UDC 664.31
DOI: 10.15587/1729-4061.2022.261473

REVEALING THE INFLUENCE OF TECHNOLOGICAL PARAMETERS ON THE PROCESS OF EXTRACTION FROM WALNUT SHELL

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The object of this study is the influence of technological parameters on the extraction process from walnut shells.

The main practical application of walnut is associated with the use of a kernel placed inside the shell. The kernel isolated after processing is used in confectionery, fat-and-oil, flour milling, pharmaceutical, and other industries. The walnut shell that remains after cleaning is waste and is usually disposed of. Analysis of studies reveals that walnut shells are rich in phenolic acids and related polyphenols, which are essentially a natural antibiotic with numerous healing effects. Along with this, crushed walnut shell is a universal organic, biodegradable, environmentally friendly, and valuable raw material with unique physical characteristics and chemical properties used in various sectors of the economy. Walnut shell is 52.3 % lignin, for comparison – almond shell contains 28.9 %, pine nut – 40 % lignin. Studies show that lignin characterizes the level of strength of the shell, and in its chemical composition is a source of antioxidants. Various methods of extraction of biologically active substances from walnut shells are used. However, the results obtained by different methods have a wide range of data. Optimization of extraction processes has been carried out and its regularity was established. By the method of mathematical modeling, optimal extraction modes were revealed under which the most complete extraction of biologically active substances is observed. Optimal extraction modes have been developed

Keywords: antioxidants walnut, biological activity, lignin, catechins, quercetin, optimization of extraction

Received date 02.06.2022
Accepted date 08.08.2022
Published date 30.08.2022

***How to Cite**: Sultanova, M., Abdrakhmanov, K., Nurysh, A., Saduaqas, A., Akzhanov, N. (2022). Revealing the influence of technological parameters on the process of extraction from a walnut shell. Eastern-European Journal of Enterprise Technologies, 4 (11 (118)), 35–42. doi: https://doi.org/10.15587/1729-4061.2022.261473*

1. Introduction

Walnuts are the fruit of the tree of the genus “Juglans” of the nut family, which are a valuable food product due to their high nutritional value and taste. About 3.8 million tons of walnuts are produced annually in the world. The main practical application of walnut is associated with the use of a kernel placed inside the shell. The kernel isolated after processing is used in confectionery, fat-and-oil, flour milling, pharmaceutical, and other industries [1].

When processing walnut fruits, a large amount of waste is generated, mainly consisting of partitions and shells. Since the walnut shell, which protects the kernel, makes up to 67 % of the total weight of the fruit [2], the volume of waste received during processing reaches 2.5 million tons on a global scale. At the same time, their applicability is extremely low, and the methods of disposal are the most primitive, that is, millions of tons of shells are burned and buried annually, which in turn leads to an increase in environmental problems.

At the same time, walnut shells, like other food products, contain a certain amount of biologically active compounds, mainly phenolic [3–8]. They have a beneficial effect on the human body due to their antioxidant, anticarcinogenic, anti-inflammatory, and antimutagenic properties [9, 10]. In addition, they play a significant role in the prevention of diseases in which free radicals are involved [11].

There is a tendency to increase the need for natural antioxidants in the production of various drugs and foods. Using agricultural and food waste to extract phenolic compounds is becoming an ideal and very attractive option for using raw materials with an initially low, even sometimes zero, cost.

It’s no secret that increased research efforts are now focused on recycling low-cost waste from the food, forestry, and agricultural industries because of environmental and economic benefits. Another important factor in favor of the use of agricultural waste is that the synthetic biologically active substances used today, which are toxic to the human body [12], can be replaced by those obtained from natural sources.

To isolate antioxidant substances, the extraction method is most often used. Antioxidant activity and the amount of extraction yield are related to the solvent used [13, 14]. Most often, methanol [15], ethyl alcohol [16], chloroform [13], water [17], N-butanol [18], and ethyl acetate [19] are used to extract antioxidant compounds while organic solvents are used for food extraction [20]. For the extraction of walnut shells, several types of solvents of different polarity (water, ethanol) were used since the choice of a suitable extractant turns out to be an important factor in the quality of the extract [21] and the level of food safety compared to other organic solvents.

Therefore, studies of the influence of technological parameters on the extraction process from walnut shells are relevant.

2. Literature review and problem statement

Paper [22] reports the results of a study into the use of walnuts as a food product. It is established that the fruit consists of four main parts: the kernel, peel, shell, and husk. The importance of walnuts is mainly due to their valuable kernels. However, their shells are now of no less interest than their kernels due to the beneficial effects of the shells. Kernels contain up to 75 % fatty oil, up to 6000 mg % amino acids, vitamin E, -carotene, vitamin C, salts K, Ca, Mg, S, P.

In work [23], the first complex identification of phenolic compounds in the husks and shells of walnuts was carried out. The results showed that the shell is a rich source of polyphenols. The dynamic trend of some polyphenols corresponded to the total number of phenols. In work [24], it was established that the iodine number of walnut oil reaches 133.59 mg I2, which indicates the presence of a significant amount of unsaturated fatty acids in it. If they are classified by drying, walnut oil can be called drying. It is established that the ratio of fatty acid groups o-3/o-6 in walnut oil is 1/10. It is concluded that walnut oil in its pure form (without the addition of other fats) is advisable to use in food. Paper [25] reports the results of a comparison of six different varieties of walnuts grown in Slovenia: Fernor, Fernette, Franquette, Sava, Krka, and Rubina. It is established that the highest content of phenols in walnut buds, followed by the shell, internal and external husks. Due to the high content of phenols, husks, buds, and walnut shells are valuable by-products of the walnut tree. It is proved that phenolic profiles can serve to determine the origin of different varieties of walnuts.

The predominant fatty acid of walnut oil is linoleic polyunsaturated fatty acid, which makes up 54.64 % of all its constituent acids, followed by oleic monounsaturated fatty acid (28.72 %). In work [26], in addition to caffeic, ferulic, p-kumaric, and synapic acids, cis- and trans-mono-caffeoylquinic, dicapheoylquinic, monoferuloylquinic, and cis- and trans-mono-p-coumaroilquinic acids were detected and quantified using liquid chromatography of ultrahigh pressure. In addition, seasonal changes in these secondary metabolites have been established.

Young walnut leaves are rich in iodine, vitamin C, and the alkaloid juglone (5 oxy-1,4-naphthoquinone), which is a good preservative. New promising iodine-containing plants were investigated [27]: young walnut leaves, honeysuckle, and feijoa in fresh, dried, and frozen forms. The studies have shown that the highest content of vitamin C and iodine was determined in young walnut leaves.

Paper [28] assessed the phenol content and antioxidant activity of various parts of the walnut, as well as the antioxidant and anti-radical activity of the prepared extracts. From the outer husk, buds, and shell of the Persian nut *J. regia*, the authors isolated 38 phenols in the husk, 57 phenols in buds, and 29 phenols in the bark. Naphthoquinones were the main identifiable phenolic compounds, approximately 75 % of all phenols analyzed in the inner husk, 85 % in the outer husk, 50 % in buds, and 80 % in the bark. The highest content of phenols was found in the buds of the walnut, followed by the shell, inner and outer husks. Due to the high content of phenols, husks, buds, and walnut shells are valuable by-products of the walnut tree. These data also show the origin-related content of phenols in varieties, and thus these phenolic profiles can serve to determine the origin of different walnut varieties.

The authors of work [29] fractionated methanol extract of the leaves of *Juglans regia L*. by using various chromatographic methods to obtain a total of 40 metabolites. Acylation of sugar units with synapine, ferulic, kumaric, benzoic, or salicylic acid was detected.

Various extraction methods [30] and process conditions [31] were used to extract polyphenols from green walnut husks, including the use of various solvents [32]. However, the disadvantage of these techniques is the hydrolysis and oxidation of compounds during extraction and the low yield of active substances in the extract. The most common is ultrasonic extraction using solvents such as water, ethanol, and methanol. The authors of work [13] report the effectiveness of ultrasonic extraction in the extraction of biologically active by-products of walnuts.

Extraction by ultrasound has gained popularity because of the simplicity, efficiency, and stability of the procedure. Ultrasound provides better penetration of solvents into cells, shorter extraction times, and higher efficiency of extraction of phenolic compounds from the plant matrix even at lower temperatures than other extraction methods. This method creates microwaves that can significantly improve the penetration of the solvent into the components of the cell and even cause the destruction of the cell, thereby provoking the release of the internal contents of the cell. Optimization of extraction processes using ultrasound is relevant.

There is extensive evidence that the consumption of walnuts has a beneficial effect on human health.

The authors of [33] found that with regular use of walnuts, many health indicators improve. For example, eating walnuts for eight weeks (43 g/day) significantly improves lipid levels in healthy people. Daily consumption of 43 grams of walnuts for eight weeks significantly affects the gut microbiome, increasing the number of species that produce probiotics and butyric acid in healthy people. Further evaluation is needed to establish whether these changes persist with longer use of walnuts and how they relate to observed changes in lipid metabolism.

The authors of [34–36] revealed a minimal effect on body weight, despite the potential additional energy consumption [34]. It is established [35] that the use of nuts contributes to weight control, including by increasing the feeling of satiety at an early stage. In addition, it is assumed that the use of nuts affects the decrease in appetite.

The authors of [37, 38] do not confirm the hypothesis that the acute postprandial peptide response of the gastrointestinal tract to food containing walnuts contributes to an increase in the feeling of satiety. However, the inclusion of walnuts weakened the postprandial insulin response.

The results of large prospective cohort studies show an association between regular consumption of nuts (140 g/week) and a reduced risk of coronary heart disease. The authors of work [39] confirmed that the consumption of walnuts protects against various cardiovascular diseases and diabetes in healthy people, but the beneficial effect of consuming walnuts on people with metabolic syndrome remains uncertain. In [40], it has been established that regular consumption of nuts is associated with better weight control and less obesity. The authors compared the effects of a low-calorie diet enriched with walnuts with a standard diet with reduced energy density on weight, risk factors for cardiovascular disease and feelings of fullness.

The authors of [41] found that when walnut oil is added to the daily diet of patients with type 2 diabetes mellitus, it improves the lipid profile. This may be due to modulation of the risk factor for coronary heart disease. In addition, walnut oil serves as a useful natural remedy for patients with hyperlipidemia and type 2 diabetes.

Based on our review, it can be argued that walnut and waste products of its processing can be used in the food, pharmaceutical industry, and cosmetology. Extracts of antioxidant substances isolated from the shell and partitions of the walnut can be widely used in medicine to treat various chronic diseases. It has been established that various methods of extracting polyphenols from plants are practiced. However, no data on the patterns of extraction processes were found. The quantity and quality of extraction is significantly influenced by the types of solvents used.

Therefore, the study into the features of the isolation of antioxidant substances from walnut shells, taking into consideration the influence of different types of solvents and technological parameters on the extraction process, can help solve the problem associated with the establishment of patterns that make it possible to optimize the extraction process.

3. The aim and objectives of the study

The purpose of this study is to identify the influence of technological parameters on the extraction process from walnut shells. This will make it possible to select the technological modes in such a way as to maximize the yield of the extract.

To accomplish the aim, the following tasks have been set:

– to establish the ratio of solvents that ensure the maximum release of antioxidant substances from the walnut shell;

– to establish the influence of rheological parameters on the extraction process.

4. Materials and methods

4. 1. Object and hypothesis of research

The object of our study is the technology of extraction of biologically active substances from walnut shells.

It is assumed that the optimal selection of solvent ratios and rheological parameters will significantly increase the efficiency of the extraction process of biologically active substances from walnut shells.

4. 2. Extraction

Extraction was carried out on a semi-automatic apparatus ASV-6 according to Soxhlet. 45 ml of solvent (water, ethanol, water+ethanol, ethyl aldehyde) was poured into the extraction flask and placed in a water bath. After reaching the set temperature, the sample was moved to a solvent, where the sample was processed for 30 minutes. The process of washing with a pure solvent is the main stage of extraction. Washing continues for 60–180 minutes; the extracted substance remains in the extraction flask.

4. 3. Grinding walnut shells

Grinding of the shell was carried out in two stages. Preliminary grinding with a crusher "Novital Magnum 4V" to fractions of 1–2.5 mm and final – to fractions of 300 μm at the laboratory ball mill "MSHL-1P". The duration of crushing varies from 20 minutes to 3 hours, depending on the size of the grind.

4. 4. Solvent concentration

The concentration of the solvent (the ratio of ethanol to water) ranged from 60 to 90 %.

4. 5. Mathematical model of the influence of technological parameters on the extraction process

To identify the parameters affecting the extraction process, a mathematical model of the technological process was built, which is a regression equation, a second-order rotatable plan (Boxing plan) was used when the number of factors K=3, the number of experiments of the plan is 20, the number of experiments at the zero point was 6, and the number of coefficients of the equation was 10.

Based on the experimental studies of the extraction process, the following factors were established: solvent concentration (C, %), grinding size (K, μm), extraction duration (t, min), which affect the optimization criteria – extract yield (B, %).

When conducting research, the confidence interval of measurement accuracy for all major indicators was determined, and the mathematical characteristics of the results obtained were calculated. Table 1 gives the values of the levels and the intervals of variation of the input factors.

Table 1

Encoding of intervals and levels of variation of input factors

Factor		Level of variation					Range of variation
Natural	Encoded	–1.68	–1	0	+1	+1.68	
C, %	x_1	50	60	70	80	90	10
K, μm	x_2	300	400	500	600	700	100
t, min	x_3	60	90	120	150	180	30

The planning matrix is given in Table 2.

The coefficient of the regression equation b_i was considered significant if its absolute value was greater than the interval Δb_i. Otherwise, it is considered insignificant and should have been excluded from the mathematical model. After screening out insignificant coefficients, the regression equation was derived in its final form and the optimum of the response function was found.

Table 2

Matrix of rotatable planning of experimental studies of the technological process of extraction

Encoded values			Natural values		
x_1	x_2	x_3	C, %	K, μm	t, min
–1	–1	–1	60	400	90
–1	–1	1	60	400	150
–1	1	–1	60	600	90
–1	1	1	60	600	150
1	–1	–1	80	400	90
1	–1	1	80	400	150
1	1	–1	80	600	90
1	1	1	80	600	150
–1.68	0	0	60	500	120
1.68	0	0	90	500	120
0	–1.68	0	70	300	120
0	1.68	0	70	700	120
0	0	–1.68	70	500	60
0	0	1.68	70	500	180
0	0	0	70	500	120
0	0	0	80	300	120
0	0	0	90	500	90
0	0	0	60	500	90
0	0	0	90	300	120
0	0	0	70	500	150

5. Results of studying the influence of technological parameters on the extraction process from walnut shells

5. 1. Dependences of extraction indicators at different solvent ratios and process parameters

Table 3 gives the results of the implementation of the experiment plan for calculating the coefficient estimates.

The resulting regression equation for the process of yielding walnut shell extract, for the coded values of the input variables, is as follows:

$$y = 3.56 + 0.18x_1 - 0.35x_2 + 0.18x_3 - 0.2x_1x_2 + \\ +0.01x_1x_3 - 0.03x_2x_3 - 0.15x_1^2 + 0.06x_2^2 - 0.5x_3^2. \quad (1)$$

After the transition from coded to natural values of independent variables, the regression equation took the following form:

$$B = 3.56 + 0.18C - 0.35K + 0.18t - 0.2CK + \\ +0.01Ct - 0.03Kt - 0.15C^2 + 0.06K^2 - 0.5t^2. \quad (2)$$

The values of the intervals for estimating the significance of the coefficients of the regression equation are of the form: Δb_0=±0.83, Δb_i=±0.55, Δb_{ii}=±0.54, Δb_{ij}=±0.72.

After the extraction of the walnut shell, 3 different ratios were obtained as the best result. These include:

1) the ratio of ethanol to water (70 %+30 %), the size of the shell grinding is 300 μm, the extraction time is 150 minutes;

2) the ratio of ethanol to water (80 %+20 %), the size of the shell grinding is 300 μm, the extraction time is 120 minutes;

3) the ratio of ethanol to water (90 %+10 %) the ratio of ethanol to water, the size of the shell grinding is 300 μm, the extraction time is 150 minutes.

Table 3

Results of the implementation of the experiment plan for the calculation of coefficient estimates

x_1	x_2	x_3	Y
–1	–1	–1	3.05
–1	–1	1	3.25
–1	1	–1	2.96
–1	1	1	3.11
1	–1	–1	3.85
1	–1	1	4.15
1	1	–1	3.02
1	1	1	3.15
–1.68	0	0	3.08
1.68	0	0	3.45
0	–1.68	0	4.67
0	1.68	0	3.09
0	0	–1.68	3.05
0	0	1.68	4.07
0	0	0	2.63
0	0	0	4.57
0	0	0	3.17
0	0	0	2.59
0	0	0	5.02
0	0	0	3.33

5. 2. The influence of process parameters on the extraction process

Fig. 1 shows response surfaces describing the values of the extraction yield of antioxidant substances from the walnut shell depending on the percentage of ethanol, the duration of extraction, and the grinding size on the extraction process.

Fig. 1 demonstrates that in the selected region of the input variables, there are optimal values thereof, providing a maximum yield of the extract. Thus, Fig. 1, *a* shows the results obtained with an extraction duration of 120 min. It follows from the chart that at a concentration value of more than 70 % of ethanol and a grinding size of less than 400 μm, an increase in the yield of the extract is observed with a maximum of 5.0 %.

It follows from Fig. 1, *b* that with a coarse grinding of K-300 μm, a duration value greater than 70 min, and an ethanol concentration above 60 %, an increase in the yield of the extract is observed, with a maximum value of 3.75 %.

It follows from Fig. 1, *c* that at an ethanol concentration of 80 %, a grinding of less than 400 μm, an extraction duration of more than 90 min, an increase in the yield of the extract is observed, with a maximum value of 4.75 %.

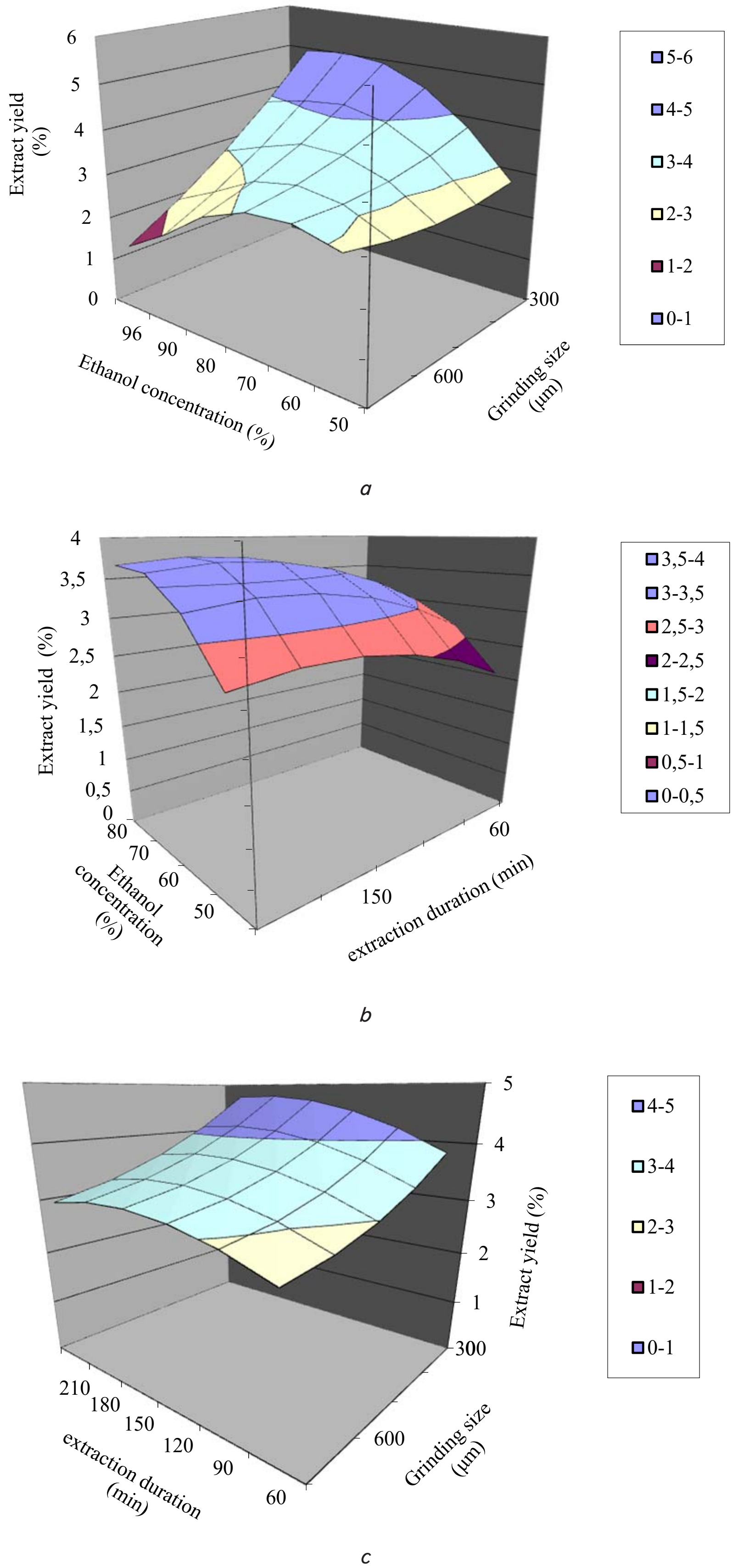

Fig. 1. Dependence of the extraction yield of antioxidant substances from walnut shells on the percentage of ethanol, extraction duration, and grinding size: *a* – extraction yield in % of ethanol concentration (%) and grinding coarseness (μm) with an extraction duration of 120 min; *b* – extraction yield in % of ethanol concentration (%) and extraction duration (min) at grinding coarseness of 300 μm; *c* – extraction yield in % of extraction duration (min) and grinding coarseness (μm) at ethanol concentration of 80 %

6. Discussion of results of studying the influence of solvents and process parameters on the extraction process

Ethanol and water as extraction solvents have been found to lead to higher extraction yields, and they are safer and less toxic than methanol and other organic solvents. For example, with an ethanol concentration of 80 %, a grinding coarseness of 300 μm, and an extraction duration of 120 min, the maximum yield value of the extract reaches 5.01 %.

With an ethanol concentration of 90 %, an extraction duration of 120 min, and a grinding coarseness of 300 μm, the maximum yield of the extract reaches 3.75 %. With an extraction duration of 150 min, a grinding size of 300 μm, and an ethanol concentration of 80 %, the maximum yield of the extract reaches 4.75 %.

With a grinding size of 300 μm, an increase in the extraction yield from 2.71 to 4.93 % was established with an increase in the ethanol concentration from 50 to 90 %, as well as an increase in the extraction yield from 3.80 to 4.81 % with an extraction duration from 60 to 180 min, but the maximum extraction value reached after 150 min.

Analysis of the effect of grinding coarseness on the duration of extraction, illustrated in Fig. 1, shows that the extraction yield decreased to 1.4 mg when the grinding coarseness was above 600 μm. However, the more time the extraction was carried out, the greater the yield of the extract.

Based on the analysis of our data, it was revealed that solvents, grinding, and duration are the basis of the regularity of the release of antioxidant substances from the walnut shell.

Knowledge of the patterns of extraction of antioxidant substances from walnut shells will make it possible to adjust the extraction process and obtain products with specified characteristics and parameters.

Our studies were conducted on the shell of a walnut of two varieties. These are the variety "Kazakhstan" and the variety "Akterek peaked", grown in a sharply continental climate with hot temperate summers and cold snowless winters. The results obtained are limited by the selected limits of variation of process parameters: ethanol concentration of 60–90 %; grinding size, 300–700 μm; extraction duration, 60–180 min.

For walnut varieties growing under other climatic conditions, additional research is needed. It is also of interest to study the influence of technological parameters on the quantitative indicators of phenols in the extraction of walnut shells.

7. Conclusions

1. The regularities of the influence of technological parameters: the percentage of ethanol, the ratio of extractants "ethanol+water", the duration of extraction, and the coarseness of grinding on the process of extracting antioxidant substances from walnut shells have been determined. For example: with an increase in the ethanol concentration from 50 to 90 %, there is an increase in the extraction yield from 2.71 to 4.93 %, and with an increase in the extraction duration from 60 to 180 min, the extraction yield increases from 3.80 to 4.81 %. These dependences are characteristic of a grinding size of 300 μm. With a grinding size of more than 300 μm, other patterns of extraction yield depending on the percentage of ethanol and extraction duration are observed. For example, with a grinding size of more than 600 μm and an extraction duration of 60 to 180 min, the maximum extraction yield is 3.1 % at a constant ethanol concentration of 80 %.

2. Optimal extraction modes have been established: grinding coarseness, 300 μm; time, 120 min; ethanol to water ratio, 90 **%** + 10 **%**.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

Acknowledgments

This study was carried out within the framework of the project funded by the Ministry of Agriculture of the Republic of Kazakhstan BR10764970-OT-21 "Use of non-traditional types of walnut waste to obtain a product with preventive properties".

In conclusion, we would like to sincerely thank all the participants of this scientific project for their assistance in conducting experimental studies. We also express our gratitude to the management and scientists of the Astana branch of KazNII of Processing and Food Industry LLP.

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