device. A similar effect will also appear in the case of a two-cell TPC device (Figure 3), in which two pairs of HFC *3* and two pairs of TC *4* are mounted, respectively, to measure the temperature and the heat flow density on the surfaces of samples *5, 6* of the research material with a thickness of $h_1 \neq h_2$.

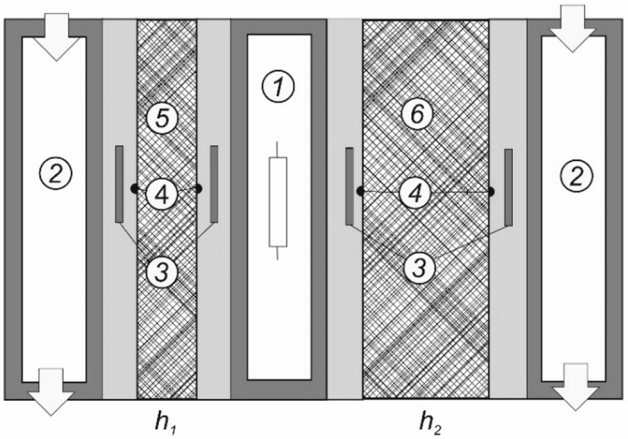


Figure 3. Two-cell device for complex determination of TPC of materials

Since the flow heat exchangers *2* are supplied with heat-transfer material with the same temperature t_2, which is lower than the temperature t_1 of the electric heater *1*, and then in the stationary thermal mode, both samples have the same average temperature $\bar{t} = (t_1 + t_2)/2$, which includes the results of the calculation of the thermal conductivity of the material according to the formula:

$$\lambda_x = \frac{\bar{q}_1 \cdot \bar{q}_2 \cdot (h_1 - h_2)}{(t_1 - t_2) \cdot (\bar{q}_1 - \bar{q}_2)} \ , \quad (18)$$

where $\bar{q}_1$ and $\bar{q}_2$ - respectively, are the density of the heat flow that passes through the sample with thickness h_1 and h_2.

To determine the volumetric heat capacity of the material, the quantities of heat Q_1 and Q_2 are measured, which are accumulated (given) by the samples in the transition mode from one stationary mode to another due to an increase (decrease) in their average temperature by the value $\delta\bar{t}$ and are calculated by the formula:

$$(c\rho)_x = \frac{Q_1 - Q_2}{(h_{x1} - h_{x2}) \cdot \delta\overline{t}} \ . \quad (19)$$

In this case, the operating coefficients of primary HFC and TC, K_q and K_t of two-cells device, as well as TPC devices of all other modifications , can be determined using special calibration stands of these converters [7–12]. We should note that in the case of the use of special calibration stands, the determination of the operating coefficients of the primary converters of the TPC device occur in conditions that are significantly different from those in which these converters operate during determination of the TPC of materials. Therefore, in some cases, the use of working calibration standards for this purpose may be more appropriate than the use of calibration stands.

At the same time, we note that the existing methods for determining the MC of TPC of device, in particular, using working calibration standards, have certain drawbacks, which include the following:

1. First and foremost it is necessary to take into account that the TPC of any materials, including those that are used as working calibration standards, are determined with certain errors that affect the accuracy of the determination of the TPC of new materials.
2. Contact thermal and volumetric resistances of samples of working calibration standards and prototypes of materials with the working surfaces of the TPC device may differ in value. This is especially true, on the one hand, for the contact resistances of such working calibration standards as, for example, glycerin or quartz glass, and on the other hand - prototypes of bulk materials. Since these contact resistances are a component of the ballast resistances of the device, depending on the type of standard material and the research material, it is likely to obtain different values of the ballast thermal and ballast volumetric resistances of the device, and, ultimately, false data on the TPC of the research material.
3. From the results of the analysis of known methods for determining the MC of devices and, in particular, devices for complex determination of TPC of materials, as well as the results of their implementation in practice, it follows that the values of the MC of device are related to each other and in the case of determining one of them with a certain error, the accuracy of determining of other characteristics can be extremely unsatisfactory. To this we add that the change in the value of one of the characteristics of the device in the process of studying the TPC of material can cause a change in the values of its other MC of device, which ultimately leads to the receipt of inaccurate information in relation to the TPC of research material. At the same time, despite the existing shortcomings, the method of determining the MC of devices for complex determination of TPC of materials with the use of working calibration standards in practice is quite common.

On the basis of the above it follows that in order to improve the accuracy of the complex determination of TPC of materials, the MC of the device should be determined by the results of experiments with the research material, and not with the standard material. At the same time, we note that in the case of using the research material as a working calibration standard, the data on the values of the MC of TPC device may in fact become unnecessary.

Graphically, the results of the determination of MC of the device for complex determination of TPC of materials for tests in two samples of standard material of known thermal conductivity λ_s and the volumetric heat capacity $(c\rho)_s$, which differ in the thickness $h_1 \neq h_2$, therefore, thermal $R_{s1} \neq R_{s2}$ and volumetric $P_{s1} \neq P_{s2}$ resistances shown in Figure 4 *a-f*.

Straight lines $(\Delta t / \bar{e}_q)$ from R_s and $(\Delta t / \bar{e}_q)$ from the thickness of the sample of the working calibration standard h_s cross the ordinate axis of their graphs (Figure 4 *a* and *b*) at point A at and have angles of inclination to the abscissa axis, respectively, $\varphi = arctq(K_q)$ and $\psi = arctq(K_q / \lambda_s)$. These lines cross the abscissa axis at $|-R_x| = R_b$, that is, at the point where the thermal resistance of the sample of the working calibration standard is equal to the ballast thermal resistance of the TPC device and at $|-h_\lambda| = \lambda_s \cdot R_b$, where $|-h_\lambda|$ is the thickness of the sample of the working calibration standard at which the thermal resistance of the sample is equal to the ballast thermal resistance of the device $|-h_\lambda| / \lambda_s = |-R_s| = R_b$.

The lines $(\Delta e_q / u_t)$ from P_s and $(\Delta e_q / u_t)$ from h_s cross the ordinate axis of their graphs (Figure 4 *d* and *e*) at point B at $(\Delta e_q / u_t) = P_b / K_q$ and have an angle of inclination to the abscissa axis, respectively, $\gamma = arctq(K_q)$ and $\theta = arctq(1 / K_q)$. These lines cross the axis of abscissa at $|-P_x| = P_b$ respectively, that is, at the point where the capacitive resistance of the sample working calibration standard is equal to the ballast capacitive resistance of the TPC device and at $|-h_{(c\rho)}| = P_b / (c\rho)_s$, where $|-h_{(c\rho)}|$ is the thickness of the sample working calibration standard at which the capacitive resistance of the sample is equal to the ballast capacitive resistance of the device $|-h_{(c\rho)}| \cdot (c\rho)_s = |-P_x| = P_b$.

Such graphs can be built on the results of experiments with any material. At the same time, since the value of the operating coefficient of the HFC of the device does not depend on the TPC of the research material, the ordinates of crossing of the lines $(\Delta t / \bar{e}_q) = f(R_x)$ and $(\Delta e_q / u_t) = f(P_x)$ with the axes of the corresponding graphs remain unchanged. The coordinates of the intersection of the lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ with the axis of the ordinates of the graphs also remain unchanged when increasing or decreasing the values λ_x and $(c\rho)_x$ of the material. At the same time, when the TPC of the material changes, due to the change in the coordinates of the concurrence of these lines with the abscissa axis, the angles of the lines to the abscissa axis of the graphs change. Therefore, for this kind of dependency $\varphi = f(\lambda_x)$, $\gamma = f(c\rho)_x$.

We should note that since the ballast resistances of the device are not a function of the TPC of the material, $R_b \neq f(\lambda)_x$ and $P_b \neq f(c\rho)_x$, then with the change of the TPC of the abscissa material $|-h_\lambda|$ and $|-h_{(c\rho)}|$, they change so that the numerical values of the ratio $|-h_\lambda| / \lambda_s = R_b$ and the product $|-h_{(c\rho)}| \cdot (c\rho)_s = P_b$ remain unchanged.

It follows from the above that for λ_x=1, the angle ψ of the slope of the straight line $(\Delta t / \bar{e}_q) = f(h)$ to the axis of the abscissa (Figure 4*b*) is equal to the angle φ of the slope of the straight line $(\Delta t / \bar{e}_q) = f(R_x)$ to R_x axis (Figure 4*a).* In this case, the lines $(\Delta t / \bar{e}_q)$ from *h* and $(\Delta t / \bar{e}_q)$ from R_x cross the abscissa axis of their graphs at $|-h_\lambda| = \lambda_x \cdot R_b = 1 \cdot R_b = R_b$ and $|-h'_\lambda| / \lambda = |-h_\lambda| / (\lambda_x \cdot 1) = R_b$. Here $|-h'_\lambda|$ is the corrected thickness of the ballast layer of thermal resistance at the thermal conductivity of the layer λ_b=1.

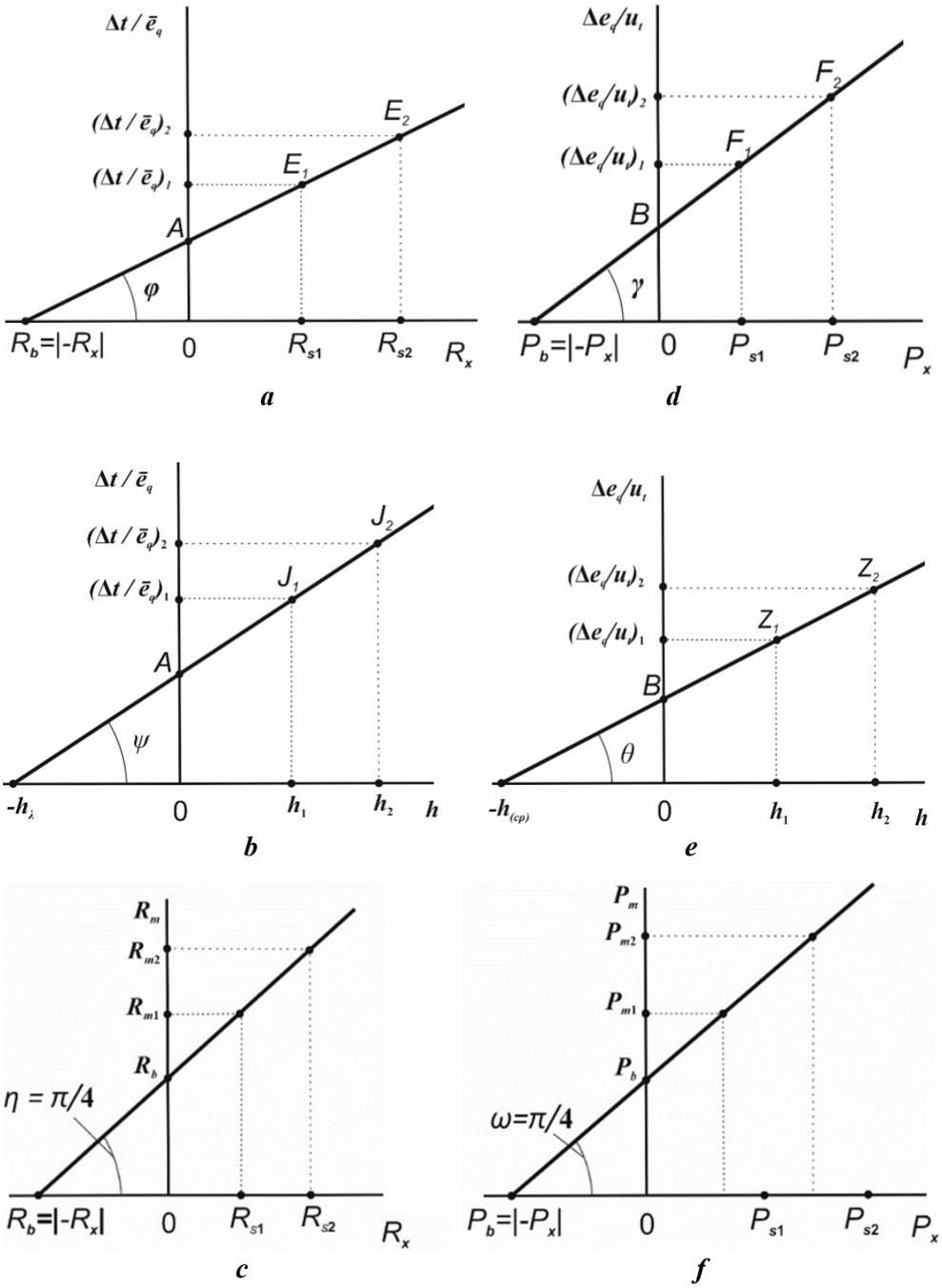


Figure 4. Results of determination of MC of the device of complex determination of TPC of materials during experiments with two samples of standard material

Similar, at $(c\rho)_x$=1, takes place with dependencies $(\Delta e_q / u_t) = f(h)$ and $(\Delta e_q / u_t) = f(P_x)$ which are shown in Figure 4 *e* and *d*. The angle θ of inclination of the line $(\Delta e_q / u_t) = f(h)$ to the axis h will be equal to the angle γ of inclination of the line $(\Delta e_q / u_t) = f(P_x)$ to the axis P_x, while the lines cross the abscissa axis of their graphs at $\left|-h_{(c\rho)}\right| = P_b / (c\rho)_x = P_b / 1 = P_b$ and $|-h'_{(c\rho)}| \cdot (c\rho) = [|-h_{(c\rho)}| \cdot (c\rho)_x] / 1 = P_b$ where $|-h'_{(c\rho)}|$ is the corrected thickness of the capacitive ballast resistance layer at the volumetric heat capacity of the layer $(c\rho)_b$=1.

Thus, at $\lambda_x=(c\rho)_x$=1 the graph $(\Delta t / \bar{e}_q)$ of h is transformered to the view $(\Delta t / \bar{e}_q)$ from R_x, and $(\Delta e_q / u_t)$ of h, respectively, to a graph $(\Delta e_q / u_t)$ of P_x. At that the sum of the angles of inclination of the lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ to the abscissa axis of the corresponding graphs is π/2, that is, for both lines $(\Delta t / \bar{e}_q) = f(R_x)$ and $(\Delta e_q / u_t) = f(P_x)$.

Subsequent to the results of determining the MC of device the dependencies R_m of R_x and P_m of P_x can be built (Figure 4 *c* and *f*). Based on the analysis of equations (8) and (9) it follows that at $\lambda = (c\rho) = 1$ these lines intersect the ordinate axis (R_x=0) and the abscissa axis (R_m=0) of their graphs at points with coordinates, respectively:

$$R_m = \Delta t / (\bar{e}_q \cdot K_q) = (K_q \cdot R_b) / K_q = R_b \text{ and } P_m = (\Delta e_q \cdot K_q) / u_t = (P_b / K_q) \cdot K_q = P_b,$$

$$|-R_m| = |-h'_\lambda| / \lambda = |-h_\lambda| / (\lambda_x \cdot 1) = R_b \text{ and}$$
$$|-P_x| = |-h'_{(c\rho)}| \cdot (c\rho) = [|-h_{(c\rho)}| \cdot (c\rho)_x] / 1 = P_b.$$

At that the angle of inclination of the lines $R_m = f(R_x)$ and $P_m = f(P_x)$ to the abscissa axis of the corresponding graph is π/4.

It follows from the above that the data on thermal conductivity λ_s and the volumetric heat capacity $(c\rho)_s$ of the working calibration standards are used to determine the MC of the TPC device. Thus, according to equations (12)–(17) to determine R_b of the device λ_s is used, to determine P_b – $(c\rho)_s$, and to determine the operating coefficient of HFC of device λ_s or $(c\rho)_s$ of working calibration standard is used.

At that the results of the analysis of expressions (12)-(17) show that:

- the system of equations (12) and equations (14) and (16) correspond to the device for determining thermal conductivity of materials;
- the system of equations (13) and equations (15) and (17) correspond to the device for determining the volumetric heat capacity of materials;
- systems of equations (12), (13) and equations (14)–(17) correspond to the device for complex determination of TPC of materials.

At the same time, we note that the method of determining the ballast resistances of the device for complex determination of TPC of materials is a combination of unrelated methods for determining the ballast resistance of the device for determining the thermal conductivity and the device for determining the volumetric heat capacity of materials.

We consider that the method of determination of MC of the device for complex determination of TPC of materials except λ_s and $(c\rho)_s$ first of all shall provide the usage of

thermal conductivity $a_s = \lambda_s / (c\rho)_s$ and thermal activity $\beta_s = \sqrt{\lambda_s \cdot (c\rho)_s}$ of working calibration standards.

Based on the above, for a combination of the six dependencies shown in Figure 4 *a-f*, it is necessary to build their combined graphs.

To build a combined graph of dependencies and $(\Delta t / \bar{e}_q)$ and $(\Delta e_q / u_t)$ on h (Figure 5), obtained from two experiments with samples of material with thickness $h_1 \neq h_2$, from systems of equations similar to (12), (13) we obtain the coordinates of the intersection of lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ with the axes of abscissa and ordinates, as well as the angles of inclination of these lines to the axis of abscissa of their graphs, respectively:

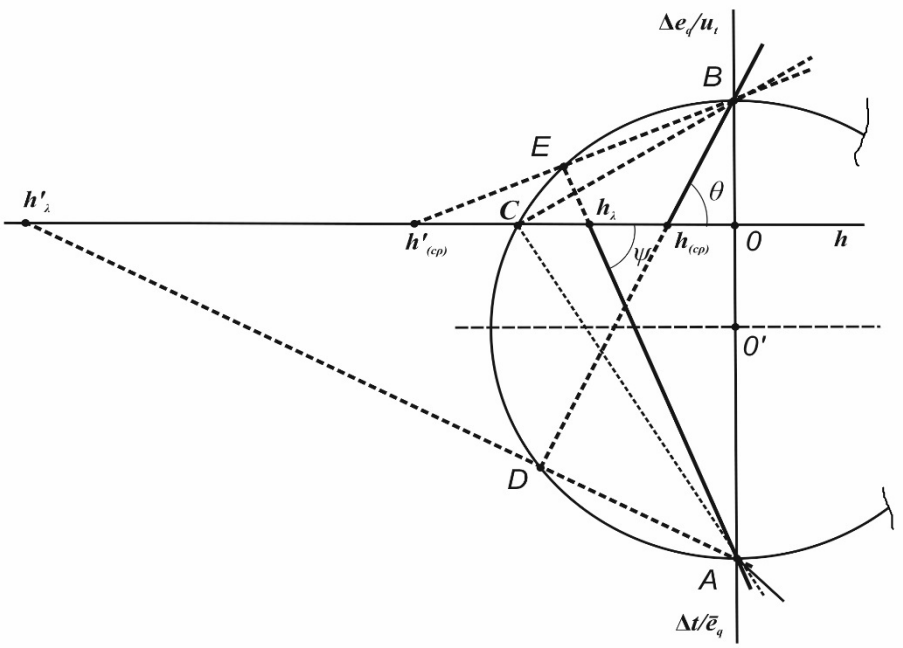


Figure 5. To the construction of a combined dependencies graph

$(\Delta t / \bar{e}_q) = f(h)$ **and** $(\Delta e_q / u_t) = f(h)$

$|-h_\lambda| = \lambda_x \cdot R_b$, $(\Delta t / \bar{e}_q) = K_q \cdot R_b = \text{A}$, $\psi = \text{arctg}(K_q / \lambda_x)$

and

$|-h_{(c\rho)}| = P_x / (c\rho)_x$, $(\Delta e_q / u_t) = P_b / K_q = \text{B}$, $\theta = arctg[(c\rho)_x / K_q]$.

On the abscissa axis in the selected scale M_h, we apply a linear scale h. To match the scales and values on the abscissa axis M_h and the axes of the ordinates $(\Delta t / \bar{e}_q) - M_{Rm}$ and $(\Delta e_q / \Delta u_t) - M_{Pm}$ from the point $-h_\lambda$ of the intersection of the line $(\Delta t / \bar{e}_q) = f(h)$ with the abscissa axis, through the third quadrant of the graph, at an angle $\psi = arctg(K_q / \lambda_x)$ and from the point $-h_{(c\rho)}$, the intersection of the line $(\Delta e_q / u_t) = f(h)$ with the abscissa axis through the second quadrant of the graph at an angle $\theta = arctg[(c\rho)_x / K_q]$ to the abscissa axis, and draw

the corresponding lines. In this case, the straight line $(\Delta t / \bar{e}_q) = f(h)$ will cross the ordinate axis at point A, which is located on the "conditionally" negative part of the ordinate axis at a distance of L_{0A} from the origin of coordinates at $(\Delta t / \bar{e}_q) = K_q \cdot R_b$, and the straight line $(\Delta e_q / u_t) = f(h)$ – at point B, which is in the positive part of the ordinate axis at a distance of L_{0B} from the origin of the combined chart at $(\Delta e_q / u_t) = P_b / K_q$.

By the values of h, R_x, P_x, the abscissa axis of the graph should be common for two pairs of lines – a pair of lines $(\Delta t / \bar{e}_q)$ from h and $(\Delta e_q / u_t)$ from h and a pair of lines $(\Delta t / \bar{e}_q)$ from R_x and $(\Delta e_q / u_t)$ from P_x. The lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ have an angle of inclination to the abscissa axis of the combined graph ψ and θ and cross this axis at points, respectively, $|-h_\lambda| = \lambda_x \cdot R_b$ and $|-h_{(c\rho)}| = P_x/(c\rho)_x$.

In contrast to the previous pair, the lines $(\Delta t / \bar{e}_q) = f(R_x)$ and $(\Delta e_q / u_t) = f(P_x)$ must have an angle of inclination to the abscissa axis of the combined graph $\varphi = arctq(K_q)$ and $\gamma = arctq(1/K_q)$ and cross the abscissa axis at points which coordinates are numerically equal:

$$\frac{|-h'_\lambda|}{\lambda_b} = \frac{|-h_\lambda| / \lambda_x}{1} = R_b \ ,$$

$$|-h'_{(c\rho)}| \cdot (c\rho)_b = [|-h_{(c\rho)}| \cdot (c\rho)_x] \cdot 1 = P_b \ ,$$

where $|-h'_\lambda|$ – effective thickness of ballast layer, which at thermal conductivity of material of this layer λ_b=1 is numerically equal to ballast thermal resistance of TFH device; $|-h'_{(c\rho)}|$ – effective thickness of the ballast layer, which, when the volume heat of the material of this layer $(c\rho)_b$=1, is numerically equal to the ballast capacitive resistance of the TFH device.

Therefore, on the abscissa axis of the combined graph, the values are intercepted, which although have the dimensionality [m], [K·m^2/W], [J/m^2·K], but numerically are equal to the thickness of the material sample. Thus, when intercepted to the abscissa axis of the graph of a uniform scale h (also R_x, also P_x), at each point of the scale, the thermal and capacitive resistances of the sample of the research material should be numerically equal to each other, R_x=P_x, that is $h / \lambda_x = h \cdot (c\rho)_x$, where $\lambda_x \cdot (c\rho)_x = \beta^2 = 1$.

Under these circumstances, for λ_b=$(c\rho)_b$=1, the coordinate of point A, $(\Delta e_t / \bar{e}_q)_A = K_q \cdot R_e$, and the ordinate of point B $(\Delta\tau \cdot \Delta e_q / \Delta e_t)_B = P_b / K_e$, the combined graph (lengths of segments L_{0A} and L_{0B}) can be given as follows:

$$\left(\frac{\Delta e_t}{\bar{e}_q}\right)_A = K_e \cdot \frac{h_{R_b}}{\lambda_b} = K_e \cdot \frac{|-h_\lambda|}{\lambda_x} = K_e \cdot \frac{|-h_\lambda| / \lambda_x}{1} = K_e \cdot |-h'_\lambda| \ ,$$

$$\left(\frac{\Delta\tau \cdot \Delta e_q}{\Delta e_t}\right)_B = \frac{h_{P_b} \cdot (c\rho)_b}{K_e} = \frac{|-h_{(c\rho)}| \cdot (c\rho)_x}{K_e} = \frac{[|-h_{(c\rho)}| \cdot (c\rho)_x] \cdot 1}{K_e} = \frac{|-h'_{(c\rho)}|}{K_e} \ .$$

As it follows from the results of the dependencies analysis shown in Figure 4 *a–f,* at $\lambda_x=(c\rho)_x=1$ each of the two pairs of lines, $(\Delta t/\bar{e}_q)=f(h)$ and $(\Delta t/\bar{e}_q)=f(R_x)$, and also $(\Delta e_q/u_t)=f(h)$ and $(\Delta e_q/u_t)=f(P_x)$ in the combined graph should be represented by the corresponding line. In turn, these lines will intersect at an angle π/2. Thus, since the lines $(\Delta t/\bar{e}_q)=f(R_x)$ and $(\Delta e_q/u_t)=f(P_x)$, as well as $(\Delta t/\bar{e}_q)=f(h)$ and $(\Delta e_q/u_t)=f(h)$, passing through the points A and B at $\lambda_x=(c\rho)_x=1$, i.e. at $a_x=1$ and $\beta_x=1$, on the combined graph should intersect at an angle π/2, we draw a circle through the points A and B with a diameter:

$$D=L_{0A}+L_{0B}=(\Delta t/\bar{e}_q)_{0A}+(\Delta e_q/u_t)_{0B}, \qquad (20)$$

the center of O′ of which is on the ordinate axis and generally does not coincide with the origin of the graph.

This circle crosses the abscissa axis of the graph at point *C*, the abscissa of which is the length of the segment 0-*C*, can be calculated as geometric mean of lengths of the segments 0-*A* and 0-*B*, which form the diameter of the circle. However, given the consistency of the scale of values that are intercepted on the axes of the graph, the abscissa of point C can be calculated as follows:

$$h_C=\sqrt{L_{0A}\cdot L_{0B}}=\sqrt{(\Delta t/\bar{e}_q)_{0A}\cdot(\Delta e/u_t)_{0B}}=\sqrt{(K_q\cdot R_b)\cdot(P_b/K_q)}=\sqrt{R_b\cdot P_b}. \qquad (21)$$

For the convenience of presentation of the material here and further the index "C" will highlight the values that refer to the point C on the combined graph.

At Figure 5 the lines $(\Delta t/\bar{e}_q)=f(h)$ and $(\Delta e_q/u_t)=f(h)$ intersect the circle in points, respectively, E and D. With the increase of λ_x, the point $|-h_\lambda|=\lambda_x\cdot R_b$ of intersection of a line $(\Delta t/\bar{e}_q)=f(h)$ with the abscissa axis of the graph moves toward point C and further in the negative section of the scale. At that the point E moves in a circle against the clock.

With decreasing of $(c\rho)_x$ the point $|-h_{(c\rho)}|=P_x/(c\rho)_x$ of intersection of the line $(\Delta e_q/u_t)=f(h)$ with the abscissa axis of the graph moves towards the point C and further into the negative section of the scale, and the point D in a circle moves clockwise, that is, to meet the point E.

The position of the points E and D on the circle coincide with the point C, when

$$|-h_C|=|-h_{C\lambda}|=|-h_{C(c\rho)}|.$$

At that the sum of the angles $\psi_C+\theta_C$ of the inclination of lines $(\Delta t/\bar{e}_q)=f(h)$ and $(\Delta e_q/u_t)=f(h)$ to the abscissa axis of the graph is equal to π/2 and hence, when $\lambda_{Cx}=(c\rho)_{Cx}$, $a_{Cx}=1$, and –

$$\frac{h_C}{\sqrt{a_{Cx}}}=\frac{h_C}{1}=\frac{h_b}{\sqrt{\lambda_b/(c\rho)_b}}=\frac{h_b}{\sqrt{a_b}}, \qquad (22)$$

where h_{Rb}, h_{Pb} and $h_b=\sqrt{h_{Rb}\cdot h_{P_b}}$ – respectively, the thickness of the layer, which causes the ballast thermal and capacitive resistances of the device and the corrected thickness of the

ballast layer; λ_b, $(c\rho)_b$ and a_b – respectively, thermal conductivity, volumetric heat capacity and temperature diffusivity of the ballast layer.

In the case where the lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ intersect each other in the area 0-C, the abscissa axis of the combined graph (Figure 6), for example, at the point F_1, where $h_1=|-h_{\lambda 1}|=|-h_{(c\rho)1}|= R_{x1}= P_{x1}$ or at the point F_2, де $h_2=|-h_{\lambda 2}|=|-h_{(c\rho)2}| = R_{x2}= P_{x2}$, in the the result of the conversion of equations $R_{x1}=\lambda_{1x}\cdot R_b=P_{x1}=P_b/(c\rho)_{1x}$ and $R_{x2}=\lambda_{2x}\cdot R_b=P_{x2}=P_b/(c\rho)_{2x}$ we get:

$$\beta_{1x} = \beta_{2x} = \sqrt{P_b / R_b} = 1 . \qquad (23)$$

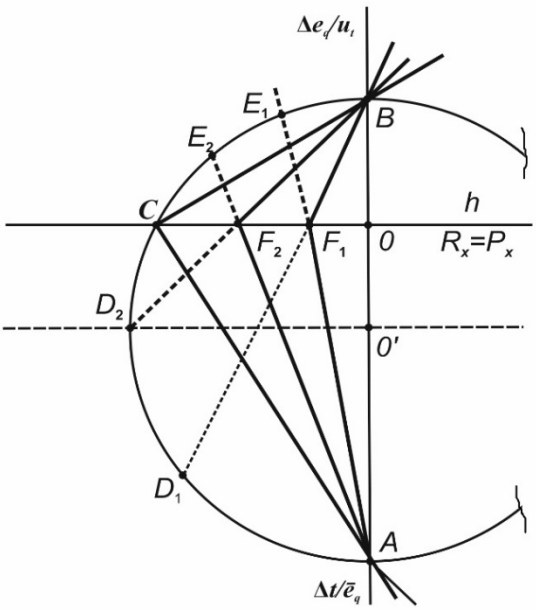


Figure 6. On the analysis of the combined dependencies graph $(\Delta t / \bar{e}_q) = f(h)$ **and** $(\Delta e_q / u_t) = f(h)$

Moving of the point F of the mutual intersection of the lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ along the h axis of the graph towards the point C (Figure 6), is accompanied by an increase in λ_x and a decrease in $(c\rho)_x$, hence an increase in the a_x of the research material. The value of the thermal activity of the material does not change, $\beta_x = 1 = const$. As in the previous case (Figure 5), the change in thermal conductivity, volumetric heat capacity and temperature diffusivity of the research material is accompanied by the movement in a circle of points E and D in the direction of the abscissa axis of the graph, that is, the point C.

The nature of the dependence of the coordinates of the cross-section of lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ with the abscissa axis of the combined graph on the thermal conductivity and volumetric heat capacity of the research material is shown in Figure 7.

Unlike intersections of lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$ on the abscissa axis at points F_1 and F_2, where $\beta_{1x} = \beta_{2x} = \sqrt{P_b / R_b} = 1$, the sum of the angles of inclination of lines to the abscissa axis is not equal to π/2, $a_{1x} \neq a_{2x} \neq 1$, $\lambda_{1x} \neq (c\rho)_{1x} \neq 1$, $\lambda_{2x} \neq (c\rho)_{2x} \neq 1$, in the case of intersection of lines at point C, the sum of angles of inclination of lines to the abscissa axis is π/2 and $\lambda_{Cx} = (c\rho)_{Cx} = a_{Cx} = \beta_{Cx} = \sqrt{P_b / R_b} = 1$ and $R_b = P_b = \sqrt{R_b \cdot P_b}$.

The procedure of plotting the combined dependencies graph of $(\Delta t / \bar{e}_q) = f(h)$, $(\Delta t / \bar{e}_q) = f(R_x)$, $(\Delta t / \bar{q}) = f(R_x)$, $R_m = R_x + R_b$, $(\Delta e_q / u_t) = f(h)$, $(\Delta e_q / u_t) = f(P_x)$, $(\Delta q / u_t) = f(P_x)$ and $P_m = P_x + P_b$, based on the results of two experiments with samples of material with thickness $h_1 \neq h_2$ (Figure 8) will be shown on the following example.

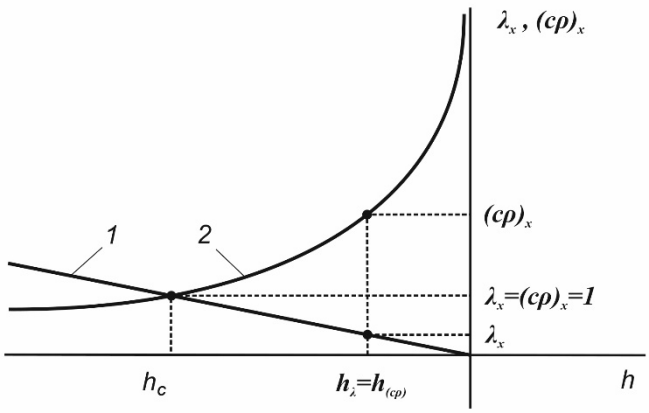


Figure 7. Dependence of thermal conductivity *1* and volumetric heat capacity of *2* experimental material on the coordinate of the intersection of straight lines $(\Delta e_t / \bar{e}_q) = f(h)$ and $(\Delta\tau \cdot \Delta e_q / \Delta e_t) = f(h)$ with the axis of the abscissus of the combined graph

Based on the results of measurement of the signals e_1, e_2, difference in signals $\Delta e = e_1 - e_2$ and the average signal $\bar{e}_q = (e_1 + e_2)/2$ of HFC device, temperature t_1, t_2 and the temperature difference $\Delta t = t_1 - t_2$ on the working surfaces of the samples, and the average temperature $\bar{t} = (t_1 + t_2)/2$ of samples and the speed of its change $u_t = \delta\bar{t} / \Delta\tau$ at heating (cooling) of the samples of research material with a thickness $h_1 \neq h_2$ in quasi-stationary mode:

1. We get the equation of a line $(\Delta t / \bar{e}_q) = (K_q / \lambda_x) \cdot h + K_q \cdot R_b$, that passes through the points J_{I} and J_{II}, with coordinates, respectively, $[h_1; (\Delta t / \bar{e}_q)_1]$ and $[h_2; (\Delta t / \bar{e}_q)_2]$, and a line $(\Delta e_q / u_t) = ((c\rho)_x / K_q) \cdot h + P_b / K_q$, that passes through the points Z_{I} and Z_{II} with coordinates, respectively, $[h_1; (\Delta e_q / u_t)_1]$ and $[h_2; (\Delta e_q / u_t)_2]$ that intersect the abscissa and the ordinate axes of their graphs (Figure 4, *b* and *e*) at $|-h_\lambda| = \lambda_x \cdot R_b$ and point $A = K_q \cdot R_b$, and $|-h_{(c\rho)}| = P_b/(c\rho)_x$ and point $B = P_b/K_q$. The angles of the lines *1* and *2* relative to the

abscissa axis of the graph are, respectively: $\psi = arctq(K_q / \lambda_x)$ and $\theta = arctq[(c\rho)_x / K_q]$.

2. By the method of plotting of the combined chart in Figure 5, we build in Figure 8 a combined graph of the line *1*, which is described by the equation $(\Delta t / \bar{e}_q) = (K_q / \lambda_x) \cdot h + K_q \cdot R_b$ and the line *2*, which is described by the equation $(\Delta e_q / u_t) = [(c\rho)_x / K_q] \cdot h + P_b / K_q$ and calculate the coordinates of the intersection of the graph circle with the abscissa axis, that is, the values of the corrected ballast thermal and capacitive resistances of the TPC device –
3. Through the points C and A, the coordinates of which are $[-R_x; 0]$ and $[0; (K_q \cdot R_b)]$, as well as the points C and B, the coordinates of which are $[-P_x; 0]$ and $[0; (P_x / K_q)]$ we draw the line 3, which is described by the equation $(\Delta t / \bar{e}_q) = K_q \cdot R_x + K_q \cdot R_b$ and the line 4, which is described by the equation $(\Delta e_q / u_t) = P_x / K_q + P_b / K_q$. At that the tangents of the angles φ and γ, the slope of the lines *3* and *4* relatively to the abscissa axis of the combined graph are, respectively: $tq\varphi = (K_q \cdot R_b) / R_b = K_q$ and $tq\gamma = (P_b / K_q) / P_b = 1 / K_q$.

$$R_b = P_b = \sqrt{(K_q \cdot R_b) \cdot (P_b / K_q)} = \sqrt{R_b \cdot P_b} \ . \quad (24)$$

4. With the center at the origin of coordinates of the combined graph, we draw the circle with radius $|-h_C| = |-R_x| = |-P_x| = R_b = P_b$ which crosses the abscissa axis of the graph at point C, and the ordinates axis at points A′ and B′,the ordinate of which is, respectively: $(\Delta t / \bar{e}_q \cdot K_q) = R_m = R_b$ and $(\Delta e_q \cdot K_q / u_t) = P_m = P_b$. In this case, the line *5*, that goes through the points *C* and *A′* is described by the equation $R_m = R_x + R_b$, and the line *6*, which goes through the points C and B' – by the equation $P_m = P_x + P_b$. On the combined chart (Figure 9), as well as in Figure 4, *c* and *f*, the angle of inclination of the lines *5* – $R_m = f(R_x)$ and 6 – $P_m = f(P_x)$ to the abscissa axis of the graph is $\pi/4$.

We should also note that when heating (cooling) material samples in a quasi-stationary thermal mode in a two-cell device (Figure 3), the equation of lines *1-6* can be obtained, and a combined graph similar to the one shown in Figure 8, can be built, for any average temperature of sample of the research material. This allows us to study the dependence of TPC of materials on temperature, taking into account the possible changes in the values of the MC of device on temperature.

Numerical values of the coefficients of the equations describing the lines *1-6*, obtained from the results of values measuring of HFC and TC of TPC device in the course of experiments from the study of TPC of material, as well as the results of calculations of TPC of this material and MC of device obtained from the equations describing the lines shown in Figure 8, are essentially corrected to $a_x = \beta_x = 1$ and, consequently to $\lambda_x = (c\rho)_x = 1$ of the research material. In principle, the MC of the device and the TPC of the research material can be calculated from any of the pairs of equations composed of those describing the line *1, 3,* or *5* and the line *2, 4* or *6.*

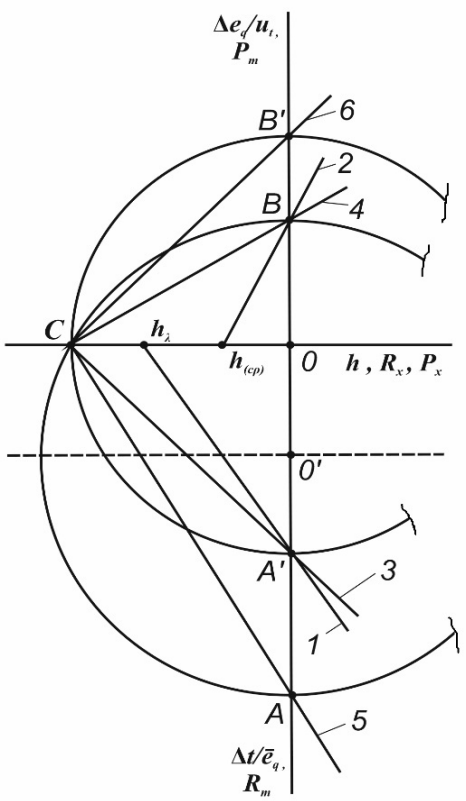


Figure 8. Combined dependencies graph:
1 – $(\Delta t / \bar{e}_q) = f(h)$,
2 – $(\Delta e_q / u_t) = f(h)$,
3 – $(\Delta t / \bar{e}_q) = f(R_x)$,
4 – $(\Delta t / \bar{q}) = f(R_x)$,
5 – $R_m = R_x + R_b$,
6 – $P_m = P_x + P_b$.

Let's show the calculation procedure of the MC of device and TPC of the research material using the equations that describe the pair of lines: 1 – $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$, 2 – $(\Delta t / \bar{e}_q) = f(R_x)$ and $(\Delta t / \bar{q}) = f(R_x)$, 3 – $R_m = R_x + R_b$ and $P_m = P_x + P_b$.

1. From equations $(\Delta t / \bar{e}_q) = (K_q / \lambda_x) \cdot h + K_q \cdot R_b$ and $(\Delta e_q / u_t) = [(c\rho)_x / K_q] \cdot h + P_b / K_q$ we obtain the absissas of lines intersection with the axis of the combined graph $|-h_\lambda| = \lambda_x \cdot R_b$ and $|h_{(c\rho)}| = P_b/(c\rho)_x$, then by equation (24) we calculate the value of the corrected thermal and capacitive resistances of the device. Also we determine the value of the operating coefficient of HFC of device, according to the equation –

$$K_q = \sqrt{\frac{K_q \cdot R_b}{P_b / K_q}}. \quad (25)$$

The easiest way to determine the TPC of research material using lines *1* and *2* of the combined graph can be calculated as follows:

$$\lambda_x = \frac{\lambda_x \cdot R_b}{R_b} = \frac{|-h_\lambda|}{|-h_C|}, \quad (26)$$

$$(c\rho)_x = \frac{P_b}{P_b / (c\rho)_x} = \frac{|-h_C|}{|-h_{(c\rho)}|}, \tag{27}$$

$$a_x = \frac{\lambda_x}{(c\rho)_x} = \frac{|-h_\lambda| \cdot |-h_{(c\rho)}|}{|-h_C|^2} \tag{28}$$

$$\beta_x = \sqrt{\lambda_x \cdot (c\rho)_x} = \sqrt{\frac{|-h_\lambda|}{|-h_{(c\rho)}|}} \tag{29}$$

It was noted above that the values of the coefficients of the equations describing the lines shown in Figure 8, including line *1* – $(\Delta t / \bar{e}_q) = (K_q / \lambda_x) \cdot h + K_q \cdot R_b$ and line *2* – $(\Delta e_q / u_t) = [(c\rho)_x / K_q] \cdot h + P_b / K_q$, are corrected to a_x=β_x=1. To substantiate this statement, we assume that the line $(\Delta t / \bar{e}_q) = f(h)$ and line $(\Delta e_q / u_t) = f(h)$, built on a combined graph (Figure 5) according to the results of experiments with samples of two materials that have temperature diffusivity a_λ=$a_{(c\rho)}$=1.

Under such conditions, we will show the procedure for determining the thermal conductivity λ_x of the material, which in Figure 5 corresponds to the line crossing the ordinate axis of the combined graph at point A, the abscissa axis at $|-h_\lambda| = \lambda_x \cdot R_b$ and the circle of the graph at point E, and the procedure of determining the volumetric heat capacity $(c\rho)_x$ of the second material, which corresponds to the line crossing the ordinate axis of the combined graph at point B, the abscissa axis at $|-h_{(c\rho)}| = P_b/(c\rho)_x$ and the circle at point D, graphically.

To do this, we draw a line $(\Delta e_q / u_t)' = f(h)$ through the points B and E, which is shown on the combined graph as a dashed line, until crossing the abscissa axis of the graph. Given that a_λ=1 abscissa of intersection of this line with the axis of the graph can be written as follows:

$$|-h'_{(c\rho)}| = \frac{P'_b}{(c\rho)_\lambda} = \frac{P'_b}{\lambda_x} = \frac{\lambda_x \cdot |-h'_{(c\rho)}|}{\lambda_x}. \tag{30}$$

Through the points A and D we draw a line $(\Delta t / \bar{e}_q)' = f(h)$, which is shown on the combined graph as a dashed line, until the intersection with the abscissa axis of the graph. Since $a_{(c\rho)}$=1, the abscissa of the intersection of this line with the axis of the graph will be:

$$|-h'_\lambda| = \lambda_{(c\rho)} \cdot R'_b = (c\rho)_x \cdot R'_b = (c\rho)_x \cdot |-h'_\lambda| / (c\rho)_x. \tag{31}$$

Taking into account the equations (30) and (31) and in accordance with (24), the equation for calculating the ballast thermal and capacitive resistances of the device, using which we carry out the determination of λ_x and $(c\rho)_x$ of materials, we write:

$$R_b = \sqrt{R_b \cdot P'_b} = h_C \text{ and } P_b = \sqrt{P_b \cdot R'_b} = h_C.$$

As a result of the transformation of these equations:

$$\frac{R_b}{\sqrt{R_b \cdot P_b'}} = \frac{\sqrt{R_b}}{\sqrt{P_b'}} = \sqrt{\frac{|-h_\lambda|}{|-h'_{(c\rho)}| \cdot \lambda_x^2}} = \frac{|-h_\lambda|}{|-h_C|} \cdot \lambda_x^{-1} = 1 \ ,$$

$$\frac{P_b}{\sqrt{P_b \cdot R_b'}} = \frac{\sqrt{P_b}}{\sqrt{R_b'}} = \sqrt{\frac{|-h_{(c\rho)}| \cdot (c\rho)_x^2}{|-h'_\lambda|}} = \frac{|-h_{(c\rho)}|}{|-h_C|} \cdot (c\rho)_x = 1 \ ,$$

we obtain equations for calculation of λ_x and $(c\rho)_x$ similar to (26) and (27).

2. The procedure for determining the MC of device using the equation $(\Delta t / \bar{e}_q) = K_q \cdot R_x + K_q \cdot R_b$, that describes the line *3* and the equation $(\Delta e_q / u_t) = P_x / K_q + P_b / K_q$, that describes the line *4*, is fundamentally the same as in the case of using the equations of lines $(\Delta t / \bar{e}_q) = f(h)$ and $(\Delta e_q / u_t) = f(h)$. At the same time, to obtain reliable results of the study of the temperature dependencies of the TPC of material, it is necessary to have information on the temperature dependencies of the MC of device. In this regard, the value of the operating coefficient of HFC $K_{q(\bar{t})}$, as well as ballast resistances $R_{b(\bar{t})}$ and $P_{b(\bar{t})}$ of the device should be calculated according to the equations (24) and (25) at the average temperature $\bar{t} = (t_2 + t_2)/2$ of the test sample, which includes the results of the calculation of the TPC of material.

Further, with the use of complexes $(\Delta t / \bar{e}_q)_{(\bar{t})}$ and $(\Delta e_q / u_t)_{(\bar{t})}$, the values of which are determined by the results of measurement of primary HFC and TC of device at the average temperature of the sample $\bar{t}$, we calculate the TPC of the material by the formulas:

$$\lambda_{x(\bar{t})} = \frac{K_{q(\bar{t})} \cdot h}{(\Delta t / \bar{e}_q)_{(\bar{t})} - (K_q \cdot R_b)_{(\bar{t})}} \ , \tag{32}$$

$$(c\rho)_{x(\bar{t})} = \frac{\left(\frac{\Delta e_q}{u_t}\right)_{(\bar{t})} - \left(\frac{P_b}{K_q}\right)_{(\bar{t})}}{K_{q(\bar{t})} \cdot h} \ , \tag{33}$$

$$a_{x(\bar{t})} = \frac{\lambda_{x(\bar{t})}}{(c\rho)_{x(\bar{t})}} = (K_{q(\bar{t})} \cdot h)^2 \cdot \left[\left(\frac{\Delta t}{\bar{e}_q}\right)_{(\bar{t})} - (K_q \cdot R_b)_{(\bar{t})}\right]^{-1} \cdot \left[\left(\frac{\Delta e_q}{u_t}\right)_{(\bar{t})} - \left(\frac{P_b}{K_q}\right)_{(\bar{t})}\right]^{-1} , \tag{34}$$

$$\beta_{x(\bar{t})} = \sqrt{\lambda_{x(\bar{t})} \cdot (c\rho)_{x(\bar{t})}} = \frac{\left(\frac{\Delta e_q}{u_t}\right)_{(\bar{t})} - \left(\frac{P_b}{K_q}\right)_{(\bar{t})}}{\left(\frac{\Delta t}{\bar{e}_q}\right)_{(\bar{t})} - (K_q \cdot R_b)_{(\bar{t})}} . \tag{35}$$

3. The procedure for calculating the TPC of material using the equations $R_m=R_x+R_b$, and $P_m=P_x+P_b$, and lines *5* and *6*, differs from the two described above, in no need to calculate the MC of TPC of device before calculating the TPC of the material by formulas:

$$\lambda_{x(\bar{t})} = \frac{h}{(\Delta t / \bar{q})_{(\bar{t})} - R_{b(\bar{t})}} \quad , \tag{36}$$

$$(c\rho)_{x(\bar{t})} = \frac{(\Delta q / u_t)_{(\bar{t})} - P_{b(\bar{t})}}{h} , \tag{37}$$

$$a_{x(\bar{t})} = \frac{\lambda_{x(\bar{t})}}{(c\rho)_{x(\bar{t})}} = h \cdot \left(\frac{\Delta t}{\bar{q}} - R_b \right)^{-1}_{(\bar{t})} \cdot \left(\frac{\Delta q}{u_t} - P_b \right)^{-1}_{(\bar{t})} , \tag{38}$$

$$\beta_{x(\bar{t})} = \sqrt{\lambda_{x(\bar{t})} \cdot (c\rho)_{x(\bar{t})}} = \frac{(\Delta q / u_t)_{(\bar{t})} - P_{b(\bar{t})}}{(\Delta t / \bar{q})_{(\bar{t})} - R_{b(\bar{t})}} . \tag{39}$$

Conclusion

Simultaneous obtaining information on the values of the thermophysical characteristics of the material and the metrological characteristics of the device can improve the accuracy of the study thanks:

- The exclusion of the use of the results of experiments with samples of reference materials for the determination of the metrological characteristics of the device, and, consequently, TFR of the research materials;
- The possibility to take into account the change of the metrological characteristics of the device from the temperature, as well as the resistance of the system "device-sample" during the expriment with the test material.

References

1. Incropera F.P., DeWitt D.P., Bergman T.L., and Lavine A.S. (2007), *Fundamentalsof Heatand Mass Transfer*, JohnWiley&Sons.
2. Eckert E. R. G., and Drake R. M. (1972), *Analysis of Heat and Mass Transfer*, McGraw-Hill, New York.
3. Touloukian Y. S., and Ho C. Y., Eds. (1972), *Thermophysical Properties of Matter*, Vol. 1, Thermal Conductivity of Metallic Solids; Vol. 2, Thermal Conductivity of Nonmetallic Solids; Vol. 4, Specific Heat of Metallic Solids; Vol. 5, Specific Heat of Nonmetallic Solids; Vol. 7, Thermal Radiative Properties of Metallic Solids; Vol. 8, Thermal Radiative Properties of Nonmetallic Solids; Vol. 9, Thermal Radiative Properties of Coatings, Plenum Press, New York.
4. Vargaftik N.B. (1975), *Tables of Thermophysical Properties of Liquids and Gases*, *2nd ed*., Hemisphere Publishing, New York.
5. Lemmon E. W., McLinden M. O., and Huber M. L. (2002), *NIST Standard Reference Database 23: Reference Fluid Thermodynamic and Transport Properties*-REFPROP,

Version 7.0 National Institute of Standards and Technology, Standard Reference Data Program, Gaithersburg.
6. Liley P. E. (1984), *Tables in SI Units, private communication*, School of Mechanical Engineering, Purdue University, West Lafayette, IN.
7. Platunov E.S., Buravoj S.E., Kurepin V.V., Petrov G.S. (1986), *Teplofizicheskie izmereniya i pribory*, Mashinostroenie, Leningrfd.
8. Hatakeyama T. and Liu Z. (1999), *Handbook of Thermal Analysis*, John Wiley.
9. Uendlandt U. (1978), *Termicheskie metody analiza,* Mir, Moscow.
10. Gerashhenko O.A., Gordov A.N. i dr. (1989), *Temperaturnye izmereniya Spravochnik,* Nauk.dumka, Kiev.
11. Gerashhenko O.A. (1971) *Osnovy Teplometrii*, Naukova dumka, Kyiv.
12. Grishhenko T.G., Dekusha L.V., Vorobev L.J. (2017), *Teplometriya: teoriya, metrologiya, praktika Knsga 1: Metody i sredstva izmereniya teplovogo potoka,* Institut texnicheskoj teplofiziki NAN Ukrainy, Kyiv.
13. Burova Z., Vorobjov L., Dekusha L., Dekusha O. (2009), Ustanovka dlya vimiryuvannya koeficiєnta teploprovidnosti budivelnix materialiv IT-7S, *Metrologiya ta priladi: Naukovo-virobnichij zhurnal*, 6, pp. 9–15.
14. HFM 436 Lambda–Heat flow meter, Available at: http://www.netzsch-thermal-analysis.
15. Instrument Line / LaserComp, Available at: http: //www. lasercomp. com/product/ default. php. – Title from the screen.
16. Anatyjchuk L.I., Luste O.Ya. (1981) *Mikrokalorimetriya,* Vishcha shkola, Lviv.
17. Dekusha O.L., Vorobev L.I. (2016) Izmerenie teploemkosti metodom poshagovogo skanirovaniya pri odnovremennom sravnenii s etalonnym obrazcom, *Ukriinskij metrologichnij zhurnal*., 4, pp. 48–51.
18. Thermocouples (2016), Available at: www.thermocoupleinfo.com/type-thermocouple.htm
19. Scientific research / heat and heat transfer measurement, Available at: https://www.hukseflux.com/applications/scientific-research-heat-and-heat-transfer-measurement.
20. Versey N.G., Gore C.J., Halson S.L., Plowman J.S., Dawson B.T. (2011), Validity and reliability of temperature measurement by heat flow thermistors, flexible thermocouple probes and thermistors in a stirred water bath, *Physiol Meas*, 32(9), pp. 1417–24.
21. Abdelaziz Y. A. (2006), Stability Characteristics of Industrial Platinum Resistance Thermometers, *MAPAN Journal of Metrology Society of India*, 21(1), pp. 3–7
22. Cvitaš Lj., Hocenski Ž. (2007), Automated Measurement System for Industrial Platinum Resistance Thermometer *Manufacturing Industry, Proceedings of the IEEE International Symposium on Industrial Electronics, ISIE*, VIGO, Spain, 4–7 June 2007, pp. 1606-1611.
23. Hocenski Ž. Cvitaš Lj. Lasinger Ž. (2008) Comparison of Methods for Nonlinearity Correction of Platinum Resistance Thermometer, *SICE Annual Conference 2008 in Chofu*, Tokyo, Japan, pp. 3151-3154.
24. Boris B. Hocenski Ž. Cvitaš Lj. (2006), Optimal Approximation Parameters of Temperature Sensor Transfer Characteristic for Implementation in Low Cost Microcontroller Systems, *IEEE ISIE* Montreal, Canada, pp. 2784-2787.
25. Xi Meng, BiaoYan, Yanna Gao, Jun Wang, Wei Zhang, Enshen Long *(2015),* Factors affecting the in situ measurement accuracy of the wall heat transfer coefficient using the heat flow meter method,. *Energy and Buildings*, 86, pp.754-765

Influence of differential heat treatment on foodstuffs with apples obtained by the convection-thermoradiation method of drying

Liudmila Strelchenko, Igor Dubkovetskyi, Ivan Malezhyk

National University of food Technologies, Kyiv, Ukraine

Keywords:

Apple
Snack
Candied fruit
Blanching
Drying

Article history:

Received 11.09.2018
Received in revised form 09.01.2019
Accepted 28.03.2019

Corresponding author:

Igor Dubkovetskyi
E-mail: dubkov78@ukr.net

DOI:
10.24263/2304-974X-2019-8-1-15

Abstract

Introduction. The influence of differential heat treatment on products from apples with different sugar content in semi-finished products is considered.

Materials and methods. Material for drying elect apples varieties «Golden Delicious». Samples of dried apples, snack and candied fruits were analyzed by organoleptic, physico-chemical and differential-thermal effects. The prepared semi-finished products were dried by the convective-thermoradiational method at a coolant temperature of 60 °C, a coolant movement speed of 5.5 m/s, a specific load of 8.8 kg/m^2, an exposure value of thermal radiation heating elements of 8 kW/m^2, and an external heating element power of 2.5 kW/m^2.

The results and discussions. Based on the drying curves, the rate of moisture removal occurred in direct proportion to the increase in the concentration of sugar in the products. Drying time of products depends on the content of dry substances (DM) in semi-finished products: which were 12% for dried apples, 18.2% for snack and 72% for candied fruits. Energy costs for dried apples, snack and candied fruits are respectively 5.9; 7.55; 19.8 kW in kg of raw materials and 1.93; 1.99; 26.0 MJ/kg of evaporated moisture. The dependence is observed, the greater the concentration of sugar in the semi-finished product, the longer it takes to remove moisture from the material.

In the presented derivatograms, when apples were heated in the T_1 = 108 °C region, a mass loss of Δm = 13.0% was observed, which was accompanied by an endothermic peak on the DTA dependence. This mass loss is due to evaporation of moisture. When the temperature increased to T_2 = 140 °C, destruction began to occur in the sample. When the snack was heated at T_1 = 108 °C, the mass was lost by Δm = 14.5%, which was accompanied by an endothermic peak on the DTA dependence. As the temperature rises to T_2 = 131 ºC, destruction begins to occur in the sample. Heating the candied fruit at T_1 = 109 °C resulted in a weight loss of Δm = 14.8% with an endothermic peak on the DTA dependence. As the temperature rises to T_2 = 125 ºC, destruction begins to occur in the sample.

Conclusions. Based on the derivatogram (T, TG, DTG, and DTA time dependencies), the prediction factor (k_0) was calculated for dried apples, snack and candied fruits to be 55; 61; 70 and also activation energy (E) for dried apples, snacks and candied fruits, respectively, 186; 203 and 236 kJ/mol.

Introduction

Based on the literature analysis, it becomes clear that a certain part of scientists is focused on finding a way to dry apple snack, a selection of technological parameters and process decisions that will have a significant impact on the quality of the finished product, and another part on the pretreatment of apple raw materials, which to some extent affects on the structural, organoleptic and physico-chemical properties of the finished product.

Preparation of apple raw materials is crucial for obtaining high-quality apple snack, because it depends on the chemical composition and nutritional value of the product.

Since 2004, studies have been conducted on the processing of raw apple material by blanching in a sugar, maltose syrup [1, 2]; immersion in a solution of $CaCl_2$ and citric acid [3]; blanching with citric acid solution [4]; treatment with a solution of ascorbic acid [5] and enrichment of apple particles with probiotics [6]. However, there are a number of problems that need to be addressed. Blanching in sugar or maltose syrup affects only the taste of the finished product; osmotic processing of raw materials in $CaCl_2$ solution with citric acid has several disadvantages, since $CaCl_2$ is a synthetic compound, affects the product's organoleptics by providing a bitter-salty flavor that is not always perceived by the consumer and has limitations for the use of various populations, including children, pregnant women, people with heart disease and high calcium in the blood. Enriching the particles of apples with probiotics can reduce the pH of the product, which is not always advisable, probiotics should not cause adverse reactions with prolonged use of the product, but special conditions are required for the storage of such a product.

Research problems remain the development of an optimal method of drying, and the creation of a fundamentally new product from apples that will be as close as possible in terms of the chemical composition of the finished product to the chemical composition of the raw material with maximum preservation of vitamin C.

The purpose of this work is the need to create apple snack that will satisfy the consumer's food preferences with pre-processing of raw materials, which focuses on the taste and physico-chemical characteristics of the product without the use of artificial flavors, dyes and preservatives.

The task of the work is to study the effect of pretreatment of apple raw materials on the process of convective-thermoradiational drying and differential thermal analysis of apple snack and their comparison with such foods as dried apples and candied fruits using the example of convective- thermoradiational drying.

Materials and methods

Investigated materials

Raw materials that were used in research are autumn and winter sorts of apples «Semerenko», «Lihold», «Champion», «Golden Delicious» and «Reinette» with the high sugar-acid index (more than 8), the high content of the original dry substances (more than 12%), large fruit size (more than 200 g) and activity of oxidative processes. In order to minimize waste, the ratio of the pulp to the inedible part was taken into account. According to these indicators apples varieties like «Champion» and «Golden Delicious» were chosen, but «Champion» has bigger peroxidase activity, that’s why the variety «Golden Delicious» was chosen as the best one.

Prior preparation for drying apples consisted of washing apples, inspecting, cleaning from inedible parts (skin and seed chamber), cutting into 3-6 mm parts and blanching in hot water with a temperature of 96–100 , then cooling and sending to drying. The production of dried apples was carried out according to the patent of Ukraine № 105128 «Method for the production of apple snack».

Preparation for apple candied fruit consisted of cooking jam in 3 stages with the same modes: boiling for 30 minutes, then cooling to room temperature, to let the sugar be absorbed into the fruit and in order to avoid overcooking the fruits of apples. The boiling ended when the dry matter in the syrup reached 78%, in the fruit 70–72%. After that, the fruit was separated from the sugar syrup, washed, cut into pieces of 15 × 15 mm. After that, they were sent for drying. Production of candied fruits was carried out according to the patent of Ukraine for the invention №. 113569 «Method of production of candied fruits».

Preparation of raw materials in the apple snack production did not differ from the preparation for apple drying apples, but blanching occurred in 30% sugar syrup, with a ratio of apples and syrup as 1:2 with the addition of citric acid 1% and ascorbic acid in the amount of 0.01%. Then it was cooled in the same syrup at a temperature of 18–20 °C. Such an operation is necessary in order that the apple pieces do not lose their shape and absorb a portion of sugar with citric acid. Sugar with ascorbic acid forms a protective shell that reduces the access of oxygen during drying, and as a result, the finished product retains the light yellow color common to the used raw material and the vitamin complex [7].The production of apple snack was carried out according to the patent of Ukraine for the invention № 113587 «Method for the production of apple snack».

Procedure of conducting researches

Prepared semi-finished products were sent for convective-thermoradiational drying. The drying parameters were established experimentally: the coolant temperature in the drying chamber was 60, the velocity of air in the chamber was 5.5 m/s; surface allowance – 8.8 kg/m^2; the amount of radiation irradiated by thermoradiation heating elements was 8 kW/m^2, the wavelength of tubular «dark» thermoradiation generators was 2.0–4.0 microns; the air was heated from an external heating element of 2.5 kW/m^2; the distance between thermoradiational heaters and the product was 14 cm.

After drying, three samples of apples were obtained: dried apples, snack and candied fruits. In order to objectively evaluate these products, a qualitative and differential thermal analysis was performed.

Methods and research facilities

Methods of analysis

The organoleptic analysis was carried out by tasting and comparing dried products using a convective-thermoradiational method with dried convection products.

During physico-chemical analysis of the mass fraction of dry substances was determined by the refractometric method [8]; moisture content – by the accelerated method of Chizhova [22]; the content of organic acids was determined by alkali titration (in terms of malic acid) [10]; sugar content – using the permanganate method [11]; the content of mineral impurities (ash) – the irrigation of a batch weight [12]; the content of pectin substances – using calcium pectate [13]; vitamin C content is potentiated by titration of 2,6-dichlorophenolindophenol [14].

Differential-thermal analysis of products was performed using a «Paulik-Paulik-Erdey Q-1500 D derivatograph». The method of differential thermal analysis (DTA) is based on a comparison of the properties of a test substance sample and a thermally inert substance accepted as a standard. The recording parameter was the temperature difference measured when the sample was heated or cooled at a constant rate, which can be represented as a change in the sample temperature or standard. A change in the temperature of a sample is caused by physical transitions or chemical reactions associated with changes in enthalpy. These include: phase transitions, melting, boiling. These transformations are accompanied by absorption or release of heat. In the genera, phase transitions are accompanied by endothermic effects, and oxidation and individual decomposition processes – by exothermic effects.

Research equipment

Convective-thermoradiational drying plant. The research of the drying process of products was carried out on a laboratory convective-thermoradiational drying unit (Figure 1), which was made in accordance with the Ukrainian patent for invention № 112348 «Radiation-convective drying unit».

The drying chamber *1*, is made of polished aluminum, which has a high coefficient of thermal radiation reflection and is 0.86, which ensures homogeneous irradiation of raw materials during drying. Air is supplied to the chamber by means of the fan blades *2*, passing gradually through the external heater *3* and the drying chamber, in which it contacts with the material, supplying heat to it and at the same time removing moisture from the product. The semi-finished product is formed into a layer on a special grid-stand in a box-shaped mesh basket *5*, which is mounted on an analytical balance *7* through the hole in the bottom wall of the drying unit. Thermoradiational irradiation is performed by emitters *4*.

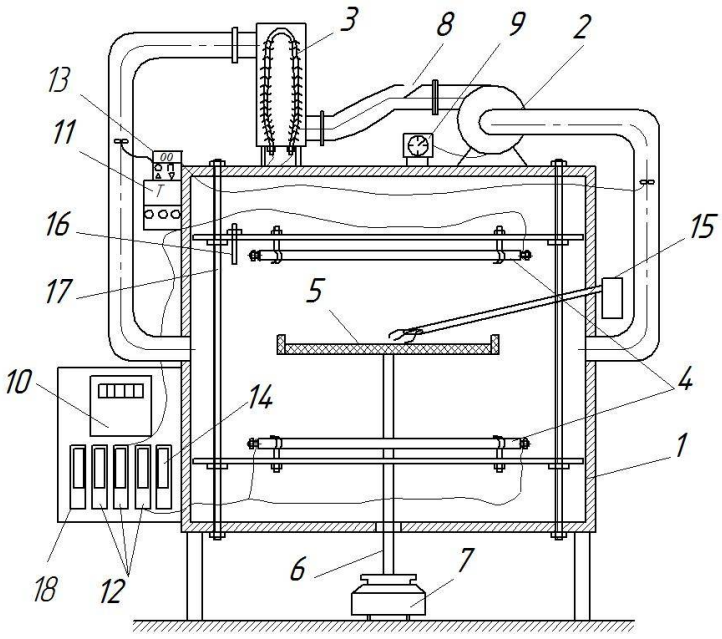


Figure 1. Schematic diagram of a convection-thermal modi-sion drying unit

With the help of vertical guides *17*, it is possible to change and fix the the nodes position of thermoradiational generators, thus changing the irradiation amount of the samples and the volume of the working chamber. The design of the sample unit of infrared radiators allows the use of «light» and «dark» thermo-radiation generators for drying raw materials. The air heated by the heater is supplied to the drying chamber at a certain speed, the value of which is controlled by the automatic control unit of the heat carrier speed *9* of the electric fan *2*. The registration unit for reducing the mass of drying material, it is a modified design of analytical weights *7*, which are connected by a bar *6* to the mesh basket *5*. Setting the temperature of the coolant is carried out by the automatic temperature control unit *11* with a minimum inaccuracy of 1 . The range of turning on and off heaters characterizes the irradiation time for semi-finished products. Energy consumption per kilogram of finished products or evaporated moisture is carried out using a counter *10*. Air recirculation in the dryer was carried out using a gate *8*. The change of air relative humidity on entrance and exit of the drying chamber was recorded using the automatic control unit of relative humidity *13*. De-energize the heater generators can be done with a help of automatic switches *12*. The temperature change in the cross section of the semi-finished product was recorded with a thermometer with thermocouples *15*. Drying was performed in a pulsed heating-cooling mode. Thermoradiational beams and external convective heating elements provided the heating. After reaching the maximum set temperature for drying, thermoradiational emitters and the external heater were switched to the pulse switching mode. The duration of the impulse and pause correlated as 1: 1, 1: 2, 1: 3, etc. The temperature was recorded by a contact temperature sensor 16, the signal of which was supplied to turn the generators on or off to the relay *18*.

Derivatograph «Paulik-Paulik-Erdey Q-1500 D». Differential thermal analysis was performed on a «Paulik-Paulik-Erdey Q-1500 D» system derivatograph.

The derivatograph (Figure 2) consists of crucible *1* with a sample, crucible *2* with a standard, ceramic tube *3,* thermowaight *4*, differential transformer *5*, magnet *6*, coil *7*.

The device can operate in two modes: simple thermometry mode and differential thermometry mode. In the first case, one simple thermocouple is used in the work, on which a crucible with a sample is placed. In the second case, a differential thermocouple is used, consisting of two simple thermocouples connected by the same poles to each other. On one of them was a crucible with a sample, on the other – with a reference substance. One of the shooting modes was chosen using the switch located on the front panel of the device.

On the second balance beam weights placed two devices with which they measured the loss and rate of weight loss of the sample. The principle of operation of these devices is based on the phenomenon of magnetic induction.

A differential transformer consisting of three coils arranged vertically on the same axis was used to measure the mass loss. The transformer is fixed at the instrument case. Inside the transformer is placed a metal core suspended on the balance arm. This core displaces when the mass of the test sample changes. When alternating current is supplied to the middle coil (primary winding of the transformer), an induction current arises in adjacent coils (secondary winding of the transformer) connected to each other (differentially). With a symmetrical arrangement of the core along the coils of the secondary winding of the transformer, the total voltage on them is almost zero (the initial position of the scale). When the core is displaced, the total voltage changes in proportion to the displacement. Thus, the differential transformer converts the movement of the weights arm into an electrical signal, which is recorded by a recording device. The differential transformer is characterized by high sensitivity and measurement accuracy.

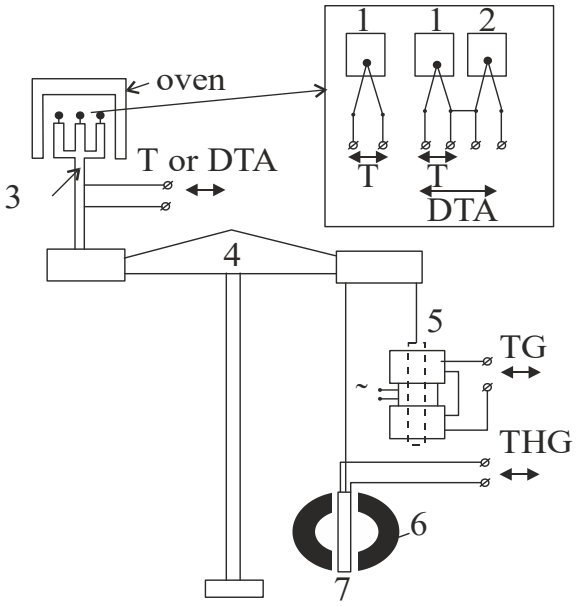


Figure 2. Schematic diagram of the derivatograph

The THG curve is obtained using a device consisting of a permanent magnet and an induction coil. The induction coil is suspended from the scale and placed in a permanent magnet field. When the sample mass changes, the coil begins to move in a homogenous magnetic field. During this movement in the coils occurs induction current, the value of which is proportional to the rate of mass change.

Signals from thermocouples, a differential transformer and an induction coil were fed through an amplifier to a computer monitor and we simultaneously received simple (T) and differential thermal (DTA) analysis curves, a mass loss curve (TG) and a mass loss rate curve (THG).

Methods for calculating the kinetic parameters of the dehydration process, such as the activation energy (E) and the preconference factor (k_0), are described in [15].

The kinetic equation of the desorption process in differential thermal analysis can be written as:

$$d\Theta/dt = -k\Theta^n;\ k = (-d\Theta/dt)/\Theta^n \quad (1)$$

The degree of coating of biopolymer molecules with water (Θ) varies from 1 – filling for the starting material to 0 – all water is dehydrated. The order of reaction (n) – is an integer from 1 to 3, it is assumed that it is known from the experiment. Constant rate of reaction

$$k = k_0\exp(-E/RT), \quad (2)$$

where R – is the universal gas constant. The value of E approaches a constant, which means the equivalence of all biopolymer molecule surface hydration centers. Substituting equation (1) into equation (2) and logarithmically we get

$$\ln k = \ln[(-d\Theta/dt)/\Theta^n] = \ln k_0 - E/RT \quad (3)$$

Bearing in mind the initial conditions $\Theta_{t=0} = 1$, $\Theta_{t=\infty} = 0$ and conducting the experiment at a constant heating rate (β), that is, the linear dependence of the temperature on time

$$T(t) = T_0 + \beta t, \quad (4)$$

the following expressions can be written:

$$\Theta(t) = S_T/S_0;\ -d\Theta/dt = \beta f_2/S_0, \quad (5)$$

where S_0 and S_T – are respectively the area on the graph f_2 under the whole peak and the peak part from T to ∞

$$S_T = \int_T^\infty f_2 dT;\ S_O = \int_0^\infty f_2 dT \quad (6)$$

If all the assumptions inherent in this method are correct and the reaction order n is chosen correctly, then the dependence of $\ln[(-d\Theta/dt)/\Theta^n]$ on the inverse temperature (equation 3) is linear over the entire temperature range. Having experimental values of f_2 and β, using expressions (5) and (6), we obtain Θ and dΘ / dt parameters of non-isothermal kinetics k_0 and E are calculated from equation (3). The advantage of the above procedure is the use of the entire array of experimental data, including the high-temperature part of the thermogram, which is especially important in determining order n, determining the reaction mechanism and the adequacy of the model.

Result and discussion

Investigation of the kinetics of convective-thermoradiation drying products from apples

On the basis of the data obtained, drying curves were constructed (Figure 3), characterizing the change in moisture content of W^c depending of time τ. The Figure shows that the warm-up period for all samples is minimal, and the rate of moisture removal was directly proportional to the increase in the concentration of sugar in the products.

Analyzing Figure 3, it can be said that the heating period for all samples of semi-finished products is minimal (about 2-3 minutes), and then there is a rapid period of moisture removal (*3 a*). From Figure *3 b* it can be seen that the process of removing moisture in the second period is long, since the sugar concentration is high and moisture in the intermediate contains osmotic bonds, which significantly complicates the drying process [19].

Approximating the data of the first and second drying periods, we derived the equation of the moisture content of W^c from the drying time τ (Table 1).

After drying, samples of finished products for which a physico-chemical analysis was performed (Table 2) were obtained.

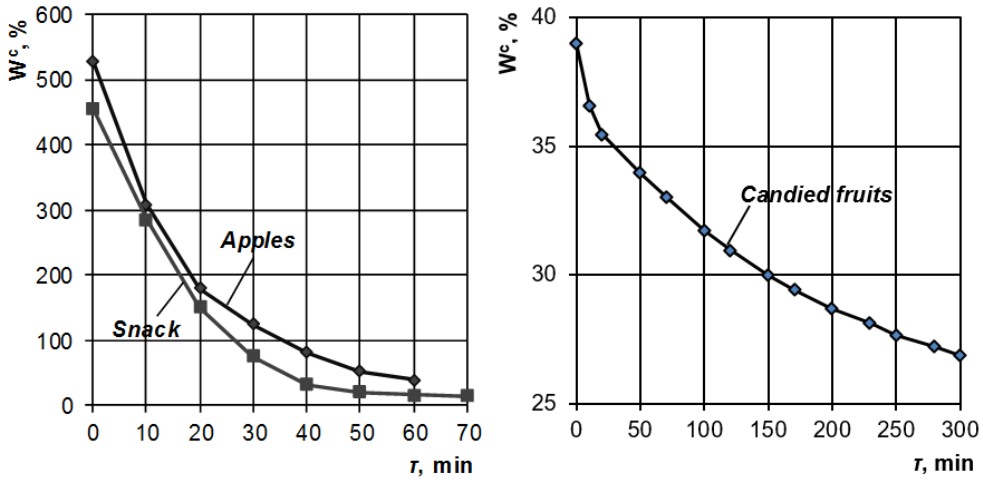


Figure 3. Drying curves of apples:
a – apples and snack; b – candied fruits

Table 1

Equation of moisture content W^c (%) from the drying time τ (min)

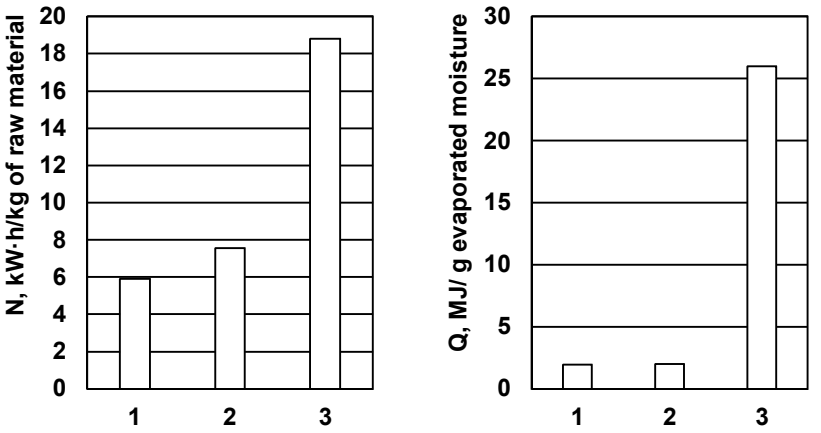
Product	1 period	2 period
Dried apples	$W^c = -22{,}06\tau + 527{,}45$ at $R^2 = 0{,}99$	$W^c = 4941{,}8\ \tau^{-1{,}137}$ at $R^2 = 0{,}95$
Snack	$W^c = -17{,}544\tau + 452{,}56$ at $R^2 = 0{,}99$	$W^c = 3418{,}2\ \tau^{-1{,}07}$ at $R^2 = 0{,}99$
Candied fruits	$W^c = -0{,}213\tau + 32{,}04$ at $R^2 = 1$	$W^c = 55{,}57\ \tau^{-0{,}26}$ at $R^2 = 0{,}99$

Table 2

Physico-chemical analysis for apple products

Indicator	Product		
	Dried apples	Snack	Candied fruits
Dry matter of fresh apples,%	12,0		
Dry substances of apples,%.	87	84,5	85,2
Mono- and bi-sugars,%.	61,5	63	76,1
Organic acids,%.	2,4	2,3	1,5
Pectin substances,%.	4,9	4,4	0,8
Dietary fiber,%.	4,3	4,3	4,2
Mineral substances,%.	2,7	2,6	0,7
Vitamin C, mg%.	4,6	8,3	6,8

When processing the drying process data, the energy consumption for all product samples in kWh in kg of raw materials (*Figure 4 a*) and in MJ/kg of evaporated moisture (*Figure 4 b*) was obtained.



a b

Figure 4. Electricity consumption per 1 kg of raw materials (4 a) and 1 kg of evaporated moisture (4 b) for apple products: 1 – dried apples; 2 – snack; 3 – candied fruits

In Figure 4, the dependence is observed, the greater the concentration of sugar in the semi-finished product, the longer it takes to remove moisture from the material.

Study of differential thermal analysis of products from apples

Figure 5, Figure 6 and Figure 7 show tree patterns (time dependences of T, TG, DTG, and DTA) for the product samples.

When the apples were heated at T_1 = 108 °C, the mass was lost by Δm = 13.0%, which was accompanied by an endothermic peak on the DTA dependence. This mass loss is due to evaporation of water. When the temperature increased to T_2 = 140 °C, destruction began to occur in the sample (Figure 5).

During heating, in the region T_1 = 108 °C, the mass was lost at Δm = 14.5%, which was accompanied by an endothermic peak at the DTA. With a temperature rise up to T_2 = 131 °C, a degradation occurs in the sample (Figure 6).

When the candied fruit was heated in the T_1 = 109 °C area, there was a loss of mass by Δm = 14.8%. with an endothermic peak on the DTA dependence. As the temperature rises to T_2 = 125 °C, destruction began to occur in the sample (Figure 7).

The table below shows the samples and the moisture content of the finished samples.

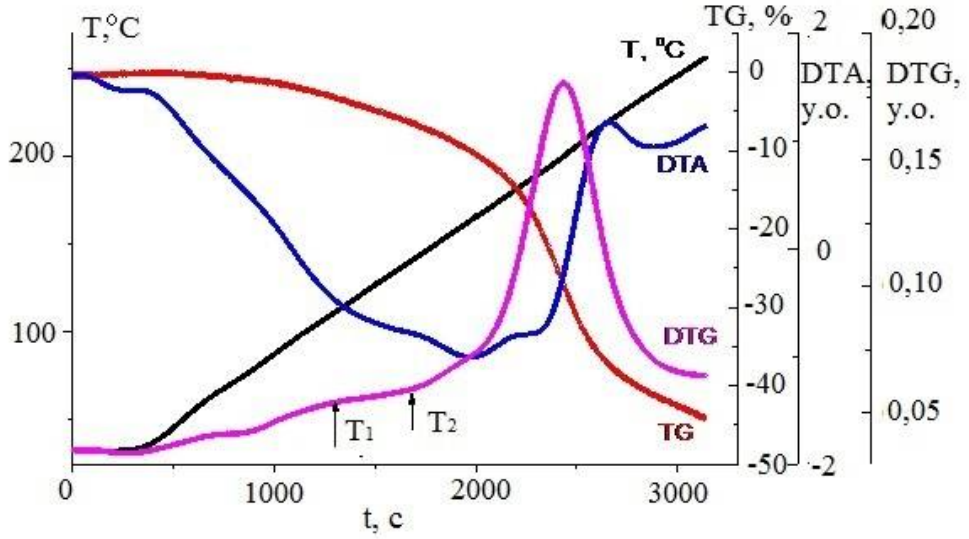


Figure 5. Derivatogram dried apples «Golden Delicious»:
T – time dependencies of temperature; TG – inward masi apple;
DTA – differential-term analysis; DTG – derivative of the mass loss

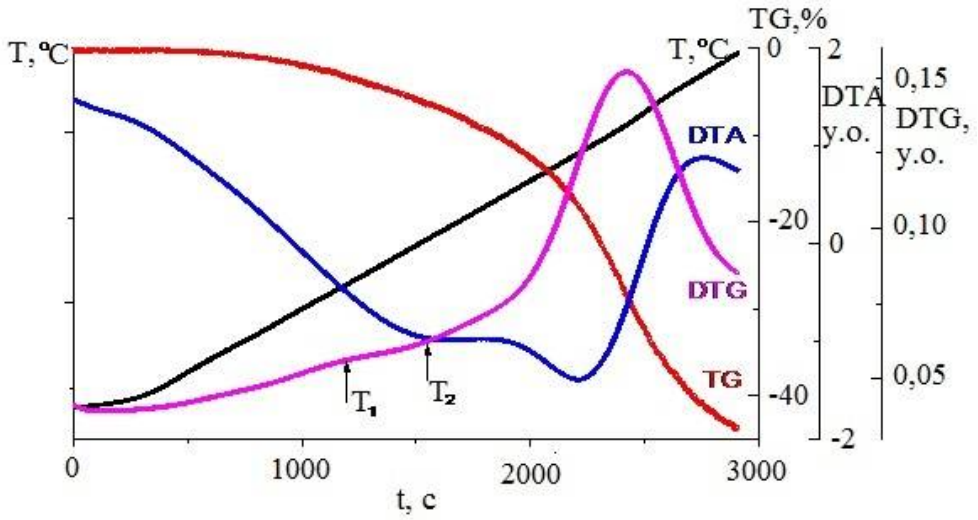


Figure 6. **Derivatogram for snack «Golden Delicious»:**
T – time dependencies of temperature; TG – inward masi apple;
DTA – differential-term analysis; DTG – derivative of the mass loss

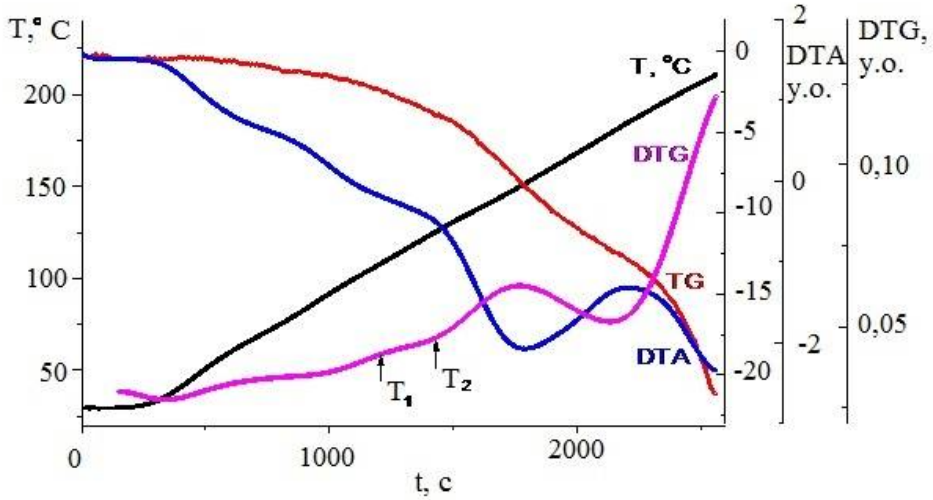


Figure 7. Derivatogram for candied fruits «Golden Delicious»: T – time dependencies of temperature; TG – inward masi apple; DTA – differential-term analysis; DTG – derivative of the mass loss

Table 3

Moisture content of the studied samples

The moisture content of the products	Product		
	Dried apples	Snack	Candied fruits
%	13.0	14.5	14.8

As it was already noted, mass loss by samples is accompanied by the appearance of endothermic peaks on the temperature dependences of the DTA (Figure 5, 6, 7). The endothermic peak is associated with the phase transition of the first stem – evaporation. That is, in the samples during heating there was evaporation of moisture in the region of 110 °C. An increase in the temperature of evaporation (in comparison with the boiling point of water) can be explained by the fact that the water in the samples was in the bound state (in the hydrate shell).

Using the described method, the activation energy (E) and the pre-exponential factor (k_0) were calculated, which are given in Table 4.

Table 4

Activation energy (E) and the preexposure factor (k_0) for the samples

Product	Dried apples	Snack	Candied fruits
k_0	55	61	70
E kJ/mol	186	203	236

Table 4 shows, that the higher the moisture content in the product, the higher the activation energy.

Explanation of the results

Scientists of the world [1] elected a winter variety of apples «Idared» as an experimental sample, which is distinguished by a large fruit size, a light flesh and an acceptable sweet-sour taste with a sugar-acid indicator of more than 4.

Based on the results of our own research, «Golden Delicious» apples were selected as raw materials for the production of apple snack due to excellent performance and low peroxidase activity.

Pre-processing of «Idared» apples [1] was to clean, cut and immerse the raw material in 40% glucose syrup at a temperature of 18–22 , but a long osmotic treatment leads to an increase in the manufacturing cycle of this product, a short-term – less than 15–20 minutes gives the desired result, that is, does not provide the sweet taste of the product. The concentration of sugar in these snack is not uniform – in the peripheral zones, its concentration is higher than in the middle. The process of internal diffusion does not reach the state of equilibrium or its complete absence in the inner layers. This can significantly affect the quality of the finished product, because during convective drying at a temperature of 105-110 , as the authors claim, Mayer reactions can form, which negatively affect both organoleptics (aroma, taste) and physical-chemistry product (reducing the content of organic acids and vitamin C). The final moisture content of snake is 6-8%. The low moisture content in the product gives a specific sound of a crunch when breaking, therefore the authors call this product chips.

Analyzing the results of research by world scientists, technology was developed taking into account certain shortcomings. Preparation of apple raw materials is focused on blanching in 30% sugar syrup with the addition of citric acid in the amount of 0.5–1% and ascorbic acid 0.05–0.1%. To avoid cooking the fruit after blanching, the apple particles are cooled in a similar syrup with a temperature of 18-20 °C to room temperature. Drying is carried out under softened conditions at a coolant temperature of 40–60 °C to a product moisture content of not more than 15%.

The world presents a large part of the scientific work on drying food products by the convective method [16, 17]; thermoraditional; microwave and temperature effects on the quality of the finished product; by the sublimation method [2], but together with the advantages of the methods there is a significant drawback – energy consumption, therefore, the creation of an energy-efficient method of drying or cheapening of already existing dryers remains urgent. There are convection units with a heat pump [18], which allow you to save energy without negative consequences for the dried products. However, it is necessary to look for new solutions and create progressive dryers.

The developed convectiv-thermoradiation method of drying is energy efficient compared to convective by 25–30%. At the same time, the final inactivation of enzymes is provided, it is positively noted on the quality of the finished product, in particular on the visual appearance, which is one of the main criteria for consumer choice of food products.

The results of research have a scientific novelty and may be of interest for the industrialization of technology in production. One of the urgent tasks of technologists will always be improving the quality of the finished product. The obtained samples of dried apples and candied fruits, made by usage of the combined method of drying, have high organoleptic and physico-chemical indicators and can be realized in commercial networks: dried apples – for the production of compotes; candied fruit – for the industrial production of cakes, muffins, cakes as fillers. Apple snacks are a quick snack without the use of artificial additives, but enriched with vitamins using ascorbic acid.

The advantages of the presented studies include the ease of operation of the pilot plant and the possibility of changing the drying parameters in a wide range: temperature, air velocity in the chamber, voltage of heating elements, distances from thermal radiation generators to the product, air recirculation, unit load on the installation, the possibility of fixing power consumption any moment of research. If necessary, the dryer can be transferred to one of the drying methods, for example, when candied fruits are blown off, only convection is used to remove excess moisture after washing the fruit.

The disadvantage of the installation is that it is periodic in action and requires additional time to load and unload a portion of the product that goes into drying.

The presented studies have a scientific novelty, and pretreatment of apple raw materials in the manufacture of apple snacks provides high organoleptic characteristics (attractive light color typical of the raw materials used; rich apple flavor; pleasant sweet-sour taste), physical and chemical indicators (high content of vitamin C). In the future, these studies will be continued and focused on the packaging of these products and physical and chemical changes during storage.

Conclusions

1. Blanching for the production of dried apples was carried out according to the actual technical instructions, and blanching of raw materials for snack production was in the recommended sugar syrup, based on previous studies, with concentration of 30%. The semi-finished product was also cooled in 30% sugar syrup with the addition of citric and ascorbic acid. Preparation in the production of candied fruit was the manufacture of jam in three stages with its periodic cooling to ambient temperature, followed by boiling
2. The process of combined convection-thermoraditional drying is investigated and drying curves and drying speed curves of dried apples, snack and candied fruits are presented. Approximation equations are derived for the first and second drying periods for all products from apple.
3. Experimentally determined the amount of electricity consumed spent on drying products from apple – in the production of dried apple costs amounted to 5.9 kW/kg of raw materials (1.93 MJ/kg of evaporated moisture) in the production of snack – 7.55 kW/kg of raw materials (1.99 MJ/kg of evaporated moisture) in the production of candied fruits – 19.8 kW/kg of raw materials (26.0 MJ/kg of evaporated moisture).
4. A differential thermal analysis of dry product samples has been performed and it has been established that the energy of water activation in candied fruits is higher by 50–236 kJ/mol, and in apple snack it is higher by 33–203 kJ/mol, in comparison with dried apples (186 kJ/mol). It is proved that higher energy of activation of moisture removal has samples with high moisture content of macro- and microcapsules; the forms of moisture bonding in the material in each product were identified.

References

1. Velickova E., Winkelhausen E., Kuzmanova S. (2014), Physical and sensory properties of ready to eat apple chips produced by osmo-convective drying, *Journal of Food Science and Technology*, 51(12), pp. 3691–3701
2. Hawkes J., Flink J.M. (2013), Osmotic concentration of fruit slices prior to freeze dehydration, *Journal of Food Processing and Preservation*, 19782(4), p. 265.

3. (2009) *Effect of different treatments on the preference of apple cubes dehydrated by a combined process*,
Available at: https://www.ncbi.nlm.nih.gov/pubmed/19817634
4. Joshi A.P.K., Rupasinghe H.P.V., Khanizadeh S. (2011) Impact of drying processes on bioactive phenolics, vitamin C and antioxidant capacity of red-fleshed apple slices, *Journal of Food Processing and Preservation*, 35(4), pp. 453–457.
5. Tavera-Quiroz M., Urriza M., Pinotti A., Bertola N., (2014), Development and Characterization of a Baked Snack from Rings of Green Apples, *Food and Bioprocess Technology*, 7(8), pp. 2218–2227.
6. Reihaneh N., Parastoo Y., Durance T. (2013), Radiant energy under vacuum (REV) technology: A novel approach for producing probiotic enriched apple snack, *Journal of Functional Foods,* 5(3), pp. 1049–1056.
7. Malejik I., Dubkovetskyy I., Bandurenko H., Strelchenko L., Levkivska T. (2016), The Use of convective-thermoradiactive method of energy supply in the apple snack technology, *Skhidno-Yevropeiskyi zhurnal peredovykh tekhnolohii*, 6/11(84), pp. 47–52.
8. Guohui Hao, Jianjun Liu, and Zhi Hong (2011), Determination of soluble solids content in apple products by terahertz time-domain spectroscopy, *International Symposium on Photoelectronic Detection and Imaging 2011: Terahertz Wave Technologies and Applications,* Proc. of SPIE Vol. 8195, 819510, DOI: 10.1117/12.900671
9. Agota Giedrė Raišienė, Artiom Volkov, Virgilijus Skulskis, Rita Vilkė (2018), Representation of agricultural producers' interests: substantiation of the research construct, *Ukrainian Food Journal*, 7(3), pp. 522-535.
10. (1998), SO 750:1998. Fruit and vegetable products - Determination of titratable acidity, Available at: https://www.iso.org/standard/22569.html.
11. ISO 751:1981. Fruit and vegetable products -- Determination of water-insoluble solids content, Available at: https://www.iso.org/standard/5007.html.
12. ISO 2173:1978. Fruit and vegetable products -- Determination of soluble solids content - Refractometric method,
Available at: https://www.iso.org/standard/6970.html.
13. Kertesz Z.I. (1957), Preparation and determination of pectic substances, Methods in Enzymology, 3, pp. 27-30.
14. ISO 6557-1:1986. Fruits, vegetables and derived products -- Determination of ascorbic acid - Part 1: Reference method, Available at: https://www.iso.org/standard/12956.html.
15. (2017), Influence of Water on the Structure and Dielectric Properties of the Microcrystalline and Nano-Kovalov etal, Nanoscale Research Letters, 12, p. 468, DOI: 10.1186/s11671-017-2231-5.
16. Chua K., Mujumdar A., Chou S., Hawlader M., Ho J. (2000), Heat pump drying of banana, guava and potato pieces: Effect of cyclical variation of air temperature on convective drying kinetics and color change, *Drying Technology*., 18(5), pp. 907–936.
17. Doymaz İ., (2010), Effect of citric acid and blanching pre-treatments on drying and rehydration of Amasya red apples, *Food and Bioproducts Processing,* 88(2–3), pp. 124–132.
18. Sunthonvit N., Srzednicki G., Craske J., (2007), Effects of Drying Treatments on the Composition of Volatile Compounds in Dried Nectarines, *Drying Technology*, 25 (95), pp. 877–881.
19. Dubkovetsky I., Malejik I., Strelchenko L. (2018), Investigation of convective-thermoradiation drying of products from apples in two stages, *Proceeding of International Conference "Modern Technologies in the food Industry", Chisinau, 2018*, p. 123.
20. Vadym Pazyuk, Zhanna Petrova, Oleksandr Chepeliuk (2018), Determination of rational modes of pumpkin seeds drying, *Ukrainian Food Journal*, 7(1), pp. 135–150
21. Galnaitytė, A., Kriščiukaitienė, I., Baležentis, T., Namiotko, V.(2017), Evaluation of Technological, Economic and Social Indicators for Different Farming Practicesin Lithuania, *Economics & Sociology*, 10(4), pp. 189–202, DOI: 10.14254/2071-789X.2017/10-4/1.
22. Drobot V.I., Arsenieva L.Yu., Bilyk O.A., Dotsenko V.F. (2006), *Laboratornyi praktykum z tekhnolohii khlibopekarskohota makaronnoho vyrobnytstva*, Kyiv.

Organizational basis of the development of innovative functional food products by the Ukrainian enterprises of deep walnut processing

Hryhoriy Kaletnik[1], Vitalii Lutsiak[1], Olha Melnichuk[1], Yuriy Dovhan[1], Marian Malicki[2]

1 – Vinnytsia National Agrarian University, Vinnytsia, Ukraine
2 – Jacob of Paradies University, Gorzów Wielkopolski, Poland

Keywords:

Walnut
Oil
Functional
Food
Marketing

Article history:

Received 26.08.2018
Received in revised form 31.10.2018
Accepted 28.03.2019

Corresponding author:

Vitalii Lutsiak
E-mail:
lutsiakvv@gmail.com

DOI: 10.24263/2304-974X-2019-8-1-16

Abstract

Introduction. Ukraine industrial enterprises, engaged in deep processing of walnuts, have a significant export potential. The lack of methodological bases for the development of innovations by domestic enterprises reduces this potential.

Materials and methods. An approach of the analysis of value chain was used, marketing functional and process approaches were used to. The research uses also: fundamental interviews with experts, laboratory experimental studies, production and implementation of the developed innovative products. Developing of innovative products was conducted on the basis of Quality Function Deployment methodology.

Results and discussion. The results of our research demonstrate that Ukraine is involved in the process of diversification of the world edible oil market and innovations development. The industry of deep walnut processing provides a kernel walnut for the confectionery and animal feeding, edible walnut oil, industrial oil and oilcake, which can be processed into food products or used to feed livestock on the domestic and foreign markets. Remains (wastes) from production can be effectively recycled or used in other industries. In addition, walnut kernels are also considered to be a product of the edible oil industry. Other by-products of walnut are being studied. In the development of the industry of deep walnut processing, as well as in the short term prospect, the focus is on the confectionery kernel and walnut oil. In the course of the industry development, innovative by-products will be developed through exploration and support of the development of alternative ways of using remains as they arise.

The value of obtained results is the possibility of using the obtained theoretical and methodological conclusions, recommendations and regulations for agrarian and food processing enterprises to solve the problem of competitiveness of the enterprise and its products when exporting in the conditions of high dynamics of business environment based on the system relations of the manufacturing enterprise in the industry of deep walnut processing.

Conclusion. The model of the value chain for the processing of walnuts and the technology of production of new products is offered.

Introduction

In a market economy, domestic producers have a goal both to increase their profits and to achieve an appropriate level of competitiveness in the market [2]. To solve these problems, the most pressing issues are the scientific definition and development of system approaches to the marketing of innovative activities of business entities [2, 14]. Such a task is complicated by the variety and difficulty of fixing the forms and manifestations of behavioral strategy and tactics in the conditions of the dynamism of the environment [3].

In our previous studies, it has been established that the competitiveness of industrial enterprises depends, in large part, on the extent to which the target markets are thoroughly investigated and specific measures to adapt products to the requirements of these markets have been developed. The methodology of enterprise competitiveness management is developed based on three key elements, namely, dynamic capabilities, innovation, quality ("DIQ"), and the dependencies between the characteristics that arise during the process of deployment of the quality function when designing innovative products are determined. Our research shows that Ukraine industrial enterprises, engaged in deep processing of walnuts have a significant export potential in the industry for deep processing of walnuts [5, 8, 11, 16, 20, 22, 24, 26, 28, 30, 32, 34,]. This is confirmed by the growing demand for products provided by walnut processing enterprises and the development of raw materials based on the unique natural conditions in Ukraine [8, 10, 15].

Establishment of constraints in the added value chains for food products based on their limited availability [1] can significantly improve the competitiveness of enterprises in the foreign markets [2], especially for transition economies such as Ukraine's [3]. Restrictions that are created by the markets can be mitigated or eliminated to a large extent based on the transformation of elements of the marketing complex of a small manufacturing enterprise into the parameters of the quality of the desired product and the process parameters [4]. The methodology [4] also allows setting requirements for equipment and for manufacturing operations that are included in the operating instructions for each step of the production process. A promising way to increase the competitiveness of the subjects of the value chain in the oil and fat industry [5, 6] for deep walnut processing is the certification according to the European quality standards [7, 8] and ensuring the recycling of waste products for biofuels [9, 36]. However, domestic agrarian and processing enterprises face a number of institutional constraints on organic certification [7]. A possible way for overcoming these constraints is to develop comprehensive and systematic measures to ensure the development of innovative products and their generalization in the form of typical provisions for domestic enterprises of the oil and fat industry.

The idea of the development is based on the investigation of the value chain for innovative functional food products from walnut kernels and to develop system measures in the activities of enterprises of industry for deep processing of walnuts aimed to increase the added value of these products and ensure its competitiveness in the foreign markets. The working hypothesis is that the lack of methodological bases for elaboration of a plan for innovative management in the conditions of the Ukrainian economy reduces the export potential of agrarian and processing enterprises of the industry for deep processing of walnuts. It is expected that the development of methodological base will increase the efficiency of enterprises in industry for deep processing of walnuts.

Materials and methods

Applied methods and theoretical approaches

The research uses such principal methodologies as fundamental interviews with experts, problem-oriented group discussions, critical literature review, results of laboratory experimental studies, results of production and implementation of research and small batches of the developed innovative products. The development of innovations in the industry for deep processing of walnuts was carried out on the basis of marketing [2, 12, 14], functional [1, 3, 17, 21, 23, 27, 31, 33] and process approaches [6, 35, 37, 19, 25, 29]. To get a complete view of the stages of product flow from the raw material base to production and to the final consumer, an approach to the analysis of the value chain was used [16, 24, 26, 32, 34]. Economic and mathematical methods [3, 18, 28] and the theory of constraints [1] have been used for the development of provisions of new products. Also, developing of innovative products was conducted on the basis of Quality Function Deployment methodology [4]. For the formulation of certain theoretical positions in the process of fulfilling the set scientific tasks, there were used general scientific methods such as scientific abstraction; morphological analysis, generalization, analysis of hierarchies.

Structure of research

This paper considers the following problem issues:

1. Research of the current state and trends of the vegetable oil market in Ukraine and in the world in the following directions: research of the global production of vegetable oils; study of the global consumption of vegetable oils; study of the features of the international vegetable oil market; research of market of high-oleic oils and oilseed crops; study of the features of the investment activity and activities of producers of vegetable oils;
2. Investigation of the value chain in the production of innovative products of the industry for deep processing of walnuts in the following areas: the study of the value chain in the oil and fat industry; determination of the value chain in the industry for deep walnut processing; support of commercialization of new types of edible oils; competitiveness of the value chain in the international business; penetration into the global agribusiness value chain;
3. Investigation of the competitiveness of the enterprise of deep processing of walnuts in the following areas: the concept of the enterprise competitiveness; methodology of enterprise competitiveness management; methodological approach to innovation development in the industry for deep processing of walnuts; development of the marketing strategy for managing enterprise's innovative activity in the industry for deep processing of walnuts; design of functional food products made from walnuts;
4. Development of innovative functional food products made of walnut based on the marketing design.

Results and discussion

Research of the current state and trends of the development of vegetable oil market

The global vegetable oil market is expected to exceed 200 million tons by 2020 due to the growing popularity of healthy, organic and unrefined vegetable oils, which is supported by high demand for vegetable oil worldwide (Figure 1) [10, 11].

From the point of view of consuming as a food product, vegetable oils are considered to be a healthier alternative than animal fats [8, 17], since they contain more unsaturated fatty acids [23, 29, 31, 33]. In particular, walnut oil is widely used in cooking, medicine, cosmetics and other industries [5, 15, 17, 21, 27, 31].

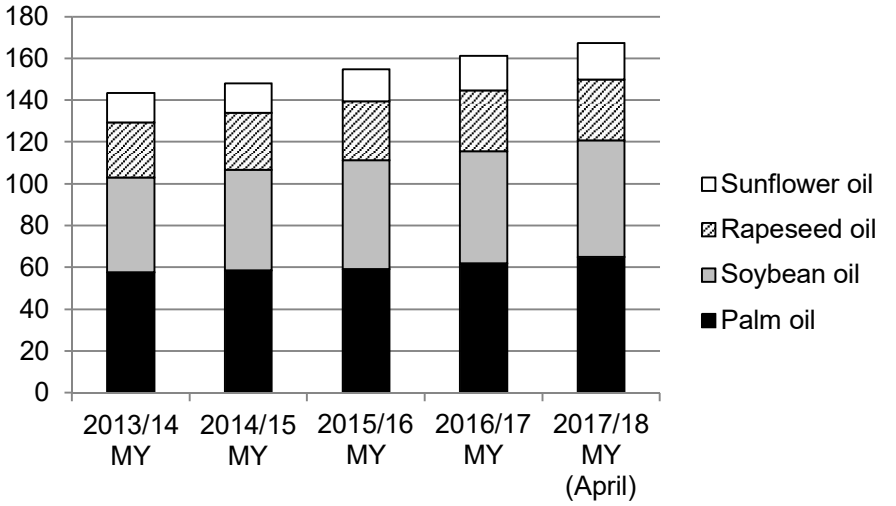


Figure 1. Dynamics of the global consumption of palm, soybean, rapeseed and sunflower oils, mln tons

Vegetable oils are oils or fats obtained from the plant. Their texture can be characterized as liquid and greasy. Most vegetable oils can be used in two ways: they can be used as cooking oil or for the production of fuels and diesel [7]. The most common types of oils include palm oil, soybean oil, rapeseed oil and sunflower oil. Palm oil is obtained from pulp of palm fruits, which are mainly found in the tropical belt of Africa, South America and Southeast Asia. It is estimated that about 90% of palm oil is used for food intake, while industrial consumption, such as cosmetic or fuel and diesel, covers the last 10% [10]. Palm oil is an industrial product with a total annual sale of approximately $50 billion. Industrial raw materials used in the production of food and confectionery products, in the cosmetics industry, for production of detergents and biofuels are also an important food product in most developing countries [11].

However, the strong growth of palm oil production creates some serious problems in the strategic perspective [13, 15, 17].

In the early 2000s, trends in the destruction of rainforests of the planet reached a critical point with the accompanying increase in greenhouse gas emissions and loss of biodiversity. It became a burden for many markets, and large multinational companies that use palm oil faced various forms of protest, including consumer boycotts [10].

Significant consumption of palm oil leads to the problems with human health [6, 17]. Palm oil has a relatively high content of saturated fats. In the research of 2003, the World Health Organization provided compelling evidence that high levels of palmatic acid increase the risk of developing cardiovascular diseases. In the countries where fat reserves in the body are low and the amount of saturated fat from animal sources is limited, this risk is more moderate. However, with the development of the economy, the habits are changing and the dietary benefits of consumers, regarding the consumption of products rich in palm oil, may disappear [7]. According to the US Academy of Sciences, the content of trans-fats in palm oil based food products is not significant and does not have any known effects on human health, but it is still highly correlated with the risk of coronary heart disease [17].

Development of the walnut raw material base and its processing into innovative functional food products with increased added value that will be able to compete in the world market is one of the main ways of solving the above-mentioned problems [16, 20, 22].

The statistics from various sources show that Ukraine holds a strong position among the world's 10 largest exporters of walnuts (Figure 2) [10, 11].

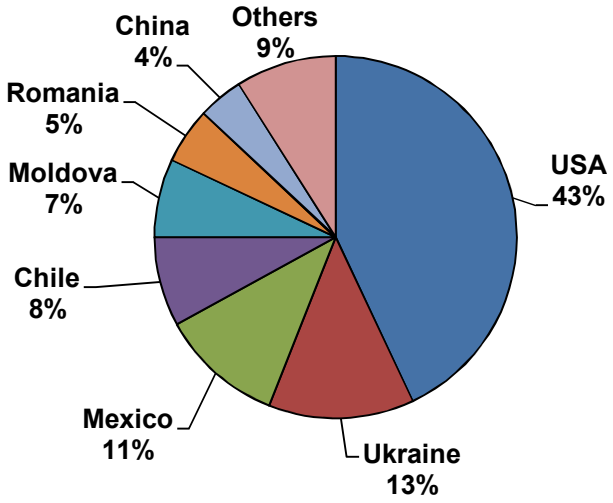


Figure 2. World walnut exports in 2014, shelled/metric tons

The world market for vegetable oil from walnut fruits is growing due to its wide range of applications [10]. Despite the fact that the world walnut market is rather specific, since production continues to be extensive and the area of land that is potentially suitable for walnut growing is very limited: walnuts usually occupy the area from 6 to 14% of the entire planet. Ukraine is lucky to be in this statistic. At the same time, walnuts do not require complicate care; the tree begins to bear fruits within 4-7 years after planting and can preserve this ability for centuries [11]. Therefore, the production of walnuts and walnut oil is very beneficial and does not cost much. According to experts, cultivation of one ton of walnuts is much cheaper than obtaining the same amount of wheat or grapes.

Data on the import and export shows that Ukraine is a net exporter of oilseeds and oils, refined edible oils, various wastes and other products (Table 1) [10, 11].

Table 1

Structure of the global oil exports by major exporting countries as of April 2017-2018 MY

Type of oil in the structure of the global exports	Ukraine's rating in the structure of the global exports	Share of Ukraine's oil exports in the structure of global exports,%
Export of soybean oil	7	1.5
Export of rapeseed oil	8	1.3
Export of sunflower oil	1	54.4

Therefore, these data justify that Ukraine will be involved in the process of diversification of the world oil and fat market and innovations development.

The supply of raw materials is only the beginning for any innovation [12]. Sale of raw materials in the world market cannot be a long-term effective solution under the lack of proper attention and investments as well as the appropriate environment [13, 14, 28].

Industry analysis

An industry can be defined either by raw materials processed by its enterprises or the end product/service intended for a particular market [5, 34]. In a broad sense, the industry includes all enterprises that are contractually bound to supply a particular set of products or services to the end users [6, 26]. The industry includes producers, processors, suppliers of raw materials and materials, exporters, retailers, etc. These entities form separate specific value chains [7, 24, 26,]. For this study and achievement of the program objectives, the value chain is defined as "a value chain of the industry of deep processing of walnuts for specific market", which primarily refers the EU market [13].

According to the definition, the industry produces a walnut kernel for the confectionery and animal feeding, edible walnut oil, industrial oil and oilcake, which can be processed into food products or used for feeding livestock in the domestic and foreign markets [3]. In addition, walnut fruits are also considered to be a product of the industry [5]. Other by-products of walnuts are being explored. In the development of the industry as well as in the short term prospect, the focus is made on the confectionery kernel and walnut oil. In the course of the industry development, innovative by-products will be created by studying and supporting the development of alternative ways of using by-products as they arise [15, 35, 37].

The system for value chain management of the production in the oil and fat industry for deep walnut processing shown in Fig. 1 contains key elements that are represented by the main entities: producers of raw materials, i.e. farmers, gardeners (1); trading enterprises engaged in the purchase and sale of raw materials. i.e. a wholesaler / wholesale buyer (2); the main processors that perform primary processing of raw materials (peeling, sorting), i.e. processors (3); processors raw materials that produce crude oil (16) and walnut oilcake (17) from walnut fruits (secondary processing), i.e. oil producer (4); after the primary and secondary processing of the walnut fruits, the waste/residues (5) and a walnut shell (6), which are the input raw materials for biofuel producers, paint and varnish industry, cosmetics industry are created (7); crude walnut oil (16) serves as an input raw material for the production of finished products in the form of oil in bottles / ingredients (11) both by producers of crude oils (4) and processors that conduct refining, blending, flavoring (8) of walnut products; walnut oil cake (17) is a feedstock for walnut flour producers (9) and producers of livestock feeds (10); walnut flour is input raw material for confectionery industry (12) producing bakery products (13) and candies, and other products (14); all products of the industry for deep walnut processing are the input product for distribution (15).

Analysis of the subjects of the value chain in the industry for deep walnut processing and the functions performed by them should consider primary and secondary subjects and their respective roles. The main subjects are those who are engaged in the principal production activities in the value chain (supply, production, processing and sale of products) [26]. Secondary subjects are involved in the main activities of the value chain not directly – indirectly, but their actions affect the activities of the main subjects of the value chain [34]. Secondary entities offer support and provide services in the value chain. Some of the secondary subjects are policy and regulatory support agencies, financial support institutions and providers of business support services [13]. Further research should be specified in accordance with these two categories of subjects of the value chain.

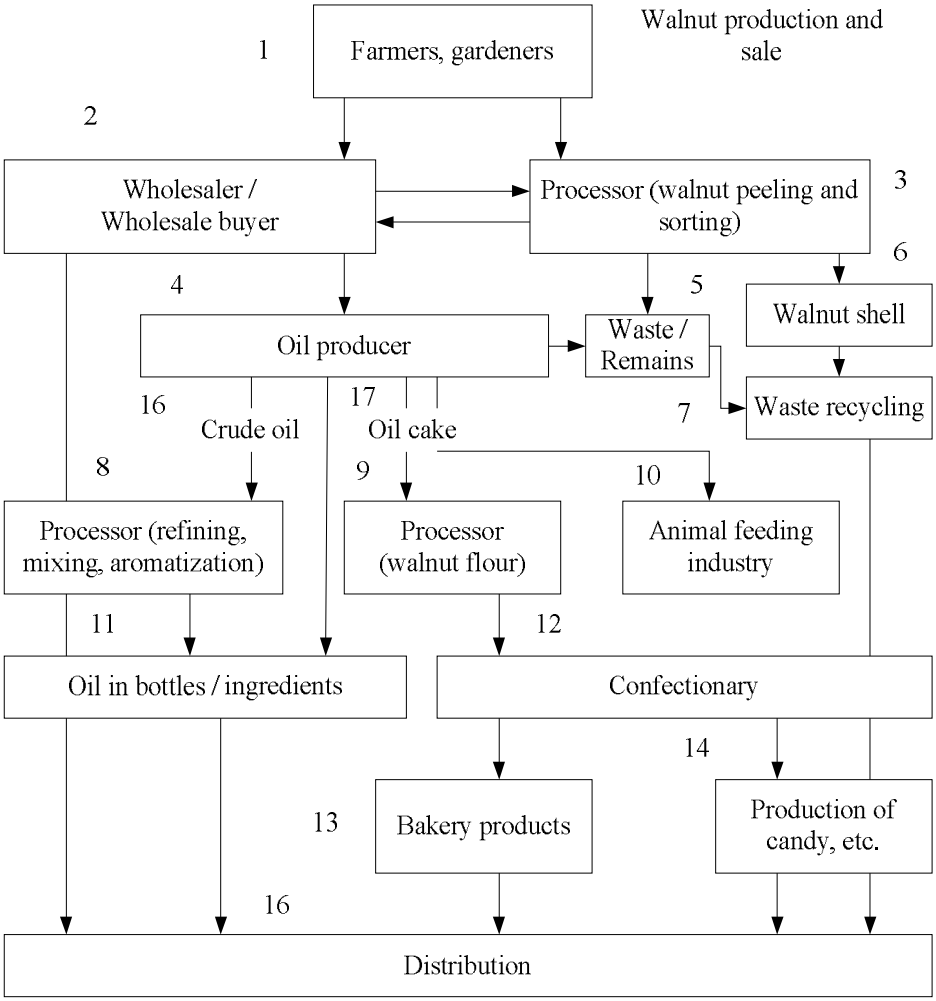


Figure 3. Cartographic model of the value chain for production in the oil and fat industry for deep walnut processing

Competitiveness of enterprises of the oil and fat industry

The current state of development of competition theories is characterized by a variety of subjects of analysis, which are the principles of activity in a competitive environment [12]. The actual difference between them is reduced to the difference in the number of variables that they cover and their predictive capacity for individual situations [13]. In addition, sectoral features are important factors of the competitiveness of enterprises [14].

Generalization of approaches to the definition of competitiveness [2, 12, 14, 22] has allowed to propose an author's approach to the defining the competitiveness of a manufacturing enterprise as a set of its capabilities and ability to produce products that are

can satisfy current social needs and abilities that lead the enterprise to a state in which its market and financial stability is provided by means of specific competitive advantages.

Figure 4 shows a non-waste competitive technology for production of innovative functional food products from walnuts, which provides comprehensive use of raw materials, processing of nut into oil and oilcake, processing of remains [15] into biofuels [9, 11], selection of the obtained products and their distribution depending on the values of qualitative indicators between such application areas as confectionery industry, livestock and poultry feeding, baking industry, spice production, biofuel production, paint and varnish industry, cosmetic industry [5, 22].

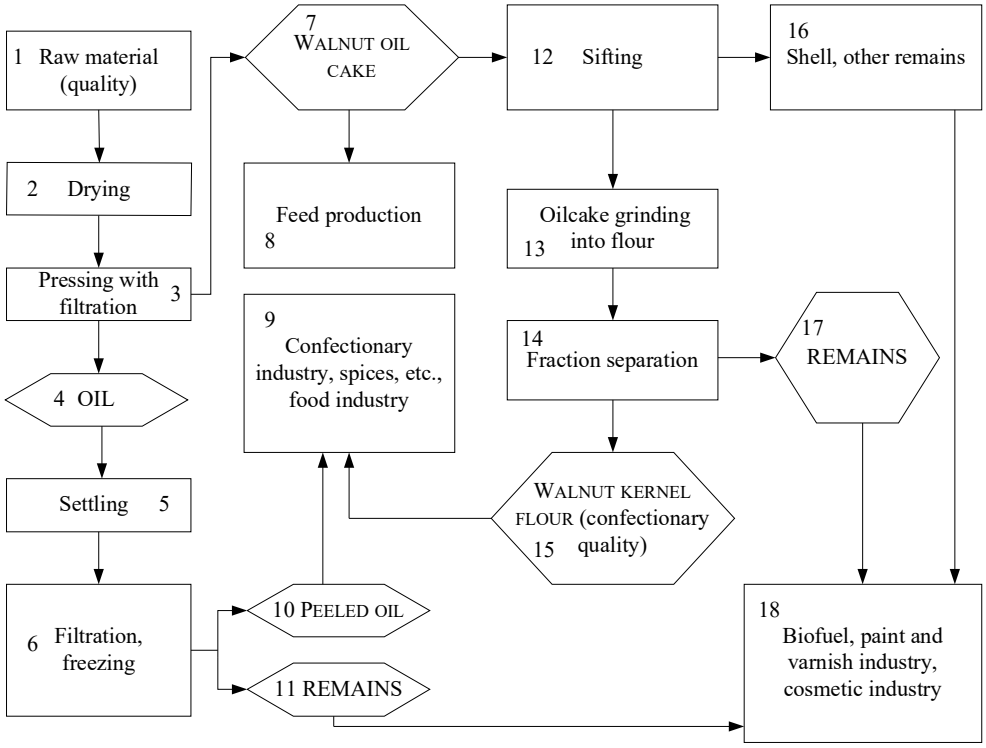


Figure 4. Non-waste competitive technology for production of innovative functional food products from walnuts

The proposed technology is implemented in the following way: raw materials and information on its qualitative characteristics serve as input resources (1); raw materials are connected with the elements of the system from its preparation to the technological process of processing – equipment for drying, operator, change in quality indicators (2); (3) – a complex of equipment and procedures for the processing of raw materials into oil (4) and oilcake (7); oil (4) serves as an input for the operation of the complex for settling (5), filtering and freezing (6); the output of these elements of the system is pure oil, information on its quality indices (10) and remains (11); walnut oil cake (7) is an input resource for the operation of the feed production complex (8) and sifting (12), grinding (13), separation of fractions

(14); the input resources for the complex operation on fraction separation (14) are the shell and other remains (16,17) and the confectionery walnut flour (15); the final elements of the system are complexes for the production of confectionery, spices and other food products (9) and production of biofuels, paint and varnish industry, cosmetic products (18).

Thus, the proposed technology involves a non-waste production cycle and it can become the basis for diversification of production, differentiation of markets and ensuring a number of unique competitive advantages for manufacturing enterprises, as well as the involvement of agricultural enterprises of the oil and fat industry in agro-industrial and agro-food chains of various levels.

Application of measures of innovative products creation

The scientific and technical levels of the developments correspond new developments in Ukraine in terms of equipment and the best world analogues in terms of products [15, 17, 19, 23, 27, 33, 35, 37]. The developed equipment and products have undergone the examination by the State Sanitary and Epidemiological Service of the Ministry of Health of Ukraine.

When designing innovative functional food products, the methodology of deployment of the quality function [4] supplemented by the authors' methodological developments has been used. There were two groups of experts that conducted evaluation of different characteristics of products and production process. The results of experts examining were statistically approved by using Kendall`s coefficient of concordance and Pearson`s coefficient of correlation. The results of designing are as follows:

- list of characteristics of the elements of the process of production of projected products in connection to consumers wants and complex of marketing of enterprise. This characteristics meet the requirements of a gradual process of Quality Function Deployment in next pair-order: customers' needs and wants → complex of enterprise marketing, customers' needs and wants → product characteristics, product characteristics → components characteristics, components characteristics → process characteristics, process characteristics → production characteristics;
- weighting coefficient for implementing Quality Function Deployment (QFD) of the product range of TM "Food of Heroes", Private Joint-Stock Company "Vinnytsia Pasta Factory", Private Joint-Stock Company "Vinnytsia Food Factory". Given coefficients were used to compare competitiveness of developed products with existed analogues (Walnut oil *M. Graham & Co. Oil Color.* United States of America; La Tourangelle artisan oil. United States of America. Virgin organic walnut oil. France, ets.) [21, 23]. In addition, the current product specification is adopted as an analogue. CAS 8024-09-7 of the Chemical Abstracts Service (American Chemical Society) for the Juglans regia l food product. seed oil Class: Oils: Vegetable Oil and Fish Oil (Marine);
- houses of quality built in the process of designing consumer innovative functional food products from walnuts. On the basis of given results there are changes made to adopt the assortment of products to customers wants and to develop corresponding marketing complex.

Conclusion

1. The results of the research meet the goals of Ukraine's sustainable development presented on September 15, 2017 by the Government of Ukraine in the National Report "Objectives

of Sustainable Development: Ukraine", which defines the basic indicators for achievement (OSD).

2. The practical value of the results of the research and developments is the possibility of using the obtained theoretical and methodological conclusions, recommendations and regulations on the creation of innovative functional food products from walnut fruits for agrarian and food processing enterprises to solve the problem of competitiveness of the enterprise and its products when exporting on the basis of the compliance of production with the modern standards and norms on the global market in conditions of high dynamics of the business environment taking into account system links of the manufacturing enterprise.
3. The developed model of the value chain for production in the oil and fat industry for deep walnut processing can be used for creation of innovative products with their own partial value management systems by studying and supporting the development of alternative ways to use by-products as they arise.
4. The developed non-waste competitive technology for production of innovative functional food products from walnuts can be used for the creation of innovative products in the industry of deep processing of walnuts. It has a non-waste cycle of combination of elements and is the basis for diversification of production, differentiation of markets and creation of a number of unique competitive advantages for production enterprises of the agro-food chain, as well as the involvement of domestic agricultural enterprises of the industry of deep processing of walnuts in global agro-industrial and agro-food chains.
5. The obtained results of marketing products modeling can be used for connecting different properties of new products and marketing complex of enterprise with characteristics of production plant.

Acknowledgements. The authors would like to thank TM "Food of Heroes", to the Ukrainian Nut Association. All major results of this research and most of the developments were obtained and implemented on the basis of Vinnytsia National Agrarian University.

References

1. Goldratt E.M., Cox J. (2014), *The Goal: A Process of Ongoing Improvement,* 30th Anniversary Edition. Great Barrington, North River Press, 362.
2. Porter, M.E. (1985), *Competitive Advantage: Creating and Sustaining Superior Performance,* New York: Simon and Schuster, Retrieved 9 September 2013.
3. Bruno S.S. (2003), *Economic Dynamics in Transitional Economies: The Four–P Governments, the EU Enlargement, and the Bruxelles Consensus,* Psychology Press.
4. Akao Y. (1972), *New Product Development and Quality Assurance – Quality Deployment System*, Standardization and Quality Control, 25(4), pp. 7–14.
5. Andreeva T.V., Ermakovam Z.A. (2011), *Formation of product value chains in the food industry,* Bulletin of the OGU, 1(120), pp. 108–113.
6. Lelieveld H. (2015), Food safety regulations based on real science, *AgroLife Scientific Journal,* 4(1), pp. 93-96.
7. Al Shamsi K.B., Compagnoni A., Timpanaro G., Cosentino S.L., Guarnaccia P. (2018), A Sustainable Organic Production Model for "Food Sovereignty" in the United Arab Emirates and Sicily-Italy, *Sustainability,* 10, 620. doi:10.3390/su10030620.

8. Shkuratov O.I., Chudovyska V.A., Vdovychenko A.V. (2015), *Organic agriculture: ecological and economic imperatives of development: Monograph*, LLC "DIA"
9. ETIP BIOENERGY SRIA 2018. (2018), *The European Biofuels Technology Platform*, Available at: http://etipbioenergy.eu/images/ETIP_SRIA_2018.pdf
10. Nuts&Dried Fruits. (2017), *Global statistical review 2015/2016.* International Nut&DriedFruit (INC).
11. Sobolev D. (2015), *Ukraine. Tree Nuts Annual. Walnuts Annual Report,* USDA Foreign Agricultural Servise, GAIN Report UP1534.
12. Porter M. (1993), *International Competition.* Translated form English; Ed. D. Shchitinin, Moscow: International conferences.
13. Sherer F.M., Ross D. (1997), *Structure of sectoral markets*. *Translated from English, T.6*, "INFRA-M",Moscow,
14. Kotler F. (2006), *Marketing Management. Express course,* Peter, St. Petersburg.
15. Otles S., Despoudi S., Bucatariu C., Kartal C. (2017), Food waste management, valorization, and sustainability in the food industry, *Food Waste Recovery. Processing Technologies and Industrial Techniques,* pp. 3-23.
16. Baldwin, R. (2012), "Trade and Industrialisation after Globalisation's Second Unbundling: How Building and Joining a Supply Chain are Different and Why it Matters", in *Globalization in an Age of Crisis: Multilateral Economic Cooperation in the Twenty-First Century*, R. Feenstra and A. Taylor (eds.), University of Chicago Press.
17. Kiritsakis A.K. (2002), Virgin olive oil composition and its effect on human health, *Inform.*, 13, pp. 237-241.
18. Barbalet F., J. Greenville W. Crook P. Gretton, R. Breunig (2015), "Exploring the Links Between Bilateral and Regional Trade Agreements and Merchandise Trade", *Asia & the Pacific Policy Studies*, 2(3), pp. 467-84.
19. Savoire, R., Lanoisellé, J. L., Vorobiev, E. (2013), Mechanical continuous oil expression from oilseeds: a review, *Food and Bioprocess Technology*, 6(1), 1-16.
20. Bas, M. and V Strauss-Kahn (2014), Does Importing More Inputs Raise Exports? Firm-Level Evidence from France, *Review of World Economics*, 150(2), pp. 241–475.
21. Matthaus, B. and Brühl, L. (2003), Quality of cold-pressed edible rapeseed oil in Germany, *Nahrung/Food*, 47(6), pp. 413-419.
22. Bojnec, S. and I. Ferto (2015), Global Agri-Food Export Competitiveness, *Paper presented at the International Conference of Agricultural Economists, Milan, Italy*, 8-14 August 2015.
23. İmer Y., Taşan M., (2018), Çeşitli soğuk pres yağların bazı mikro ve makro element içeriklerinin belirlenmesi, *Tekirdağ Ziraat Fakültesi Dergisi*, 15(01), p. 15.
24. Cadestin C., J. Gourdon, P. Kowalski (2016), "Participation in Global Value Chains in Latin America: Implications for Trade and Trade-Related Policy", *OECD Trade Policy Papers*, No. 192, OECD Publishing, Paris, Doi: 10.1787/5jlpq80ts8f2-en.
25. Ionesu M., Vladut V., Ungureanu N., Dınca M., Zabava B. S., Stefan M. (2017), Methods for oil obtaining from oleagınous materials, Annals of the University of Craiova-Agriculture, *Montanology, Cadastre Series*, 46(2), pp. 411-417.
26. De Backer K. and S. Miroudot (2013), Mapping Global Value Chains, *OECD Trade Policy Papers*, 159, OECD Publishing, Paris, DOI: 10.1787/5k3v1trgnbr4-en.
27. Kirschenbauer H. G., (1944), *Fats and Oils, Reinhold Publishing*, New York, pp. 122–123.
28. Diaz-Bonilla E., Orden D., Kwieciński A. (2014), Enabling Environment for Agricultural Growth and Competitiveness: Evaluation, Indicators and Indices, *OECD Food,*

Agriculture and Fisheries Papers, 67, OECD Publishing, Paris, DOI: 10.1787/5jz48305h4vd-en.
29. Niranjan K., Hanmoungja P. (2004), *Nutritionally Enhanced Edible Oil and Oilseed Processing*, Chapter 5- Enzyme-Aided Aqueous Extraction, AOCS Press, USA.
30. Drabenstott M, (1995), "Agricultural Industrialization: Implications for Economic Development and Public Policy", *Journal of Agricultural and Applied Economics*, 27(1), pp. 13–20.
31. Choo W. S., Birch J., Dufour J.P. (2007), Physicochemical and quality characteristics of cold-pressed flaxseed oils, *Journal of Food Composition and Analysis*, 20(3-4), pp. 202-211.
32. Ferrantino M.J. (2012), Using Supply-Chain Analysis to Analyse the Costs of NTMs and the Benefits of Trade Facilitation, *World Trade Organization Working Paper,* ERSD 2012–02.
33. Siger A., Nogala-alucka M., Lampart-Szczapa E. (2008), The content and antioxidant activity of phenolic compounds in cold-pressed plant oils, *Journal of Food Lipids*, 15(2), pp. 137-149.
34. Gereffi G., Fernandez-Stark K. (2011), *Global Value Chain Analysis: A Primer,* Center on Globalization, Governance & Competitiveness (CGGC), Duke University, Durham, NC.
35. Maskus K.E., Otsuki T., Wilson J.S. (2005), The Cost of Compliance with Product Standards for Firms in Developing Countries: An Econometric Study, *World Bank Policy Research Working Paper*, No. 3590.
36. Manuela Tvaronavičienė, Dalia Prakapienė, Kristina Garškaitė-Milvydienė, Romas Prakapas, Łukasz Nawrot (2018), Energy Efficiency in the Long-Run in the Selected European Countries, *Economics & Sociology*, 11(1), pp. 245-256, DOI: 0.14254/2071-789X.2018/11-1/16
37. Otsuki T. (2011), Effect of International Standards Certification on Firm-Level Exports: An Application of the Control Function Approach, *Osaka School of International Public Policy Discussion Paper,* No. 11E005.

Lithuanian carrot market: production, foreign trade, and price transmission issues

Nelė Jurkėnaitė[1], Dimitrios Paparas[2]

1 – Lithuanian Institute of Agrarian Economics, Vilnius, Lithuania
2 – Harper Adams University, Shropshire, the United Kingdom

Keywords:

Agriculture
Carrot
Price transmission
Supply chain
Trade

Article history:

Received 05.08.2018
Received in revised form 24.11.2018
Accepted 28.03.2019

Corresponding author:

Nelė Jurkėnaitė
E-mail:
nele@laei.lt

DOI: 10.24263/2304-974X-2019-8-1-17

Abstract

Introduction. The paper aims to analyse the situation in Lithuanian carrot market and focus on the changes of agricultural production, structure of foreign trade, and vertical price transmission along the supply chain over the period of 2011–2017.

Materials and methods. Methods of comparative and graphical analysis of the main indicators of carrot production and foreign trade development rely on the secondary data of Statistics Lithuania. The research on the vertical price transmission along the supply chain of domestic carrots relies on price series collected by SE 'Agricultural Information and Rural Business Centre' and employs unit root tests, cointegration, causality and asymmetry tests.

Results and discussion. The conducted research evidences that over the analysed seven years carrot production in Lithuania has shrank. Harvested area dropped from 2,400 to 1,800 ha, while the harvest reduced by more than 1/3. The key driving forces behind the negative development trends were prolonged unfavourable weather conditions and the Russian import ban of 2014. The transformation of the export structure took place during the unsuccessful harvesting years and contributed to the worsening of farmers' welfare.

The analysis of the farm-retail prices shows that the examined series are integrated of I(1). Thus, we found a significant structural break in April 2015, which has been included as a dummy variable in the cointegration approaches. The Engle-Granger cointegration test confirms the presence of the long-run relationship between the analysed price series. The Granger causality test shows that the causality is running from retail to farm prices. Finally, there is a strong evidence of symmetric price behaviour, i.e. positive and negative shocks are transmitted from retailers to farmers with the same intensity.

Conclusions. The study shows that the prolonged unfavourable weather conditions and the Russian import ban were among the main contributors determining price fluctuations on the Lithuanian carrot market in 2011–2017. The analysis of vertical price transmission of domestic carrots indicates that retailers may experience some market power.

Introduction

In Lithuanian agriculture carrots remain an important vegetable, which has demand on domestic and foreign markets. Although over the last seven years carrots have represented almost the same share in the total harvested area of Lithuanian vegetables (in 2011 accounted for 16.44%, in 2017–15.65% [30]), the changes in carrot production were more dramatic than the alteration of average indicators of the EU agriculture and witnessed the shrinking of the domestic production.

This research investigates carrot price as one of the factors which could contribute to market inefficiencies and encourage farmers to leave carrot production if they believe that their welfare is violated. Such behaviour is explained by cobweb theory arguing that production depends on the price during the previous period [28]. The paper also contributes to the academic literature which states that a traditional markup concept often does not explain price behaviour in the market, because we often observe the lagged or incomplete price transmission among the supply chain [16]. Conclusions on asymmetric price behaviour in the supply chains of agricultural commodities are common. The divergence from the markup concept to some extent could be explained by the theoretical model [11] investigating the changes in state-of-the-art of consumers' demand, marketing services or farm supply. However, it is argued that empirical research enrich our knowledge about price asymmetry [2], while most of the current theoretical models face limitations and cannot explain price behaviour properly.

According to previous academic studies, the dissonance with the markup concept could be explained by different factors affecting vertical or spatial price transmission and market efficiency. Some of these factors are commodity- or country-specific, while another could be used as an explanatory variable for a wider phenomenon. The main factors which influence price behaviour on the market of agricultural commodities are level of perishability [2, 4, 21, 22, 25, 27], market power and concentration related indicators [2, 4, 20, 21, 24, 25, 29], selected risk-management and pricing strategies [19, 20], imperfect information [20, 21, 24, 27] and search costs [4], adjustment or menu costs [4, 21, 24, 25], the shortage of stored commodities or speculation by the shortage [14, 18, 20, 24], the expectation of price change [2, 12], outbreaks of diseases related to agricultural production [22], trade restrictions [22, 25], interventions of policy makers [2, 24], annual and long-term climate changes [14, 22, 29], seasonality [4, 29], the shortage of water resources [14], structural and economic crises [22], the presence of competitive proposals, including the substitution by imported products [20], and etc. The complexity of the phenomenon confirms the statement that each case of the price transmission in the supply chain is unique as the country of origin, industry, and the selected for the analysis time period make difference [2].

This statement is supported by many studies with contradicting results for different countries and supply chains. It should be noted that carrots as an object of price transmission research were selected only in a few studies [4, 6, 12, 15, 17, 24, 27], while the problems of vertical price transmission in the vegetable sector generated an impressively high number of publications (reviews on such studies are provided in [1, 3, 23, 24, 26]). The aforementioned studies on carrot price transmission investigate cases of the USA [6, 15, 27], Italy [24], the United Kingdom [17], Austria [17], Germany [12, 17], Ireland [17], and Hungary [4]. Most of the mentioned papers analyse the short- and long-term relationships between prices along the supply chain of carrots. These studies differ by number of stakeholders in the supply chain and employ various data frequency. Findings confirm both the asymmetric and symmetric price transmission along the supply chain of carrots, but the cases of the

symmetric transmission are more often. The price leading supply chain stakeholders also differ.

However, three of the aforementioned studies select less common research directions. Two papers investigate the differences in behaviour of organic and conventional carrot prices in the USA and find the evidence of symmetric [15] and asymmetric behaviour [6]. These studies also show the different adjustment to the long-run equilibrium. Another paper estimates the impact of Euro introduction on vertical price transmission of carrots and concludes that there is no evidence of significant impact on changes of retail prices [12].

The paper aims to analyse the situation in the Lithuanian carrot market and focuses on the changes of agricultural production, structure of foreign trade, and vertical price transmission along the supply chain over the period of 2011–2017. The research contributes to the scientific discourse on vertical price transmission adding the case of Lithuanian domestic carrot supply chain, which has not attracted an in-depth analysis until now.

Materials and methods

In order to achieve the aim, the paper combines two subtasks and applies different research methods. The first subtask analyses the changes of domestic carrot production and the structure of foreign trade. The comparative and graphical analysis of the secondary data collected by Statistics Lithuania is conducted. The analysis identifies the most important aftermaths of these changes for the period from 2011 to 2017 and discusses the development of carrot price.

The second subtask investigates the vertical price transmission along the supply chain of carrots of Lithuanian origin on domestic market during the period from 2011 to 2017. This subtask relies on the prices derived from the secondary data collected by the SE 'Agricultural Information and Rural Business Centre'. The farm price is calculated as the average of minimum and maximum price of carrots on farms. The retail price shows the average price of seven Lithuanian counties for not prepacked carrots in the main network supermarkets. The weekly data is aggregated into monthly frequency using average values.

Monthly prices in Figure 1 demonstrate that both farm and retail prices experience twofold impact of seasonal changes. First, the early start of the harvesting season is characterized by the sharp increase in price level, while the nearing to the end of the season shows the decrease of prices. Second, the introduced price series advise of a remarkable success solving the problem of domestic carrot availability for the consumer throughout the year. An uninterrupted supply of domestic carrots at the retail level became available only after 2013. However, during the analysed period farm price series have repetitive gaps as the selling of the last year harvest on farm is done earlier than the early season carrots appear on the shelves of supermarkets.

The review of the literature on vertical price transmission in vegetable sector allowed to select econometric tests for the analysis of relationships between farm and retail prices. Thus, the judgement about the efficiency of the domestic supply chain and the functioning of carrot market could be done. First, the analysed price series are tested for the stationarity in order to select the appropriate research methods. The augmented Dickey-Fuller (ADF) test [7] is run to classify the price series as stationary or non-stationary.

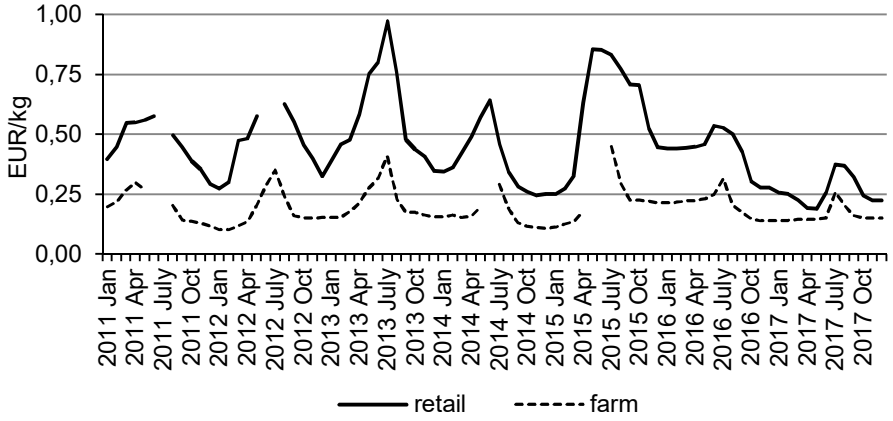


Figure 1. Average carrot prices: retail and farm levels
Source: own calculations on the basis of the data collected by SE 'Agricultural Information and Rural Business Centre'

The long-run relationship between two tested variables can be affected by the presence of structural breaks in the data. These possible breaks can be a result of economic regime or a change in the factors (support system or taxation, population, etc.) that determine and affect the tested series. Hence, if structural breaks are not taken into account when investigating the existence of a long-run relationship, there is a possibility that linear methods may fail to confirm the relationship when in fact it does exist.

Second, the short-run and long-run relationships between farm and retail price series are investigated. The nature of the long-run relationship is investigated applying two-step Engle-Granger cointegration technique [8], because time series are integrated of order one. This approach is based on the idea that if there is a cointegration between the variables, the residuals obtained from ordinary least squares equations have to be stationary. So, in order to test for the long-run relationship between retail and farm prices, we are testing the stationarity of residuals with the help of ADF.

The Granger causality test [13] reports about the short-run relations and characterizes the direction of the farm and retail price causality along the vertical supply chain of carrots and the nature of this feedback. The Granger causality tests if one price series of carrots can be predicted by historical data of another supply chain level's price series better than by carrot prices data in the past. The Granger causality test investigates two null hypotheses (H_0): 1) 'Carrot retail price does not Granger cause carrot farm price'; 2) 'Carrot farm price does not Granger cause carrot retail price'. The estimation of these two H_0 will assist in setting the direction of causality and identifying the price leading stakeholder along the carrot supply chain.

The aforementioned tests have assumed that there is linearity and symmetry within the long-run relationship. However, the previous research on price transmission in carrot sector confirms that asymmetric price behaviour is often. Therefore, a momentum Threshold Autoregressive model [9, 10] is applied in order to ensure that asymmetries within the transmission of prices have been accounted for. The first step tests the price series for the cointegration. If cointegration is found, the next step tests for asymmetry within the

relationship. This will identify if the price series reacts differently to an increase or decrease from the long run equilibrium or if the price behaviour is symmetric.

Results and discussion

Production

In 2016, carrots were on the top three list of the most important vegetables in the European Union (EU) [31]. Over the past seven years the EU carrot production has not demonstrated significant changes in cultivated area and harvested production. According to Eurostat [31], the main carrot producing countries were Poland (14.71%), the United Kingdom (12.96%), and Germany (11.48%), while the highest shares of cultivated area were in Poland (19.17%), France (10.87%), and Italy (9.88%). In 2016, Lithuania produced 0.77% of the EU carrots and the cultivated area accounted for 1.56% of the land related to the EU carrot production.

The carrot is one of the most popular vegetables grown in open fields of Lithuania. The largest share of carrots is harvested on farmer and family farms (Table 1). In 2017, the harvest of carrots on farmer and family farms accounted for 95.23% of the total harvest on all farms compared to 93.33% in 2011 [30]. Although the structure of stakeholders involved in carrot production remains stable, the changes of the main indicators of carrot production are visible.

In 2017, the harvested area was 1,800 ha and this indicator demonstrated a decrease by 25.00% compared to 2011 [30]. According to Statistics Lithuania, the average yield on farmer and family farms is lower than at agricultural companies and enterprises. During the analysed period, the minimum and maximum average yields fluctuated from 22.69 to 33.17 tonnes per ha [30]. However, starting from the year 2015 the average yields were extremely low and reached the bottom in 2017.

Table 1

Main indicators of the Lithuanian carrot production

	Harvest, thou tonnes		Average yield, tonnes per ha		Harvested area, thou ha	
	Farmer and family farms	All farms	Farmer and family farms	All farms	Farmer and family farms	All farms
2011	65.80	70.50	28.26	29.32	2.30	2.40
2012	65.30	67.80	32.14	32.31	2.00	2.10
2013	54.40	57.50	25.87	25.99	2.10	2.20
2014	64.90	68.10	32.74	33.17	2.00	2.10
2015	39.30	43.80	22.27	23.57	1.80	1.90
2016	46.20	49.30	22.64	23.10	2.00	2.10
2017	39.90	41.90	22.15	22.69	1.80	1.80
2017 compared with 2011 (2011=100%)	*60.64*	*59.43*	*78.38*	*77.39*	*78.26*	*75.00*

Source: Statistics Lithuania [30], own calculations.

Table 1 demonstrates that Lithuanian farmers have faced significant difficulties over the analysed period and the number of low harvest years dominates over the successful years. The first challenging year was 2013. After the cold, rainy spring and dry summer the average yield accounted for 80.44% of the level of 2012. The late start of the harvesting season in many EU countries and the early shortage of the previous year harvest at Lithuanian storage houses contributed to the sharp growth of both retail and farm prices (Figure 1). However, the most challenging for farmers was the unfavourable weather period starting from 2015. The decreased carrot supply on domestic market and the growth of prices for the imported carrots determined a spike of carrot price in 2015. Although policy targeted to control the most important prices after the introduction of Euro in January 2015, the change of currency resulted in the growth of input prices and had an impact on farmers' welfare during the low harvest period. In 2016 and 2017, farm prices were not beneficial for the long-standing low harvest situation in carrot production.

It should be noted that the shrinking production of vegetables in Lithuanian agriculture was a talking-point during the national planning of the direct payment model for the period of 2014–2020. As a result, Lithuania identified this niche as experiencing difficulties and included the voluntary support, coupled to production, into the model of direct payments starting from the year 2015. The payment for field vegetables amounted to 324.2 EUR/ha^2 in 2015, 310.9 EUR/ha^2 in 2016, and 381.8 EUR/ha^2 in 2017.

During the analysed period, additional policy interventions were made to support communication and sales at vegetable market, promote consumption of vegetables at pre-school institutions and primary classes. The stabilization of supply and price on the carrot market after the Russian ban was ensured introducing support for market withdrawals and harvesting measures. This type of support remained relevant for carrot producers even in 2017. However, the changes of the harvested area for the period 2015–2017 show that unfavourable weather conditions and reduced margins were more important arguments than policy interventions.

Foreign trade

Another important driving force encouraging or discouraging to develop carrot production could be the situation on the market, i. e. both export/import situation and market efficiency problems in the domestic supply chain. The comparisons of import, re-export, and export indicators during the period of 2011–2017 evidence the important changes of the foreign trade situation (Figure 2). The analysis of the foreign trade situation is conducted using the aggregated indicator of carrots and turnips (CN code: 07061000). It is important to note that the production of turnips is not significant and the aggregated indicator could be used to monitor changes on the carrot market.

The impressive decrease of import from 2016 is explained by a significant decline of re-export to Russia and Belarus. To be more specific, the drastic changes in the structure of export and import were aftermaths of the response to the EU sanctions over the Ukraine crisis. In August 2014, Russia imposed an import ban on a list of agricultural commodities of the EU origin, and trade restrictions included the vegetable sector. As a result, during the analysed period, export and re-export of carrots and turnips reduced twice, and Lithuanian producers of carrots switched the trade from third countries to the EU market (Table 2).

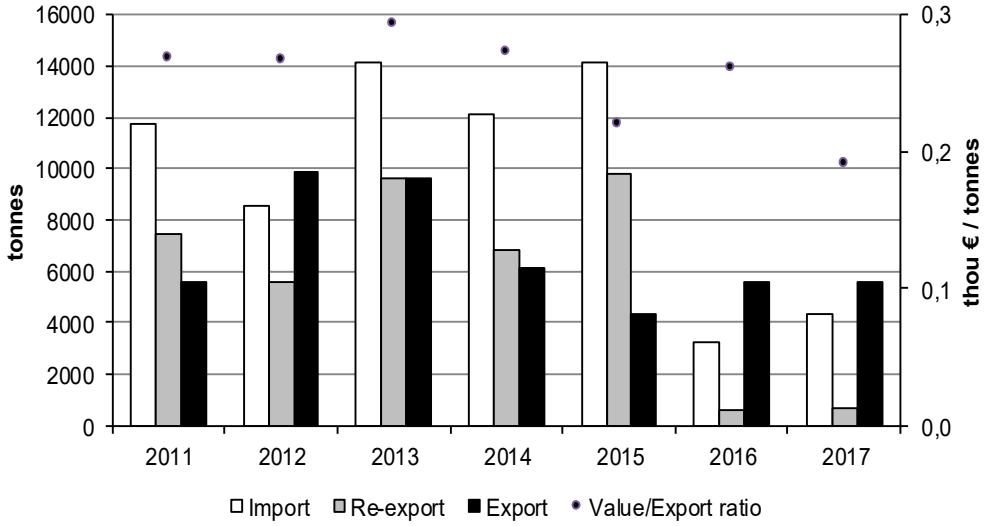


Figure 2. Developments of export, re-export, and import of carrots and turnips in 2011–2017
Source: Statistics Lithuania [32], own calculations.

During the analysed period, the share of third countries in the structure of export dropped from 92.10 to 7.77%. The structure of Lithuanian carrot export became more diversified, and the Baltic countries occupied the position of main trading partners. Although the decrease of the total amount of exported carrots and turnips was dramatic, the amount of the exported domestic carrots and turnips had increased and was slightly higher than the level of 2011.

However, the comparison of the annual indicator of value per tonne over the period of 2011–2017 shows that access to the market of third countries allowed to ensure higher and more stable indicator for domestic carrots and turnips until the period of import ban (Figure 2). The changes in the structure of foreign trade reduced the annual indicator of value per tonne and had an impact on farmers' welfare.

Domestic supply chain

Resuming the results for the period of 2011–2017, the development of carrot production in Lithuania was heavily dependent on weather conditions and trade restrictions. However, market inefficiency issues related to price changes along the supply chain of carrots could be an important contributor to the shrinking of domestic carrot production too. In order to identify market development problems the nature of the long-run and short-run relations between farm and retail prices of carrots is investigated.

First, the unit root test is conducted. The results of ADF test at level show that for both farm and retail prices absolute values of *t*-statistic is lower than critical values at 5.00% significance level (Table 3). Thus, the H_0 that data series contain a unit root cannot be rejected. However, ADF tests at first differences show that the H_0 could be rejected.

Table 2

Structure of carrot and turnip export in 2011–2017

Year	Export structure by country (%)	Total amount of exported carrots and turnips, tonnes
2011	**RU***(51.18), LV*(4.95), **BY***(0.82), EE*(0.15), PL*(0.09), DK*(0.00). **RU**(39.15), LV(2.70), **BY**(0.95), GB(0.01), DK(0.00)	13,002.80
2012	**RU***(30.11), LV*(4.83), EE*(0.54), **BY***(0.35), BG*(0.19), NL*(0.14), PL*(0.03), GB*(0.00). **RU**(59.59), LV(4.07), **BY**(0.13), GB(0.02), IE(0.00).	15,430.13
2013	**RU***(40.20), LV*(6.28), **BY***(2.38), EE*(0.44), NL*(0.29), GB*(0.13), PL*(0.11). **RU**(43.54), LV(6.36), **BY**(0.25), GB(0.02), IE(0.00), FR(0.00).	19,269.66
2014	**RU***(28.50), **BY***(21.04), LV*(1.81), EE*(0.95), **KZ***(0.07), NL*(0.05), **MN***(0.01), **AF***(0.01), GB*(0.00). **RU**(33.94), LV(11.41), **BY**(2.07), **KZ**(0.10), GB(0.04), IE(0.00).	12,973.89
2015	**BY***(59.13), **RU***(5.59), LV*(3.34), PL*(0.20), EE*(0.64), NL*(0.45), **KZ***(0.01), GB*(0.00), IT*(0.00). LV(17.14), **BY**(5.98), PT(2.77), CZ(2.49), EE(1.12), NL(1.11), GB(0.03).	14,144.94
2016	GB*(4.22), **BY***(2.83), LV*(2.56), **RU***(0.55), EE*(0.24), **KZ***(0.02), IT*(0.02), PL*(0.01), ES*(0.00). LV(66.87), EE(14.54), **BY**(5.55), NL(2.27), GB(0.14), PL(0.09), **KZ**(0.04), DK(0.01), **AF**(0.04), BG(0.00).	6,259.17
2017	**BY***(6.38), LV*(3.55), EE*(0.38), PL*(0.13), IT*(0.06), **RU***(0.03), GB*(0.00). LV(62.58), EE(24.35), **BY**(1.34), NL(0.67), GB(0.49), **KZ**(0.02), DK(0.02), PL(0.00), FI(0.00)	6,290.05

The asterisk refers to re-export of carrots and turnips. Bold font signifies export of carrots and turnips to third countries. ISO standard is applied to code countries.

Source: own calculations on the basis of the data collected by Statistics Lithuania [32].

Table 3

ADF unit root test results for the retail and farm carrot prices

Variables		ADF *t*-statistic	Test critical value at 5.00% significance level	MacKinnon (1996) one-sided *p*-values
Farm price (0)		0.09	-1.95	0.71
Retail price (2)		-1.05	-1.95	0.26
D (Retail price (1))*		-5.13	-1.95	0.00
D (Farm price (1))*		-3.97	-3.47	0.01
Unit root with single break				
Variables	**Break date**	**ADF *t*-statistic**	**Test critical value at 1.00% significance level**	**Vogelsang (1993) asymptotic one-sided *p*-values**
D (Farm price (0))*	2012M05	-6.60	-4.95	<0.01
D (Retail price (1))*	2015M04	-5.96	-4.95	<0.01

The lag length (based on Schwarz Information Criterion, maxlag = 11) for the price series is provided in parentheses. * means that H_0 can be rejected. [Source: own calculations].

The similar results of price stationarity at first difference were found for carrots on markets in the USA [6, 15], while in the United Kingdom [17], Germany [17], and Hungary [3] price series were stationary. The study of Austrian market showed that producer prices were stationary, but retail prices were stationary only at first difference.

The ADF test with single break shows that in case of both farm and retail prices at first difference the break dates, significant at 1.00% level, could be identified. Farm price series show break date in May 2012, while the break in retail prices is dated by April 2015. The latter break could be related to the aftermaths of the Russian ban and changes in export structure as this market closed alternative routings gradually. The situation also could be explained by the introduction of Euro as the German study of structural breaks in retail price series of carrots and cucumbers witnesses the similar behaviour [12].

Engle-Granger two-steps technique for price series including breaks in 2012 and 2015 is conducted in order to identify the nature of the long-run relations between prices. However, the coefficient for 2012 break is not significant. The results of Engle-Granger test with the single break in 2015 are provided in Table 4. The first step shows that derived coefficients are significant at 1.00% level.

Table 4

Results of the Engle-Granger technique (1st step)

	Coefficient	***t*-statistic**	**Std. Error**	**Prob.**
Retail price	0.71	12.20	0.06	0.00
D2015	0.19	4.43	0.04	0.00
Constant	-1.17	-20.99	0.06	0.00
R-squared	0.67	Durbin-Watson		0.65
Adjusted *R*-squared	0.66	*F*-statistic		75.76

Source: own calculations.

The testing of the residuals for the presence of a unit root is the second step of the Engle-Granger unit root test. The results report that we can reject the H_0 that there is a unit root for the tested period, because the computed absolute *t*-statistic value for the first period (-7.65) is higher than the critical value (-4.11). We conclude that the residuals are stationary as the H_0 could be rejected at 1.00% significance level. As a result, the derived equation is a cointegrated and non-spurious regression. The results of Engle-Granger test prove the evidence of the long-run relationship between carrot prices on farm and retail levels.

The next step is to analyse the causality and feedback. The Granger causality test refers to the short-run relations between the analysed price series. The test empowers answering the question 'Do changes in retail price series of carrots cause changes in farm price series of carrots, and vice versa?' and setting the direction of these causalities (Table 5). If lagged farm prices explain the retail prices, and vice versa, the conclusions on the leading role of farmers or retailers could be made. However, this test does not help to predict future movements of variables [5].

The first H_0 'Carrot retail price does not Granger cause carrot farm price' can be rejected at 5.00% significance level, and retail prices do lead farm prices of carrots in the short run. The second H_0 'Carrot farm price does not Granger cause carrot retail price' cannot be rejected, and farm prices do not lead retail prices in the short run.

Table 5

Results of Granger causality test for carrot prices

H_0	*F*-Statistic	Prob.
Carrot retail price does not Granger cause carrot farm price	4.15*	0.02
Carrot farm price does not Granger cause carrot retail price	1.47	0.24

Lags: 2 // * indicate rejection of the H_0 at the 5.00% level of significance.

Source: own calculations.

According to the findings of the previous research [3, 17, 27], the situation in the Lithuanian carrot market is not typical and this commodity could demonstrate both one-direction and bi-directional causality, while the leading stakeholder could differ from country to country. It should be noted that important causality links could be found even outside the supply chain of the commodity. For example, the study of the relationship between prices of organic and conventional carrots in the USA argues that organic market Granger cause price level of conventional carrots [15].

Finally, we test the selected price series for asymmetry applying Threshold Autoregressive Model. The residuals obtained from the Engle-Granger procedure were decomposed and tested for cointegration and asymmetry. The results of the estimation are provided in Table 6.

Table 6

Threshold Autoregressive Model for carrot prices

	Coefficient	Std. Error
Above threshold	-0.47	0.12
Below threshold	-0.15	0.15
Threshold Value	0.00	
F-equal	2.98	(3.52)*
T-max value	-1.00	(-1.65)*
F-joint (phi)	7.55	(4.70)*

* Simulated critical values for 5.00% significance level.

Source: own calculations.

The comparision of *F*-joint with critical value suggests that there is a cointegration between farm and retail prices. However, *F*-joint is higher than critical value and the H_0 of asymmetry is rejected. Thus, positive and negative shocks from retailer prices affected farmer prices in the same magnitude, and the results do not confirm related market inefficiency problems.

Although the results of the conducted study witness in favour of the symmetry, the nature of the asymmetric price behaviour in vegetable markets is widely discussed [23, 24, 27]. Even research with a specific focus on carrot market [17, 23, 24, 27] provides an evidence of both symmetric and asymmetric price transmission in the long run. Thus, it is important to mention that the introduction of the more detailed stakeholders' analysis could have an impact on findings in the Lithuanian carrot market.

Conclusion

The analysis of the main indicators of carrot production for the period from 2011 to 2017 shows that the harvested area declined by 1/4, while the reduction of harvest was even higher than by 1/3. During the analysed period, the important factors determining farmers' welfare and contributing to the shrinking of carrot production were weather conditions and the import ban for vegetables imposed by Russia.

The introduction of trade ban in 2014 negatively affected farmers' welfare and dramatically changed the structure of foreign trade by carrots in Lithuania. The study allows to state that national policy should encourage the diversification of export structure in the individual sectors of agriculture in order to avoid dependence on trading partners with the dominant share of the market. The absence of this strategy leads to the imminent losses of welfare for farmers and could be an expensive burden for taxpayers in case if the dominant partner impose trade restrictions.

The analysis of the Granger causality in domestic carrot supply chain shows that in the short-run retailers lead carrot price and have an impact on farmers' welfare situation, while causality runs from the retail prices to the farm prices. This result shows that retailers may exercise some market power and could influence the level of farm prices. The research results confirm the presence of the long-run relationship between farm and retail prices. Finally, there is a strong evidence of symmetric price behaviour along the carrot chain, i. e. positive and negative shocks are transmitted from retailers to farmers, and vice versa, with the same intensity.

References

1. Ahmed O. (2018), Vertical Price Transmission in Egyptian Tomato Sector after the Arab Spring, *Applied Economics*, pp. 1–16.
2. Aquiar D.R.D., Santana J.A. (2002), Asymmetry in Farm to Retail Price Transmission: Evidence from Brazil, *Agribusiness*, 18(1), pp. 37–48.
3. Bakucs Z., Fałkowski J., Fertő I. (2013), What causes asymmetric price transmission in agro-food sector? Meta-analysis perspective, *Discussion Paper No MT-DP – 2013/3*, Institute of Economics, Research Centre for Economic and Regional Studies, Hungarian Academy of Sciences, 23 p.
4. Bakucs L.Z., Fertő I., Szabó G.G. (2007), Price transmission in the Hungarian vegetable sector, *Studies in Agricultural Economics*, 106, pp. 23–40.
5. Brooks C. (2008), *Introductory Econometrics for Finance*, Cambridge, Cambridge University Press.
6. Darbandi E., Saghaian S. (2016), An Empirical Comparison of Price Transmission between Conventional and Organic Products: The Case of Fresh Carrots, *2016 Annual Meeting, February 6–9, 2016, San Antonio, Texas,* available at: https://ideas.repec.org/p/ags/saea16/230124.html.
7. Dickey D.A., Fuller W.A. (1979), Distribution of the Estimators for Autoregressive Time Series with a Unit Root, *Journal of the American Statistical Association*, 74(366), pp. 427–431.
8. Engle, R.F.; Granger, C.W.J. (1987), Co-integration and error correction: Representation, estimation and testing, Econometrica, 55(2), pp. 251–276.

9. Enders, W., & Granger, C.W.J. (1998), Unit-Root Tests and Asymmetric Adjustment with an Example Using the Term Structure of Interest Rates, *Journal of Business & Economic Statistics*, 16(3), pp. 304–311.
10. Enders, W., & Siklos, P.L. (2001), Cointegration and Threshold Adjustment, *Journal of Business & Economic Statistics*, 19(2), pp. 166–176.
11. Gardner B.L. (1975), The Farm-Retail Price Spread in a Competitive Food Industry, *American Journal of Agricultural Economics*, August, pp. 399–409.
12. Glauben T., Loy J.P., Meyer J. (2005), The impact of Euro introduction on the vertical price transmission in the German food market – Does money illusion matter? *XIth Congress the EAAE, 'The Future of Rural Europe in the Global Agri-Food System', Copenhagen, Denmark, August 24–27, 2005*.
13. Granger C.W.J. (1969), Investigating Causal Relations by Econometric Models and Cross-spectral Methods, *Econometrica*, 37(3), pp. 424–438.
14. Jeder H., Naimi A., Oueslati A. (2017), Transmission between Retail and Producer Prices for Main Vegetable Crops in Lithuania, *International Journal of Food and Agricultural Economics*, 5(1), pp. 19–28.
15. Kim G., Seok J.H., Mark T.B., Reed M.R. (2018), The price relationship between organic and non-organic vegetables in the U.S.: evidence from Nielsen scanner data, *Applied Economics*, pp. 1–15.
16. Li C., Sexton R.J. (2013), Grocery-Retailer Pricing Behavior with Implications for Farmer Welfare, *Journal of Agricultural and Resource Economics*, 38(2), pp. 141–158.
17. London Economics. (2004), *Investigation of the determinants of farm-retail price spreads: final report to DEFRA*, London Economics.
18. Maître d'Hôtel E., Le Cotty T. (2018), Why does on-farm storage fail to mitigate price volatility? *Agricultural Economics*, 49(2018), pp. 71–82.
19. Napaporn G., VanSickle J.J., Renwick A. (2003), Price Asymmetry in the United States Fresh Tomato Market, *Journal of Food Distribution Research*, 34(3), pp. 1–9.
20. Parrot S.D., Eastwood D.B., Brooker J.R. (2001), Testing for Symmetry in Price Transmission: An Extension of the Shiller Lag Structure with an Application to Fresh Tomatoes, *Journal of Agribusiness*, 19(1), pp. 35–49.
21. Pérez Mesa J.C., Galdeano Gómez E. (2011). Asymmetric Margines in Prices and retail Supply Chain Integration: The Spanish Vegetable Case, *Journal of International Food and Agribusiness Marketing*, 23, pp. 211–230.
22. Santeramo F.G., Cioffi A. (2012), Transmission of Market Crises in the European Vegetable Sector, *PAGRI*, 2(2012), pp. 37–46.
23. Santeramo F.G., Gioia L.D. (2018), A Review of Supply Chain Prices Analyses with Emphasis on Perishable Markets, in *Agricultural Value Chain*, IntechOpen, London.
24. Santeramo F.G., von Cramon-Taubadel S. (2016), On Perishability and Vertical Price Transmission: Empirical Evidence from Italy, *Bio-based and Applied Economics*, 5(2), pp. 199–214.
25. Verreth D.M.I., Emvalomatis G., Bunte F., Kemp R., Oude Lansink A.G.J.M. (2015), Price Transmission, International Trade, and Asymmetric Relationships in the Dutch Agri-Food Chain, *Agribusiness*, 31(4), pp. 521–542.
26. Von Cramon-Taubadel S. (2017), The analysis of market integration and price transmission – results and implications in an African context, *Agrekon*, 56(2), pp. 83–96.
27. Ward R.W. (1982), Asymmetry in Retail, Wholesale, and Shipping Point Pricing for Fresh Vegetables, *American Journal of Agricultural Economics*, May, pp. 205–212.

28. Xie H., Wang B. (2017), An Empirical Analysis of the Impact of Agricultural Product Price Fluctuations on China's Grain Yield, *Sustainability*, 9, 906, pp. 1–14.
29. Zainalabidin M., Iliyasu A. (2017), Analysis of Asymmetric Price Transmission of Selected Vegetables in Peninsular Malaysia, *Malaysian Journal of Mathematical Sciences*, 11(S) February, 87–100.
30. (2018), *Agriculture, Crop Production*, available at: https://osp.stat.gov.lt/statistiniu-rodikliu-analize?theme=all#/.
31. (2018), *Crop production in national humidity*, available at: http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=apro_cpnh1&lang=en.
32. (2018), *Foreign Trade, Detailed foreign trade indicators*, available at: https://osp.stat.gov.lt/statistiniu-rodikliu-analize#/.

Анотації

Харчові технології

Якісні показники зернових пластівців функціонального призначення

Світлана Бажай-Жежерун, Галина Сімахіна,
Людмила Береза-Кіндзерська, Наталка Науменко
Національний університет харчових технологій, Київ, Україна

Вступ. Обґрунтовано використання цільного біологічно активованого зерна злакових культур з метою створення функціональних харчових продуктів.

Матеріали і методи. Досліджено рецептури зернових сумішей пластівців, готові продукти на їх основі. Вміст білка визначали біуретовим методом, вміст крохмалю – поляриметричним методом. Вміст жиру визначали методом вичерпного екстрагування хімічно чистим гексаном. Вміст вітаміну Е, речовини з Р-вітамінною активністю визначати колориметрично. Вміст вітаміну С визначали титриметричним методом. Мікробіологічні показники досліджуваних зразків визначали висіванням їх поверхнево на агаризовані поживні середовища.

Результати і обговорення. Визначено вплив температурного режиму та тривалості холодного кондиціонування зерна на його біологічну цінність. Зі зміною температурного режиму до 12–18 ºС і тривалістю кондиціювання 24–30 год вміст у зерні усіх водорозчинних вітамінів збільшується у 2–2,5 рази, кількість токоферолу зростає у 5–7 разів, кількість речовин з Р-вітамінною активністю збільшується у 2,5 рази.

Досліджено залежність основних фізико-технологічних показників зернових пластівців та органолептичних властивостей дослідних зразків готових продуктів від їх компонентного складу. Збільшення масової частки вівса до 50% зумовлює підвищення в'язкості каші, внаслідок збільшення вмісту геміцелюлози знижується розсипчастість. Підвищення масової частки зерна пшениці до 35% зумовлює більш жорстку структуру каші, що пояснюється вищою щільністю оболонкових частин зерна пшениці, порівняно з іншими складовими.

Ступінь забезпечення добової потреби дорослого населення в макронутрієнтах за рахунок споживання 100 г пластівців складає: білки – 18–22%, жири – 5–7%, вуглеводи – 13–16%, харчові волокна – 13,5%. Враховуючи добову потребу дорослого населення у вітамінах, 100 г пластівців дає змогу задовольнити потребу у вітаміні Е на 67–76% та у вітаміні Р на 17,4%.

Загальна кількість колонієутворювальних одиниць мезофільних аеробних і факультативно-анаеробних мікроорганізмів у свіжих зразках сумішей пластівців та після їх зберігання не перевищує $2 \cdot 10^3$ на г продукту, плісняві гриби та патогенні мікроорганізми відсутні.

Висновки. Біологічно активоване зерно злакових культур пшениці, голозерного вівса та тритикале є джерелом цінних нутрієнтів для створення сумішей пластівців функціонального призначення.

Ключові слова: *активування, пшениця, тритикале, овес, пластівці.*

Морфологічна характеристика крохмальних гранул східно- та центральноєвропейських сортів картоплі (Solanum tuberosum)

Анастасія Заболотєц[1], Владімір Літвяк[1], Аляксєй Єрмаков[2], Гулназим Оспанкулова[3]

1 – Республіканське унітарне підприємство «Науково-практичний центр Національної академії наук Білорусі з продовольства», Мінськ, Республіка Білорусь
2 – Білоруський національний технічний університет, Мінськ, Республіка Білорусь
3 – Казахський агротехнічний університет ім. С. Сейфулліна, Нур-Султан, Казахстан

Вступ. Метою дослідження виявлення впливу розміру крохмальних гранул на якість крохмалю, отриманого із східно- та центральноєвропейських сортів картоплі.

Матеріали і методи. Об'єкт дослідження – нативний картопляний крохмаль, отриманий з 15 сортів картоплі: білоруської, російської, української та німецької селекції. Морфологічна структура гранул крохмалю досліджена на скануючому електронному мікроскопі LEO 1420. Контрастність знімків досягалася за рахунок металізації препаратів, здійсненої золотом у вакуумній установці EMITECH K 550X.

Результати і обговорення. Зерна нативного крохмалю, отриманого із східно- та центральноєвропейських сортів картоплі, значно відрізняються як за формою, так і за розмірами, про що свідчать технологічні особливості отримання крохмалю. Досліджено, що залежно від селекції білоруської, німецької, російської, української, розміри зерен нативного картопляного крохмалю східно- та центральноєвропейських сортів картоплі коливаються в межах: 5-90,26; 8,38-83,47; 5,3-88,7; 12,36-70 мкм відповідно.

Найвищий вміст крохмалю в зернах, отриманого зі східно- та центральноєвропейських сортів картоплі, виявлено в сорті німецької селекції «Kormoran» – 23%. Серед розглянутих сортів саме він має найвищу крахмалистість, але при цьому дрібні крохмальні гранули (середній розмір гранул дорівнює 24,0 мкм). Найнижчий вміст крохмалю в зернах було встановлено в сорті російської селекції «*Крепыш*» – 11,1%, при цьому середній розмір гранул дорівнює 30,1 мкм. З огляду на отримані дані можна зробити припущення, що при більшому середньому розмірі крохмальних зерен вміст крохмалю знижується, а при меншому – відсоток вмісту крохмалю зростає.

Згідно з отриманими результатами, а також відомими даними крохмальні зерна мають овальну, сферичну або неправильну форму, їхній діаметр коливається в межах 0,001-0,2 мм. Крохмальні зерна поділяються на прості та складні: прості зерна – це однорідні утворення; складні – поєднання більш дрібних частинок. Щільність крохмалю дорівнює в середньому 1,5 кг/м^3.

Висновки. Морфологічна структура гранул нативного крохмалю залежить від сорту картоплі та може змінюватися в діапазоні від 5-7 мкм до 80-90 мкм, що впливає на якість отриманого крохмалю.

Ключові слова: *крохмаль, нативний, морфологія, зерно, картопля.*

Екстракти на основі листя фізалісу (Physalis peruviana L.) та їх застосування в медичній і косметичній галузях

Тетьяна Іванова[1], Венеліна Попова[1], Надєжда Мазова[1],
Албена Стоянова[1], Станка Дамянова[2]
1 – Університет харчових технологій, Пловдив, Болгарія
2 – Русенський університет «Ангел Канчев», філія Разград, Болгарія

Вступ. Метою дослідження є визначення хімічного складу листя фізалісу та отримання на його основі екстрактів, що містять біологічно активні речовини з подальшою можливістю застосування в медичній і косметичній галузях.

Матеріали і методи. Екстрагування висушених листів фізалісу проводили за таких умов: гідромодуль – 1:10 (w/v), екстрагент – 95, 70, 50 та 30-відсотковий етанол, температура – 20, 40 та 60 °C, тривалість екстрагування – 1, 3 та 5 год. Вміст поліфенолів, флавоноїдів і тритерпенів у листі та отриманих екстрактах визначали за допомогою HPLC.

Результати обговорення. Листя фізалісу обох генотипів має вологість 8,32% для генотипу Пловдив та 8,79% – для біофармацевтичного. Вміст дубильних речовин в обох видах становить 9,62% та 10,58% відповідно. Колір екстрактів змінюється залежно від концентрації екстрагента: жовто-помаранчевий (30-відсотковий етанол), жовтувато-коричневий (50-відсотковий етанол), зеленувато-коричневий (70-відсотковий етанол) та коричневий (95-відсотковий етанол). Експериментальні дані й отримані рівняння показують, що два основних фактори екстракції – температура і тривалість, мають суттєвий вплив на вміст дубильних речовин. Оптимальними умовами процесу є: екстракція тривалістю 5 год за температури 60 °C, 30- та 50-відсотковим етанолом для листя генотипу Пловдив та з етанолом 50- і 70-відсотковим відповідно для листя біофармацевтичного. У листі та екстрактах генотипу Пловдив ідентифіковано 12 фенольних сполук, а в листі біофармацевтичному – 10. Основний флавоноїд у листі та екстрактах з двох генотипів рутин, переважають тритерпени – олеонова кислота та бетулін.

Екстракти листя фізалісу багаті біологічно активними речовинами (фенольними сполуками, флавоноїдами, тритерпенами) та мають потенціал для можливого застосування в медичних і косметичних продуктах.

Висновки. Вперше представлені дані про оптимальні умови отримання екстракту листя *Physalis peruviana*, а також інформацію про вміст деяких біологічно активних речовин у листі та отриманих екстрактах. Це перші результати генотипів фізалісу, вирощеного в Болгарії.

Ключові слова: *Physalis peruviana* L., *екстракт, поліфеноли, флавоноїд, тритерпен.*

Вплив структури таутомерів куркуміну на його активність: огляд

Самеера А. Реге, Мегха Ар'я, Шамім А. Момін
Інститут хімічної технології, Мумбай, Індія

Вступ. Метою цього огляду є розгляд унікальної хімії куркуміну для пояснення його різноманітної поведінки у різних середовищах.

Матеріали і методи. Проаналізовано статті, які присвячені вивченню структурної активності взаємовідносин куркуміну як антиоксиданту або прооксиданту. Огляд об'єднує досягнення, зроблені у цьому напрямі з 1980 року.

Результати і обговорення. Куркумін – це основний куркуміноїд, знайдений у куркумі, який вважається найбільш активним компонентом куркуми порівняно з іншими куркуміноїдами. Велика кількість досліджень присвячена доведенню корисності його активності. Описується використання куркуміну для лікування таких хвороб, як рак, альцгеймер, діабет, алергії, артрити тощо. Куркумін також має антиоксидантні властивості при низький концентрації. Отже, залежно від своєї структурної форми куркумін може діяти як антиоксидант або як прооксидант. Куркумін існує у двох таутометричних формах – кето та енол. В кетоформі куркумін проявляє антиоксидантні властивості. Енольна форма схильна до деградації. Тому бажано тримати куркумін у кетоформі. В поляризованому та кислому середовищі куркумін існує в кетоформі, тоді як в неполяризованому або лужному середовищі він підлягає деградації. Обговорюється механізм деградації куркуміну в різних середовищах. В лужних умовах відбувається нуклеофільна атака на гідроксильну групу, а в умовах неполяризації діє механізм вільних радикалів. Деградація в лужних умовах призводить до повного розкладу молекули, в той час як в неполяризованих умовах деградація відбувається через утворення проміжного пероксиду й обумовлює прооксидантний ефект куркуміну. В обох випадках ванілін є продуктом деградації, крім інших продуктів деградації.

У проаналізованих працях приділяється увага двом іншим видам куркуміноїдів – диметоксикуркуміну і біс-диметоксикуркуміну. Антиоксидантна активність куркуміну вища, тоді як бісдиметоксикуркумін має найменшу антиоксидантну активність серед куркуноїдів. Однак швидкість деградації куркуміну також максимальна серед куркуминоїдів, за ним іде диметоксикуркумін і бісдиметоксикуркумін. Це свідчить, що донор електронів – метоксильна група, впливає на активність куркуминоїдів. Отже, структура речовини відповідає за її активність.

Висновок. Підтверджено важливість конкретного середовища для забезпечення бажаної активності куркуміну.

Ключові слова: *куркумін, метилен, розкладення, нуклеофільний, радикал.*

Характеристика протеолітичних процесів при виділенні природних казеїнових фосфопептидів

Володимир Юкало, Ольга Крупа, Людмила Сторож
Тернопільський національний технічний університет імені Івана Пулюя, Тернопіль, Україна

Вступ. Метою дослідження є вивчення особливостей протеолітичних процесів під час отримання природних фосфопептидів білків казеїнового комплексу молока.

Матеріали і методи. Казеїновий субстрат виділяли ізоелектричним осадженням із знежиреного молока. Протеоліз проводили з використанням панкреатину. Ступінь протеолізу визначали спектрофотометрично за абсорбцією низькомолекулярних продуктів протеолізу при λ=280 нм. Вихід фосфопептидів визначали гравіметрично після осадження їх етанолом за наявності іонів кальцію. Електрофорез фосфопротеїнів

субстрату і продуктів протеолізу проводили в лужній системі однорідного поліакриламідного гелю за наявності сечовини.

Результати і обговорення. Природні фосфопептиди отримували протеолізом фосфопротеїнів казеїнового комплексу панкреатином (E:S=1:100) при фізіологічних умовах (37 ºС, pH 7,9). На різних етапах протеолізу було визначено вихід фосфопептидів і пептидів, розчинних в десяти- і трихлороцтовій кислоті. Ступінь протеолізу монотонно зростає протягом усього досліджуваного періоду. Вихід фосфопептидів досягає максимуму на 90-й хвилині протеолізу і далі постійно зменшується. Вихід фосфопептидів менший, ніж при використанні протеолітичних препаратів мікробного походження. Результати, отримані з допомогою гель-фільтрації і електрофорезу в поліакриламідному гелі, свідчать, що зменшення виходу фосфопептидів після 90-ї хвилини протеолізу може бути пов'язане із зменшенням молекулярної маси фосфопептидів. Більшість фосфопептидів, отриманих на 90-й хвилині протеолізу мають молекулярну масу до 2000 Да, яка характерна для відомих біологічно активних фосфопептидів.

Висновки. Під час протеолізу казеїну панкреатином у фізіологічних умовах загальний вихід продуктів протеолізу монотонно зростає. Вихід фосфопептидів має максимум. Дані гель-фільтрації і електрофорезу свідчать, що це пов'язано із зменшенням молекулярної маси.

Ключові слова: *казеїн, фосфопептид, протеоліз.*

Морфологія поверхні білкових ізолятів сої, квасолі і молочної сироватки та їх висушених драглів

Максим Полумбрик[1], Олена Шестель[2], Ольга Яценко[1],
Наталія Ющенко[1], Ульяна Кузьмик[1]
1 – Національний університет харчових технологій, Київ, Україна
2 – Черкаський державний технологічний університет, Черкаси, Україна

Вступ. Гідроколоїди рослинного походження широко використовуються в харчових технологіях, особливо у виробництві м'ясних і молочних продуктів. Мета цієї статті – дослідити морфологію поверхні ряду білкових ізолятів рослинного і тваринного походження.

Матеріали і методи. Використані білкові ізоляти сої, квасолі, молочної сироватки, а також к-карагінан та гуарова камідь. Виготовлено драглі зазначених білкових ізолятів із знежиреним молоком у співвідношенні 1:6 та 1:8. Структурні зміни білків сої, квасолі та молочної сироватки та їх висушених гідратів досліджено за допомогою скануючого електронного мікроскопа JSM-6700F.

Результати і обговорення. Наявність грубодисперсних часточок спостерігалась у зразках ізолятів сої і квасолі, в той час як у зразку молочної сироватки наявні лише часточки сферичної будови. Встановлено, що ізоляти сої та квасолі істотно не відрізнялись за розмірами часточок, які становлять приблизно 40 μm. Низький ступінь гідратації досліджених ізолятів у співвідношенні 1:5 призводить до утворення рівної поверхні із значною кількістю пустот. Залишки великих глобул можуть бути ідентифіковані в зразках ізоляту квасолі за своєю сферичною формою, розміри якої подібні до вихідних ізолятів. Підвищення рівня гідратації до 1:8 призводить до зшивання білків макромолекул й утворення каркасу. Включення полісахаридів до

суміші стабілізаторів приводить до утворення поверхні драглів, морфологічні властивості яких є подібними властивостям ізолятів сої, квасолі та молочної сироватки. Однак це призводить до утворення структур з незначним зшиванням

Висновки. Підвищений вміст молока у драглях спричиняє утворення тривимірної структури, як і очікувалось. Ізолят квасолі характеризувався підвищеною здатністю до зшивання молекул білків.

Ключові слова: *морфологія, білки, молочна сироватка, соя, квасоля.*

Вплив попередньої обробки гідролітичними ферментами на вилучення олії з насіння гарбузів

Тамара Носенко, Ганна Вовк, Тамара Королюк
Національний університет харчових технологій, Київ, Україна

Вступ. Ензимні технології використовують для переробки олійного насіння. Про вилучення олії з насіння гарбузів із використанням попередньої ензимної обробки у науковій літературі результати не наведені. Мета дослідження – оцінити дію суміші протеаз і целюлаз на цілісність клітин, вихід олії холодного пресування та динаміку екстрагування олії розчинником із насіння гарбузів.

Матеріали і методи. Protolad, лужна та кисла протеази, Cellulad (ENZIME, Україна) використовувались для попередньої обробки насіння гарбузів. Цілісність клітин оцінювали методом "моментального збовтування". Холодне пресування здійснювали на лабораторному шнековому пресі. Швидкість екстрагування олії розчинником визначали в апараті Сокслета як кількість вилученої олії з насіння після кожного циклу екстрагування.

Результати і обговорення. Виявлено, що основне збільшення руйнування цілісності клітин насіння гарбузів порівняно з контролем відбувалось протягом 2 годин обробки. Подальше інкубування подрібненого насіння з ферментами не викликало суттєвого збільшення "розкритих" клітин у суміші. Показано, що використання різних протеолітичних ферментів для попередньої обробки гарбузового насіння призводило до збільшення кількості зруйнованих клітин – від 3 до 10,4% порівняно із контролем. Використання суміші протеаз і целюлаз для попередньої обробки гарбузового насіння викликало подальше збільшення кількості зруйнованих клітин на 10%. Вихід олії "холодного" пресування після попередньої обробки сумішшю ферментних препаратів із протеазною (70%) та целюлазною (30%) активністю збільшився від 62,3 до 70,0% від загального вмісту олії в насінні. Швидкість екстрагування олії розчинником із гарбузового насіння після ферментативної обробки зростала – 25,4 і 17,7% олії було екстраговано після 80 хвилин екстрагування від маси ферментативно обробленого насіння та контролю відповідно. За вмістом пероксидів досліджувані зразки олії не відрізнялись.

Висновки. Внаслідок використання суміші ферментних препаратів із протеазною та целюлазною активністю для попередньої обробки гарбузового насіння кількість зруйнованих клітин зростає, що призводить до підвищення виходу пресової гарбузової олії та швидкості екстрагування олії розчинником.

Ключові слова: *насіння, гарбуз, олія, протеаза, целюлаза, пресування.*

Обґрунтування раціональних режимів заморожування напівфабрикатів молочно-рослинних фаршів

Тетяна Юдіна[1], Вікторія Гніцевич[1], Ірина Назаренко[2]
1 – Київський національний торговельно-економічний університет, Київ, Україна,
2 – Донецький національний університет економіки і торгівлі імені Михайла Туган-Барановського, Кривий Ріг, Україна

Вступ. Обґрунтовано та експериментально підтверджено раціональні режими заморожування напівфабрикатів молочно-рослинних фаршів на основі концентрату зі сколотин.

Матеріали і методи. Досліджувались молочно-морквяний, молочно-гарбузовий і молочно-кабачковий фарші та контрольний зразок – фарш із кислого сиру. Дослідження проводили на спектрометрі високого розв'язання Tesla BS 567A з робочою частотою 100 мГц на протонах за методом Кюнтца

Результати і обговорення. Встановлено, що за температури 20°С у спектрі ядерного магнітного резонансу напівфабрикату молочно-рослинного фаршу реєструється інтенсивний сигнал води, що при охолодженні зразка до температури -25°С значно зменшується за інтенсивністю. Наявність сигналу ядерного магнітного резонансу свідчить, що при охолодженні зразка до температури -25°С незамерзаюча вода в ньому повністю не зникає, що передбачає можливість перебігу у фарші біохімічних реакцій. Визначено, що для розроблених напівфабрикатів молочно-рослинних фаршів потрібно більш глибоке переохолодження, ніж для контрольного зразка, оскільки у зразках розроблених фаршів відбувається зв’язування води вуглеводами.

Досліджено, що за -25°С у контрольному зразку утримується 0,21 г H_2O на г сухої речовини, а у напівфабрикаті молочно-морквяного фаршу – 0,40 г H_2O на г сухої речовини, молочно-гарбузового – 0,39 г H_2O на г сухої речовини, молочно-кабачкового – 0,37 г H_2O на г сухої речовини за тієї ж температури. За -30 °С у контрольному зразку утримується 0,20 г H_2O на г сухої речовини, а у напівфабрикаті молочно-морквяного фаршу – 0,32 г H_2O на г сухої речовини, молочно-гарбузового – 0,25 г H_2O на г сухої речовини, молочно-кабачкового – 0,24 г H_2O на г сухої речовини за тієї ж температури. Доведено, що найменша кількість незамерзаючої води в фаршах утримується за температури -25...-30 °С та складає 0,26...0,40 г H_20 на 1 г сухої речовини, що дозволяє рекомендувати такий діапазон температур для швидкого їх заморожування.

Висновки. Обґрунтовано температуру заморожування молочно-рослинних фаршів (-25...-30°С). Доведено можливість зберігання заморожених фаршів при температурі -18...-19°С, що є нормативною температурою промислових морозильних камер.

Ключові слова: *фарш, напівфабрикат, заморожування, зберігання.*

Біотехнологія, мікробіологія

Перспективи використання поверхнево-активних речовин *Nocardia vaccinii* IMB B-7405 для обробки овочів

Тетяна Пирог[1,2], Христина Берегова[1], Богдана Гейченко[1], Віктор Стабніков[1]
1 – Національний університет харчових технологій, Київ, Україна
2 – Інститут мікробіології і вірусології НАН України, Київ, Україна

Вступ. Вивчали використання поверхнево-активних речовин (ПАР) *Nocardia vaccinii* IMV B-7405 для продовження терміну зберігання овочів.

Матеріали і методи. Органічні овочі, такі як томати, огірки та кабачки, обробляли розчинами ПАР, що утворюються *N. vaccinii* IMV B-7405, з концентрацією 0,25 або 0,5 г/л. Мікробіологічний аналіз проводили до початку зберігання овочів. Оцінку якості овочів проводили візуально протягом терміну зберігання.

Результати і обговорення. Результати наших досліджень показали ефективність застосування біосурфактанту, який синтезується *Nocardia vaccinii* IMV B-7405 при рості на промислових відходах, для продовження терміну зберігання овочів. Результати візуальних спостережень, а також мікробіологічні дослідження показали, що обробка овочів розчинами ПАР у концентрації 0,25–0,5 г/л була ефективнішою порівняно з миттям їх водою. Загальна кількість гетеротрофних бактерій і грибів після обробки овочів розчинами ПАР *N. vaccinii* IMB B-7405 знизилась у 16–34 та 3–14 разів відповідно, у той час як після миття водою загальна кількість мікроорганізмів знизилась лише у 2–2,5 рази. Було показано, що овочі, які мили тільки водою, псувалися швидше, ніж ті, що були оброблені розчинами ПАР. Перевагами використання біосурфактанту для обробки овочів є те, що (1) він може бути застосований в нижчій в 2-6 разів концентрації порівняно з іншими відомими з літератури мікробними ПАР, та (2) він може бути вироблений при застосуванні промислових відходів, що знижує вартість його виробництва.

Висновки. Біосурфактант *N. vaccinii* IMB B-7405 може бути використаний для для обробки овочів з метою подовження терміну їх зберігання.

Ключові слова: біосурфактант, *Nocardia vaccinii* IMV B-7405, термін зберігання, овочі.

Вплив наночастинок на сольвентогенез бактерій *Clostridium beijerinckii* IMB B-7806, *Clostridium acetobutylicum* IMB B-7807

Сергій Скроцький, Сергій Войчук, Людмила Хоменко,
Ольга Василюк, Валентин Підгорський
Інститут мікробіології і вірусології НАН України, Київ, Україна

Вступ. Зважаючи на те, що наночастинки (НЧ) використовуються самою природою, актуальним залишається питання застосування альтернативних, спроектованих людиною НЧ, для отримання бажаного біологічного чи медичного ефекту. В галузі біотехнології НЧ можуть виявитися як каталізаторами біохімічних процесів, так і протекторами (кріо-, осмо-), сорбентами токсичних метаболітів, провідниками і медіаторами, сигнальними молекулами тощо.

Матеріали і методи. Вивчали вплив наночасток металів (оксиди заліза, церію, срібла, золота та гадолінію) на біосинтез бутанолу штамами ацетоно-бутилових бактерій *Clostridium beijerinckii* IMB B-7806 і *C. acetobutylicum* IMB B-7807.

Результати і обговорення. Синтез основних продуктів АБЕ-ферментації (ацетону, бутанолу і етанолу) змінювався при наявності НЧ в середовищі культивування. Встановлено, що для штаму *Clostridium beijerinckii* IMB B-7806 всі НЧ пригнічували синтез бутанолу порівняно з контролем. А при використанні НЧ оксиду срібла для синтезу бутанолу штамом *C. acetobutylicum* IMB B-7807 спостерігали тенденцію ($p \leq 0,16$) до збільшення виходу бутанолу з 9,0±0,6 г/л у контролі до 11,1±1,8 г/л і 11,1±1,1 г/л при концентраціях НЧ 0,1 і 10 ηМ відповідно.

Характер змін залежав від типу НЧ та їх концентрації. Підібрано оптимальні концентрації вивчених НЧ, а також висловлені припущення щодо можливих механізмів дії НЧ на процес АБЕ-ферментації. Вивчено регулюючий потенціал НЧ для координування процесів АБЕ-ферментації і синтезу жирних кислот з метою збільшення виходу цільового продукту.

Висновки. Ефект дії НЧ вказує на те, що синтез органічних розчинників ацетоно-бутиловими бактеріями має штамову специфічність та визначається не тільки ростовими характеристиками, але й роботою самих ферментних систем незалежно від росту бактерій.

Ключові слова: *бактерії, Clostridium, біобутанол, наночастинка.*

Процеси і обладнання

Вплив робочого тиску на опір шару концентраційної поляризації при зворотному осмосі

Сергій Гулієнко, Оксана Лещенко
Національний технічний університет України «Київський політехнічний інститут імені Ігоря Сікорського», Київ, Україна

Вступ. Проведено експериментальну перевірку гіпотези щодо лінійної залежності опору шару концентраційної поляризації від тиску та визначено вплив гідродинамічних умов на величну цього опору.

Матеріали і методи. Дослідження проводилися з використанням комерційно доступних мембранних модулів типу TFC-75. Вимірювання продуктивності проводилися для знесоленої води (загальний солевміст 5-15 мг/дм^3), а також для розчинів NaCl. Для вимірювання витрат використовувався об'ємний метод, концентрації вимірювалися кондуктометричним методом.

Результати і обговорення. Опір мембрани під час зворотного осмосу знесоленої води не змінювався з тиском в умовах експерименту і дорівнює R_m=7,549·10^{13} м$^{-1}$.

Опір шару концентраційної поляризації (R_{cp}) зростав від 0,65–1,29·10^{13} м$^{-1}$ до 1,46–1,83·10^{13} м$^{-1}$ зі збільшенням робочого тиску від 0,2 МПа до 0,6 та від 0,65–1,46·10^{13} м$^{-1}$ до 1,2-–1,83·10^{13} м$^{-1}$ зі зростанням концентрації розділюваного розчину від 100 мг/дм^3 до 600 мг/дм^3. Зростання величини R_{cp} з тиском відбувається за лінійним законом, що узгоджується з раніше опублікованими результатами для процесу ультрафільтрації. Крім того, в розглядуваному діапазоні тисків експоненційна залежність індексу концентраційної поляризації від тиску може бути апроксимована лінійним рівнянням

з коефіцієнтом кореляції понад 0,93. Тому припущення щодо лінійної залежності опору шару концентраційної поляризації від тиску є обґрунтованим і може бути розповсюджене на процес зворотного осмосу для вказаних умов.

Зростання опору шару концентраційної поляризації зі збільшенням тиску обумовлене більшими значеннями потоку речовини через мембрану та меншими значеннями коефіцієнта масовіддачі при вищих значеннях робочого тиску в розглядуваній системі. Такі результати узгоджуються з плівковою теорією концентраційної поляризації.

Висновки. Досліджувана гіпотеза підтверджується для зворотного осмосу в розглядуваному діапазоні зміни робочого тиску. Існує кореляція між опором шару концентраційної поляризації і індексом концентраційної поляризації

Ключові слова: *осмос, опір, поляризація, потік, тиск.*

Спосіб термометричного визначення теплофізичних характеристик термолабільних матеріалів

Ольга Мазуренко, Лілія Харченко, Дмитро Коломієць, Олександр Мазуренко
Національний університет харчових технологій, Київ, Україна

Вступ. Проведено аналітичні дослідження способів і приладів визначення теплофізичних властивостей складних за структурою та хімічним складом продуктів переробної галузі з метою підвищення точності вимірювань.

Матеріали і методи. Основа досліджуваних приладів – термоелектричні перетворювачі температури та теплового потоку у вигляді "допоміжної стінки". Для підвищення точності вимірювання теплофізичних характеристик матеріалів використано графо-аналітичний метод градуювання приладів.

Результати і обговорення. Для вивчення теплофізичних характеристик термолабільних матеріалів найбільш придатні теплометричні засоби їх комплексного визначення, які дають змогу проводити дослідження при наявності або відсутності в матеріалі фазових перетворень його складових. Теплометричні засоби вимірювання засновані на вимірюванні температури теплових потоків, які пронизують зразок за різних теплових умов. Теплові, електричні та інші процеси передачі в термометричних матеріалах визначають нестабільність функції перетворення і формують інструментальну похибку. Тому більшість методів визначення теплофізичних характеристик матеріалів передбачають попередні досліди з еталонними матеріалами, за відомими характеристиками яких обчислюють метрологічні характеристики пристрою. Саме використання еталонних матеріалів є основним недоліком цих методів. На основі розрахунково-графічного аналізу теплового та ємнісного опору системи "пристрій-зразок" розроблено принципово новий спосіб, за яким теплофізичні характеристики матеріалу та метрологічні характеристики пристрою визначаються в комплексі і одночасно за результатами експерименту зі зразками тільки досліджуваного матеріалу.

Висновок. Одночасне отримання інформації про значення теплофізичних характеристик матеріалу і метрологічних характеристик пристрою дає змогупідвищити точність визначення теплофізичних характеристик матеріалів.

Ключові слова: *продукти, теплообмін, характеристики, метрологія, точність.*

Вплив диференційно-термічної обробки на харчові продукти з яблук, отриманих конвективно-терморадіаційним сушінням

Людмила Стрельченко, Ігор Дубковецький, Іван Малежик
Національний університет харчових технологій, Київ

Вступ. Розглядається вплив диференційно-термічної обробки на продукти з яблук з різним вмістом цукрів у напівфабрикатах.

Матеріали і методи. Матеріалом для сушіння обрано яблука сорту «Голден Делішес». Зразки сушених яблук, снеків і цукатів аналізували органолептично, фізико-хімічно та диференційно-термічно. Підготовлені напівфабрикати сушили конвективно-терморадіаційним способом при температурі теплоносія 60 °С, швидкості руху теплоносія 5,5 м/с, питомому навантаженні 8,8 кг/м^2, величині опромінення терморадіаційних ТЕНів 8 кВт/м^2, потужності зовнішнього ТЕНу 2,5 кВт/м^2.

Результати і обговорення. На основі кривих сушіння швидкість видалення вологи відбувалось прямо пропорційно збільшенню концентрації цукру в продуктах. Час сушіння продуктів залежить від вмісту сухих речовин (СР) у напівфабрикатах: які для сушених яблук складали 12%, для снеків 18,2% і для цукатів 72%. Витрати енергії для сушених яблук, снеків і цукатів становлять, відповідно, 5,9; 7,55; 19,8 кВт·год на кг вихідної сировини та 1,93; 1,99; 26,0 МДж/кг випареної вологи. Спостерігається така залежність: чим більша концентрація цукру в напівфабрикаті, тим більше потрібно її для видалення вологи з матеріалу.

На представлених дериватограмах при нагріванні яблук в області T_1=108 °С спостерігалась втрата маси Δm=13,0%, яка супроводжувалась ендотермічним піком на залежності DTA. Ця втрата маси пов'язана з випаровуванням вологи. При зростанні температури до T_2=140 °С в зразку починала відбуватись деструкція. При нагріванні снеку в області T_1=108 °С маса втрачалась на Δm=14,5%, яка супроводжувалась ендотермічним піком на залежності DTA. При зростанні температури до T_2=131 °С в зразку почала відбуватися деструкція. Нагрівання цукатів в області T_1=109 °С призводило до втрати маси на Δm= 14,8% з ендотермічним піком на залежності DTA. При зростанні температури до T_2=125 °С в зразку почала відбуватися деструкція.

Висновки. На основі дериватограм (часових залежностей Т, ТГ, ДТГ та ДТА) розраховано передекспоненційний фактор (k_0) для сушених яблук, снеків і цукатів, що становить 55; 61; 70, а також енергію активації (Е) для сушених яблук, снеків і цукатів – 186; 203 та 236 кДж/моль відповідно.

Ключові слова: *яблуко, снек, цукат, бланшування, сушіння.*

Економіка і управління

Організаційні засади створення інноваційної функціональної харчової продукції підприємствами глибокої переробки горіха волоського

Григорій Калетнік[1], Віталій Луцяк[1], Ольга Мельничук[1], Юрій Довгань[1], Маріан Малікі[2]
1 – Вінницький національний аграрний університет, Вінниця, Україна
2 – Університет Якоба Парадизького, Гожув-Велькопольський, Польща

Вступ. Українські промислові підприємства, що здійснюють глибоку переробку горіха волоського, мають значний експортний потенціал. Однак відсутність методичних напрацювань для розроблення інновацій у цій сфері знижує потенціал.

Матеріали і методи. Використано підхід до аналізу підкомплексу, маркетинговий, функціональний і процесний підходи. Також застосовано фундаментальні інтерв'ю з експертами, проаналізовано результати лабораторних досліджень, випуску та реалізації інноваційної продукції. Розробка продукції проводилася на основі методології розгортання функцій якості.

Результати і обговорення. Результати дослідження демонструють те, що Україна залучається до процесу диверсифікації світового олійно-жирового ринку та створення інновацій. Підкомплекс з глибокої переробки горіха волоського створює ядро горіху волоського для кондитерської промисловості та годування тварин, олію горіхову харчову, олію технічну та макух, що може перероблятись у харчові продукти або використовуватись для годування худоби. Залишки від виробництва можуть бути ефективно перероблені на біопаливо. Крім того, плоди горіха волоського теж вважаються продуктом підкомплексу. Інші похідні продукти з горіха волоського досліджуються. У розвитку підкомплексу, а також у короткостроковій перспективі акцент робиться на кондитерське ядро та горіхову олію. З розвитком підкомплексу будуть створюватись інноваційні похідні продукти шляхом дослідження та підтримки розвитку альтернативних способів використання залишків у міру їх виникнення.

Цінність результатів дослідження та виконання розробок полягає у можливості використання отриманих теоретико-методологічних висновків, рекомендацій і положень аграрними та харчовими підприємствами для вирішення завдання щодо забезпечення конкурентоспроможності підприємства та його продукції в умовах високої динаміки ділового середовища з урахуванням системних зв'язків виробничого підприємства в олійно-жировому підкомплексі.

Висновки. Запропоновано модель ланцюга створення вартості при переробці горіха волоського та технологія виробництва нових продуктів.

Ключові слова: *інновації, промисловість, маркетинг, горіх волоський.*

Литовський ринок моркви: виробництво, зовнішня торгівля та зміни цін

Нелі Юркенайте[1], Дімітріос Папарас[2]

1 – Литовський інститут аграрної економіки, Вільнюс, Литва

2 – Університет Харпера Адамса, Шропшир, Великобританія

Вступ. Проаналізовано ситуацію на ринку моркви Литви з огляду на зміни аграрного виробництва, структури зовнішньої торгівлі та вертикальної передачі цін у ланцюжку поставок за 2011-2017 роки.

Матеріали і методи. У дослідженнях використані методи порівняльного та графічного аналізу основних показників виробництва моркви і розвитку зовнішньої торгівлі, які спираються на вторинні дані Департаменту статистики Литви. Дослідження вертикальної передачі цін у ланцюжку поставок вирощеної в Литві моркви ґрунтується на даних ДП «Центр сільськогосподарської інформації і сільського бізнесу». Надано результати тесту на стаціонарність, коінтеграцію, Гренджера на причинність і тесту на асиметрію.

Результати і обговорення. Результати дослідження свідчать, що за семирічний період у Литві скоротилися обсяги виробництва моркви. Посівні площі морквяних

полів скоротилися з 2,4 до 1,8 тис. га, а врожай зменшився більш ніж на 1/3. Негативні зміни обумовлені тривалими несприятливими погодними умовами та забороною на імпорт до Росії, що, у свою чергу, спричинило зміни в структурі експорту та погіршення добробуту селян.

Аналіз цін фермерів і роздрібної торгівлі показав порядок інтеграції I (1). Виявлено структурний розрив у квітні 2015 року, який був включений в аналіз як фіктивна змінна. Тест Енгл-Гренджер на коінтеграцію підтвердив наявність зв'язку між цінами в довгостроковій перспективі. Тест Гренджер на причинність виявив наявність причинного зв'язку від роздрібних до фермерських цін. Позитивні та негативні шоки цін передаються від роздрібної торгівлі до фермерів з однаковою інтенсивністю, що свідчить про симетричну поведінку цін.

Висновки. Дослідження показало, що тривалі несприятливі погодні умови та заборона на імпорт були важливими факторами, що визначили зміни цін на литовському ринку моркви в 2011-2017 роках. Аналіз вертикальної передачі цін свідчить, що роздрібні ціни можуть мати вплив на формування цін.

Ключові слова: *сільське господарство, морква, трансмісія ціни, ланцюжок постачання, торгівля.*

Instructions for authors

Dear colleagues!

The Editorial Board of scientific periodical
"**Ukrainian Food Journal**"
invites you for publication of your research results.

Requirements to all texts:
Language – English.
Size of the article – 10–15 pages in Microsoft Word 2003 and earlier versions with filename extension *.doc (!)
Times New Roman, font size 14, 1 line intervals, margins on both sides – 2 cm.

The structure of the article:

1. The title of the article
2. Authors (full name and surname)
3. Institution, where the work has been performed.
4. Abstract (2/3 of a page). The structure of the abstract should correspond to the structure of the article (Introduction, Materials and methods, Results and discussion, Conclusion).
5. Keywords.
6. The main body of the article should contain the following parts:
   - Introduction
   - Materials and methods
   - Results and discussion
   - Conclusion
   - References

   If you need you can add another parts and/or divide them into subparts.
7. The information about the author (Name, surname, scientific degree, place of work, email and contact phone number).

All figures should be made in graphic editor, the font size 14.

The background of the graphs and charts should be only in white color. The color of the figure elements (lines, grid, text) – in black color.
Figures and EXCEL format files with graphs additionally should be submitted in separate files.
Photos are not recommended to be used as graphical materials.

Website of Ukrainian Food Journal: http://ufj.ho.ua

Email for all submissions and other inquiries: ufj_nuft@meta.ua

Шановні колеги!

Редакційна колегія наукового періодичного видання «**Ukrainian Food Journal**» запрошує Вас до публікації результатів наукових досліджень.

Вимоги до оформлення статей

Мова статей – англійська.

Мінімальний обсяг статті – **8 сторінок** формату А4 (без врахування анотацій і списку літератури)**.**

Стаття виконується в текстовому редакторі Microsoft Word 2003, в форматі *.doc.

Для всіх елементів статті шрифт – **Times New Roman**, кегль – **14**, інтервал – 1.

Всі поля сторінки – по 2 см.

Структура статті:

1**.** УДК**.**
2. **Назва статті.**
3. Автори статті (ім'я та прізвище повністю, приклад: Денис Озерянко).
4. *Установа, в якій виконана робота.*
5. Анотація. **Обов'язкова** структура анотації:
   - Вступ (2–3 рядки).
   - Матеріали та методи (до 5 рядків)
   - Результати та обговорення (пів сторінки).
   - Висновки (2–3 рядки).
6. Ключові слова (3–5 слів, але не словосполучень).

Пункти 2–6 виконати англійською і українською мовами.

7. Основний текст статті. Має включати такі обов'язкові розділи:
   - Вступ
   - Матеріали та методи
   - Результати та обговорення
   - Висновки
   - Література.

За необхідності можна додавати інші розділи та розбивати їх на підрозділи.

8. Авторська довідка (Прізвище, ім'я та по батькові, вчений ступінь та звання, місце роботи, електронна адреса або телефон).
9. Контактні дані автора, до якого за необхідності буде звертатись редакція журналу.

Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути **співрозмірним** (!) тексту статті. **Фотографії можна використовувати лише за їх значної наукової цінності.**

Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

Рисунки та графіки EXCEL з графіками додатково подаються в окремих файлах.

Скорочені назви фізичних величин в тексті та на графіках позначаються латинськими літерами відповідно до системи СІ.

В списку літератури повинні переважати англомовні статті та монографії, які опубліковані після 2000 року.

Правила оформлення списку літератури

В Ukrainian Food Journalвзято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

1. Посилання на статтю:

Автори А.А. (рік видання), Назва статті, Назва журналу (курсивом), Том (номер), сторінки.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

1. Приклад:
   Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (Juglans regia L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies,* 2(2), pp. 104–108.

2. Посилання на книгу:

Автори (рік), Назва книги (курсивом), Видавництво, Місто.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

Посилання на електронний ресурс:

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова ***Available at:*** та вказується електронна адреса.

Приклади:

1. (2013), *Svitovi naukovometrychni bazy*, available at: http://www1.nas.gov.ua/publications/q_a /Pages/scopus.aspx
2. Cheung T. (2011), *World's 50 most delicious drinks [Text]*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт, а з російської – стандарт МВД (в цих стандартах використовуються символи лише англійського алфавіту, без хвостиків, апострофів та ін).

Зручні сайти для транслітерації:

З української мови – http://translit.kh.ua/#lat/passport

З російської мови – http://ru.translit.net/?account=mvd

Додаткова інформація та приклад оформлення статті – на сайті

http://ufj.ho.ua

Стаття надсилається за електронною адресою: ufj_nuft@meta.ua

УДК 663/664

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

Тематика публікацій в Ukrainian Food Journal:

Харчова інженерія
Харчова хімія
Мікробіологія
Фізичні властивості харчових продуктів
Якість та безпека харчових продуктів
Процеси та обладнання
Нанотехнології
Економіка та управління
Автоматизація процесів
Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

Ukrainian Food Journal індексується наукометричними базами:
Index Copernicus (2012)
EBSCO (2013)
Google Scholar (2013)
UlrichsWeb (2013)
Global Impact Factor (2014)
Online Library of University of Southern Denmark (2014)
CABI full text (2014)
Directory of Research Journals Indexing (DRJI) (2014)
Universal Impact Factor (2014)
Directory of Open Access scholarly Resources (ROAD) (2014)
European Reference Index for the Humanities and the Social Sciences (ERIH PLUS) (2014)
Directory of Open Access Journals (DOAJ) (2015)
InfoBase Index (2015)
Chemical Abstracts Service Source Index (CASSI) (2016)
Emerging Sourses Citaton Index (2018)

Рецензія рукопису статті. Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний учений.

Авторське право. Автори статей гарантують, що робота не є порушенням будь-яких авторських прав, та відшкодовують видавцю порушення даної гарантії. Опубліковані матеріали є правовою власністю видавця «**Ukrainian Food Journal**», якщо не узгоджено інше.

Політика академічної етики. Редакція «Ukrainian Food Journal» користується правилами академічної етики, викладених в роботі Miguel Roig (2003, 2006) "Avoiding plagiarism, self-plagiarism, and other questionable writing practices. A guide to ethical writing". Редакція пропонує авторам статей і рецензентам прямо слідувати цьому керівництву, щоб уникнути помилок у науковій літературі.

Інструкції для авторів та інша корисна інформація розміщені на сайті
http://ufj.ho.ua

Редакційна колегія

Наукове видання

Ukrainian Food Journal

Volume 8, Issue 1
2019

Том 8, № 1
2019

Підп. до друку 28.03.2019 р. Формат 70x100/16.
Обл.-вид. арк. 16.46. Ум. друк. арк. 16.11.
Гарнітура Times New Roman. Друк офсетний.
Наклад 100 прим. Вид. № 02н/19.

НУХТ. 01601 Київ–33, вул. Володимирська, 68

Свідоцтво про державну реєстрацію
друкованого засобу масової інформації
КВ 18964–7754Р
видане 26 березня 2012 року.