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PHYSICAL FACTORS RELEVANT FOR EFFICIENT HAWTHORN FRUIT EXTRACTION

Ivan Fiodorovich Gorlov, Olga Vyacheslavovna Drucker, Vera Vasilievna Kryuchkova, Marina Ivanovna Slozhenkina, Natalya Ivanovna Mosolova, Olga Andreevna Knyazhechenko

ABSTRACT

Today, the healthy nutrition market is one of the most promising market niches; modern consumers increasingly claim fortified products for their diets. One of the ways to increase the biological and physiological value of products is their enrichment with extracts of plant origin. The aim of the work was to study the influence of various factors on the process of hawthorn fruit extraction and determine the optimal parameters of the technological process. The study objects were hawthorn fruit extracts produced by the statistical method of maceration (with stirring). In the extracts obtained, there were determined the quantitative content of tannins and pectin substances, dietary fiber and vitamins. Currently, the global health and wellness food market is steadily growing due to changes in consumer behaviour patterns and developing of healthy self-consciousness. The studies conducted by the authors have shown that extracting of plant materials and the efficiency of biologically active substances extraction are influenced by the following factors: the extractant pH, grinding type and size of raw materials and process parameters. The optimal technological regimes have been established. They are the extraction temperature of 60 °C and extraction time of 30 minutes. The appropriate grinding size of dry hawthorn fruit has been determined to be up to 2.8 mm of a particle. In case of milk being an extractant, a high extraction dynamic of pectic substances and dietary fibers was observed. So, the hawthorn milk extract has been revealed to have higher organoleptic characteristics.

Keywords: dietary fiber; extraction; fermented milk product; hawthorn fruit; pectic substances; tannins

INTRODUCTION

Currently, the global health and wellness food market is steadily growing due to changes in consumer behaviour patterns and developing of healthy self-consciousness **(Nikolaev, 2012)**. Food products, combining a fairly complete set of vitamins and minerals with other biologically active substances, such as dietary fiber, pectic substances and phospholipids, are appearing on the market. Sometimes the content of some enriching additives in one product is undesirable or impossible due to their taste incompatibility, instability or undesirable interactions with each other **(Kukharenko et al., 2008)**. One solution to this problem is to use food fortification with plant extracts. Extraction is the most acceptable way to obtain biologically active substances from plant materials with respect to efficiency and rationality and provides the maximum yield of biologically active substances that increase the physiological value of the product enriched **(Lyapin et al., 2009)**.

The extraction process is caused by a complex of interacting factors that make it difficult to establish general principles for intensifying this process **(Khalanskaya, Lodygin and Kurchenko, 2017)**.

To obtain various types of extracts, fresh and dried raw materials are applied. Fresh raw material is usually used in small quantities and on rare occasions because of its storage and transportation being complicated, as well as rapid decomposition of biologically active substances in it **(Aliyev and Stepanov, 2006)**. The raw materials subjected to drying undergo considerable changes, i.e., dry residue is obtained from cellular fluid; the inside of the cell is filled with air; after drying, the cell wall and membranes of the cell organelles acquire the properties of porous membranes. Therefore, depending on the raw materials used and the cell wall's physiological state, there are some features of the extraction of biologically active substances **(Tikhonov and Yarnykh, 2002)**. A number of physicochemical processes take place inside the cell of the dried raw material and on its surface under the action of the extractant, i.e., diffusion, desorption, dissolution, dialysis and leaching **(Leonova and Klimochkin, 2012)**.

Diffusion is the main physical and chemical process. The presence of a porous membrane of dry plant material during extraction leads to the movement of the substance in both directions and the nature of diffusion through it constitutes a dialysis process. Biologically active substances whose molecules do not exceed the pore size can diffuse through the porous membranes of the cell wall, so, the vast majority of extraction preparations are obtained from dried plant materials.

In terms of the nature of diffusion, the main stages of extraction are distinguished **(Tretyakova, 2014)**. They are diffusion of extractable substances from the inside of the cells to their surface; diffusion of substances through the laminar sublayer, surrounding the particle and arising due to viscous forces of the extractant during the flow of raw materials through the layer; and convective diffusion of the extracted substances from the outer surface of the laminar sublayer into total extractant flow. Convective diffusion is the more effective, the more intense the hydrodynamic regime (mixing) is. The thickness of the laminar sublayer depends on the hydrodynamic regime.

Desorption is a part of the extraction process and takes place in the cells when the extractant penetrates them, which results in a concentrated solution ("primary juice") in cells. Due to the difference in osmotic pressures, soluble substances leave the cells, and the extractant penetrates into them; "colliding" the processes of osmosis and dialysis leads to swelling of plant material **(Zyubr and Vasiliev, 2008)**. Diffusion processes are mass transfer processes, occur spontaneously and proceed until true dynamic phase equilibrium is established between the phases under certain conditions. Equalizing the concentration on both sides of the cell membrane to achieve a state of mobile diffusion equilibrium means the completion of the process at this extraction mode **(Ishanhodzhaeva, 2012)**.

The phytocomponents extraction dynamics is influenced by a number of factors, i.e., the pH of the extractant, types of the extractant and raw material, its grinding size, ratio between the weight of the raw material and volume of the extractant, the temperature regime of extraction and duration of extraction.

So, when selecting the technological parameters of the extraction process, the following factors affecting the released biologically active substances should be taken into account: the acidic pH of the extractant helps to transfer difficultly soluble alkaloid compounds (complexes with tannins) into easily soluble alkaloid salts and dynamics of tannins extraction; temperature drop causes decrease in the solubility of tannins; while drying raw material, muciform substances destruct, which contributes to the increased extraction of pectin; in the extraction process, the difference between concentrations of the extracted substance in the liquid and solid phases gradually decreases, so it is necessary to determine the dynamic equilibrium of the extraction process at the appropriate time **(Terletskaya, Planer and Zinchenko, 2013)**.

An important intensifying factor in extracting biologically active substances is continuous mixing of raw material and extractants, which ensures the best hydrodynamic conditions of the process, i.e., the particles of the raw material do not become compressed and are constantly and intensively washed with extractants. So, the conditions of mass transfer improve, the driving force of the extraction process increases, and greater yield of extracting substances is reached **(Oorzhak, Ushanova and Repyakh, 2003; Sorokopud, Mustafina and Fedyaev, 2012)**.

Scientific hypothesis

Study the influence of various factors on the process of hawthorn fruit extraction and determine the optimal parameters of the technological process.

MATERIAL AND METHODOLOGY

The objects of the study are hawthorn fruit (*Crataégus*) extracts produced by the maceration (with mixing). To obtain extracts of hawthorn fruit (*Crataégus*), the following equipment was used: a laboratory mill LZM-1 (LAB-AGRO, Barnaul), laboratory scales VLT-1500-P (SARTOGOSM, St. Petersburg), infusing device AI-3 (Kiev production association MEDPARATURA, Kiev).

Hawthorn fruit (*Crataégus*) was extracted by the method of maceration with stirring. To study the factors influencing the efficiency of the extraction of tannins, pectic substances and dietary fiber, the following parameters of the extraction process were chosen:

- types of extractants, i.e., distilled water, whey, milk and cream;
- extraction temperature of 50 ±2 °C; 60 ±2 °C; and 70 ±2 °C;
- duration of extraction of 20 min, 30 min, 40 min and 50 min; and
- grinding size: up to 1.0; up to 2.8; and up to 5.6 mm.

The experiment was repeated three times; 185 prototypes were studied.

Figure 1 Photo of hawthorn fruit (*Crataégus*): fresh and dried.

Quantitative determination of tanning substances in the objects was carried out by the spectrophotometric method, pectic substances by the titrimetric method and dietary fiber by the enzymatic-gravimetric method **(Fedoseeva, 2005)**. Quantitative determination of vitamins in extracts was performed by the high-performance liquid chromatography.

Statistic analysis

Statistical data were processed using the Statistical program (StatSoft, version 9.0 (Dell, USA). The data are presented as averages. The differences between the samples were assessed using unpaired *t*-test. Correlation analysis with calculation of pair correlation coefficient, for establish the dependence of parameters was used. The significance of differences was determined by the Student's criterion (t). The level was considered significant at $p \leq 0.05$. The study was repeated three times.

RESULTS AND DISCUSSION

Under laboratory conditions, hawthorn fruit extracts were produced according to the technology that included the following steps: dried hawthorn fruit was ground in a cutting mill; the ground vegetable raw material (5%) was placed in a pre-warmed porcelain mug and filled with extractant; distilled water (pH of 6.46), whey (pH of 6.12), milk (pH of 6.54 and weight fraction of fat content of 2.3%) and cream (pH of 6.68 and weight fraction of fat content 10%) were used as extractants; the mug was capped, heated in a water bath to a temperature of 50 – 70 °C and kept for 30 minutes, periodically stirred; the resulting mass was filtered and squeezed; and the quantitative contents of extractable substances were determined with respect to the extractant used. The research results are presented in Figure 3.

Viscosity of the extractant had a great influence on the solubility and the substances' diffusion rate. The extraction efficiency depends on a large number of parameters, i.e., extraction method, extractant and technological parameters of extraction. In this regard, it is very important to find the optimal extraction parameters for obtaining extracts with the highest content of biologically active compounds **(Lincheva et al., 2017)**.

The experiments have shown that the nature of the extractant, temperature regime of the extraction process have a considerable impact on the yield of extractable substances (Figure 3). According to the Fik-Shchukarev's law, the amount of dissolved substance, diffusing through an extractant layer was directly proportional to the difference in concentrations of this layer, time and surface area of the layer and inversely proportional to the thickness of the diffusion layer. So, less viscous extractants had greater diffusion capacity. Increasing temperature of the extractant reduced the kinematic viscosity. Therefore, heating was used to reduce the viscosity during extraction **(Kňazovická et al., 2015)**. In cream, high viscosity was caused by milk fat and particularly by the formation of clusters of fat globules; structural viscosity was due to the interaction between protein and water. In cream, it was not casein that interacted, but shell protein that had larger contact surface with water, which reduced the diffusion coefficient; and mixing the extracting raw materials lead to mechanical destruction of hawthorn fruit tissue and mixing colloidal particles, which made the process of separation of the mixture after extraction more difficult. Therefore, the extract on the basis of cream had low values of extractable substances. Thus, whey and milk were established to be the most suitable extractants for the extraction of biologically active substances.

Some researchers extract hawthorn fruit using acidic methods for efficient pectin extraction **(Yenipinar and Yildirim, 2014)**.

A number of scientists also emphasize the influence of plant materials grinding and choice of temperature parameters on the extraction process **(Ivanova et al., 2008; Titova and Aleksanyan, 2013; Terletskaya et al., 2013)**.

The conducted experiments showed that the type of extractant and temperature regime of the extraction process had a significant impact on the yield of extractable substances. Compared with other extracts obtained, the whey extracts had an excess of tannins due to the low value of its active acidity, contributing to the intensification of the extraction process by converting low soluble compounds into highly soluble ones. High extraction dynamics of pectic substances was observed when milk being used as an extractant due to the interaction of pectin with casein and free calcium ions. The use of milk as an extractant also made it possible to obtain a high quantitative content of dietary fiber.

The study showed a direct relationship between the changes in the temperature regime of the extraction process and yield of extracted substances. The smallest quantitative content of extractable biologically active substances was observed at a temperature of 50 ºC. An increase in temperature caused their content increasing in direct proportion until the onset of dynamic equilibrium. The temperature of 60 ºC contributed to better separation of plant tissues and tearing of the cellular walls of hawthorn fruit, which led to the highest yield of extractable substances. So, in milk extract, the content of pectic substances was 62.98 $mg.100g^{-1}$, dietary fiber 176.08 $mg.100g^{-1}$. In whey extract contained tannins of 63.91 $mg.100g^{-1}$. These exceeded the weight fractions of tannins by 4.17%, pectic substances by 3.22% and cellulose by 3.82% compared with the extracts produced at a temperature of 50 °C.

The 70 °C temperature regime applied in the hawthorn fruit extraction led to peptization of substances, the extracts became slimy, and the extraction dynamics of biologically active substances decreased, the extractable substances in the obtained extracts had lower values than in extracts produced at 60 °C. The conducted studies showed that the most appropriate temperature regime for extracting hawthorn fruit was 60 °C.

Next, we identified the organoleptic characteristics of whey and milk 5% hawthorn fruit extracts. Tannins were characterized by a special tart, astringent taste, so the high content of these substances was unlikely to have the best effect on the organoleptic characteristics of the extracts, and further deteriorate the quality indicators of the product; therefore, it was important to conduct these studies. Organoleptic characteristics of the extracts are shown in Table 1.

The analysis of the indicators presented in Table 1 showed that the milk extract had higher organoleptic characteristics that allowed milk to be chosen as an extractant for further research. Despite the high content of biologically active substances, whey extract was inferior to milk extract with respect to its organoleptic characteristics because of its inharmonious, sour, astringent taste of whey and tannins in it.

The influence of the duration of the extraction process on the intensity of hawthorn fruit extraction was studied. For this purpose, 5% hawthorn fruit plant material was extracted with milk at a temperature of 60 ºC for 20, 30, 40 and 50 minutes. The obtained extracts were examined to determine the quantitative content of biologically active substances; the results are presented in Figure 3. Figure 3 shows that the increase dynamics of the extractable substances content in the milk extract was observed in the period of 20 – 30 minutes; during this period, biologically active substances had time to dissolve and diffuse before they coagulated or swelled. A longer extraction process within 40 – 50 minutes decreased their concentration; the extracts became too viscous; the dissolved substances diffused into the extractant more slowly; and the yield of substances decreased. Thus, during the 30 minutes extraction, the diffusion and osmosis of biologically active substances proceed at the greatest speed; their maximum extraction occurred, so, this time mode of extraction was established to be the most acceptable.

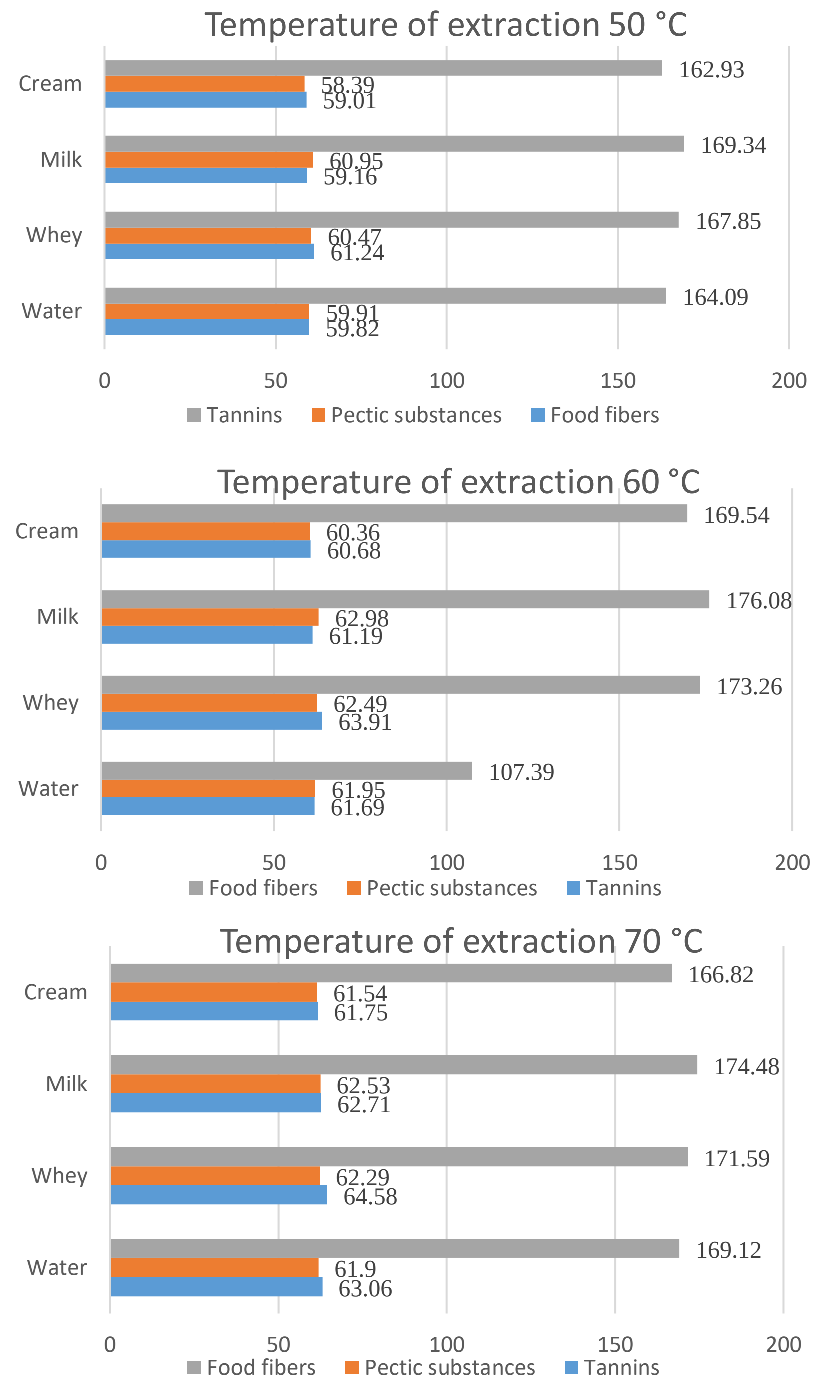

Figure 2 Weight fraction of extractable substances in the extract with respect to the extractant.

Table 1 Analysis of the indicators.

Indicator	Whey extract	Milk extract
Appearance and Consistency	Homogeneous consistency	Homogeneous consistency
Taste and smell	Inharmonic, sour, astringent taste of the extract with light aroma of hawthorn fruit	Milky taste and smell with pleasant aroma and sweetish taste of hawthorn fruit
Color	Brown, uniform throughout the mass	Cream, uniform throughout the mass

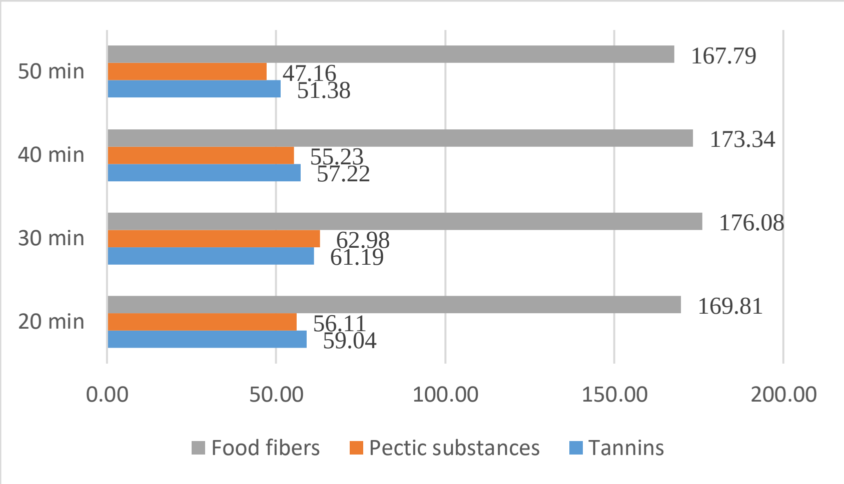

Figure 3 Weight fraction of extractable substances in the extractant (milk) with respect to the duration of extraction, mg.100g^{-1}.

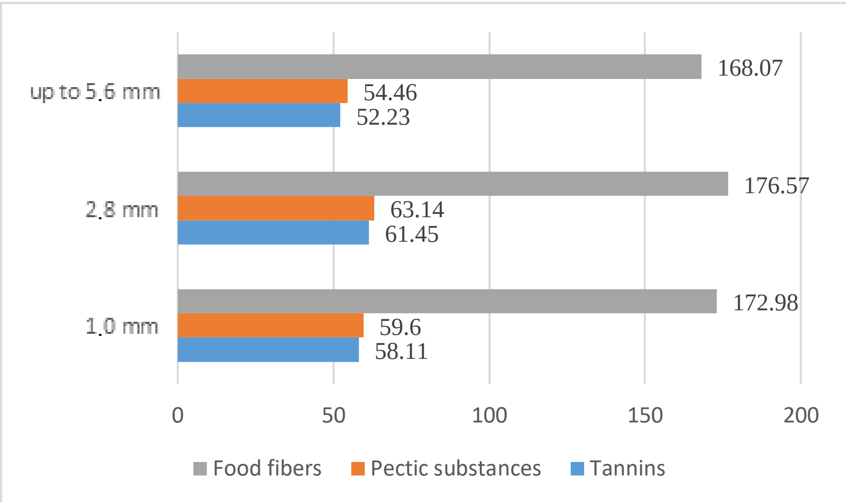

Figure 4 Weight fraction of extractable substances in the extractant (milk) with respect to the duration of extractionmg.100g^{-1}.

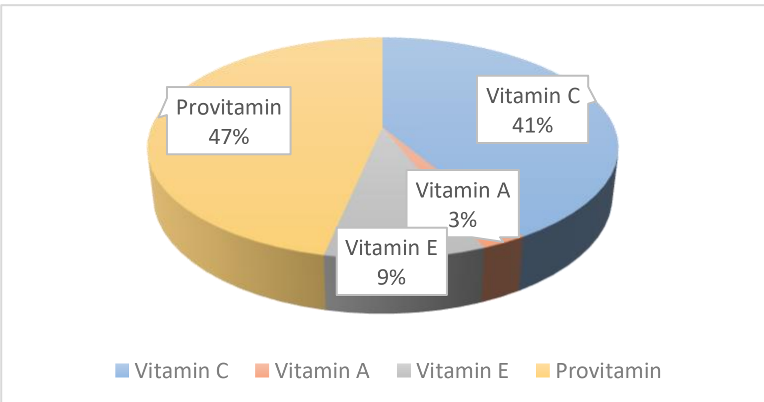

Figure 5 Vitamin composition of hawthorn fruit extract, mg.100g^{-1}.

To study the influence of the grinding size of dry hawthorn fruit on the yield of substances extracted, the raw material was ground before particles passed through a sieve with a hole diameter of 1.0, 2.8 and 5.6 mm and extraction with milk at a temperature of 60 °C for 30 minutes (Figure 4). The influence of the particle size of plant materials on the effectiveness of extraction was confirmed by the studies of some scientists; so, a decrease in the particle size of crushed raw materials (up to a certain minimum size) causes an increase in internal and external diffusion as well as mass exchanging, but the minimum size of raw materials must be accurately determined **(Iudina, 2015; Okhrimenko et al., 2011)**.

The data presented indicates that the appropriate grinding size for this type of raw material was a particle size of up to 2.8 mm, this grinding size contributed to the enlargement of the contact surface of the raw material with the extractor, its rapid penetration into the cells and hence the acceleration of the extraction process. The yield of extractable substances turned out to be maximum; the extract contained 61.45 mg.100g^{-1} of tannins, 63.14 mg.100g^{-1} of pectic substances and 176.57 mg.100g^{-1} of fiber.

Experimental studies indicated that a strong mechanical effect on the phytocomponent, i.e., grinding up to 1.0 mm in particle size was impractical and contributed to excessive cell destruction. An increase in grinding size of plant material enhanced the number of cells with a destroyed membrane and accelerated the leaching process. Fine powder of plant materials got easier compressed. In extracting, substances rapidly dissolved and swelled, and lumps were formed due to the adhesion of the slimy cells, settling to the bottom of the mug. All this greatly slowed down the extraction process. When grinding up to the size of 5.6 mm, active substances were incompletely extracted; in this extract, the pectic substances concentrated less and decreased by 8.68 mg.100g^{-1}, tannins by 9.22 mg.100g^{-1} and dietary fiber by 8.56 mg.100g^{-1} compared to the milk extract with raw material of a particle size being up to 2.8 mm. The vitamin composition of hawthorn fruit milk extract was determined. The results are presented in Figure 5.

The studies of the vitamin composition showed that the 5% hawthorn fruit milk extract contained the highest concentration of provitamin A (0.575 mg.100g^{-1}) that had antioxidant, adaptogenic and immunomodulatory properties and vitamin C (0.511 mg.100g^{-1}). The need for vitamin C especially increases in diseases of the gastrointestinal tract; ascorbic acid helps to strengthen the immune system and restore intestinal cells; it accelerates the elimination of toxins and toxins and improves digestion.

The optimal parameters of the extraction process are the extraction temperature of 60 °C and extraction time of 30 minutes. The decrease in these parameters was directly proportional to the yield of extracted biologically active substances; the increase in the temperature and duration of extraction also had a negative tendency, namely, the extracts became slimy and viscous, solutes diffused more slowly, and the dynamics of their extraction decreased.

The grinding size of dry hawthorn fruit was also important for the extraction; with respect to the high content of biologically active substances in the extract, the appropriate grinding size of the phytocomponent was the particle size up to 2.8 mm. The resulting extract contained vitamins C and E, provitamin A and vitamin A that increase the physiological value of the fermented milk product developed.

CONCLUSION

The experimental studies showed that the extraction is a complex process, combining diffusion, desorption, dissolution, dialysis and leaching. Plant material extracting is influenced by a number of factors that must be taken into account, i.e., the pH of the extractant, the types of extractant and raw material, grinding size of raw material and parameters of the technological process. The studies of the influence of various factors on the extraction efficiency of tannins, pectic substances and dietary fibers showed that milk was the optimal extractant with a high extraction dynamics of pectic substances and dietary fiber; the milk extract had high sensory characteristics, i.e., a homogeneous consistency, milky taste and smell with pleasant aroma and sweetish taste of hawthorn fruit, cream colour and uniform throughout the mass. Thus, the identified factors influencing the extraction process made it possible to establish that the statistical method of maceration (with stirring) with grinded hawthorn fruit being extracted allows obtaining an extract not only with a high content of pectin, tannins, dietary fibers and some vitamins, but with great sensory parameters. The introduction of the extract in food production will improve the quality indices and nutritional and biological values.

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Contact addresses:

*****Gorlov Ivan Fyodorovich, Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, Rokossovsky Str., 6, Volgograd, 400131 Russia; Volgograd State Technical University, Lenin Avenue, 28, 400050 Volgograd, Russia, Tel: +78442391048,
E-mail: nniimmp@mailru
ORCID: https://orcid.org/0000-0002-8683-8159

Olga Vyacheslavovna Drucker, Don State Agrarian University, Kryvoshlukova, 26, p. Persyanovsky, Rostov-on-Don, Russia, Tel: +79882509672,
E-mail: olgadruker@ya.ru
ORCID: https://orcid.org/0000-0002-9746-6338

Vera Vasilievna Kryuchkova, Don State Agrarian University, Kryvoshlukova, 26, p. Persyanovsky, Rostov-on-Don, Russia, Tel: +79882509672,
E-mail: kverav@yandex.ru
ORCID: https://orcid.org/0000-0003-2058-2370

Marina Ivanovna Slozhenkina,Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, Rokossovsky Str., 6, Volgograd, 400131 Russia; Volgograd State Technical University, Lenin Avenue, 28, 400050 Volgograd, Russia, Tel: +79047729999,
E-mail: niimmp@mail.ru
ORCID: https://orcid.org/0000-0001-9542-5893

Natalya Ivanovna Mosolova, Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, Rokossovsky Str., 6, Volgograd, 400131 Russia, Tel.: +79033735182,
ORCID: https://orcid.org/0000-0001-6559-6595
E-mail: natalyniimmp@yandex.ru

Olga Andreevna Knyazhechenko, Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, Rokossovsky Str., 6, Volgograd, 400131 Russia; Volgograd State Technical University, Lenin Avenue, 28, 400050 Volgograd, Russia, Tel: +78442391048, +79616696630
E-mail: knyazhechenko71@gmail.com
ORCID: : https://orcid.org/0000-0003-1508-2179

Corresponding author: *