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THE STUDY AND SCIENTIFICAL SUBSTANTIATION OF CRITICAL CONTROL POINTS IN THE LIFE CYCLE OF IMMUNOSTIMULATING PRODUCTS SUCH AS PASTILA AND MARMALADE

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Based on the study results, this paper reports a technology to produce natural fruit and berry pastila and marmalade of improved nutritional and biological value with a long shelf life. Critical control points of product safety have been identified and scientifically substantiated. The technological process to prepare pastila and marmalade involves exposing the raw materials to high and low temperatures while maintaining certain indicators of the content of dry substances. The identification feature of marmalade products is the content of a certain type of fruit raw materials, which poses risks throughout the life cycle of product manufacturing despite the fact that the preparation and storage of the raw materials implies the temperature range from 0 °C to minus 15 °C. Problematic issues of mold occurrence have been considered. It was established that the moisture content in the product above 15 % leads to an increase in the content of mold fungi and significantly reduces its shelf life. This paper gives the results of a study to identify the preservation of vitamin C in products prepared from fresh berries and from berries subjected to freezing. It was revealed that in the process of storing the raw materials at a temperature of minus 15 °C, the amount of vitamin C in the resulting product decreased by 11.3 % compared to the product prepared from fresh raw materials. The maximum limit of the high preparation temperatures of 108 °C did not significantly affect the loss of vitamin C. Studies were conducted to determine the sugar content in products whereby a high fructose content was identified. Critical control points at all stages of production were determined, which has made it possible to choose the optimal technological modes and parameters for the safety and quality of the product

Keywords: critical control points, marmalade-pastila products, vitamin C, reducing substances

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1. Introduction

Confectionery products made on the basis of fruit raw materials include marmalade and pastila products, sweets, caramel with fruit fillings, chocolate with fruit additives and fillings, etc. A distinctive feature of marmalade and pastila products is their gel-like structure. At the same time, marmalade has a solid structure while pastila products have a cellular structure in the form of foam fixed with a gel-like mass. According to the quantitative content of nutrient extractive substances, marmalade and pastila products are more concentrated products than the original fruits and berries since they contain 3–5 times less water (15–20 % instead of 75–90 %). They are also high in sugar, at 60–75 %, compared to fresh fruits and berries (5–15 %) [1].

The main source of energy in natural marmalade-pastila products are carbohydrates introduced into the product with such raw materials as fruit and berry puree and fructose.

Raw materials of carbohydrate nature are divided into two types: digestible and indigestible carbohydrates.

Digestible carbohydrates include mono- and oligosaccharides, starch, glycogen. Indigestible carbohydrates are cellulose, hemicellulose, pectin, inulin, etc. The studied fruit pastila receives digestible carbohydrates with fruit and berry puree, including sucrose, glucose, fructose. The indigestible carbohydrate is pectin [2].

Monosaccharides and disaccharides, especially sucrose, cause a rapid rise in glucose content. Therefore, excessive consumption of simple sugars is harmful to health: it can cause atherosclerosis, obesity, and diabetes [2]. At the same time, the number of people leading a healthy lifestyle is growing and, therefore, the demand for special-purpose products is increasing [3].

In Europe and the United States, jelly sweets with biologically active additives of natural origin are produced, aimed at improving digestion, eliminating the effects of stress. Vitaminized jelly sweets, dragees and lozenges aimed at weight loss, for dietary and baby food are also produced. Analysis of consumer demand shows that confectionery products with functional therapeutic and prophylactic properties are popular with more than half of the population, mainly among young women. There is a decrease in interest in such products with an increase in the age of the respondent [4, 5].

In this regard, it is a very relevant and important area of research to devise the technology of low-calorie confectionery products for functional purposes and to study their critical control points in the process of the life cycle of production.

2. Literature review and problem statement

The authors of [6] conducted research work on the development of a formulation of marmalade with an extract of viburnum vulgaris, which is a pharmacopeial raw material with preventive and restorative properties, due to its rich chemical composition. However, they did not study the safety criteria of finished products, the change in their properties during storage. The reason for this is likely the specific focus of the study. Most likely, the authors continue to investigate the properties and safety assessment of finished products, which could be reflected in their next works.

Work [7] reports the marmalade with pectin-containing pumpkin concentrate, developed in order to reduce the content of dry substances in the finished product and increase its nutritional value. However, the cited work does not study the dependence of the mass fraction of moisture in the finished product and the growth of mold fungi. Further research in this area is needed.

Technologies to produce jelly marmalade enriched with phyto fillers (decoction of herbs of the root of lapchatka, herbs aralia, parmelia, and dry cranberries) [8], as well as persimmon puree and sourdough from *Lactobacillus bulgaricus* and *acidophilus* [9] have been developed. In the cited works, little attention was paid to the study of storage modes of fruit and berry raw materials for the safety of vitamin C in finished products. It would be interesting to study the effect of herbal extracts on the dynamics of changes and preservation of vitamin C during processing and storage, as well as their effect on the contamination of the product.

The use of vegetable additives, such as cabbage with sesame, carrots with apple-strawberry puree [10], as well as beet puree [11], is proposed to give the dietary and functional properties to the finished products. When developing the technology of jelly marmalades, the authors paid little attention to identifying the effect of heat treatment time and the amount of dry matter on the consistency of the jelly. Most likely, this is due to the study's focus on determining the dietary and functional properties in the finished products. It would be interesting to expand the range of research with the identification of the influence of the introduced components on gel formation.

The author of [12] has developed a recipe for marmalade from nanostructured Jerusalem artichoke to give therapeutic and prophylactic properties to finished confectionery. However, when devising a formulation of marmalade from nanostructured Jerusalem artichoke, the dependence of various factors on the growth of mold fungi in finished products during storage has not been studied. This is most likely due to the fact that the author paid special attention to the development of the component composition of the marmalade recipe. And the next stage of the area of research for the author could be determining the safety criteria and the effect of nanostructured Jerusalem artichoke on the bacterial load of the product.

Scientists [13] have well studied the field of application of tropical fruits in the production of jams and marmalades; their results demonstrate high indicators of the quality characteristics of the use of lemon in the production of marmalade. However, the issue of studying the factors of gel formation is poorly covered. And it will be interesting if the authors continue their research in this area.

Studies into the properties of marmalade with vegetable cryo additives during storage are underway. To improve the organoleptic parameters and antioxidant properties of marmalade, cryogenic additives were used [14]. However, despite the vastness of the study, the authors did not study the dynamics of changes in vitamin C due to heat treatment and during storage. The reason for this may be the incompleteness of their study and, possibly, these results will be demonstrated in the following works.

Paper [15] reports the results of optimization of the recipe for plum marmalade containing date syrup. In this case, the issue of using date syrup as a substitute for sucrose in the formulation of marmalade was studied. The authors did not discuss in detail the influence of the use of date syrup on the rheological properties of marmalade.

Paper [16] reports the results of studying the physical-chemical and qualitative properties of pumpkin (*Cucurbita Moschata Duch.*) in the production of jam, marmalade, and pastila. In the cited study, the authors paid special attention to the content of hydroxymethylfurfural (HMF), total phenol content, total antioxidant capacity, total sugar, organic acid composition, pH, water-soluble solids, and humidity. In the study of safety criteria, the authors did not conduct research in the field of studying the influence of film selection and its thickness on the safety of finished products during storage. The reason for this may be the construction of other prerogatives of research that have a high cost. In the future, the authors could pay attention to the choice of packaging material, in order to increase the shelf life of finished products.

The authors of [17] present the physical-chemical characteristics, sensory properties, and antioxidant activity of marmalade made from the peel of black plum.

Paper [18] reports the results of studying the antimicrobial and antioxidant properties of garaghurt: the traditional dogwood marmalade (*Cornus mas*). However, no data are given on the safety of vitamin C and other representatives of the antioxidant group during the processing and storage period. It would be interesting to continue that study with the disclosure of the preservation of ardent representatives of the antioxidant group during the processing and storage period.

The authors of [19] conducted research to determine the quality and nutritional value of functional strawberry marmalade enriched with Chia seeds (*Salvia hispanica L.*). However, there are no data on the effect of Chia seeds on the product bacterial load. Most likely, this would be reflected in their next works since this area of research is very important and deserves attention.

Paper [20] reports the results of a study to identify the possibility of using orange by-products (dry peel) as an alternative gelling agent to produce marmalade. That study evaluated the antioxidant activity and inhibition of HMF formation at different storage temperatures. However, the authors do not give examples of the use of packaging material and its impact on the safety of the product. Most likely, this is due to the fact that the studies conducted by the authors were expensive due to the repeated analyses in the study of the preservation of antioxidant properties during storage.

Paper [21] reports the results of a study on optimizing the formulation composition of jelly masses using a Scheffe's simplex plan. In the cited paper, the authors do not give the results of the influence of heat treatment on the safety of quality indicators of the optimized formulation components. Most likely, research is ongoing, and the next works would provide extended results.

The authors of [22] conducted research on the study of safety and the identification of basic, secondary, and toxic elements using the ICP-OES method, and other ultra-high-frequency methods in tropical fruits used for the preparation of marmalade. However, the authors do not report the results of studying the microbiological safety of finished marmalade products. The reason for this may be the narrow focus of the research conducted, which is costly in implementation. An option for overcoming the relevant difficulties is the separation of research areas.

The enrichment of fruit and berry, melon juices with medicinal herbs was tackled by scientists from different countries [23]. In this case, the optimization of the components of blended juices from melon and berries was performed using the simplex centroid method [24].

Those studies may be interesting for the purpose of using the developed types of juices as a basis for marmalade.

Based on the conducted literary review, one can conclude that it is advisable to conduct a study on improving the technology of confectionery products for diabetic, therapeutic and prophylactic, functional purposes with the identification of the main control critical points of production. Such a study could preserve the native properties of berries, fruits, vegetables, medicinal herbs. In this regard, the proposed area of research is of great importance and relevance for the development of the confectionery industry in the field of healthy nutrition.

3. The aim and objectives of the study

The purpose of this study is to identify and scientifically substantiate the critical safety control points in the production of pastila-marmalade products with immunostimulating properties.

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

– to identify the dependence of the mass fraction of moisture and the growth of mold fungi;

– to study the storage modes of fruit and berry raw materials and the preservation of vitamin C in finished products during storage;

– to investigate the effect of heat treatment time and the amount of dry matter on the consistency of the jelly;

– to determine the optimal thickness of the film for storing finished products.

4. The study materials and methods

4. 1. Materials

To produce pastila and marmalade, the following raw materials are used:

– strawberries of the "Black Prince" variety, selected according to the principle of preserving taste and aroma in the resulting product in line with GOST 33953-2016 "Fresh strawberries. Specifications";

– apples of the "Aport" variety were selected according to GOST 27572-2017 "Fresh apples for industrial processing. Specifications", based on studies reported in [25], this variety contains the largest amount of pectin substances, which helps reduce the amount of pectin introduced;

– dry apple pectin was selected as a thickener according to GOST 29186-91 "Pectin. Specifications";

– fructose was selected as a sweetener, while the quantity and quality of fructose was chosen in accordance with the results of tests for the total amount of sugars.

4. 2. Experimental setup and methods of analysis

The study consisted of 7 main processes. These include the selection of raw materials, compilation and optimization of recipes, the selection of technological parameters, control over critical points, analysis, adjustment of the formulation and technological parameters, the study of the physical-chemical and microbiological properties of the finished product.

Sampling was carried out by aa quartering method in accordance with GOST 5904-2019 "Confectionery products. Acceptance Rules and Sampling Methods".

Organoleptic indicators were determined by the sensory method at the tasting room of Almaty Technological University.

We studied the nutritional and biological value of pastila and marmalade at the certified testing laboratory "Food Safety" of the Almaty Technological University and the laboratory of the University of Santiago de Compostela (Spain).

Sucrose, glucose, fructose, and maltose were detected using high-performance liquid chromatography (HPLC) with a refractometric detector and a thermostatically controlled chromatographic column (GOST 31669-2012).

The mass fractions of moisture, vitamin C were determined using standard methods (GOST R EN 14130-2010).

4. 3. Experiment planning

In order to reduce the number of our experiments and optimize the study, samples were selected in accordance with marketing research and market analysis, which showed the best result.

To study the factors of influence of technological parameters as a source and indicator of inaccuracy of the experiment setup, the storage process was preliminary studied and the optimal modes and formulation components for the object of our study were selected.

Critical control points are determined by the risk assessment method, which makes it possible to identify the structure of hazard identification and their limits, relative to the point of no return. The main risk or criticality limit (the first critical control point) is the amount of moisture in the product, which poses a microbiological hazard and the impossibility of changing further processes on the way to the formation of a safe product ready for use.

5. Results of studying the safety points of immunostimulating products such as pastila and marmalade

5. 1. The dependence of the relative share of moisture content in the product and the growth of mold fungi

In accordance with the objectives set, according to the study results, the moisture content limits in the finished product and the content of dry substances were established, when preparing to optimize the technological process and achieve the level of microbiological safety of the product under study.

To investigate the dependence of the relative mass fraction of moisture and the growth of mold fungi on microbiological load, samples with a moisture content of 31 %, 26 %, 21 %, 18 %, 16 %, and 11 % were studied (Fig. 1).

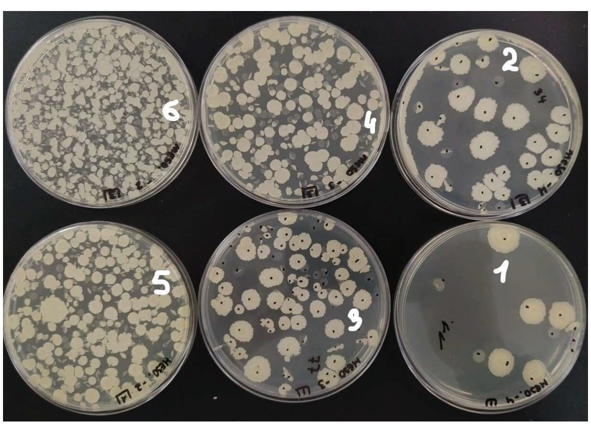

Fig. 1. Microbiological contamination of marmalade product samples with a moisture content from 11 to 31 %: 1 – moisture content, 11 %; 2 – moisture content, 16 %; 3 – moisture content, 18 %; 4 – moisture content, 21 %; 5 – moisture content, 26 %; 6 – moisture content, 31 %

Counting the bacteria showed the following results: at a moisture content in the product of 11 % – $1*10^3$ CFU; 16 % – $2*10^3$ CFU; 18 % – $2.2*10^3$ CFU; 21 % – $3*10^3$ CFU; 26 % – $4*10^3$ CFU; 31 % – $5*10^3$ CFU.

Fig. 2 shows a plot of the dependence of bacterial growth on the moisture content and shelf life of strawberry marmalade.

According to Fig. 2, which shows a plot of the response of bacterial growth depending on moisture content and shelf life, demonstrating a linear relationship, the following regression equation (formula (1)) is calculated:

$$z = -8667.857 + 1157.143x + 250y, \quad (1)$$

where x is the shelf life in days, y is the moisture content as a percentage.

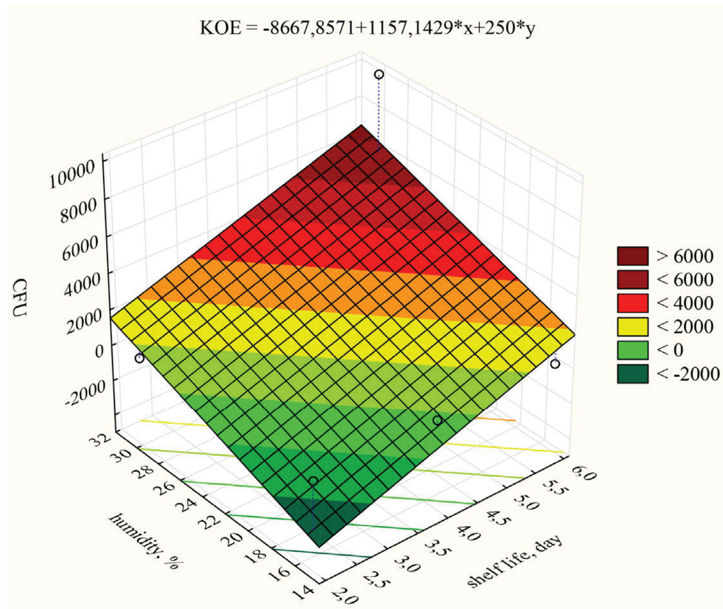

Fig. 2. Dependence of bacterial growth on moisture content and shelf life

Additionally, Fig. 1 shows that the contamination with microorganisms of the developed product increases as the moisture content in the product increases. The lower the moisture content, the less contamination of the product in terms of time, while the lowest result in the growth of bacteria is observed at a moisture content of the product under study not exceeding 16 %. With this moisture content, the consistency of fruit slices is not disturbed.

Based on the conducted studies, the recipe was optimized as regards the moisture content of products, in strict intervals from 15 % to 16 %. At the strict observance of the specified moisture content interval, the consistency of the finished products turns out to be plastic, well preserving the shape, not hard, without fractures at bending. When biting off the product, its integrity is easily disrupted, while the consistency is not brittle, slightly tightened, without a crust on the surface, evenly dense over the entire surface of the product.

The next stage of the study was to identify the dependence of the mass fraction of moisture and the growth of mold fungi (CFU) in the experimental and control samples of strawberry marmalade. As an experimental sample, the devised strawberry marmalade with a moisture content above 18 % was used; the developed strawberry marmalade with a moisture content not exceeding 16 % was the control. The results of this study are demonstrated in Fig. 3.

Fig. 3 demonstrates that the scale x is the mass fraction of moisture, the scale y is the growth of mold fungi, CFU.

This plot (Fig. 3) shows that, depending on the humidity indicators, the microbiological contamination of the experimental sample is higher than that of the control sample up to a certain point.

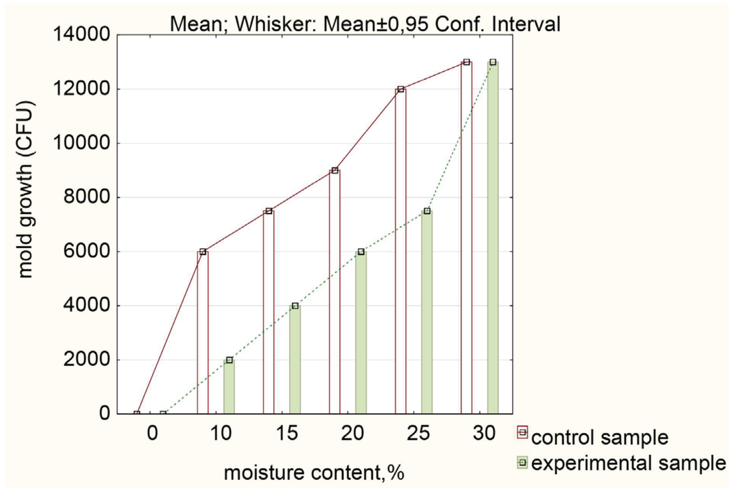

Fig. 3. Results of studying the dependence of the mass fraction of moisture, %, and growth of mold fungi (CFU) in the experimental and control samples

In interstate standards for marmalade, it is recommended that moisture content should not exceed 30 %; in interstate standards for pastila – should not exceed 25 %.

In accordance with our study, the critical control points for a given product were identified to establish the moisture level and prevent the growth of mold fungi – moisture content should not exceed 15 %.

5. 2. Changing the amount of vitamin C in the product depending on the storage modes of berry raw materials

The second critical control point in the technological chain of a given product preparation is compliance with the storage regimes of fruit and berry raw materials with the preservation of vitamin C.

The scientific substantiation of the critical control point in a natural immunostimulatory product such as pastila is the study that investigated the content of vitamin C in a product with various technological (temperature) storage regimes of raw materials. Based on our study, there was a difference in the content of vitamin C in the product the raw materials for which were stored under sub-zero temperatures and the product prepared from freshly harvested raw materials (Fig. 4). As an experimental raw material, freshly picked strawberries of the "Black Prince" variety were taken.

Fig. 4 shows that the level of vitamin C in marmalade depends on the condition, terms, and storage conditions of the raw materials used. The marmalade made from fresh raw materials demonstrated a higher content of vitamin C. The loss of vitamin C is 10.7 % when using frozen raw materials after a month of storage at a temperature of minus 18 °C. After three months of storage of the finished product, the loss of vitamin C is 11.9 % when using frozen raw materials stored at a temperature of minus 18 °C.

Thus, when investigating the second critical control point in the technological chain of preparation of this product, the storage modes of fruit and berry raw materials with the maximum preservation of vitamin C were studied and established.

In this case, the losses are 10.7 % and 11.9 % when using frozen raw materials, respectively, after one and three months of storage at a temperature of minus 18 °C.

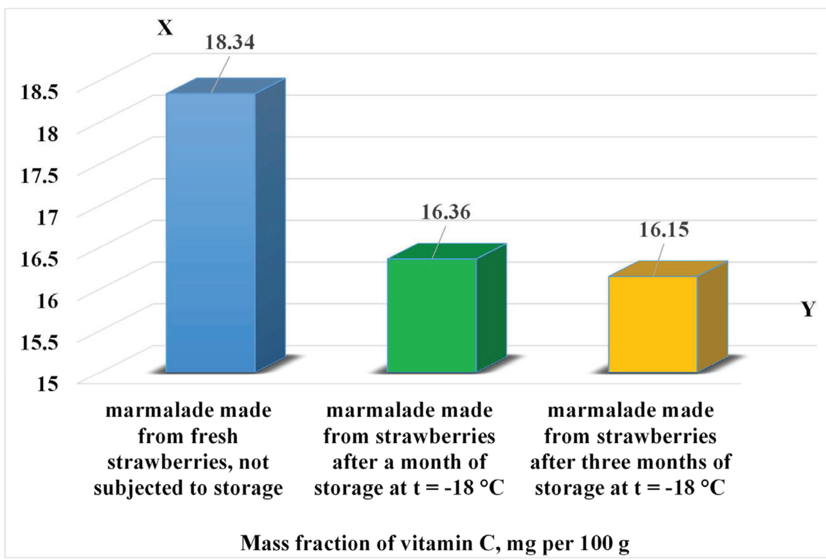

Fig. 4. Change in the content of vitamin C in the finished strawberry marmalade, depending on the period and storage conditions of strawberries of the "Black Prince" variety

5. 3. Studying the influence of heat treatment time, dry matter content on the consistency of jelly

The third critical control point refers to the time limits of heat treatment and the point of control for the amount of dry matter in the process of manufacturing the products under study.

The influence of heat treatment time and the amount of dry matter in the developed products on the consistency of jelly was investigated. Within that framework, the optimum for a jelly of good consistency was clearly shown, 20–22 minutes of heat treatment at a temperature not exceeding 105 °C and the amount of dry matter of 59 % (Fig. 5).

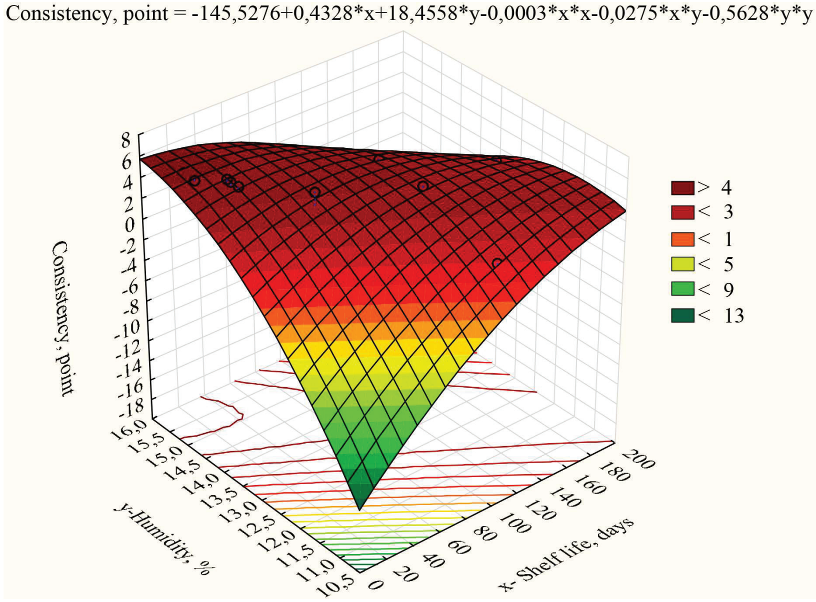

Fig. 5. Dependence of consistency on the mass fraction of dry matter (DM, %) and heat treatment time (x_1, min)

As a result of this study, the following equation (2) was built:

$$Y=198.5-1.4x_1+4x_2. \quad (2)$$

The function is linear, SD±1.96, where x_1 is the heat treatment time, min; x_2 – the amount of dry matter determined by a branded refractometer, **%**.

The x axis is the time in minutes, the y axis is the amount of dry matter. The region with the best result is marked in red, where the most suitable parameters of consistency, moisture content, and time indicators coincide.

The influence of heat treatment time and the amount of dry matter on the consistency of the jelly was investigated: the optimum for a jelly of good consistency is clearly shown, 20–22 minutes of heat treatment with an amount of dry matter of 59 %. It has been experimentally proven that with an amount of dry matter of less than 59 %, the jelly is weak in strength; over 59 %, the jelly cloaks (it solidifies unevenly in speed).

Thus, the third critical control point in the technological chain of preparation of a given product is the setting of control measures for measuring dry matter and heat treatment time.

5. 4. Studying the dependence of the quality characteristics of the product on the μm quantity in the film for storage

The fourth critical control point is to establish and control the μm quantity of the selected film for storage. This point is also a point of no return for quality indicators.

An erroneous change in this indicator would entail premature damage to the product.

For the experiment, a biaxyl-oriented polypropylene three-layer metallized food film was used.

The results of this study are demonstrated in Fig. 6, 7.

Fig. 6 shows that the interdependence of factors reveals that with a film thickness of 60 μm, the longest shelf life without loss of quality is achieved – 9 months. With a film thickness of 40 μm, one can store the product for up to 100 days, at room temperature at that.

When using a package with a thickness of 20 μm, the shelf life without loss of quality is, according to the plot, from 40 to 60 days.

Fig. 7 demonstrates that it is optimal to use of a film with a thickness of 60 μm.

Given this, the fourth critical control point in the production chain of preparation of a given product is the control point during the packaging and storage of products. It also refers to the process of acceptance control since the acceptance of raw materials and packaging materials is carried out at the input control and is controlled during packaging and storage. This procedure includes control over the thickness of the film, which should not be less than 60 μm.

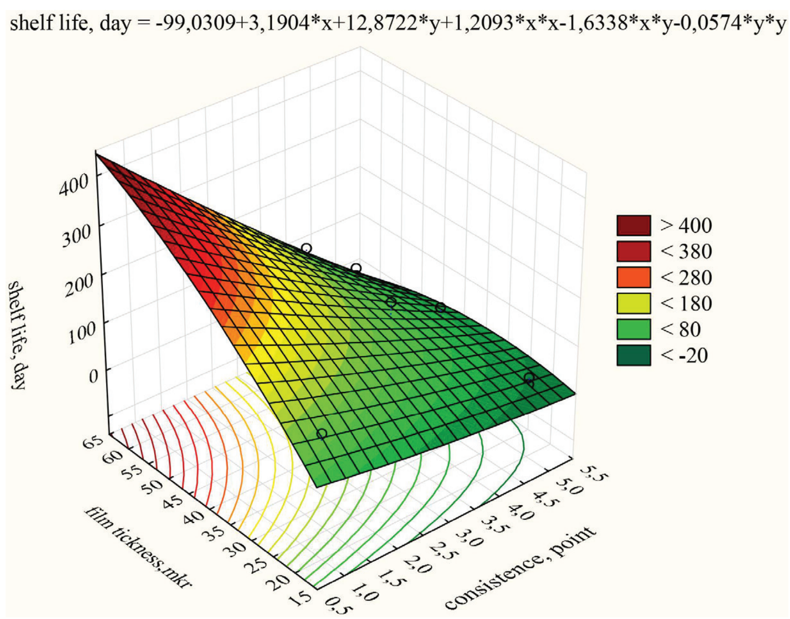

Fig. 6. Dependence of shelf life on film thickness

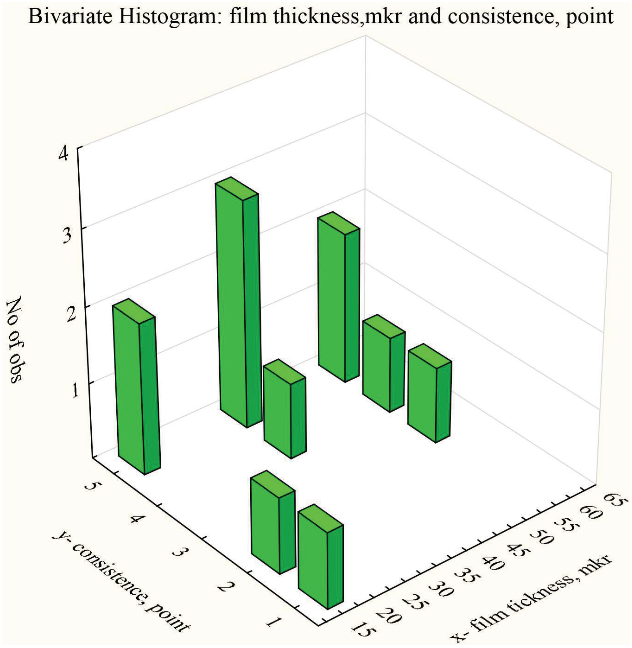

Fig. 7. Selecting the optimal storage solution point according to the planning matrix

6. Discussion of results of studying the safety points of immunostimulating products such as pastila and marmalade

All microbiological studies were repeated twice. When determining the microbiological contamination of finished pastila-marmalade products with a moisture content of 31 %, 26 %, 21 %, 18 %, 16 %, 11 % (Fig. 1), it was revealed that the lowest contamination is observed at a product moisture content of 11 %. However, at a product moisture content of 11–14 %, the products obtained are rigid, brittle, and dark, that is, have inappropriate organoleptic indicators. With the pastila-marmalade products' moisture content of 16 %, the level of contamination reaches $2*10^3$ CFU, which is within the permissible limits of microbiological contamination for sweets and confectionery, non-glazed, diabetic, pastila-marmalade products. At this moisture content, the best organoleptic indicators are observed, including the undisturbed consistency of fruit slices. With a moisture content above 16 %, the growth of mold fungi increases sharply, which is clearly demonstrated by Fig. 2, 3.

The results of the change in the amount of vitamin C in the product, depending on the storage modes of berry raw materials, are clearly shown in Fig. 3. According to Fig. 3, the marmalade made from fresh raw materials showed a higher content of vitamin C. Its losses are 10.7 % and 11.9 %, when using frozen raw materials, after one and three months of storage, respectively. This indicates the possibility of using frozen raw materials since the loss of vitamin C is within the limits that make it possible to enrich the product. It is known that vitamin C is an antioxidant; in addition, according to the authors of [26], it follows that vitamin C is involved in preventing/neutralizing the inflammatory response, reducing oxidative stress, and stimulating interferons and other antiviral cytokines.

The dependence of the consistency on the mass fraction of dry matter and the time of heat treatment is illustrated in Fig. 4. Our data make it possible to optimize the formulation for the moisture content not exceeding 16 % and not less than 15 %, with a duration of heat treatment in the range of 20–22 minutes, at a temperature not exceeding 105 °C. At this ratio of the moisture content, the consistency of the finished products is plastic, it retains its shape, it is not solid, there are no fractures at bending, it is slightly tightened.

Fig. 5, 6 show the results of studying the dependence of the quality characteristics of the product on the µm quantity in the film for storage. As these figures demonstrate, it is optimal, for maintaining the quality indicators of finished products during long-term storage, to use a biaxyl-oriented polypropylene three-layer metallized food film, with a thickness of at least 60 µm.

The entire process is based on the main principles of food safety and the integration of such quality management systems as HACCP and GMP.

Our results could be used in the implementation of pastila and marmalade production technology at confectionery enterprises.

In this study, the vitamin-mineral complex in the developed products and the effect of heat treatment on its preservation, except for vitamin C, was not fully studied. The influence of using medicinal plants with immunostimulating effect on the quality of finished products, their safety, and bacteria contamination has also not been investigated. Given this, these areas of research should be addressed in the future.

7. Conclusions

1. It has been established that the moisture content in the product above 15 % leads to an increase in the content of mold fungi and significantly reduces its shelf life. The study was conducted with the indication of qualitative and quantitative indicators. Qualitative indicators include organoleptic indicators. Quantitative indicators include the percentage of moisture content in the finished product, selected experimentally, and the number of CFU mold fungi relative to the moisture content of the product. At the same time, according to organoleptic indicators, the product is characterized by a pleasant taste and aroma, with a plastic enough consistency, corresponding to this type of product.

2. It has been found that in the process of storing raw materials at a temperature of minus 15 °C, the amount of vitamin C in the resulting product decreased by 10.7 % compared to the product prepared from fresh raw materials.

3. We have selected modes and defined quantitative indicators of dry matter involved in gelling processes. For the best gelling result, it is necessary to maintain the moisture content in the range of 15–16 % with the following heat treatment modes: temperature, not higher than 105 °C; duration, 20–22 minutes.

4. The terms of the optimal storage of pastila and marmalade in a biaxyl-oriented polypropylene three-layer metallized food film, with a thickness of 60 µm, up to 9 months, at a temperature of 25 °C, have been established.

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