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INVESTIGATION OF THE SAFETY GRAPESEED POWDER AS AN ALTERNATIVE TO COCOA-POWDER IN A CONFECTIONERY GLAZE

O. Gorodyska, PhD student*, *E-mail*: gorelena84@gmail.com
N. Grevtseva, PhD, associate Professor*, *E-mail*: grevtseva66@gmail.com
O. Samokhvalova, PhD, associate Professor*, *E-mail*: sam55ov@gmail.com
O. Savchenko, PhD, associate Professor **, *E-mail*: savchenkolm68@ukr.net
A. Grygorenko, PhD***, *E-mail*: anzhegrig@yandex.ru
*Department of Bakery, Confectionary, Pasta and Food Concentrates Technology
Kharkiv State University of Food Technology and Trade, Klochkivska str., 333, Kharkiv, Ukraine, 61051
** Department of Food technology
Chernihiv National University of Technology, Shevchenko str., 95, Chernigiv, Ukraine 14027
*** Chief technologist TM «Magik Mosaik»
21-a Avtostradnaya Naberezhnaya St., Kharkiv, Ukraine, 61038

Abstract. The article considers the safety and environmental cleanliness of grapeseed powders compared to the natural and alkalized cocoa powders. The content of heavy metals in the investigated powders has been determined by the atomic adsorption method; radionuclide activity has been determined by the spectrometric method; the presence of mycotoxins B1, T-2-toxin, zearalenone, and deoxynivalenol (vomitoxin) has been determined by thin-layer chromatography; the amount of nitrates has been determined by the ionometric method; contamination of powders with organophosphorous and organochlorine pesticides has been determined by chromatographic method.

According to the results of experiments, the content of heavy metals in all investigated powders is within the limits of the maximum permissible concentration, whilst powders of grapeseeds are notable for a significantly lower mass content of plumbum, zinc, copper salts. As for the radioactivity of grapeseed powders, the activity of radionuclides is significantly below the permissible level. Presence of aflatoxin B_1 has been detected in the alkalized cocoa powder sample, of zearalenone – in the grapeseed powder oilcake sample. Their concentration does not exceed the allowable concentration. Mycotoxines are absent in other samples investigated. Concentration of nitrates in the natural and alkalized cocoa powder samples is by 20–30 times higher than that found in grapeseed powders. The content of organophosphorous and organochlorine pesticides in all cocoa powder samples and all grapeseed containing powders is below the method's threshold of detectability. The glaze samples containing the additives investigated have a considerably lower content of heavy metals compared to the reference samples.

Glaze with cocoa powder partially replaced with grapeseed powders has high organoleptic quality parameters. During the glazing process, it is applied on the surface of products in thin layers and cools down in small waves. The results obtained prove that domestic raw materials are safe for human health, and confectionery glaze containing grapeseed powders as partial replacement of the cocoa powder has high safety characteristics.

Keywords: safety characteristics, xenobiotics, grapeseed powders, natural and alkalized cocoa powder.

ДОСЛІДЖЕННЯ БЕЗПЕКИ ВИНОГРАДНИХ ПОРОШКІВ ЯК АЛЬТЕРНАТИВИ КАКАО-ПОРОШКУ У КОНДИТЕРСЬКІЙ ГЛАЗУРІ

О.В. Городиська, аспірант*, *E-mail*: gorelena84@gmail.com
Н.В. Гревцева, кандидат технічних наук, доцент*, *E-mail*: grevtseva66@gmail.com
О.В. Самохвалова, кандидат технічних наук, професор*, *E-mail*: sam55ov@gmail.com
О.М. Савченко, кандидат технічних наук, доцент**, *E-mail*: savchenkolm68@ukr.net
А.М. Григоренко, кандидат технічних наук***, *E-mail*: anzhegrig@yandex.ru,
* Кафедра технології хліба, кондитерських, макаронних виробів і харчоконцентратів
Харківський державний університет харчування та торгівлі, вул. Клочківська, 333, м. Харків, Україна, 61051
** Кафедра харчових технологій
Чернігівський національний технологічний університет, вул. Шевченка, 95, м. Чернігів, Україна, 14027
***Головний технолог ТОВ «Чарівна мозаїка», Автострадна набережна, 21-А, м. Харків, Україна, 61038

Анотація. У статті досліджено показники безпеки та екологічної чистоти порошків з виноградних кісточок порівняно з какао-порошками натуральним та алкалізованим. У досліджуваних порошках визначали вміст важких металів атомно-адсорбційним методом; активність радіонуклідів спектрометричним методом; наявність мікотоксинів B_1, Т-2-токсину, зеараленону та дезоксиніваленолу (вомітоксину) методом тонкошарової хроматографії; кількість нітратів іонометричним методом та забруднення порошків фосфорорганічними і хлорорганічними пестицидами хроматографічним методом.

За результатами проведених експериментів встановлено, що у всіх досліджуваних порошках вміст важких металів знаходиться у межах гранично допустимої концентрації, причому порошки з виноградних кісточок відрізняються значно меншим вмістом солей плюмбуму, цинку, купруму. Активність радіонуклідів у виноградних порошках за радіаційною ознакою значно нижче межі допустимої концентрації. Наявність афлатоксину B_1 виявлено у пробі какао алкалізованого, зеараленону – у пробі порошку з макухи виноградних кісточок. Їхній вміст не перевищує рівні допустимої

концентрації. В інших дослідних зразках мікотоксини відсутні. Вміст нітратів у зразках какао-порошків натурального та алкалізованого перевищує такий у виноградних порошках у 20–30 разів. Кількість фосфороорганічних та хлороорганічних пестицидів у всіх зразках какао-порошків та порошків з виноградних кісточок – нижче межі визначення методу. Зразки глазурі з додаванням дослідних добавок мають значно нижчий вміст важких металів порівняно з контролем.

Глазур з частковою заміною какао порошку на виноградні порошки має високі органолептичні показники якості, в процесі глазурування вона наноситься на поверхню виробів тонким шаром та остигає дрібними хвилями під час охолодження. Отримані результати свідчать, що сировина вітчизняного походження є безпечною для здоров'я людини, а кондитерська глазур, вироблена з частковою заміною какао-порошку порошком з кісточок винограду, має високі показники безпеки.

Ключові слова: показники безпеки, ксенобіотики, порошок з виноградних кісточок, какао-порошок натуральний та алкалізований.

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Introduction. Formulation of the problem

Food products safety is a topical issue due to the following factors: problematic ecological situation both in Ukraine and worldwide; extensive use of genetically modified organisms; intensification of crop and livestock farming; enhanced elevated pollution of the environment with household and industrial wastes, resulting in a higher concentration of nitrate and nitrite forms of nitrogen in water, soils and, thus, in food; presence of heavy metals, toxic elements, radionuclides, etc. in raw materials and final products.

Continuous consumption of such products results in deterioration of people's health and in spreading of serious illnesses: cardiovascular, allergic, gastrointestinal, oncological, etc. Plenty of low-quality products, adulterated and therefore dangerous for human health, are permanently supplied to the retail chain. Ensuring safety and quality of food products is one of major tasks of the modern society. People's health and preservation of our genetic resources depend on the successful solution to these problems [1-3].

Careful attention to the food products safety and quality is also related to the Ukraine's accession to the World Trade Organization (WTO) and adapting the products' quality and safety control systems to the requirements of the European Union (EU).

The food products' safety has to be controlled during every phase of their lifecycle, starting from the cultivation of agricultural raw materials and up to the packing, storage, and transportation of the final product [4,5].

Nowadays, to prevent nutritional diseases, developing the technologies for dietary, preventive, and medical products is becoming ever more relevant. First of all, it concerns confectionary products, which are especially popular with kids. Using vegetable raw materials, including the second-generation materials, is the priority direction for the development of the confectionery industry. We know that plants contain a lot of biologically active substances able to impact the human organism in the protective, preventive, and health-improving manner. However, it should be emphasized that due to their cumulative ability, plants could be a source of substances not only useful, but dangerous as well, which they absorb in the ecologically polluted areas. This is also due to the fact that plants are the key element of the environmental chain linking all the objects of the biosphere [6]. Further, during the processing of raw materials, toxic matters pass over into the food products and thus into the human body and affect internal organs, as well as physiological processes in them. That is why, when developing new technologies for health-improving products, the focus should be on the safety characteristics of vegetable raw materials included into traditional recipes.

Analysis of recent research and publications

Development of confectionary production technologies involving products of processing vegetable raw materials cannot go without studying how these raw materials effect on the safety characteristics of the final product. The amount and origin of substances accumulated in them can depend on different conditions. For example, a research of the heavy metal content of peanuts has shown that the extent of their accumulation depends on the environmental conditions, on their mineral nutrition, and on particular characteristics of the cultivar [7]. The content of heavy metals in different types of vegetable raw materials has been investigated – in black chokeberry, rose hips and cranberries, ginger and liquorice roots, and extracts based on these raw products. The results of the experiment have shown that even when the content of heavy metals exceeds the levels determined for the raw materials, extracts of those materials contain heavy metals within the maximally permissible concentrations [6]. It has been investigated how adding extracts of basil, cinnamon, sage, cloves, spirulina, and that of pomegranate peel and pips effects on the content of cuprum, cadmium, zinc, mercury, and arsenic in the candy caramel. It has been shown that their number in the product samples does not exceed allowable concentrations, and that the changes in the plumbum content are inversely proportional to the amounts of the additives investigated [8].

Herbal additives can be an unwanted source of nitrates, nitrites, pesticides, mycotoxines, radionuclides. The content of nitrate-ions was investigated in eight samples of the most common food products (apples, potatoes, carrots, tomatoes, cabbages, beets, oranges, bananas) in the period from 2010 to 2015. The investigation results show that the content of NO_3^- ions in different kinds of apples, oranges, potatoes, and carrots is within the maximally concentrations (MPC). In bananas and tomatoes, it exceeds the MPC by 40–55 and 30–68 mg/kg, respectively, in cabbages and beetroots by 1.25–2.46 and 2.37–3.74 times [9].

In fact, 30–80% of contaminants are absorbed by the human body with food. To prevent this, the Codex Alimentarius has been introduced in the developed Western countries. It is a set of legislative acts concerning the composition, properties, and quality of food products. Processing facilities are introducing the system for the Hazard Analysis and Critical Control Points (HACCP), a quality control system for the production of food with regard to the risks criteria (so called safety technology of the resulting product).

Cocoa products (natural and/or alkalized cocoa powder, crude chocolate, crushed cocoa, cocoa butter) are considered to be one of the main types of raw materials in the confectionary glaze recipe. These raw materials are supplied exclusively by foreign suppliers, because cocoa beans only grow in the subtropical climatic zone countries. That is why the output of cocoa products remains practically unchanged, but the demand grows constantly. For example, consumption of chocolate in the developing countries (India, China, Saudi Arabia) over the past years has grown considerably. Increased demand for cocoa products results in prices raising, in a considerable quality decrease, and in the products' counterfeiting. That is why the search for alternative substitutes of cocoa-products is essential. Grapeseed powder (GSP) is one of those products. Using grapeseed powders could not only decrease the prime cost of the glaze, but also enrich it with biologically active substances, first of all phenol compounds with high antioxidative activity [10], so as to increase the anti-oxidative properties of confectionary products. Many researches are dedicated to studying phenols contained in grapes, wine, grapeseeds [11-13]; their positive impact on the human health is proved [14,15], especially considering oxidative stress conditions.

The experience of using grapeseeds in glazing technology to replace partially the cocoa powder dates back to the Soviet Union period. Then, grape stones were roasted, ground with sugar, and used instead of cocoa products. However, a processing temperature of 100°C and even higher resulted in changes of the chemical composition of the product and in loss of valuable compounds.

We have developed a confectionary glaze technology that involves partial replacement of the cocoa powder through incorporating grapeseeds or grapeseed oil cake powders. Grapeseed powders were received in gentle conditions by double-phased drying at a temperature below 60°C. Thus, it allowed preserving biologically active components as much as possible, especially polyphenol ones [16]. The recipe and technology of the traditional fat glazing has been used as a basis.

As for the organoleptic characteristics of the glazing developed, it is a little lighter compared to the reference sample. Concerning its physical and chemical characteristics, such as moisture content, viscosity, flowability, the glazing does not differ from the reference sample.

Replacing cocoa powder with grapeseed powder allows solving two tasks: on the one hand, this can enrich the powder with essential substances, including micro- and macroelements, antioxidants, which is important under the current conditions, and on the other hand, it would allow lowering the prime cost of the final product, taking into account that GSP is a second-generation raw materials and is available in Ukraine in commercial quantities.

Researches performed earlier prove that according to their microbiological characteristics, grapeseed powders are much safer than cocoa powder. Moreover, model systems have been used to study and prove that grapeseed powders have a bactericide effect. Due to their antimicrobial action [17], the microbiological stability increases considerably, and improves the quality of glazing itself and of glazed products as well, which, in turn, allows extending the shelf life of products [18].

The purpose of the article is to study the safety of grapeseed-containing powders in comparison with cocoa powders (natural and alkalized), to confirm the possibility of equivalent replacement of cocoa products to produce high-quality confectionary glaze according to safety indicators.

Tasks of the research:

1. To study and analyse safety characteristics of grapeseed powders and cocoa powders.

2. To study safety characteristics of the developed confectionary glaze with cocoa powder partially replaced with grapeseed powders, and to compare their safety parameters with those of the glaze reference sample.

Research Materials and Methods

As an alternative to cocoa powder, grapeseed-containing powders have been chosen, obtained from winemaking afterproducts at the factory 'Orion' (Odessa, Ukraine) under the trademark 'Oleo Vita.' Powders investigated as to their texture and technological properties are very similar to cocoa powder and could replace it. Two grapeseed powder samples were used during the investigation:

1) powder obtained by grinding grapeseeds separated from the dejuiced grapes (GSP);

2) powder obtained by grinding the grapeseed oil-cake resulting from pressing the grapeseed oil (GSOCP).

As for the natural and alkalized cocoa powder, four samples from different producers were selected: two samples from wholesale suppliers of raw materials for the production needs of confectionery enterprises (samples 1, 2), and two sold in the retail network (samples 3, 4), namely:

1) Natural cocoa powder (Poland);

2) Alkalised cocoa powder (Spain);

3) Natural cocoa powder from the TM «Rozumnyy vybir»;

4) Natural cocoa powder from the TM «Mriya».

The confectionery glaze manufactured according to the DSTU 4660:2017 'Glazes and masses for moulding. General technical requirements' was used as a reference sample. In the tested samples, the cocoa powder was replaced with GSP (sample 2) and GSOCP (sample 3) in the amount of 3.0% of the total mass of the glaze.

Concentration of heavy metals was studied by the atomic adsorption method. The lead, cadmium, copper, and zinc concentrations was measured in the ashes of the source raw material by the atomic-adsorption method with the spectrophotometer Semy C-115 M1 (Ukraine); the mercury concentration was measured by the cold vapour method with the spectrophotometer GRG-107 Cortec; the arsenic concentration was measured with the thermal atomic adsorption spectrophotometer Varian Spectr AA 240 Z (Australia) according to the procedure described in GOST 26929-86.

The activity of radionuclides was evaluated by the spectrometric method. Radiation activity of strontium Cs-137 and Sr-90 was measured in GSP and GSOCP samples by the spectrometric method with a multipurpose spectrometry complex "Gamma Plus" (Russia) according to the 'Methodics of measuring radionuclide's activity by applying the scintillation beta-spectrometer with the "Progress" software, Mendeleevo, 2004' and 'Methodics of measuring radionuclide's activity by applying scintillation gamma-spectrometer with the "Progress" software, Mendeleevo, 2003.'

Testing the samples for the concentration of mycotoxins B1, T-2-toxine, zearalenone, and deoxynivalenol (vomitoxin) was performed by the thin-layer chromatography method according to the MV15-14/73-98 'Guidelines for sanitary and mycological assessment and feed quality improvement.' The prepared extracts of the tested cacao and grapeseed powder samples were applied on chromatographic plates Sorbfil and observed in UV-rays with a wavelength of 365 nm.

The content of nitrogenous compounds in cacao and grapeseed powder samples was studied according to the DSTU 4948:2008, MV 15-14/248-2000. The concentration of nitrates was evaluated by the ionometric method.

The concentration of organophosphorous and organochlorine pesticides in the powders under study was analysed by the chromatography method based on the methodology described in [19].

Results of the research and their discussion

Heavy metals affect adversely the human organism. With their high biological accumulation, they can produce mutagenic, carcinogenic, teratogenic, embryogenic, and gonadotoxic effects. Mercury, cadmium, lead, arsenic can cause the disruption of methabolic function of an organism, when applied even in small doses. The above metals, as well as copper, strontium, zinc, and iron, have been included by the Codex Alimentarius Commission in the list of components, concentration of which is to be controlled in the international trade of food products. Another six elements are to be controlled in Ukraine and CIS – antimony, nickel, chromium, aluminium, fluorine, iodine [1].

Concentration of toxic elements in cocoa powders shall not exceed the maximum permissible concentrations provided for by the State Sanitary Rules and Standards (SSanRS) 42-123-4089. The results of the performed research are presented in Table 1.

Table 1 – Concentration of heavy metals in the cocoa and grapeseed powder samples

Sample No	Primary product	Concentration of salts of heavy metals, mg/kg					
		Hg	Pb	Cd	Zn	Cu	As
	Standard (allowable concentration, mg/kg)	0.10000	1.00000	0.50000	70.00000	50.00000	1.0000
1	Natural cocoa powder	0.01260	0.70223	0.08229	28.81319	16.17743	0.0027
2	Alkalised cocoa powder	0.00971	0.72624	0.03717	1.58521	0.15576	0.0016
3	Natural cocoa powder, TM 'Rozumnyy vybir'	0.00203	0.28376	0.00149	10.45093	0.21091	0.0265
4	Natural cocoa powder, TM 'Mriya'	0.00408	0.48082	0.00559	21.36480	0.85597	0.0238
5	GSP	0.00000	0.22901	0.00000	7.50357	7.40949	0.0017
6	GSOCP	0.00922	0.34912	0.03437	6.82057	6.37509	0.0016

Based on the investigation results, a conclusion can be made that the content of heavy metals in all tested powders remains within the maximally permissible concentration. However, it is obvious that the grapeseed powders are distinguished by a considerably lower content of plumbum salts (on the level 0.2–0.3 mg/kg), while in the natural and alkalized cocoa powder samples it reaches 0.7 mg/kg, which is more than

twofold higher. A relatively high content of cadmium salts was shown by the natural cocoa sample No. 1 (0.08 mg/kg). A similar cadmium content (0.03 mg/kg) was detected in the alkalized cocoa powder (No. 2) and GSOCP (No. 6); it was practically absent in other samples. A high content of zinc salts has been detected in all the natural cocoa powder samples, with samples No. 1 and No. 4 showing a 3–4 times higher level compared to grapeseeds powders. Natural cocoa-powder sample No. 1 is worth mentioning, its cuprum content being 16.2 mg/kg. The same parameter for GSP and GSOCP is 6.8 and 7.5 mg/kg, respectively. Other samples contain cuprum salts in concentrations below 1.0 mg/kg. The content of arsenic salts in all samples investigated is considerably below the regulatory level. Thus, the lowest content of heavy metal salts has been detected in grapeseed powders, the highestin the natural cocoa sample No. 1.

Basing on the data received, one could assume that in the confectionary glaze where the cocoa powder is partially replaced with grapeseed powder, the toxicity indicators will be better as compared to the reference samples.

For many regions of the planet, and especially for Ukraine, ionizing radiation is one of major environmental toxicants. Up to 90% of the ionizing radiation exposure doze is absorbed by people from radionuclides, which enter the body with food products [1]. So monitoring their raw materials content and the final product is an overriding need. Raw materials supplied to our internal market undergo a thorough radiological examination; that is why the content of radionuclides has been investigated only in non-traditional raw material, which includes the products resulting from processing dejuiced grapes.

According to the experiment results, the Cs-137 activity in the tested GSP and GSOCP samples is < 2.2, and <4 Bq\kg, and Sr-90 < 4.8, and <5.5 Bq\kg, respectively. Basing on these results, a conclusion can be made that the activity of radionuclides in grapeseed powders is below the allowable concentration level. According to the 'Allowable Concentration of Radionuclides Cs-137 and Sr-90 in Food Products and Potable Water', the required activity of Cs-137 and Sr-90 should not be higher than 200 and 50 Bq\kg, respectively.

Another dangerous factor that could toxically impact the human body through food products is accumulation of mycotoxins, which are the secondary metabolites of microfungi. They are the most dangerous natural ecotoxicants for the human health and for animals.

So far, about 250 types of microscopic fungi have been identified, which produce ca. 200 mycotoxines, many of them causing alimentary toxicosis of humans and animals. A significant number of mycotoxines have immunosuppressive, mutagenic, allergenic, teratogenic properties, contribute to the reduction of the general resistance of an organism, to the development of infectious and non-contagious diseases, produce cancerogenic effect [1].

One of the most wide-spread types of microscopic fungi is the fungi of the families *Fusarium* and *Aspergillus*, in particular *Fusarium sporotrichioides, Fusarium graminearum, Aspergillus flavus, Aspergillus parasiticus*, which produce mycotoxins dangerous for animals and humans: T-2-toxin, zearalenone and deoxynivalenol (vomitoxin), aflatoxins B_1, B_2, G_l, G_2, M_l.

Micromyces can contaminate vegetable raw products during all phases of their production, storage, processing, and transportation. Mycotoxines in the samples under study can be generated due to the propagation of mold-line fungi caused by non-observance of the proper temperature and humidity while planting, storing, or transporting a certain raw material. So the investigation of the mycotoxines content in the selected samples of raw material was important.

None of the samples have shown the turquoise and blue colour glowing that would evidence the presence of the T-2-toxine and of the desoxynivalenol (vomitoxin), respectively. In the alkalized cocoa powder, glowing shaped like a dark-blue spot and comparable to the standard has been observed, confirming the presence of aflatoxin B_1. To prove the result obtained, the plate was treated with nitrate acid; thereafter, the reference spot and the cocoa sample changed their colour to yellow. Calculations showed the presence of 0.001 mg/kg of aflatoxin B_1 in the mentioned cocoa-powder sample. Thus, the presence of aflatoxin B_1 in 0.001 mg/kg concentration has been evidenced in the alkalized cocoa powder sample while its normal content in food products should not exceed 0.005 mg/kg.

The dark-blue glow on the same level as the standard has been detected in GSOCP. After the repeated investigation of the sample, the availability of zearalenone in the amount of 0.19 mg/kg has been confirmed while the normal content should not exceed 1.0 mg/kg.

The investigations have identified the presence of aflatoxin B_1 in the alkalized cocoa powder sample and of zearalenone in the GSOCP sample. The content of identified mycotoxines does not exceed the maximum permissible concentration levels. Mycotoxines were absent in other samples inspected.

One of important problems of our time is the enhanced content of nitrogen compounds in food products, especially in products of vegetable origin. The reason for that is the excessive use of mineral fertilizers and violation of the technology of their application. Excessive content of nitrates in the human diet is highly dangerous for human health. Long-lasting and systematic consumption of these compounds with food can have a carcinogenic effect.

The results of studying the content of nitrogen compounds are presented in Table 2.

The presented data evidence that the content of nitrates in the natural and alkalized cocoa powder samples exceeds by 20–30 times that of grapeseed pow-

ders, and approximately by 10–12 times of the natural cocoa powder samples.

Table 2 – Concentration of nitrates in the samples analysed

Concentration of nitrates NO_3^-, mg/kg			
Natural cocoa powder	Alkalised cocoa powder	GSP	GSOCP
270.58	364.56	11.403	13.650

Besides fertilizers, different chemical compositions with pesticide activity are used in plant cultivation to control pests and preserve the crop. These can also be accumulated in products of vegetable raw materials processing. On studying the content of organophosphorous and organochlorine pesticides in the powders under analysis, it has been established that all cocoa powder samples and all grapeseed powders contain less than 0.005 mg/kg of substances with pesticidal potential, i. e. below the method detection threshold.

The heavy metals content has been tested in glaze samples. The glaze recipe includes natural and alkalized cocoa powder, and 3 % of its total quantity has been replaced by GSP and GSOCP. The investigation results are presented in Table 3 below.

Table 3 – Content of heavy metals in the confectionary glaze samples with grape seeds

Sample No.	Confectionary glaze samples	Content of salts of heavy metals, mg/kg					
		Hg	Pb	Cd	Zn	Cu	As
	Standard (allowable concentration level, mg/kg)	0.10000	1.00000	0.50000	70.00000	50.00000	1.0000
1	Reference sample	0.00486	0.31005	0.02291	5.92748	3.88687	0.0004
2	3.0% of GSP added	0.00000	0.15893	0.02296	5.45021	2.93047	0.0002
3	30 % of GSOCP added	0.00340	0.24005	0.02140	5.29336	2.99840	0.0002

The results prove a high safety level of confectionary glaze that includes grapeseed powder. This is explained by the fact that GSP and GSOCP have a considerably lower content of heavy metals compared to that of cocoa powders. So, partial replacement of cocoa powder by grapeseed powder will allow lessening the content of heavy metals in the glaze, which is confirmed by the results of our research.

The glaze with cocoa powder partially replaced by GSP (sample 2) and by GSOCP (sample 3) looks like finely powdered smooth dark-brown mass, with the taste and smell peculiar to the glaze, without foreign flavours and odours. At temperatures between 0 °C and 18 °C, the glaze texture is hard; within the range between 28°C and 40°C, it is fluent. In the glazing process, glaze is applied on the surface of products in thin layers and cools down in small waves.

Conclusions

1. Investigation of safety indexes showed that, compared to the cocoa powder samples being a traditional raw material for the production of confectionary glaze, grapeseeds and grapeseed oilcake powders intended to be used as an alternative to cocoa powder in the confectionary glaze technology have a considerably lower content of heavy metals, nitrates, nitrites, and mycotoxines.

2. Determination of safety indexes of the invented confectionary glaze with the cocoa powder partially replaced by grapeseeds powders has shown that the content of heavy metals in the samples under study is lower compared to those in the reference sample. Thus, powders containing grapeseeds are a promising raw material to be used in the confectionery production.

The results of the research show that the content of toxic elements, mycotoxines, pesticides, microbiological indicators, and radionuclides in the raw material (cocoa powder, GSP, GSOCP) does not exceed the maximum allowable levels accepted by the requirements of the Order of the Ministry of Healthcare (MHC) of Ukraine No.368 dd. 13.05.2013, the Order of the MHC of Ukraine No.1140 dd. 29.12.2012, the Order of the MHC of Ukraine No. 548 dd. 19.07.2012, DSanPiN 8.8.1.2.3.4–000 and the GN 6.6.1.1–130.

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