Determination of Optimal Technological Parameters of Obtaining Stevia Extract in Technology of Sour Dairy Desserts

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1. Introduction

In the top diseases of our time, provoked by metabolic disorders, the leading positions are occupied by obesity and diabetes mellitus (DM), and the number of patients with which is constantly growing. Obesity and overweight affect more than 50% of the population of 19 out of 34 countries from the Organization for Economic Co-operation and Development. Even though the economic system encourages diets that are their causes [1]. According to data from the International Diabetes Federation (IDF), about 250 million patients with DM are registered in the world. According to forecasts for 2030, their number will vary on average from 320 million to 530 million and even more. In the case of counting together patients with impaired glucose tolerance – 480 million (in the world). And this is a registered violation of the incremental function of the pancreas in every 8–10 people. Such a high prevalence of diabetes in developed countries brings the disease to several social diseases that require widespread treatment and preventive measures.

In this regard, back in 2007, the UN appealed to create programs at the state level to solve several problems related to DM. And attribute it to significant medical and social problems [2].

On the recommendation of nutritionists, people with DM and obesity need to reduce the glycemic index of the diet by introducing nutrients into the diet. They reduce postprandial and basal glycemia, as well as exclude dessert products that contain sugar [3].

The use of a sweetener of natural origin with zero calorie content – Stevia rebaudiana Bertoni [4] has become common in food technologies. A significantly higher degree of sweetness, which is inherent in stevia compared to sucrose (by 250–300 times), is caused by a glycoside molecule (consisting of glucose, sophorose, and steviol), the breakdown of which in the human body occurs without the participation...
of insulin [5]. These organic compounds in their chemical structure are devoid of glucose groups. The content of glycosides in dry stevia leaves ranges within 6–13 % (stevioside; rebaudioside A, B, C, D, E, F; rubioside; steviolmonoside; stevioside H, b-Gic) depending on the climatic conditions of cultivation [6]. It is the use of stevioside that leads to a decrease in insulin resistance [7].

Stevia at normal concentrations of its components does not provoke any side effects on the body, does not have teratogenic and mutagenic properties, and also does not cause allergic reactions [8]. Similar data on the absence of toxic effects of stevia and stevioside were also confirmed in work [9]. It is worth noting that since 2004, WHO experts have approved stevia as a safe food additive with an indicator of permissible daily intake of 5 mg/kg [10]. Significant importance in the diet of people with impaired carbohydrate metabolism belongs to fermented dairy products. In particular, desserts based on fermented milk cheese can be an ideal alternative to the usual sweets because, in addition to the pleasure of eating, consumers receive several useful micronutrients, vitamins, and a large amount of easily digestible milk protein [11].

Therefore, the development and improvement of existing technologies for obtaining food sweeteners from natural plant materials is a relevant task in science. The results of such studies are needed to expand the range of desserts based on fermented milk cheese, which will correspond to the concept of functional (healthy) nutrition. In addition, the combination of animal and plant components will create a positive biological effect from the consumption of this symbiotic product.

2. Literature review and problem statement

The expediency of using stevia to replace sugar in the technologies of fermented milk desserts (based on cottage cheese), which undergo a mandatory stage of heat treatment, is related to the heat resistance of stevioside. In addition, it is not fermented by microorganisms, it does not react with melanoidin formation (unlike sugar), and also does not provoke darkening of the product during its production and storage [12]. It has been proven that glycosides and stevia leaves remain thermostable in ranges up to 200 °C and can be used natively or after heat treatment. That is, rebaudioside A and stevioside during heating in technological processes of food production are not amenable to darkening and caramelization [13]. In addition, a positive effect was observed from the use of steviol glycoside on organoleptic parameters of sweet dairy products and yogurt, along with the ability to stimulate the growth of lactic acid microflora [14].

The possibility of using stevia in ice cream technology has been studied. According to the results of physicochemical and organoleptic studies, the possibility of combining stevia with a milk base was confirmed. The product is characterized by qualitative rheological properties, and according to the organoleptic assessment, it has the highest indicator. Analysis of trace elements showed that the product with vegetable filler is non-toxic [15]. However, the authors have not investigated the possibility of using stevia as an extract.

The purpose of study [16] was to investigate the technology of yogurt with a natural sweetener. The results demonstrated that adding stevia improves the texture and syneresis of natural yogurt. However, the use of mathematical and statistical methods aimed at modeling processes and finding rational combinations of experimental data is not considered.

The technology of dessert [17] based on fermented milk cheese in combination with (obtained in its production) whey enriched with stevia extract and fruit and berry fillers is known. However, the technological parameters for obtaining stevia extract have not been investigated. Also necessary is the additional introduction of gelatin, accompanied by the use of additional operations. It also involves the addition of chocolate and fruit and berry extracts, which significantly reduces the profitability of the product and complicates the technological process of its production.

In [18], the influence of different concentrations of the sweetener on the physicochemical properties and microbiological parameters of drinking yogurt from camel milk was investigated. The addition of a sweetener significantly affected the active acidity index and viscosity of the product. The control sample without the addition of stevia had a higher rate of active acidity and lower viscosity. Adding stevia before fermentation does not affect the development of microorganisms S. thermophilus, L. bulgaricus, and the probiotic culture of L. acidophilus. Nevertheless, the dispersed composition of stevia has not been studied.

The possibility of replacing sugar with stevia by 50 % in chocolate milk has been investigated in [19]. However, the use of mathematical and statistical methods has not been considered. The development and further application of mathematical models make it possible not only to give an objective assessment of the product but also to predict the dynamics of a complex indicator of its quality under variable conditions.

It is proved [20] that the use of stevia in the technology of flavored cheeses does not affect the development and viability of sourdough and probiotic cultures. Thus, a product with a reduced calorie content is obtained. However, nothing was said about getting rid of the main drawback of stevia, namely bitter taste.

The authors of [21] considered the latest developments on the use of stevia extract in the technology of fermented milk drinks, cheeses, and ice cream. However, the issues related to the study of the technological parameters of the extract remained unresolved.

Thus, according to the review of literary data, it can be argued that the use of stevia in food technologies is now quite common. However, the possibility of application and the form of administration of stevia in the technologies for obtaining fermented milk desserts require further thorough scientific research.

The expediency of using stevia in the form of extracts is to deprive the bitter taste caused by glycosides. It was noted that aqueous and alcoholic extracts of stevia show antibacterial activity against some strains of bacteria of the genus Lactobacillus and Streptococcus. They are also able to have a detrimental effect on microorganisms Streptococcus mutans and Streptococcus sanguis [22].

Most proposed technologies for obtaining sweet extracts of stevia involve the use of extractants such as aqueous or alcohol solutions. It is worth noting that in water extracts from dry stevia leaves, in addition to steviol glycosides, mono- and oligosaccharides, proteins, starch, etc. are present. Thus, in work [23], data are given on the extraction of various groups of organic substances from stevia leaves, subject to the use of various extractants. Demethanol, ethanol,
and methyl acetate extracts largely had greater antioxidant activity and phenolic content as opposed to aqueous extracts. The fractions of hexane-ethyl acetate and chloroform were characterized by a low content of steviol glycosides in relation to the fractions of isobutanol, which extracted them in an amount of 60.3%.

There is a known technique for obtaining concentrate from dry stevia leaves (mass fraction of solids, 60–65%). Purified water (hydro module, 1:5) was used as an extractant at a temperature of 60–70°C for 4.5–5 hours. With the subsequent separation of the extract from the meal, cleaning, concentration, cooling, and packaging [24]. The problem area in this work is a too bulky technological scheme, including several operations that lead to an increase in the scale of production. There are also increases in the economic cost and energy consumption of the process.

Thus, the use of whey to obtain stevia extracts makes it possible not only to obtain a quality product but also to create a range of fermented milk desserts, the production of which is waste-free. And solve the problem of processing whey, its complex and rational use. It is worth noting that stevia extracts based on whey from sour milk cottage cheese will additionally contain almost half of the valuable components of milk. Moreover, the amount of pyridoxine, choline, and riboflavin in whey exceeds their content in milk, which is due to the vital activity of lactic acid microorganisms [25].

Our review of scientific information has shown that it is expedient to conduct a study to determine the optimal technological parameters for obtaining stevia extract.

3. The aim and objectives of the study

The aim of this work is to determine the optimal technological parameters for obtaining stevia leaf sweet extract. This will provide an opportunity to improve the technology of obtaining stevia extracts by intensifying the process and increasing the percentage of extractive substances (ES), as well as optimizing the technique for obtaining sweet extracts for food industry enterprises.

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

– to investigate the dependence of ES yield on the temperature and duration of the process of extraction for plant materials;

– to analyze the response surfaces for the presence of experimental values of variables that provide the maximum response function (degree of extraction of extractive substances).

4. The study materials and methods

According to the results of theoretical studies, at the first stage of setting up experiments to obtain a sweet extract of stevia, distilled water, and whey from cottage cheese were chosen as extract agents. The most appropriate is the thermoacid technique of coagulation of milk proteins, which makes it possible to obtain a curd product containing all protein milk fractions, as well as clarified whey (protein-free), which will later be used to extract the dry leaf mass of stevia.

According to the conducted organoleptic assessment, we used whey from the production of fermented milk cheese in a series of experiments, which meets the requirements of DSTU 7515:2014 “Milk whey. Specifications”. According to the results of several previous studies, dry stevia leaves of a large fraction of the TM “Steviasun” brand were used. The leaves were pre-crushed in the laboratory using the hammer crusher A1-DM2R, produced in Ukraine (rotation frequency, 1500 rpm; power, – 200 kW) for T=15±2 s. The crushed stevia leaves were sifted through a set of wire sieves (sieves of the fourth (Ø6.5), third (Ø5), and second (Ø3) groups) to obtain a homogeneous fraction with an average size of 3.9±0.1 mm. All model samples of stevia extracts were prepared at a hydro module of 1:15.

At the second stage of the experiments, the process of obtaining model samples of extracts was reproduced under the condition of the variability of two procedural factors of influence – the temperature and duration of extraction for the quantitative content of ES. Extraction was carried out in two ways – by maceration and using the rotary-pulse apparatus (RPA) “Flora-120” (Latvia), which is characterized by high efficiency to ensure the intensification of technological processes. It is worth noting that the use of RPA will provide an opportunity to introduce energy-efficient technologies in food production [26].

The volume of solids in the model samples of extracts was determined by the generally accepted refractometric method (DSTU 8402:2015 “Products of processing fruits and vegetables. Refractometric method for determining the content of soluble solids”). This method involves determining the mass fraction of solids of the test liquid on a refractometer scale, at a temperature of 20°C, after carrying out a complete inversion in the product sample.

All experimental studies were performed in three repetitions, and the results obtained and given are the results of these repeated values, taking into consideration the standard deviation. Statistical analysis was carried out using the Microsoft Excel software.

Mathematical and graphical modeling of technological extraction processes for variable factors (temperature and duration) was performed in the environment of the mathematical package “Mathecad 15” and “Statistica”. The mathematical and graphical representation of response functions is carried out using the methodology of the response surface, which is a set of mathematical and statistical methods aimed at modeling processes and finding rational combinations of experimental data.

The general view of the response function is described by a polynomial:

\[ \hat{y}(x,b) = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} b_{ij} x_i x_j \]

where \( x \in R^n \) – the vector of variables, \( b \) is the vector of parameters.

In order to establish the dependence of the yield of ES on the duration of the extraction process and temperature, the geometric form of the approximating function for these specified discrete functions (results of experimental studies) is given. The approximating function is represented as a two-dimensional polynomial of the second power; polynomial coefficients were found by the least squares method (LSM).

To estimate the unknown parameters \( b_0, b_1, b_2, b_{11}, b_{22}, b_{12} \) LSM was used, according to which the unknown parameters of the function are chosen so that the sum of the squares of empirical values (\( Q \)) from their calculated (\( Y_{\text{obs}} \)) was minimal, namely:

\[ Q = \sum (Y_{\text{obs}} - \hat{y}(x,b))^2 \]
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\[ S = \sum_{i=1}^{n} (Y_i - \bar{Y})^2 = \sum_{i=1}^{n} (Y_i - \hat{g}(X, b_1, b_2, \ldots, b))^2 \rightarrow \min. \quad (2) \]

To determine the accuracy of the model, we used standard deviation:

\[ \sigma = \sqrt{\frac{\sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2}{n-1}}, \quad (3) \]

where \( \hat{Y}_i \) is the values calculated using the regression equation, \( Y_i \) – the values of the experimental data.

In order to describe the dependence of the degree of extraction of ES (Q, %) from dry stevia leaves on the technological parameters of the extraction process: \( x \) – temperature (variation interval, 20–100 °C, in increments of 20 °C), and \( y \) – extraction duration (varying interval, 5–30 min.), a second-power polynomial was used. General view of a two-dimensional polynomial:

\[ Q = b_0 + b_1\cdot x + b_2\cdot y + b_3\cdot x^2 + b_4\cdot y^2 + b_5\cdot x\cdot y. \quad (4) \]

The resulting polynomial equations of the second power with calculated coefficients take the following form:

- subject to the use of distilled water as an extracting agent:
  \[ Q_1 = 1.694 + 0.0725\cdot x - 0.00363\cdot x^2 + 0.0014\cdot y - 0.00101\cdot x\cdot y; \quad (5) \]

- subject to the use of cottage cheese whey:
  \[ Q_2 = 1.563 + 0.073\cdot x - 0.0036\cdot x^2 + 0.0014\cdot y - 0.00113\cdot x\cdot y; \quad (6) \]

- subject to obtaining stevia extract using RPA:
  \[ Q_3 = 1.235 + 0.072\cdot x - 0.0035\cdot x^2 + 0.0012\cdot y - 0.00104\cdot x\cdot y; \quad (7) \]

where \( x \) is the temperature, °C; \( y \) – duration of extraction, min; \( Q_1, Q_2, Q_3 \) – mass fraction of extracted substances, %.

The specified polynomial is a mathematical model of a process whose results are valid within the limits of changing its arguments, namely in the interval of variation of factors \( x \) and \( y \). The mean-square deviation (approximation error) of experimental data from mathematical models for two systems does not exceed, \( \sigma Q_1 = 0.01 \% \), \( \sigma Q_2 = 0.02 \% \), \( \sigma Q_3 = 0.01 \% \), calculated from formula (3).

To compare the results of experimental data determined under the condition of using different extractants, the coefficient of variation (relative value, which is the ratio of the mean quadratic deviation to the arithmetic mean), was used.

The coefficient of variation was calculated from the formula:

\[ \delta = \sqrt{\frac{\sum_{i=1}^{n} (\eta_i^2 - \eta^2)}{44 - 1}}, \quad (8) \]

\[ K_{\text{var}} = \frac{\delta}{\text{mean}(\eta^2)} \times 100, \quad (9) \]

where \( \delta \) is the standard deviation.

5. Results of experimental studies to determine the optimal technological parameters for obtaining stevia extract

5.1. Results of studying the dependence of the yield of extractive substances on the temperature and duration of the extraction process

In order to establish the optimal technological parameters for obtaining an extract (from previously prepared dry stevia leaves), several experiments were carried out first of all to study the multiple correlation coefficient. Determining the relationship between the percentage of extraction of ES, temperature, and duration of the extraction process. The results are shown in Fig. 1.

![Fig. 1. Dependence of the degree of extraction of extractive substances from stevia under variable technological parameters of the maceration extraction process (extract agent, distilled water)](image)

In accordance with our results (Fig. 1), the most effective is the implementation of the process of extraction by maceration under temperature conditions of 80–90 °C, the duration of the process of 20–30 minutes, for maximum extraction of ES in the amount of 2.9–3.1 %. Moreover, a further change in the indicators of two factors of the experiment has a negative value for the course of the extraction process.

In order to intensify the extraction process and increase the percentage of extraction of ES from dry stevia leaves, it is proposed to use whey from the production of fermented milk cheese. Data from experimental studies of the degree of extraction of ES and variable technological parameters of extraction are shown in Fig. 2.

Analyzing Fig. 2, an increase in the percentage of extraction of ES from dry stevia leaves was noted, subject to the use of whey. In particular, the largest mass fraction of DM of 3.2–3.4 % is typical for model samples of sweet extracts obtained at a temperature of 80–90 °C, with an exposure of 20–30 minutes.
To intensify the extraction process and increase the percentage of extraction of ES, model samples of sweet extracts were obtained using RPA with whey from the production of fermented milk cheese as an extracting agent. The results of a series of experimental studies are shown in Fig. 3.

According to the constructed 3D graphic models (Fig. 4) of extraction processes, the extraction of the largest percentage of ES is observed with the following technological parameters:

- temperature, 80±5 °C; extraction duration, 25–30 min.; ES, 2.9–3.1%; provided that distilled water is used as an extracting agent;
- temperature, 80±5 °C; extraction duration, 25–30 min.; ES, 3.1–3.4 %, provided that whey from the production of fermented milk cheese is used as an extracting agent.

In order to choose a rational extracting agent (distilled water/whey) for the production of sweet extracts of stevia, the comparison of the obtained results of experimental values was carried out using the coefficient of variation (9). According to the calculated data ($K_{var} = 12.129$), the use of whey from the production of fermented milk cheese increases the degree of extraction of ES by 12.1 %, compared with distilled water (Fig. 5).

After the mathematical processing of the obtained extraction results (Fig. 3), using the “Mathcad15” environment, a polynomial equation of the second power was derived, as well as a 3D plane of dependence of the percentage of extraction of ES in the specified ranges of changes in technological parameters (Fig. 6).

Based on the analysis of the graphic dependence in Fig. 6, one can see that the rational technological parameters of extraction using RPA are as follows: temperature, 80±5 °C; extraction time, 20–25 min; the volume of ES removed, 3.4–3.5 %.

The use of RPA extractors makes it possible to intensify the process of extraction of ES plant materials, compared with traditional methods, by 0.4 %, and the tightness of the circuit will minimize the risk of additional microbiological contamination of the obtained extracts.

5.2. Results of the analysis of the response surface for the presence of experimental values of variables

Our mathematical models (3D graphic models) of obtaining sweet extracts (Fig. 4) make it possible to predict the efficiency of mass exchange processes in order to maximize the extraction of ES from dry stevia leaves.

Fig. 2. Dependence of the degree of extraction of extractive substances from stevia under variable technological parameters of the process of extraction by maceration (extract agent, whey)

Fig. 3. Dependence of the degree of extraction of extractive substances from stevia using a rotary-pulse apparatus

Fig. 4. 3D graphic model of the dependence of the degree of extraction of extractive substances from stevia by maceration with different extract agents: $a$ — whey; $b$ — distilled water
Our experimental results (Fig. 1–3) of the processes of accumulation of solutes in model samples of extracts are well consistent with the known regularities of mass exchange processes. They are characterized by the transition of one or more components of the initial substance from one phase to another. Moreover, the driving force of mass exchange processes is the difference in their concentrations. It is worth noting that the content of soluble substances in the extract is influenced by the ratio extractant:raw materials, the degree of grinding, raw materials, the surface area of their contact, as well as the temperature and duration of extraction [27].

Papers [22–25] investigated the possibility of using stevia in the form of extracts but the use of mathematical and statistical methods that make it possible to predict the dynamics of a complex quality indicator under variable conditions has not been considered.

The reported dynamics, obtained from the results of mathematical calculations, are well consistent with the dynamics obtained from the results of experimental studies. Analysis of the obtained dependences (Fig. 4, 5, 6) shows that the yield of ES from stevia leaves is almost equally significantly influenced by both temperature and duration, and the influence of both factors is nonlinear in nature. In accordance with the specified conditions for the extraction process, the equilibrium state is achieved over a period of 25–30 minutes. After this, the efficiency of extraction of ES decreases and remains constant even if the technological parameters of the process change.

Based on our results of scientific research, it should be noted that maceration does not provide a sufficient mechanical opening of leaf tissue cells, which causes the presence of a significant content of ES (sweet glycosides) in the pressed leaf pulp of stevia. In this regard, the use of RPA to obtain sweet extracts is more appropriate. Comparing the reported results of the processes of obtaining extracts in Fig. 2, 3, it can be argued that the use of RPA makes it possible to increase the yield of ES by 0.1–0.4 %.

The disadvantages of this study are a narrow circle of investigated extract agents that can be used in the technology of fermented milk desserts. The sweet extracts obtained by the proposed technology should be used over a brief period. They are in a liquid form and do not contain preservatives in their composition, and therefore, after long-term storage, they can become an additional source of bacterial seeding.

Our data create scientific prerequisites for the development of dietary food technologies with a complete or partial replacement of the sweet component. In particular, fermented milk desserts, or a dessert series of products with low sugar content. The introduction of such technological schemes of production at enterprises in the food industry will have social and economic effects due to an increase in the range of products for the intended purpose and minimization of the percentage of the introduction of the extract due to the high degree of sweetness.

Along with this, it should be noted that the quality of stevia extracts depends on several factors that are not considered in the framework of the experiments conducted. This leads to a potentially interesting scientific and practical direction of several novel studies. In particular, further advancement can be focused on:

- studying the potential for the use of sweet extracts of stevia, made according to the proposed technology, in the production of food products for the intended purpose;
The analysis of the above results shows that the temperature and duration of extraction are almost equivalent factors influencing the yield of ES. The optimal technological parameters for obtaining stevia extracts at a 1:15 hydro module have been scientifically substantiated:

1) extraction (maceration) subject to the use of distilled water as an extracting agent; temperature, 80±5 °C; duration, 25–30 minutes; ES yield, 2.9–3.1 %;
2) extraction (maceration) subject to the use of whey as an extracting agent; temperature, 80±5 °C; duration, 25–30 minutes; ES yield, 3.1–3.4 %;
3) extraction (RPA) subject to the use of whey as an extracting agent: 85±5 °C, duration, 20–25 minutes; ES yield, 3.4–3.5 %.

All obtained data are under the conditions of existing restrictions imposed by the limits of variation of input data.

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.

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References


