Optimization of extraction parameters of diterpene glycosides from stevia leaves by mathematical modeling

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Abstract. The parameters and modes of extraction of stevioside – hydromodule, temperature and duration of the process were selected in the work. The object of the study was dried stevia leaves of the Ramon Sweetmeat variety. The determination of diterpene glycosides in the extract was carried out by the modified Komissarenko method. To optimize the technological parameters of extraction, regression models of the second order were built using orthogonal central composition planning. The degree of crushing of stevia leaves was 1-3 mm. The extraction at a temperature below 76 ° C reduces the amount of diterpene glycosides in the aqueous extract, and an increase in the extraction temperature above 92 ° C does not increase the yield of glycoside. The maximum extraction of diterpene glycosides is achieved at an extraction temperature of 92 ° C. When selecting a hydromodule, extraction was carried out at the ratio of stevia leaves and water 1:5, 1:10, 1:15. The highest yield of extractive substances was observed with a hydromodule of 1:10. With an increase in the duration of extraction from 60 to 180 minutes, an increase in the extract is observed. The optimal duration of the extraction process is 120 min. Based on the experimental data obtained, a second-order mathematical model was constructed, suitable for optimizing the process of obtaining stevia extract. The main factors influencing the process of stevioside transition from leaves to solution were considered: extraction temperature, °C; extraction duration, min; hydromodule. The criteria for evaluating the efficiency of the extraction process were: the yield of diterpene glycosides % by weight of stevia and energy consumption kW/dm^3 extract. The first criterion is desirable to maximize, the second – to minimize. Optimal extraction modes are obtained:hydromodule - 1:10.8, temperature - 79.8 ° C, duration - 94.1 min.

1 Introduction

The problem of creating functional products is urgent, one of the goals of which is to increase the adaptive capabilities of the body [1, 2].Consumers' interest in healthy food and demand for healthy food products have increased significantly recently. Chronic diseases such as heart disease, cancer, diabetes cause serious public concern. They are often caused by a diet characterized by a relatively high content of fat, refined sugar, salt and cholesterol. Functional foods have attracted considerable attention from researchers in the field of food health and technological innovation.

The ratio and amount of glycosides contained in the plant vary depending on the type of plant and the main agricultural technologies [1, 2, 3].

The popularity of stevia as a sweetener is growing, as it has no calories and can be consumed by diabetics. The sweetness of S. rebaudiana is due to the enteric triterpene glycosides contained in it, commonly known as steviolglycosides.Its main glycosides are stevioside (4%-13%) and rebaudioside A (≈2-4%), which make up at least 90% of the mass of all glycosides present in the leaves. Its main glycosides are stevioside (4%-13%) and rebaudioside A (≈2-4%), which make up at least 90% of the mass of all glycosides present in the leaves. Rebaudioside C (1,51.5%) and dulcoside A (≈0.5%) are found in lower concentrations, while there are also traces of a number of additional steviol-based glycosides.According to [8], the content of diterpene glycosides in stevia ranges from 16.8 to 17.2%. Structurally, steviol glycosides consist of an aglycone part (trunk), which is connected in C4 and C-13 with mono-, di- or trisaccharides (containing mainly glucose and/or rhamnose).Rebaudioside A is the active component with the highest sweetness index, while stevioside, the main component of glycosides, has a bitter taste. Diterpene glycosides are highly soluble in water, are characterized by thermal stability, stability of properties during long-term storage [9, 13]. Stevia leaf extracts containing such secondary metabolites as steviolglycosides and polyphenols are known to have potentially important biologically active effects - antidiabetic, antikaryogenic, antioxidant, hypotensive, anti-inflammatory and antitumor [4, 5, 6].

Steviol glycosides are 250-300 times sweeter than sucrose, but they do not provide energy. Stevia supplements were approved by the European Union in 2011 as a low-calorie and low-toxic natural sweetener in the food and beverage industry. [7, 10] Stevioside is

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prescribed to patients with impaired carbohydrate metabolism, such as diabetes, obesity and hypertension, but its use is limited due to the smell and bitter astringent taste associated with certain types of glycosides and alkaloids in the extract, while rebaudioside A has a good taste without bitterness. Consequently, the quality and taste of S. rebaudiana leaf extract depend on the proportion of steviol glycosides in the productConsequently, the quality and taste of S. rebaudiana leaf extract depend on the proportion of steviol glycosides in the productConsequently, the quality and taste of S. rebaudiana leaf extract depend on the proportion of steviol glycosides in the product. Stevia leaves also contain other important phytochemicals that can be co-extracted with steviol glycosides, such as phenolic compounds, flavonoids and carotenoids. These substances have antioxidant activity and attract increasing attention of scientific community to the production of low-calorie sweetening products containing biologically active compounds. Steviosides are cleaved by intestinal bacterial flora to aglycone (steviol) and are not absorbed in the large intestine, therefore steviolglycosides have zero energy value [12, 13].

Stevia is usually sweetened with tea, soft drinks, including kvass to improve the taste characteristics of the product, as well as reduce the energy value. It is known that diterpene glycosides can enhance the smell and taste of soft drinks and other foods [9, 13].

For the extraction of steviolglycosides from stevia leaves, there are several analytical techniques, usually based on the use of aqueous or alcoholic solvents, sometimes using an auxiliary energy source (for example, ultrasound, microwaves, liquid pressure), followed by clarification and purification stages. However, most of these procedures are time-consuming and require the use of organic solvents, which are unacceptable in the food industry.As an alternative, membrane purification technologies are proposed, which are usually used in the pharmaceutical industry, as well as nuclear magnetic resonance [5-7], infrared spectroscopy with Fourier transform, capillary electrophoresis and liquid chromatography. Among them, liquid chromatography combined with matrix detection of ultraviolet photodiodes (LC-DAD) or mass spectrometry (LC-MS) are the most commonly used tools for the analysis of these compounds due to their accuracy and wide range of applicability. Also, some researchers have used soxlet extraction (using chloroform and methanol), ultrasound, high-pressure liquid extraction with methanol, supercritical liquid extraction. In recent years, developments have been carried out to extract bioactive molecules from plants using enzymatic processes. Enzymes that destroy the cell wall, such as cellulose, pectinase and hemicellulase, were used to facilitate extraction.However, all these methods are significantly time-consuming, expensive, which affects the cost of the final product and require imported high-tech equipment and are not environmentally friendly [5, 6, 11].

To extract stevioside from the leaves of S. rebaudiana, water or alcohol can be used.However, the use of organic extraction solvents such as methanol can lead to the formation of dangerous volatile residues, greenhouse gas pollution and subsequent health problems. Water extraction is a safe and inexpensive method.

One of the technological tasks in obtaining an extract of a natural sweetener is the maximum extraction of diterpene glycosides from stevia leaves with minimal energy costs [8, 9].

2 Materials and methods

The object of the study is the leaf mass of stevia of the "Ramon Sweetmeat" variety, obtained in the conditions of the Voronezh region. The leaves were dried in an air-ventilated room at a temperature of 25 ± 2 ° C for two weeks, and then stored at room temperature in plastic bags to have a constant quality raw material. Requirements for crushed stevia leaves - at least 95% must pass through a sieve with a hole diameter of 1 mm.

The determination of diterpene glycosides in the extract was carried out by the modified Komissarenko method. Modification of the method consists in isolation of diterpene glycosides, extraction with butanol and quantitative determination of diterpene glycosides by glucose in an aqueous solution. The essence of the modified method consists in the quantitative determination of glucose equivalent to the mass of diterpene glycosides [4]. The study was carried out in the following order: crushed stevia leaves were extracted with a 10-fold amount of ethyl alcohol with a concentration of 80% vol. The extraction lasted for 2 hours. The resulting extract was evaporated to an oil-like residue in a drying cabinet, which was then thoroughly mixed with hot distilled water (in a ratio of 1:0.4), filtered and the cooled mixture was treated with butanol. Butanol extract was dried in a drying cabinet at a temperature not higher than 90 °C. The dry residue was dissolved with distilled water in a measuring flask per 100 cm^3. Next, the optical density of the solution was measured after adding an antron reagent. The glycoside content was calculated using a glucose calibration graph

To optimize the technological parameters of extraction, regression models of the second order were built using orthogonal central compositional planning. Its main feature is that it allows you to apply methods of sequential planning of the experiment. First, a complete factorial experiment is constructed to analyze the response surface. Further, if the results of the analysis do not satisfy the researcher, then the full factorial experiment is completed to the central compositional planning and a more complete study of the response surface is carried out. In this case, compositional plans give a gain in the number of experiments compared to other plans.

3 Results and discussion

Extraction is an important technological operation when extracting diterpene glycosides from the green mass of stevia. The main factors influencing mass transfer from raw materials of diterpene glycosides are: the size of crushing, temperature, the ratio of water and dry leaves, the duration of mass transfer and the number of drains. The size of the crushing of the leaves was 1 - 3 mm.

At the first stage of research, the temperature of water extraction was selected by experiment. At different temperature values in the range from 72 to 100 °C and a hydromodule of 1:10, mass transfer of glycosides from crushed leaves was carried out (Table 1).

Table 1. Mass transfer efficiency at different temperatures

Temperature, °C	The ratio of the amount of extractive substances to the weight of stevia leaves, %	The ratio of the sum of diterpene glycosides in the extract to the mass of the original leaves, %	The yield of dry substances, in % to the initial raw material
72	37.87	6.67	8.47
76	38.18	7.23	9.23
80	38.62	8.01	9.76
84	41.73	8.45	10.73
88	45.95	8.87	11.29
92	47.95	8.90	11.30
96	49.22	8.89	11.31
100	49.22	8.89	11.31

The extraction of stevia leaves at a temperature of 72-100 °C allows to induce the destruction of the cell walls of plant material, and therefore to promote the transition of diterpene glycosides. At temperatures below 76 °C, the yield of dry substances decreases. An increase in temperature above 92 °C does not increase the yield of the product. During the extraction process, an increase in temperature induces the destruction of the cell walls of plant material, providing mass transfer and the release of intracellular compounds, which leads to high yields of valuable substances.

Thus, it was found that the greatest mass transfer of glycosides takes place at 92 °C, which is probably due to the maximum rupture of the cell membrane and surrounding structures of the material.The hydromodule (the ratio of leaf mass and polar solvent) was determined at the second stage of research. The ratios of crushed stevia leaves and water were 1:5, 1:10 and 1:15. The results obtained are shown in table 2.

With an increase in the hydromodule, the difference between the concentration of the biologically active extractable substance in water and in the starting material increases, which leads to an increase in the amount of the transferred extractable substance into the solution.Table 2 shows that with an increase in the hydromodule, the extract yield increases, but with a hydromodule of 1:15, this increase was insignificant, therefore, the ratio of crushed stevia leaves and water 1:10 is a rational hydromodule.

Table 2. The yield of biologically active substances in the extract depending on the different hydromodule

Hydro-module	The total content of bioactive compounds in the extract, %	The content of the sum of diterpene glycosides in the extract, %	Mass fraction of dry substances, %
1:5	45.94	8.61	11.01
1:10	46.73	8.82	11.28
1:15	46.76	8.83	11.29

Next, the duration of extraction was selected. Extraction was carried out for 1, 2 and 3 hours. The results are shown in Table 3. During extraction, the concentration of the extracted substance in the extract increased from 1 to 3 hours. The rational extraction time of bioactive substances was 2 hours, since at 2.5 hours the yield of stevioglycosides increased by only 0.35%, which is insignificant compared to energy consumption.

Table 3 Effect of extraction duration on the yield of extractable substances

Duration of extraction, h	The total content of extractive substances in the water extract, in % by weight of stevia	The content of the sum of diterpene glycosides in an water extract, % by weight of stevia	Dry product yield, % by weight of stevia
1	40.34	7.75	10.23
2	45.95	8.83	11.29
2.5	46.11	8.86	11.33

Thus, during water extraction, 11.29% of stevia glycosides were extracted within 2 hours. Based on the experimental data obtained, a second-order mathematical model was constructed.The main factors influencing the diffusion of stevioside into the solution: X_1 – temperature, ° C; X_2 –duration, min; X_3 – hydromodule. The criteria for evaluating the efficiency of the extraction process were: the yield of diterpene glycosides Y_1 (% by weight of stevia) and energy consumption Y_2 (kW / dm^3 extract). It is desirable to maximize the first criterion, and minimize the second.

To search for optimal modes of the extraction process, a series of experiments were carried out, as a result of which mathematical models were constructed in the form of multivariate regression equations linking the values of criteria and input factors of the process. A composite uniform rotatable plan of the 2nd order was used (Tables 4), since it allows us to obtain a model with a minimum error in the central area of the plan [9, 10]. The point of the assumed maximum Y1 was chosen as the center of the plan.

Table 4 Limits of changes in factors during water extraction

Planningconditions	X_1, °C	X_2, mines	X_3
Basic level	85	90	1:10
Variation interval	5	15	5
Upper level	90	105	1:15
Lower level	80	75	1:5
The upper "star" point	93,4	115,2	1:18,4
The lower "star" point	76,6	64,8	1:1,6

Regression equations of the 2nd order were obtained in coded variables, which, after excluding insignificant terms, took the form:

$$Y_1 = 8.2026 + 0.39353X_2 - 0.43319\,X_1^2 - 0.62937\,X_2^2 - -0.47738\,X_3^2 \quad (1)$$

$$Y_2 = 0.093608 + 0.014316\,X_1 + 0.0021011\,X_2 - -0.0011588\,X_2^2 - 0.00085836\,X_3^2 \quad (2)$$

Characteristics of the first equation: residual variance s^2 = 0.498015, evaluation of correctness according to the Fisher criterion $F_{\text{calculated}} = 6.004$ at $F_{\text{critical}} = 3.056$.

Characteristics of the second equation: residual variancess^2 = 1.85316×10^{-6}, evaluation according to the Fisher criterion $F_{\text{calculated}} = 389.5$ at $F_{\text{critical}} = 3.056$.

With the help of optimization programs of the MathCAD PRIME 3.1 computer mathematics system, the optimum points of each criterion were found within the boundaries of the uniform rotatable plan.

Experiments were carried out at each point. As a result, the point x_1 = 79.8 °C, x_2 = 94.1 min, x_3 = 1:10.8 was selected. The experimental values of the criteria in it were Y_1 = 8.23% of the stevia mass, Y_2 = 0.0682 kWh / 1dm^3 of the extract.

4 Conclusion

Thus, using the experimental design methodology to obtain an extract from stevia leaves, the optimal parameters and modes are as follows: hydromodule - 1:10.8, temperature - 79.8 ° C, duration - 94.1 min. The resulting stevia extract can be recommended as a complete or partial substitute for sucrose in combination with additives such as fillers, gelling agents, stabilizers, flavorings for the development of functional products - dairy products, soft drinks, fruit nectars, bakery products, flour confectionery, jellies, jams and others. The aim of the study was to optimize the parameters of the process of environmentally friendly extraction of diterpene glycosides from stevia leaves directly suitable for use in the food industry.

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