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ANALYSIS OF THE INFLUENCE OF TECHNOLOGICAL PARAMETERS OF THE CHLOROGENIC ACID EXTRACTION PROCESS FROM SUNFLOWER MEAL ON THE DEGREE OF ITS EXTRACTION

Об'єктом дослідження є ефективність вилучення хлорогенової кислоти – природного антиоксиданту, в залежності від умов її екстрагування зі соняшникового шроту. Основною проблемою данного питання є той факт, що на ступінь екстрагування вказаної фенольної сполуки впливає багато факторів, таких як ступінь подрібнення продукту, вид сировини, спосіб екстрагування, природа розчинника-екстрагента, температура та тривалість процесу екстрагування, гідромодуль в системі «сировина – екстрагент» тощо. Вплив кожного з цих параметрів потребує детального розгляду та відповідних досліджень. Це дасть змогу визначити оптимальні значення вказаних параметрів процесу екстрагування та підвищити ефективність вилучення хлорогенової кислоти.

В даній роботі сировиною для отримання хлорогенової кислоти обрано шрот з насіння соняшнику – дешеву вторину сировину олієжирових виробництв. У попередніх дослідженнях визначено, що високоефективним екстрагентом вказаного антиоксиданту виступає розчин етилового спирту з концентрацією 60 %, а оптимальною температурою процесу екстрагування хлорогенової кислоти зі соняшникового шроту є температура кипіння екстрагенту. В результаті даного дослідження вивчено закономірність впливу на ступінь вилучення хлорогенової кислоти таких технологічних параметрів, як гідромодуль в системі «шрот – розчин етилового спирту 60 %» (далі за текстом – «шрот – екстрагент») та тривалість процесу екстрагування. Експерименти по визначенню залежності ступеня вилучення хлорогенової кислоти від гідромодулю в системі «шрот – екстрагент» та тривалості екстрагування проведено відповідно до плану повного факторного експерименту. Для планування експерименту та обробки даних застосовані математичні методи з використанням програмних пакетів Microsoft Office Excel 2003 (USA) та Stat Soft Statistica v6.0 (USA).

Вказана в роботі залежність являє собою квадратичну функцію, яка прогнозує збільшення вмісту хлорогенової кислоти під час екстрагування за умови збільшення гідромодуля в системі «шрот – екстрагент» з 1:5 до 1:10 та зменшення часу екстрагування з 60 хв. до 30 хв. Встановлено, що для максимально можливого збільшення ступеня вилучення хлорогенової кислоти зі шроту насіння соняшнику, оптимальна величина гідромодуля екстрагування – 1:10, тривалість екстрагування – 30 хв. Отримані результати дають змогу збільшити ступінь вилучення хлорогенової кислоти зі соняшникового шроту з 2,46 % до 5,58 %. Це вказує на можливість збільшення ефективності вилучення антиоксиданту більше ніж у 2 рази.

Ключові слова: *екстрагування соняшникового шроту, хлорогенова кислота, гідромодуль, ефективний антиоксидант, тривалість екстрагування.*

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1. Introduction

Chlorogenic acid [1, 2] and its derivatives belong to polyphenolic compounds. They are also effective natural antioxidants [3, 4]. Among other properties are the pronounced antibacterial, antiviral, membrane-protective, hypoglycemic activities, as well as the ability to inhibit a number of enzymes [5, 6].

It should also be noted that chlorogenic acid is found in sunflower seeds in a sufficiently large amount, and the meal is, in turn, a secondary raw material with low cost [7].

The studies presented in [8, 9] outline a range of innovative technical solutions for investigating the effectiveness of a number of solvents for extracting chlorogenic acid from sunflower meal. As well as its further chemical modification for use as an antioxidant in the food and cosmetic industries. Based on the results of the experimental studies, the effectiveness of the proposed technological solutions in comparison with the existing ones is proved.

An important further development in this direction is the analysis of the influence of technological parameters of the extraction of chlorogenic acid process from sunflower meal, in particular the value of the hydromodule in the system «meal – extractant». As well as the duration of extraction with an aqueous solution of ethyl alcohol at a concentration of 60 %, the extraction degree of chlorogenic acid. The results obtained should increase the efficiency and reduce the cost of the developed technological solutions for the extraction of antioxidants from the secondary products of oil and fat production, which emphasizes the relevance of the chosen direction of research.

2. The object of research and its technological audit

The object of research is the efficiency of extraction of chlorogenic acid – a natural antioxidant, depending on the conditions of its extraction from sunflower meal. To identify the features of this dependence, a technological audit was conducted to determine the change in the content of chlorogenic acid in the extractant, depending on the technological parameters of the process of its extraction from sunflower meal.

The fundamental possibility of extracting chlorogenic acid from sunflower meal depends essentially on the value of the hydromodule in the system «meal – extractant», as well as the duration of treatment of sunflower meal with an aqueous solution of ethyl alcohol with a concentration of 60 %. Therefore, the main direction of improvement of the process of extraction of chlorogenic acid is to determine the optimal parameters of the process, which will increase the efficiency of antioxidants production from such cheap secondary raw oil and fat production, such as sunflower meal.

3. The aim and objectives of research

The aim of research is to improve the technological process of extraction of chlorogenic acid from sunflower meal on the basis of modeling and optimization of physicochemical processes of extraction of the specified phenolic compound with an aqueous solution of ethyl alcohol with a concentration of 60 %. This will increase the efficiency of the release of chlorogenic acid.

To achieve this aim it is necessary to:

1. Study the regularities of the influence of the hydromodule size in the system «meal – extractant» and the duration of treatment of sunflower meal with an aqueous solution of ethyl alcohol with a concentration of 60 % on the extraction degree of chlorogenic acid.

2. Determine the optimal range of the selected factors values of processing sunflower meal for the maximum possible extraction of chlorogenic acid.

4. Research of existing solutions to the problem

Among the main directions of chlorogenic acid extraction from vegetable raw materials found in the resources of the world scientific periodicals, the following can be distinguished:

– extraction with organic solvents and aqueous-alcoholic solutions [10];
– ultrasound extraction [11, 12];
– removal using microwave generators (ultra-high frequency radiation) [13, 14].

When comparing the above methods of chlorogenic acid extracting, it should be noted that each of these methods has advantages and disadvantages. The cost and, often, the toxicity (e. g. methanol [15]) should be taken into account when using organic solvents. In addition, traditional techniques tend to take considerable time. The transition to the use of ultrasonic methods and microwave generators can significantly reduce the time. According to [13, 16], reduction of extraction time is achieved by changing the structure of the plant matrix as a result of ultrasonic and microwave waves. However, this requires additional costs to modernize the experimental base, which many scientists are unable to afford. This fact explains why most researchers, in their experiments on the extraction of chlorogenic acid, still prefer to use organic solvents and aqueous-alcoholic solutions [17]. With respect to the latter, their low toxicity and moderate cost should be noted. In addition, attention should be paid to a very important point. Alcohol and its solutions are quite selective solvents. During the extraction of chlorogenic acid, other related substances, including proteins, pass into its extract. Meal from sunflower seeds contains very little alcohol-soluble proteins, so more of them still remain in the meal. Such meal can be used to produce plant-based protein and then use it to increase the biological value of food [18, 19].

It should also be noted that alcoholic solutions allow the separation of pure chlorogenic acid in crystalline form, unlike many other organic solvents, such as the succinic and hydrochloric acid solutions reported in studies [20]. For example, the results of studies in [20] show the high efficiency of weak succinic acid solutions, however, the disadvantage of this method is the inability to further separate the succinic and chlorogenic acid complex, which makes it impossible to obtain pure chlorogenic acid.

In the present work, according to the results of previous studies [21], an ethanol alcohol solution with a concentration of 60 % was selected as an effective chlorogenic acid extractant.

In the process of extracting chlorogenic acid from sunflower meal, it should be borne in mind that the technological process of obtaining this phenolic compound, in addition to the solvent-extractant composition, is also influenced by a number of other various factors, such as:

– temperature and duration of the process;
– mass ratio of components in the meal-extractant system – hydromodule;
– product grinding degree, etc.

In [22], the following technological parameters of the process of extraction of chlorogenic acid in the cavitation unit were determined: hydromodule – 1:5, process temperature – 60 °C, duration – 18 min. At the same time as the raw material used sunflower seeds cake, and as the extractant – the combined solvent (ethanol-hexane). If to look at such indicators as the hydromodule and the extraction time, it can be noted that they are quite economical, but specific equipment is used in the work. Of interest are also the results of [23], where it is stated that the optimal parameters of the process of extraction of chlorogenic acid from the drug lily are the following indicators: hydromodule – 1:20, duration – 60 minutes, extractant – ethyl alcohol solution with a concentration of 70 %. However, the obtained results can't be called economically promising.

Thus, the results of the analysis of existing solutions to the problem suggest that there is no consensus on the possibility of increasing the efficiency of the release of chlorogenic acid from vegetable raw materials. The choice of the most appropriate technical solution for each individual object may be based on the results obtained in the model systems under study. However, in terms of technological and economic aspects, variation of such technological parameters as extraction hydromodule and process duration is promising [22, 23].

5. Methods of research

The following materials and reagents were used for the research:

– sunflower meal according to DSTU 4638:2006;

– water distilled in accordance with GOST 6709-72. Clear liquid without color and odor. M_r=18.0 g/mol; *Melting point*=0 °C; *Boiling point*=100 °C; d_{420}=1 g/cm^3;

– ethyl alcohol according to GOST 18300-87. Colorless liquid with a faint «alcoholic» odor. M_r=46.07 g/mol; *Melting point*=–114.3 °C; *Boiling point*=78.4 °C; d_{420}==0.789 g/cm^3; n_{D15}=1.3614.

Physico-chemical parameters of sunflower meal were determined by standard methods. The process of extraction of chlorogenic acid was carried out from sunflower meal with the following quality indicators: meal moisture – 10.6 %, oilseed meal – 0.4 %, mass fraction of crude protein – 39.82 %, mass fraction of crude fiber – 22.26 %. The content of chlorogenic acid in the extract was determined by the method of titration with potassium permanganate [22]. The results obtained for the extraction degree of chlorogenic acid were in the range of (2.46–5.58) %.

Experiments to determine the dependence of the extraction degree of chlorogenic acid on the hydromodule in the system «meal – extractant» and the duration of extraction were carried out in accordance with the plan of the full factor experiment. Mathematical methods using the Microsoft Office Excel 2003 (USA) and Stat Soft Statistica v6.0 (USA) software packages have been applied for experiment planning and data processing. To study the dependence of the extraction degree of chlorogenic acid from sunflower meal on the value of the hydromodule in the meal-extractant system and the duration of extraction with an aqueous solution of ethyl alcohol with a concentration of 60 % used a three-level plan for two-factor response function. The studies were performed in triplicate. For a given degree of probability P=95 %, the relative error in determining the content of chlorogenic acid in sunflower meal and in the ethyl alcohol extract did not exceed 2 %.

6. Research results

The data on the effect of the hydromodule in the «meal – extractant» system and the duration of extraction on the efficiency of chlorogenic acid extraction from sunflower meal are given in the Table 1.

The results of the studies are presented in Fig. 1.

The graph in Fig. 1 illustrates the mutual effect of the hydromodule and the duration of extraction on the extraction degree of chlorogenic acid as a result of extraction from sunflower meal.

On the basis of the experimental studies (Fig. 1), statistical model (1) was created for the dependence of the extraction degree of chlorogenic acid from the sunflower meal on the hydromodule (HM) and the duration of extraction (τ) at an approximation reliability value of R>0.975. With this model it is possible to predict the extraction degree of chlorogenic acid from sunflower meal from these factors. The main characteristics of the meal are as follows: the moisture content of the meal – 10.6 %, the oilseed meal – 0.4 %, the mass fraction of crude protein 39.82 %, the mass fraction of crude fiber – 22.26 %.

$$Y(HM,\tau)=-0.5089+1.3033\cdot HM-$$
$$-0.0437\cdot\tau-0.0656\cdot HM^2+0.0027\cdot HM\cdot\tau+$$
$$+7.4074E-6\cdot\tau^2, \qquad (1)$$

where $Y(HM, \tau)$ – extraction degree of chlorogenic acid from sunflower meal, %; HM – hydromodule, units; τ – extraction time, min.

Table 1

The dependence of the efficiency of chlorogenic acid extraction from sunflower meal from the hydromodule in the «meal – extractant» system and the duration of extraction

Experiment no.	Moisture content, *W*, %	Oil content in the meal, *O*, %	Hydromodule, *HM*, units	Extraction time, τ, min	Extraction degree of chlorogenic acid, *Y*, %
1	10.6	0.4	1:5	30	3.45
2				45	3.15
3				60	2.46
4			1:8	30	4.96
5				45	4.68
6				60	4.56
7			1:10	30	5.58
8				45	5.14
9				60	4.94

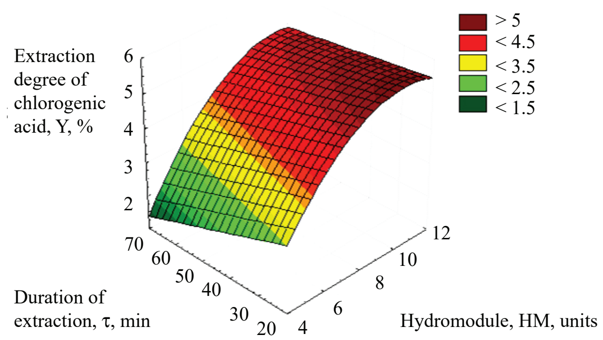

Fig. 1. The dependence of the extraction degree of chlorogenic acid from sunflower meal on the hydromodule in the system «meal – extractant» and the duration of extraction

The coefficients of this regression equation were determined using the least squares method. The significance of the individual regression coefficients was determined using Student's t test (t) by testing the hypothesis that the null parameter of the equation was equal. The absolute value of the Student's t (6) criterion for the estimation of individual regression coefficients was compared with its critical table value t_{table} (6)=2.447 at the significance level p=0.05 and the number of degrees of freedom for multiple regression df=6. If the absolute value of the Student's criterion was greater than its critical tabular value, then the null hypothesis was rejected and a probability of 0.95 (or 95 %) recognized the value of the corresponding regression coefficient significant. Otherwise, the regression coefficient was considered insignificant and excluded from the equation (Table 2).

Table 2

Data and conclusions regarding the determination of the significance of the regression coefficients of the dependence of the extraction degree of chlorogenic acid from the sunflower meal on the hydromodule and the extraction duration

Coefficient of regression equation	The value of the coefficient in natural quantities	The value of the Student test		Estimated probability of the null hypothesis for the regression coefficient (p-level)	Conclusion on the significance of the coefficient
		t (6)	t_{table} (6)		
Intercept	–0.5089	3.95411	2.447	0.007502	Meaningful
HM	1.3033	10.33891		0.000048	Meaningful
T	–0.0437	–3.08640		0.021486	Meaningful

In order to evaluate the quality of the model and the completeness of the influence of the selected factors, the determination coefficient R^2 was determined. The obtained value of $R^2=0.95$ makes it possible to conclude that the variations of the hydromodule and the duration of extraction on the variations in the extraction degree of chlorogenic acid are very significant (greater than 95 %). To determine the significance of the regression model, Fisher's criterion (F) was calculated based on the assumption that the equation was not statistically significant ($R^2=0$; null hypothesis). The calculated value of the Fisher criterion was $F(2, 6)=58.209$ and was greater than its critical table value $F_{table}(2, 6)=5.14$ at the significance level $p=0.05$ and the number of degrees of freedom $df_1=2$ and $df_2=6$. This result allows to reject the null hypothesis and, at a probability of 0.95 (or 95 %), recognize the value of the coefficient of determination $R^2=0.95$ significant, and the model is meaningful. It should be noted that the equation obtained by approximating the data adequately describes the dependence in the range of values of the hydromodule – 1:5–1:10 and extraction duration – 30–60 minutes.

The experimental data show that there is an increase in the extraction degree of chlorogenic acid during its extraction from sunflower meal from (2.46–3.45) % to (4.94–5.58) %. This is observed by increasing the extraction hydromodule in the «meal – solvent» system from 1:5 to 1:10 and by reducing the extraction time from 60 minutes up to 30 minutes. The obtained data indicate that in order to increase the efficiency of the chlorogenic acid recovery process, a cost-effective ratio of the hydromodule to the extraction time should be found.

Noteworthy is the fact that the extraction degree of chlorogenic acid reaches the maximum value in this experiment – 5.58 % under the following conditions: hydromodule – 1:10, extraction duration – 30 min. The obtained process parameters allow to conclude that in the first 30 min. the maximum amount of chlorogenic acid goes into the alcohol solution, so further extraction is pointless. On the contrary, with the continuation of the experiment, part of the alcohol solution evaporates, which leads to the condensation of the extract and subsequently to the delay of part of chlorogenic acid in the meal. This is especially good with the low value of the hydromodule 1:5. The cause of this phenomenon is the high hygroscopicity of the meal, so when in contact with water or various solutions, the meal will swell and increase in size several times. As the value of the hydromodule increases and the process duration remains constant, the extraction degree of chlorogenic acid increases, which can be explained by the increase in the amount of alcohol solution for the treatment of the swollen meal.

Based on the analysis of equation (1) and graphical dependence, optimal parameters of technological process of extraction of chlorogenic acid from sunflower meal were determined: hydromodule – 1:10, extraction duration – 30 min. This will increase the efficiency of processing sunflower meal to extract valuable biologically active substances and, accordingly, reduce the cost of obtaining chlorogenic acid.

7. SWOT-analysis of research results

Strengths. Among the strengths of this research are the results obtained by the optimal ranges of technological parameters of processing sunflower meal – hydromodule in the system «meal – extractant» and the duration of extraction of chlorogenic acid. According to the results of the analysis of modern scientific literature, such results have not been revealed to date. It is for this reason that it is difficult to choose a priority method for the extraction of chlorogenic acid in the technology of recycling by-products of oil and fat production. Using the obtained data allows to solve the problem of choosing the optimal extraction conditions of this biologically active compound in order to increase the efficiency of the process and reduce its cost. It is worth noting the economic attractiveness of the selected method of processing sunflower meal for food technology.

Weaknesses. The weakness of this research is that the choice of optimal parameters of extraction of chlorogenic acid from sunflower meal, still depends on a number of characteristics of the feedstock (e. g., physico-chemical parameters). And, accordingly, will vary when changing these raw material characteristics. Therefore, in order to prevent this shortcoming, special attention should be paid to the quality of the sunflower meal, which imposes special obligations on the manufacturer.

Opportunities. Research on the features of extraction of chlorogenic acid from sunflower meal can be developed in the pharmaceutical industry, as well as in the processing of vegetable raw materials containing chlorogenic acid into protein products (isolates, concentrates, low-fat flour, textured proteins). This is how the problem of increasing the organoleptic characteristics of plant protein products can be solved.

Threats. Difficulties in implementing the research results may be related to such factors as the management of the food industry. The investment of additional funds, even small ones, in the purchase of the necessary equipment and the lack of tangible results affect the position of decision makers. This risk has every reason, since the obtained statistical model of the process of extraction of chlorogenic acid from the sunflower meal, depending on the hydromodule and the duration of the process, as stated above, requires the standardization of a number of raw materials.

Thus, SWOT analysis of the research results allows to determine the main directions for successful achievement of the set research aim. Including:

– development of scientifically grounded recommendations for standardization of sunflower meal indicators for the chlorogenic acid production;
– assessment of the efficiency of chlorogenic acid extraction from sunflower meal under grounded parameters of the technological process in industrial conditions;
– development of technological solution for obtaining chlorogenic acid from sunflower meal.

8. Conclusions

1. In the course of experimental studies, the influence regularities of the hydromodule in the «meal – extractant» system and the duration of extraction of chlorogenic acid from sunflower meal with an aqueous solution of ethanol at the concentration of 60 % on the degree of product extraction are studied. This dependence is a quadratic function that predicts an increase in the content of chlorogenic acid during extraction, provided that the hydromodule extraction in the «meal – solvent» system is increased from 1:5 to 1:10 and the extraction time is reduced from 60 minutes up to 30 minutes.

2. The optimal range of values of the selected factors of technological processing is established for the maximum possible increase of the extraction degree of chlorogenic acid from the raw material. The optimal value of the extraction hydromodule is 1:10, the extraction time is 30 minutes.

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