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Meat industry by-products for berry crops and food production quality improvement

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Abstract. This paper describes the problem of obtaining a hydrolysate from animal industry by-products. A new innovative protein-containing product has been created to stimulate the growth and development of berry and fruit crops. The paper describes a technique for a plant treatment with a hydrolysate invented, its concentrations being determined. We have studied the chemical composition of fruit and berry raw materials in a native form after rapid freezing and refregiration. The possibility of creating a new confectionery product made from quick-frozen berries treated with a stimulator is predetermined**.**

Key words: protein hydrolysate, stimulator of plant growth and development, quick-frozen berries, anthocyanins

INTRODUCTION

Beef cattle farming can be made more cost-effective by complex meat by-products and waste processing. This will decrease the meat waste volume, which improve environmental situation and broaden the range of food and feed products available on the market. New products with tailored properties for a particular application can be produced in presence of chemical catalyst in micro concentration (Uspenskaya et al., 2016). This technology enables to produce protein hydrolysate, which can be used as a stimulator of plant growth and development for cultivation of berries with increased phenol compounds. The vegetation period is decreased by 10–14 days for the climate conditions of Russian north-west regions. Further berries quick-freezing using fre-flo freezer allows to produce semi-products of high quality. Its application in confectionery with agar or starch embedding makes products competitive. (Garrido et al., 2016; Arfat et al., 2017).

The purpose of the work was to determine the optimum concentrations of treatment of garden plants by hydrolysate of meat processing by-product – beef split and possibilities in usage of fruit and berry fast-frozen products.

MATERIALS AND METHODS

Canned beef split was used to receive the protein hydrolysate as the stimulator. Beef collagen tissue was preserved with calcium hydroxide, treated with hydrochloric acid to neutralize calcium hydroxide and then rinsed with running water. Then two-stage hydrolysis was performed. The first stage included water hydrolysis at 100 °C for 1.0–1.5 hours, decantation of liquid phase, homogenization of solid phase and mixing of both phases into suspension. At the second stage the suspension was hydrolysed with 0.012 – 0.250% hydrochloric acid solution under 130–150 °C and hydro modulus of 1: (1.5–2.0) with a pressure of 3 atm during 4–6 hours, followed by drying in the drying machine with counter-curved flows of inert support with 180 °C input and 95 °C output temperature (Kutsakova, 2004).

Amino acid composition of the modified protein products was determined by the method of ion exchange chromatography on the automatic amino acid Analyser (Japan). The research results are presented, and also the characteristics of collagen hydrolysate are given below. Dry matter content was determined to the constant weight by the drying method at a temperature 105 ± 0.5 ºC. Calcium ions content was determined by the method based on measuring the electromotive force of the element that consists of a reference electrode with a known potential value and a calcium-selective electrode, which potential is determined by concentration of calcium ions in the examinee solution. The electromotive force is fixed by the converter, the operation principle of which is based on the transformation of ion activity to electro moving force values (mV), which is linearly dependent on ion activity in the analysed solution and its temperature. The electrode system includes an auxiliary reference electrode (silver chloride) and a measured electrode – calcium-selective. The temperature compensator is also applied for measurements. Active acidity (pH) of the hydrolysate solution with a mass fraction of 1% was determined by the potentiometric method. The mass fraction of sodium chloride was determined by the method of protein substances precipitating by solution of nitric acid and titration of the chlorides in an acidic extract according to Folhard.

Wide research of treated plants (meadow and green grasses, berry, fruit crops) in the North-West region of Russia was conducted. Objects of research were treated once (at acrospire and blossoming stage) with water solutions of hydrolysate with 50 to 800 mg l^{-1} concentration using atomizing nozzle. Studies connected with treatment of sweet cherry trees and black currant bushes are presented in this paper. Single-factor experiment was used. A step of varying the concentration of 200 mg l^{-1} to establish the limiting, stationary and inhibitory area was established. The perennial bushes and fruit trees without stimulator treatment were the control group. The objects treatment was carried out in triplicate with the water solution the stimulator concentration of 200, 400, 600, 800 and 1000 mg l^{-1}. Leaves research was presented for better stimultor concentration for black currant bushes (600 mg l^{-1}) at vegetation period.

Concentration of chlorophyll and carotenoids were used to determine the level of photosynthesis activity and concentration of photosynthesis products. Chlorophylls and carotenoids concentration in leaves, phenolic compounds in fruit and berries were determined by spectrophotometric method. The aboveground mass in the blossoming and fructification phase was collected. Selection was made from the area of 30x2 m, allocating 5 platforms of 1x1 m for each species of plants. Leaf samples for the research were taken triply. The pigment content was determined in an acetone extract (100%

concentration) using a spectrophotometer, in cuvettes with a layer thickness of 10mm by the absorption centers on lengths of waves of 644, 662 and 440.5 nm for chlorophyll a, b and carotenoids (k) respectively. The pigment concentration was calculated by Wettstein equations:

$$C_a = 9.78 \times D_{662} - 0.99 \times D_{644};$$
$$C_b = 21.42 \times D_{644} - 0.99 \times D_{662};$$
$$C_{a+b} = 5.13 \times D_{662} + 20.43 \times D_{644};$$
$$C_k = 4.69 \times D_{440.5} - 0.268(C_{a+b}),$$

where: $C_{(a;\ b;\ a+b;\ k)}$ – pigment concentration, mg l^{-1}; D – optical density in the centre of pigment absorption on the set wavelength.

Pigment content was calculated by the equation:

$$X = (C_{(a;b;a+b;k)} \times V)/(P \times 1000),$$

where: X – pigment content in mg per 1g of crude batch weight; V – volume of pigment extraction, ml; P – leaf batch weight, g. (Shlyk, 1971).

Leaf juice drying was performed in the drying unit (Kutsakova, 2004) at an air inlet temperature of 140 °C and an outlet of 90 °C. An average sample weight was 0.3 kg. In dry leaf juice the following parameters of the product safety were determined: the quantitative content of toxic elements – lead, cadmium, copper, zinc, mercury, and arsenic – by the method of inversion voltammetry. The method is based on the determination of the mass concentrations of elements in the sample solution that are defined by the method of adding certified mixtures of the determined elements, which do not demand a calibration curve construction. Using this method the voltammogram cycles are recorded. Residual amount of organochlorine pesticides – aldrin, hexachlorane, DDT and its metabolites were detected by method of chromatography in a thin layer – by gas-liquid chromatography.

Fruit sampling was performed by the method of medium samples extraction that implies taking the samples from no less than 10 trees and bushes located on the diagonal sections. The average sample mass was 0.2 kg to study each option.

To study the parameters of fresh, chilled, frozen fruit as well as the ones at fruit refrigeration storage, the following methods were applied. To determine phenolic compounds from fruitan alcohol extract was obtained. (Katserikova, 1998) To determine the total content of phenolic compounds (phenol carbonic and oxycinnamic acids), 0.3 cm^3 of the Folin-Denis reagent was added to 1 cm^3 of alcohol extract. After stirring the mixture for 20 sec. 5 cm^3 of a 20% solution of Na_2CO_3 was added. After 30 sec. the optical density at the wavelength of 725 nm was measured. The number of phenolic compounds was calibrated according to the curve that was constructed according to chlorogenic acid. The calculation was performed by equation:

$$X = aVpm^{-1},$$

where: X – number of phenolic connections of mg per 100 of crude weight; a – content of chlorogenic acid according to the calibration curve, mg cm^{-3}; V – volume of alcohol extract, cm^3; p – extent of dilutions; m – mass of a hinge plate, g. (Katserikova, 1999).

Determining the sum of flavonols of the initial alcohol extract 2 cm^3 of a 2% solution of $AlCl_3$ and 6 cm^3 of a 5% solution of sodium acetate were added. To a control sample 2 cm^3 of H_2O was added instead of 2 cm^3 of 2% solution of $AlCl_3$. If solutions became turbid, they were filtered. After 2.5 hours after the beginning of the reaction the optical density at a wavelength of 440 nm was measured. The content of the sum of flavonols (mg per 100g in terms of rutin) was found by equation (Ermakov & Arasenovich, 1987):

$$X = K(D - D_1)Vpm^{-1} \times 100,$$

where: K – recalculation coefficient on the calibration curve constructed on a routine (0.655); D – optical density of a test solution; D_1 – optical density of a control solution; V – volume of a spirit extract, cm^3; p – extent of dilutions; m – mass of a hinge plate of plant material, g (Katserikova, 1999).

To find the number of anthocyanins according to the calibration curve needs to define their optical density (Tanchev, 1980) in alcoholic extracts at a wavelength of 529 nm. The quantity of anthocyanins (mg per 100g) is found by the equation:

$$X = KDVm^{-1} \times 100,$$

where: K – coefficient calculated on a calibration curve; D – optical density of solution; V – volume of extract, cm^3; m – mass of a hinge plate, g (Durmishidze et al., 1981).

The content of the tannin-catechin complex was conducted by the titration method with $KMnO_4$ solution recalculating results by equation:

$$X = (a - b)m^{-1} \times 5.82 \times 100 \times 100 \times 10^{-1},$$

where: a and b – quantity of $KMnO_4$ of cm^3 for test and control titration; m – a hinge plate of a dye, g (Bokuchava et al., 1976).

The activity of peroxidase and polyphenol oxidase determination was performed by standard iodine solution titration methods. (Ermakov, 1987) The results of the studies are represented on the example of sweet cherry and black currant fruit. The trees were sprayed with protein hydrolysate water solutions in concentrations of 200, 400, 600 and 800 mg l^{-1}. The black currant bushes were treated by the solutions with the concentrations of 400, 600 and 800 mg l^{-1}. Then both control and test fruit samples were frozen by fluidization method.

Fruit with initial temperature of 19–22 °C were frozen in an industrial quick–freezing machine with directed fluidized layer. Raw material was continuously fed into the machine and subjected to jets of air cooled to temperature of -28–-35 °C. Air coolant speed was 2.5–2.9 m s^{-1}. Quick–freezer design features provided placing fruit uniformly throughout the apparatus. That allowed to freeze each berry from its surface to the centre while moving at constant rate along the apparatus. The processed product was held inside the machine. The product layer was regulated to be 5 to 8 cm by a restraining device. Maximum throughput of an experimental apparatus for quick-frozen berries production was 20 kg h^{-1}.

Statistical data processing was carried out by using the software package of Microsoft Word 2010, STATISTICA Application (Confidence interval was 0.95).

RESULTS AND DISCUSSION

High quality protein ingredients (hydrolysates) used as the stimulator of plant growth and development (the stimulator, growth-promoting agents) consist of finely dispersed powder of white or light-yellow colour with dry solids weigh ratio as much as 95%. Weight fraction of Ca^{2+} on dry product basis is as much as 0.14%. pH of the hydrolysate solution with a mass fraction of hydrolysate of 1% is 5–7, the mass fraction of sodium chloride is 4%. Essential active substances of hydrolysate are amino acids in following quantities (%): glycine – 9.01; proline – 6.56; alanine – 5.17; glutamine acid – 6.03; lysin – 3.36; leucine – 1.57; aspargine acid – 2.06; valine – 1.64; serine – 0.84; histidine –0.84; isoleucine – 0.45; arginine – 0.31; methionine – 0.03 and trace amounts of threonine.

Using the hydrolysate as the stimulator for cropping, the specific combination of amino acids, particularly α- and β-amylases, results in accelerated plant growth acceleration. In north-western region of Russia ripening of treated plants starts 10–14 days earlier compared to the control group. The stimulators of plant growth and development are used in hydrolysed form under carefully selected component concentration specific to a particular plant. If the concentration is less than that of an optimal solution, the lack of sufficient number of units of active ingredients prevents enzyme synthesis. If the concentration is greater than optimal the growth rate of the plants becomes impeded as a result of fermentation inhibition. (Berg & Gundersen, 2003; Burey et al., 2008; Roussos et al., 2009; Taştan et al., 2012; Rahman et al., 2013)

Chlorophyll is the main pigment responsible for leaves colour. Chlorophyll concentration in leaves can be used to judge about the level of photosynthetic activity and degree of plant saturation with its products. Aside from that chlorophyll is also a K provitamin. Due to the fact that black currant leaves can be used as raw material in pharmaceutical and cosmetic industry the research also included determination of content of *a* and *b* chlorophyll and carotenoids. Leaves were gathered from bushes both untreated (control group) and treated with the stimulator. The acquired data is provided in Table 1.

As seen from the data acquired, chlorophyll concentration in treated bushes is always higher during the vegetation period compared to the untreated bushes from the control group. Total chlorophyll content in bushes from the control group is at its peak during harvest time. Increased content of chlorophyll and carotenoids are observed in leaves of treated plants. This can be explained by the fact that amino acids participating in metabolic reactions cause protein synthesis, which result in increase in chlorophyll concentration. Thus, proper plant treatment with the stimulator increases its photosynthetic activity.

Such intensive chlorophyll synthesis during the most active period of the plant growth (fast growth period preceding blossoming) can be explained by high productivity of assimilating organs producing and providing high quantity of assimilates to the plant.

During blossoming and fruiting periods the significant depletion of leaf yellow pigment is observed. However, during the activation of bio production process, chlorophyll amplification and carotenoids biosynthesis occurred. It might be necessary

to supply activated process of organic substance formation of the vegetative parts of the plant with assimilates. Until the end of vegetation there was a trend of total chlorophyll content decreasing, which relates to the process of pigment suppression. The second stage of carotenoids biosynthesis activation is accompanied by the weakening of bio production process. Such dependence between the content of pigments in leaf tissue and growth function of plant development was previously observed. As for the ontogenetic rhythms of green pigment accumulation within leaf vegetation period, the proportion of its different forms is observed. At the time of the most intense chlorophyll biosynthesis ratio of *a*-chlorophyll to *b*-chlorophyll decreased. Obviously, *b*-chlorophyll is synthesized at higher rate during plant blooming preparation and intense growth phases.

Table 1. Measurement of pigment content in treated black currant leaves by stimulator at vegetation period

Month of research, sample	Black currant content*, mg g^{-1}			
	Chlorophyll *a*	Chlorophyll *b*	Sum of chlorophyll	Carotenoids
May, control sample	1.062 ± 0.008	0.134 ± 0.007	1.196 ± 0.015	0.659 ± 0.007
May, stimulated sample	1.261 ± 0.007	0.244 ± 0.005	1.505 ± 0.012	0.767 ± 0.008
June, control sample	1.287 ± 0.006	0.438 ± 0.003	1.725 ± 0.009	0.559 ± 0.003
June, stimulated sample	1.328 ± 0.008	0.707 ± 0.004	2.035 ± 0.012	0.525 ± 0.005
July, control sample	1.509 ± 0.005	0.826 ± 0.008	2.335 ± 0.013	0.409 ± 0.008
July, stimulated sample	1.652 ± 0.007	1.032 ± 0.007	2.684 ± 0.014	0.397 ± 0.004
August, control sample	0.552 ± 0.004	0.269 ± 0.007	0.821 ± 0.011	0.363 ± 0.007
August, stimulated sample	0.582 ± 0.007	0.272 ± 0.005	0.854 ± 0.012	0.379 ± 0.008
September, control sample	0.028 ± 0.006	0.008 ± 0.006	0.036 ± 0.012	0.266 ± 0.004
September, stimulated sample	0.142 ± 0.008	0.017 ± 0.004	0.159 ± 0.012	0.261 ± 0.004

* – Confidence interval ≥ 0.95

Thus, seasonal dynamics of pigment accumulation in black currant leaves shows a trend specific to particular plant species. The range of chlorophyll and carotenoid oscillations and rate of their accumulation was narrower in depleted agrochemical background conditions (control group).

Dry powder with solubility of 98% was obtained from black currant leaves juice. The powder contained the following amounts of toxic elements (mg kg^{-1}): Pb (5.2), Cd (0.31), Cu (80.0), Zn (120.0), Hg (0.03), As (< 0.1); trace amounts of pesticides were (mg kg^{-1}): aldrin (not detected), heptachlor (not detected), hexachlorane (sum of isomers) (< 0.001), DDT and its metabolites (< 0.002).

The stone fruit and berry crops (plum, sweet cherry) treatment by protein hydrolysate accelerated the beginning of fructification. The 10–14 day increase in the rate of plant development made it possible to form a crop, 15–20% bigger in comparison with control group.

The stone fruit cultures treatment is performed by a stimulator water solution at a concentration of 600 mg l^{-1} (stationary area) If concentrations are below optimal level (less than 600 mg l^{-1}) the activity of amylases is insufficient to obtain a higher yield of fruit (limiting area). When using concentrations exceeding the optimal level (more than 600 mg l^{-1}), the activity of amylase decreases slowing the rate of organogenesis stages (inhibitory area). Plant organogenesis is responsible for accelerating development, beginning of fruiting and achieving maximum productivity.

Experimentally established relationship between the concentrations of the stimulator (a protein hydrolysate), the content of polyphenol compounds (in particular anthocyanins responsible for staining stone fruit and berry crops, possessing P-vitamin activity and antioxidant properties) and the activity of oxidative enzymes-polyphenol oxidase (PFO) and peroxidase (PO). The corresponding relationships are shown in Figs 5 and 6.

At protein hydrolysate concentrations, less than 600 mg l^{-1}, the total number of amino acids supplied to plant cells with a stimulator and nitrogen fertilizers is insufficient for intensive activity and proteolytic and oxidative enzymes. Therefore, when using the stimulator concentrations below 600 mg l^{-1}, it is impossible to achieve neither an increase in the content of polyphenol compounds, in particular anthocyanins, nor an increase in yield.

At stimulator concentrations bigger than 600 mg l^{-1}, the plant has a significant number of amino acids for the synthesis of enzymes. As a result of high activity of proteolytic enzymes (α- and β-amylases), there is an active formation and outflow of nutrients to fruit, which lead to 15–20% increase in yield by. However, high activity of oxidizing PFO and PO enzymes causes a decrease in the content of polyphenolic compounds, including anthocyanins preventing the production of fruit with specified properties.

At optimal concentration of protein hydrolysate 600 mg l^{-1}, a stone fruit had the maximum content of anthocyanins.

Thus, treating stone fruit at optimal concentration, the application of a protein hydrolysate promotes the production of a high fruit yield with more intense colouring substances of fruit caused by the increased content of polyphenolic compounds, in particular anthocyanins.

An advantage of plants is their ability to effectively use amino acids, supplied by the stimulator in strictly defined development phases, established according to the stages of organogenesis. Hence, the treatment of stone fruit crops is performed during the blossoming phase. The results of the study allowed to develop a technique for the use of the stimulator for berry and stone fruit crops.

The main way of processing stone fruit crops is spraying by injectors. The flow rate of the working fluid depends on the quality of the spray (uniform and complete wetting of a surface of plants are crucial), weather conditions (the possibility of flushing the stimulator from the plants in case of rain should be considered), and the vegetative phase. Plants should be sprayed either in the morning or in the evening hours, so that the stimulator gradually penetrated into the plant with the most efficiency. The working solution is prepared immediately before the use and cannot be stored. This invention advantage is in the usage of the stimulator for fruit growth and development. The protein stimulator consists of polyphenolic connections, in particular anthocyanins having P-vitamin activity and antioxidant properties, to accelerate the beginning of fruiting by 10–14 days.

Moreover, double stimulation with a protein hydrolysate of 400 mg l^{-1} is possible and positively affects the accumulation of biologically active agents of berry raw materials. Further research allowed to determine the optimum concentrations of the stimulator in a single treatment of plants. For sweet cherry, cherry, plums trees; gooseberries, black and red currant bushes water solution of stimulator with a concentration of 600 mg l^{-1} is recommended.

In this work we have studied the effect of the stimulator concentration on the quality of grown berries and fruit in a single treatment of bushes and trees, for example, on the content of substances with P-vitamin activity. Fruit trees and bushes were processed in the flowering phase with the solutions of the stimulator in various concentrations.

All mentioned grades are zoned and recommended for the cultivation in the North-West region of Russia.

As the harvest of fruit and berry raw materials occur within 2–3 summer months in the North-West region of Russia, it is desirable to prolong the possibility of fruit consumption all year round. Therefore, it is necessary to develop a technology for their refrigeration processing and storage. For fruit processing with freezing, raw material collecting is recommended at the stage of semimature. By this time, the process of accumulation of reserve substances and ripening of seeds is basically completed, a fruit acquires a required size, appearance, consistence, colour and taste, so they are suitable for technical processing and transportation. Fruit collected at the stage of full maturity are recommended for fresh consumption as they are not suitable for processing.

It is necessary to analyse the changes in the content of biologically active substances in native products and in their refrigerated storage.

The influence of the stimulator concentration on the content of phenolic compounds of fruit and berry raw materials. The aim of the experiment was to select fruit and berry raw materials, most suitable for the production of confectionery products, sauces, and other products, including stone fruit and berry crops.

Due to the fact that the anthocyanins content in treated fruit and berry raw materials with the stimulator of 1,000 mg l^{-1} concentration is practically equal to the parameter content in the control group, fruit and berries were treated with water solutions of the stimulator of 200, 400, 600 and 800 mg l^{-1} concentrations. The stimulator concentration increasing (1,000 mg l^{-1}) does not stimulating action on plant facilities and deteriorate its chemical composition. Figs 1–6 show the changes in sweet cherry fruit depending on the concentration of the stimulator collected at the stage of semimature.

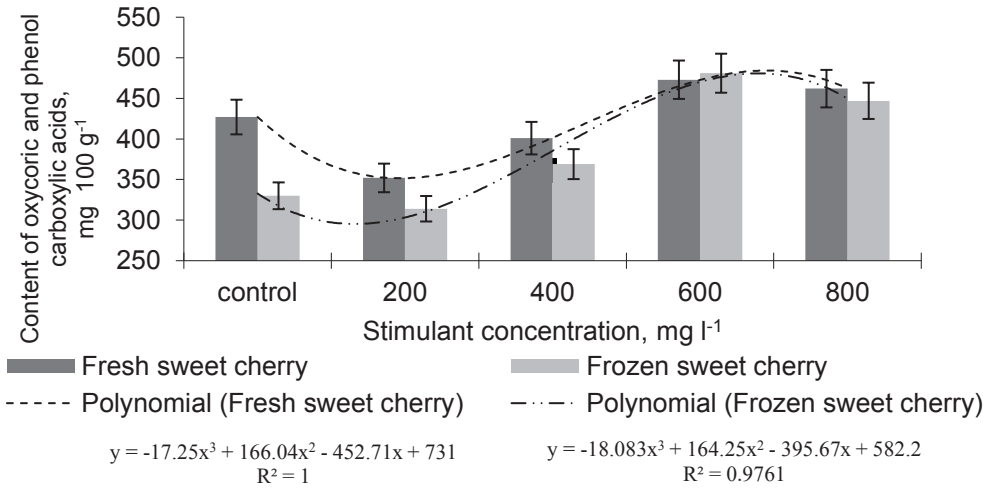

Figure 1. Dependence of the content of oxycoric and phenol carboxylic acids in sweet cherry fruit fresh and frozen on the concentration of protein hydrolysate.

As seen from the dependencies represented in Figs 1–6, the content of flavonols and anthocyanins in all experimental samples exceeds the values of the investigated parameters with respect to the control ones. In fruit, treated with a concentration of 200 mg l^{-1}, the highest content of flavonols was found (Fig. 2) with respect to other experimental samples, maximum polyphenol oxidase activity (Fig. 5) and peroxidase (Fig. 6) was observed. The low content of oxycoric and phenol carboxylic acids can be explained only by the fact that most of them are spent on the synthesis of flavonols and tannic-catechol complex (Fig. 3). This results in the incomplete maturation of the fetus.

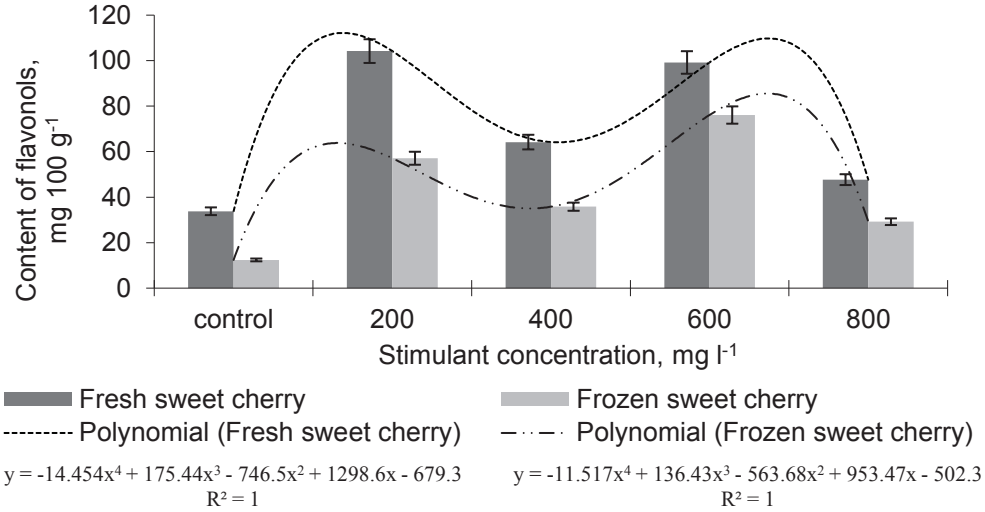

Figure 2. Dependence of the content of flavonols in sweet cherry fresh and frozen on the concentration of protein hydrolysate.

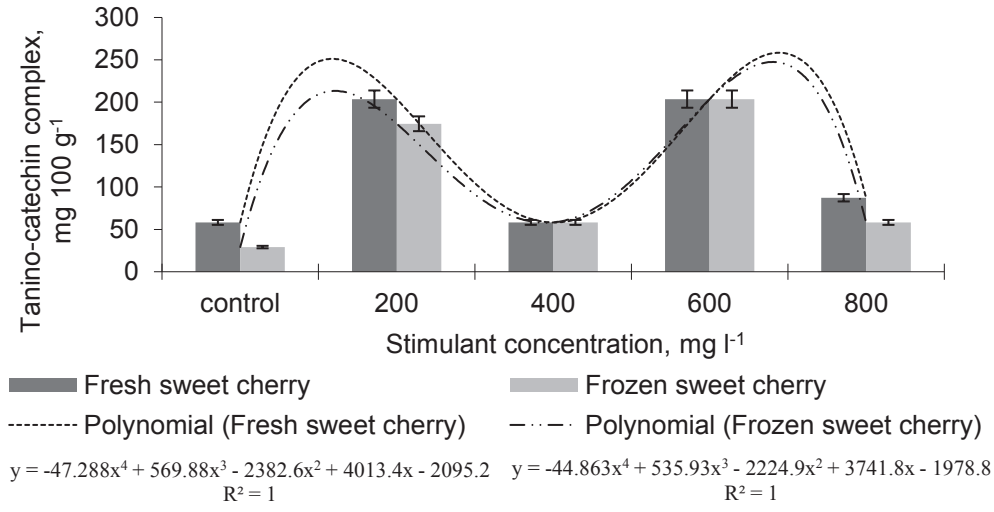

Figure 3. Dependence of the content of the tannic-catechol complex in sweet cherry fruit fresh and frozen from the concentration of protein hydrolysate.

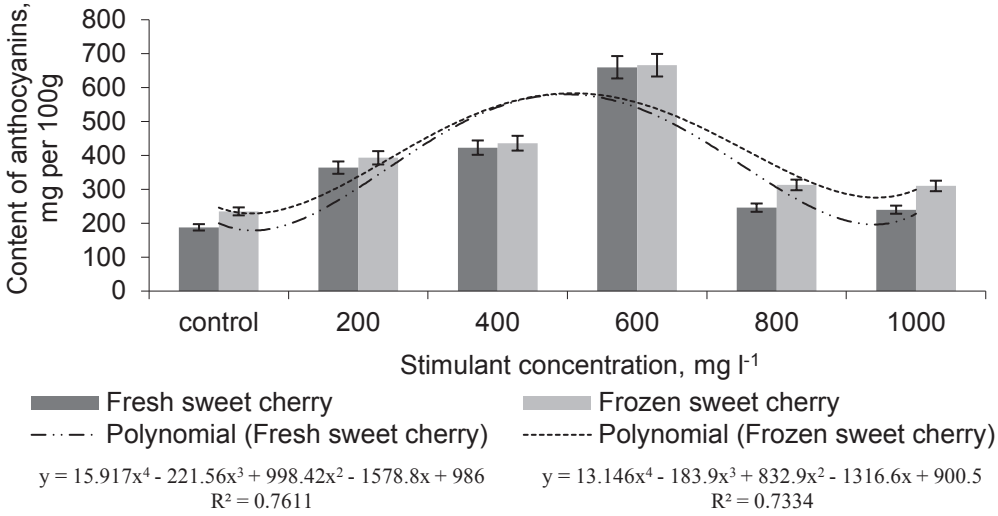

Figure 4. Dependence of the content of anthocyanins in sweet cherry fruit fresh and frozen on the concentration of protein hydrolysate.

The unfinished maturation process was also observed in fetuses that were treated with the stimulator of the concentration of 400 mg l^{-1}: the synthesis of phenolic compounds was not completed, their content was lower than in other samples. However, the activity of oxidizing enzymes (Figs 5 and 6) is close to zero, the content of the tannic-catechol complex is numerically equal to the content in the control sample and is 58.2 mg 100 g^{-1}.

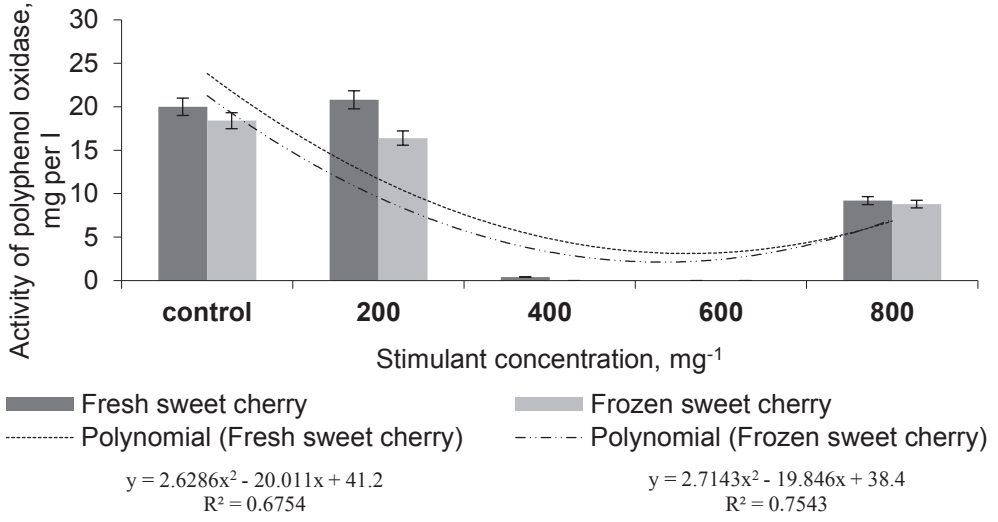

Figure 5. Change in the activity of polyphenol oxidase in fruit of sweet cherry fresh and frozen from the concentration of protein hydrolysate.

The highest content of anthocyanins (660 mg 100 g^{-1}, Fig. 4), oxycoric and phenol carboxylic acids (Fig. 1), as well as tannins (Fig. 3) was in sweet cherry samples treated with a stimulator concentration of 600 mg l^{-1}. At the same time, the activity of polyphenol oxidase (Fig. 5) and peroxidase (Fig. 6) is small. In other words, the concentration of the growth stimulant affects the rate of accumulation of phenolic compounds.

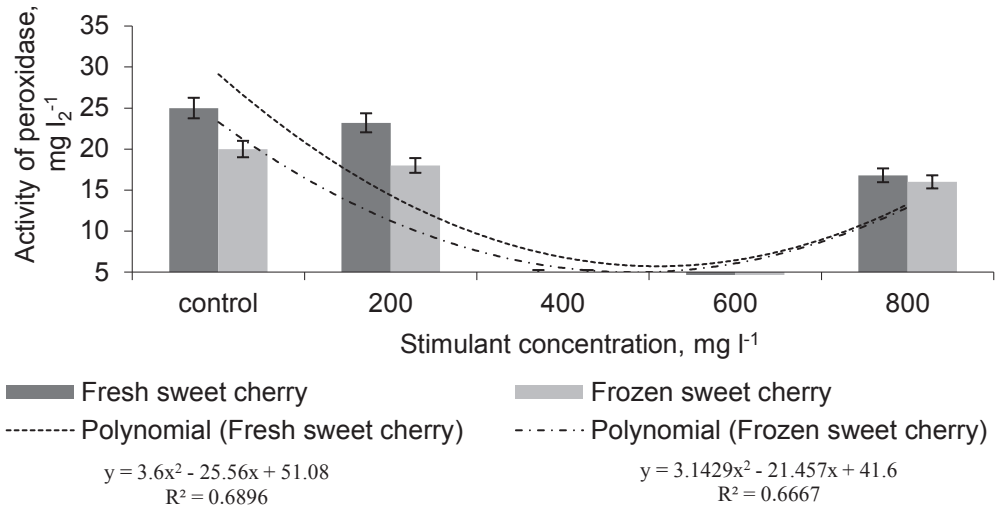

Figure 6. Change in the activity of peroxidase in fresh and frozen sweet cherry fruit from the concentration of protein hydrolysate.

It is possible to assume that the inhibition of enzymes is influenced by tannins; their content at the time of sweet cherry harvesting is sufficient for PFO and PO oppression. With further maturation, the activity of the enzymes increases and leads to the oxidation of various groups of phenolic compounds. Hence, the accumulation of phenolic substances with a lower molecular weight happen. With the concentration of the stimulator to 800 mg l^{-1}, no noticeable increase in phenolic connections is observed, their concentrations are close in value to the control sample.

Biosynthesis of phenolic compounds is conducted on the shikimate and acetate-malonate pathways. The predecessor of ring B is shikimic acid (or L-phenylalanine), and the predecessor of ring A is acetate (malonate). The sources of shikimic acid formation are the products of glycolytic decomposition of sugars and pentose phosphate cycle: phosphoenolpyruvic acid and erythrose-4-phosphate. The initial connection of the acetate-malonate pathway – acetyl-coenzyme A, which is formed either by oxidative decarboxylation of pyruvic acid, or in a thiokinase reaction containing a macroergicthioether bond. There are reasons to suppose that the C_6-C_3 fragment, which was formed by shikimat path, participates in the synthesis of flavonoids in the form of a corresponding ester with coenzyme A, performing the function of a matrix for the configure of activated acetate (malonate) residues. Chalcones formed during these reactions are the precursors of phenolic compounds. As known the hydroxylation of ring B occurs at earlier stages of biosynthesis, i.e. at the stage of cinnamic acids. Moreover,

it should be noted there is no enzyme that is capable to introduce of the third hydroxyl group into the ortho position to the two molecules of flavonoids already present in B rings. In the shikimic acid molecule 3,4,5-trioxygrouping already exists, and probably higher plants have a mechanism that is able to preserve this substitution during the biosynthesis of flavonoid compounds.

The dynamics of phenolic connections accumulation shows that the stimulated berries are active metabolites. The quantitative ratios of oxycoric and phenolic carboxylic acids, flavonols, anthocyanins, and tannic-catechol complex, as well as the activity of PFO and PO, which vary during plant vegetation, are most likely associated with the formation of either more oxidized or reduced forms of phenolic compounds.

Changes in phenol compounds in black currant berries at the stage of semimature intended for industrial processing (quick freezing) gathered on 20th of July (sample no. 1) and berries at the stage of full maturity, gathered on 25th of July (treated with the stimulator) and 2nd of August (the control group) (sample no. 2) are shown in Table 2. It should be noted that samples no. 2 were gathered at different time, because at the moment of harvesting both berries treated and berries untreated did not reach full maturity condition according to organoleptic estimation.

Table 2. Content of phenol compounds in black currant berries

Conc. of stimulator, mg l^{-1}	Polyphenolic compounds, mg 100 g^{-1}					
	Flavonols		Phenol compounds		Anthocyanins	
	Sample no. 1	Sample No. 2	Sample no. 1	Sample no. 2	Sample no. 1	Sample no. 2
Control	171.5 ± 0.3	211.3 ± 0.2	120.0 ± 0.4	120.9 ± 0.2	1.415 ± 0.002	1.860 ± 0.004
400	179.3 ± 0.2	231.1 ± 0.3	126.4 ± 0.2	136.1 ± 0.3	1.333 ± 0.005	2.368 ± 0.002
600	187.9 ± 0.4	258.6 ± 0.4	145.9 ± 0.3	154.6 ± 0.4	1.923 ± 0.003	2.602 ± 0.002
800	173.9 ± 0.3	228.6 ± 0.2	134.4 ± 0.4	146.4 ± 0.3	1.934 ± 0.004	2.573 ± 0.003

The data demonstrates the concentration of chlorogenic acid, quercetin and anthocyanin increase as the berries of black currant grew while being treated with the stimulator with concentrations of 400, 600 and 800 mg l^{-1}.

The greatest content of phenol compounds was found in berries treated with the stimulator with the concentration of 600 mg l^{-1} gathered at both semimature and full maturity stages. Moreover, at the same concentration the lowest enzymatic activity was observed (Table 3). Relative to the control group samples, samples gathered at the stage of semimature showed a higher concentration of oxycinnamic and phenolic carboxylic acids by 21.6%, flavonols by 9.6%, anthocyanins by 35.9%. Samples gathered at the stage of full maturity also demonstrated increased concentrations of these components: the content of oxycinnamic and phenolic carboxylic acids was higher by 27.8%, flavonols by 22.4%, anthocyanins by 39.9%. As seen from the data provided, relatively to the control group samples the accumulation of phenol compounds is much more intense for berries gathered at the stage of full maturity rather than for the ones gathered at the stage of semimature. Treated berries reached the stage of full maturity 10 days earlier than the control samples and berries had a higher concentration of phenol compounds (Bushkov et al., 2016; Rodriguez-Furlán et al., 2016).

Thus, it can be concluded that the processing of storage fruit and berry crops grown with the use of the stimulator with the concentration of 600 mg l^{-1} is advantageous.

The value of black currant berries as a source of P-active substances is usually determined by the high content of anthocyanins, represented by the derivatives of cyanidin and delphinidin: cyanidin-3-rutinoside, delphinidin-3-monoglycoside and delphinidin-3-rutinoside. During the vegetation period, the total content of colouring substances gradually increases, the periods of active growth of fruit and maturation being characterised by the most intensive accumulation. Quantitatively, in the most anthocyanins fruit and berries, colouring substances predominate over other flavonoids, especially flavonols.

Table 3. Changes in the activity of polyphenol oxidase (PPO) and peroxidase (PO) in black currant berries collected in stage of semimature

Enzyme	Concentration of stimulator, mg l^{-1}			
	control	400	600	800
Activity of PPO, mg I_2^{-1}	63.8 ± 0.4	55.6 ± 0.2	20.4 ± 0.2	23.2 ± 0.5
Activity of PO, mg I_2^{-1}	30.5 ± 0.1	16.3 ± 0.3	1.0 ± 0.1	26.5 ± 0.4

The content of anthocyanins in blackcurrant berries collected at the stage of full maturity reaches 2602.9 mg 100 g^{-1}, and at the semimature stage – 1923.4 mg 100g^{-1}. Freezing is recommendable for berries collected at the technical stage. Maturity, since during the subsequent refrigeration a number of enzymatic reactions occur. These processes approximate a product properties to the stage of full maturity.

It should also be noted that a high content of phenolic compounds in all samples of black currant berries may be connected with weather conditions. So high temperatures (up to 30 °C) in the first half of the month had a positive effect on the accumulation of flavonols and phenols, while temperature decrease in the second half of July raise the content of anthocyanins.

Protein hydrolysate treated black currant berries, being fresh, frozen and 9 months of cold stored, had the content of phenols (oxycoric and phenol carboxylic acids) respectively: 145.9, 146.8, 197.2 mg 100g^{-1}; flavonols – 187.9, 188.1, 189.9 mg 100g^{-1}; anthocyans – 1923, 1972, 187 mg 100g^{-1}. In control samples, respectively, phenols – 120.0, 121.2, 193.4 mg 100g^{-1}; flavonols – 171.5, 173.8, 170.2 mg 100g^{-1}; anthocyanins 1415, 1482, 1375 mg 100g^{-1}.

In red currant berries, treated with stimulator in the concentration of 600 mg l^{-1}, had the maximum accumulation of essential substances – flavonols (162.8 mg 100g^{-1}), phenols (75.2 mg 100g^{-1}) and anthocyanins (235.6 mg 100g^{-1}).

In addition, the sugar-acid index in the samples treated with the stimulator is 600 mg l^{-1} higher in terms of the other samples considered, it affects their taste characteristics.

Thus, the processing of fruit and berry raw materials grown using the stimulator at a concentration of 600 mg l^{-1} seems promising and allows obtaining raw materials with a high content of essential substances not only for its direct consumption, but also for the subsequent freezing.

Freezing effect on phenolic compounds of fruit and berry raw materials and on their changes during subsequent refrigerated storage. The quality preservation of fruit and berries for a long time is possible with the creation of modern technologies of rapid freezing and plants that ensure high speed of the freezing process.

To research the freezing effect on fruit and berry raw materials grown with the stimulator treatment, studies have been performed on the biochemical composition of raw materials during the freezing and in the process of refrigerated storage in a frozen state.

As sweet cherry fruit treated with the stimulator have the highest content of oxycoric and phenolic carboxylic acids, flavonols, anthocyanins and tannin catechol complex, they were used to examine the influence of low temperatures on phenolic compounds.

When frozen in relation to the content in the native berry, the content of phenols is increased by 2% (Fig. 7), the content of flavonols reduced by 23% (Fig. 8) and the content of anthocyanins increases by 1% (Fig. 9).

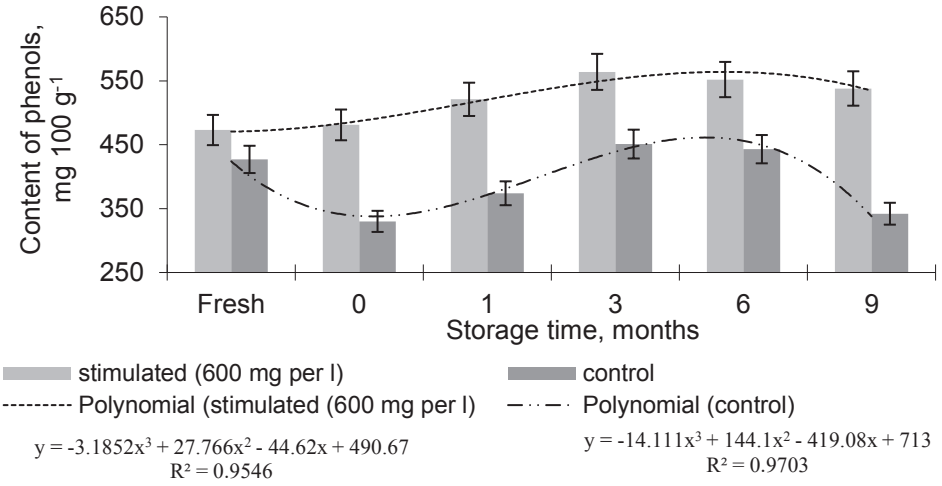

Figure 7. Dependence of the content of oxycoric and phenol carboxylic acids in sweet cherry fruit from the duration of storage in the frozen state.

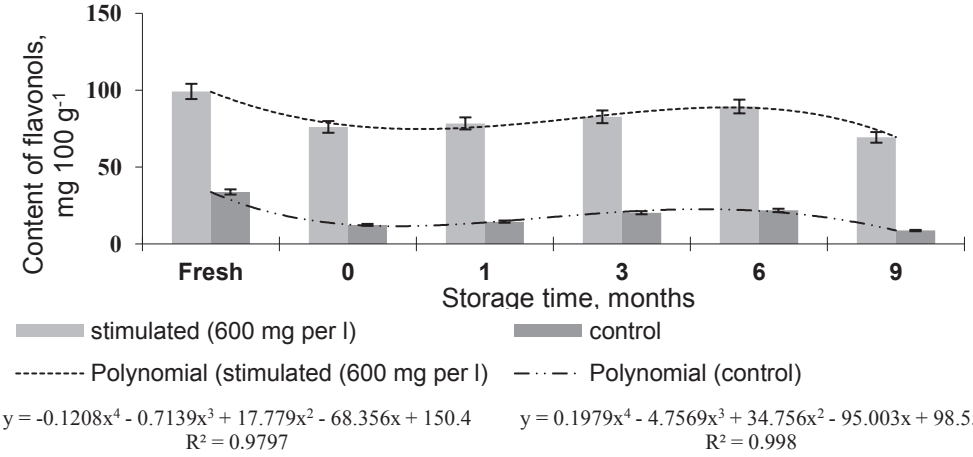

Figure 8. Dependence of the content of flavonols in sweet cherry fruit from the duration of storage in the frozen state.

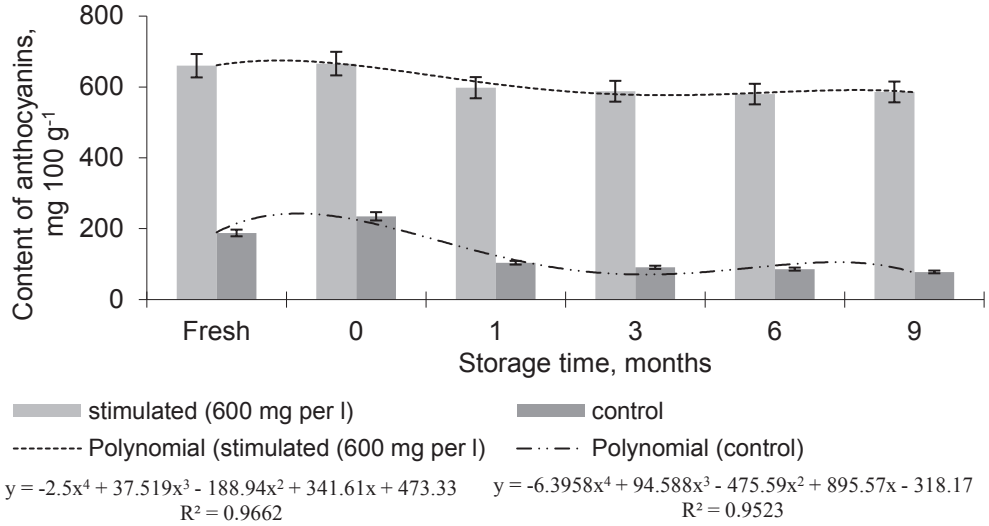

Figure 9. Dependence of the content of anthocyanins in sweet cherry fruit on the duration of storage in the frozen state.

To study the content of oxycinnamic and phenolic carboxylic acids (Fig. 9), a technique to determine these components only in a free, but not in a bound state was applied. Basically, the oxycoric and phenolic carboxylic acids of the fruit are either esterified or glycosidase. In the case of fruit refrigeration, the decomposition of oxycoric and phenol carboxylic acids with the elimination of sugar residues, the destruction of ester bonds causing the appearance of acids in a free form, and the accumulation due to the destruction of flavonoids of complex structures (anthocyanins, flavonols) to simpler compounds occur in fruit.

During the refrigeration the content of oxycoric and phenol carboxylic acids in sweet cherry increased by 12% comparing with the content obtained immediately after the freezing.

In fruit the flavonols (Fig. 8) as glycosides and aglycone forms in a smaller quantity are found. The technique used allows to determine only the aglycone forms of flavonols. During the freezing and refrigeration there is the decomposition of flavonols with the formation of simple precursors (oxycoric and phenolic carboxylic acids), and the conversion the flavonols glycosidic form into aglyconic form occurs. Due to this fact the content of flavonols after 6 months refrigeration was increased, however, the initial fresh fruit content was not reached. The content of flavonols in refrigerated sweet cherry decreased comparing with the content obtained immediately after the freezing by 9%.

As known not all moisture turns into ice in the process of freezing. A small part of it does not freeze providing enzymatic activity oxidoreductase and hydrolase primarily.

Probably, air oxygen and enzymatic activity cause the oxidation and decomposition of condensed tannins to simpler precursors – catechins and leucoanthocyanins.

The oxidative enzymes decomposition to simpler components (oxycoric and phenolic carboxylic acids), resulted in decreasing content of authocyanins in the course of refrigeration by 11% comparing with the content obtained immediately after freezing (Fig. 9).

There is a drop in the amount of biologically active substance in the fruit during freezing and further refrigeration. But the usage of the stimulator makes it possible to obtain a higher-quality product for confectionery, sauces, etc.

The biochemical composition of berries and fruit was measured with a fivefold. Confidence interval was 0.95 and Student's *t* test coefficient was 2.7764 (as fivefold measurement). The statistical part of the work was calculated for stimulated black currant leaves and berries with a concentration of 600 ml l^{-1} collected in July (Table 4).

Table 4. Describing statistics for stimulated black currant leaves and berries with a concentration of 600 ml l^{-1} collected in July

	Standard deviations			Statistical significance	
	Arithmetical average	Variance	Standard deviation	Absolute error	Comparative error, %.
Chlorophyll *a*	1.652	$3.32\ 10^{-5}$	0.00576	0.007	0.43
Chlorophyll *b*	1.032	$3.25\ 10^{-5}$	0.00570	0.007	0.69
Carotenoids	0.397	$1.00\ 10^{-5}$	0.00316	0.004	0.99
Flavonols	187.9	$8.50\ 10^{-2}$	0.292	0.362	0.19
Phenol compounds	145.9	$6.50\ 10^{-2}$	0.255	0.317	0.22
Anthocyanins	1.923	$0.65\ 10^{-5}$	0.00255	0.003	0.16
Activity of PPO	20.4	$2.3\ 10^{-2}$	0.152	0.188	0.92
Activity of PO	1.0	10^{-2}	0.1	0.124	12.42

CONCLUSIONS

The improvement of technologies for the complex processing of by-products of collagen-containing raw materials new food products with the specified composition and properties is importance in meat, poultry, fish processing industries, in various sectors of canning (thermal and refrigeration) production, fat and oil production, confectionery production and others.

The semi-product of beef cattle derma collagen tissue is used in pharmacy and hospital line of products as well as in feedstuff. It is used as raw material to produce absorbable wound healing collagen haemostatic sponges, absorbable collagen burn sponges, absorbable collagen haemostatic sponges and finds its use in haemostatic and wound healing preparation and gel wound dressing. In food industry this semi-product is used to produce natural shells for a large variety of sausages. The usage of by-products of animal origin for the development of new technologies producing high-quality protein ingredients for various purposes is also possible. Agriculture in severe weather conditions demands an innovative stimulator of growth and development of plants to provide ample harvests. We have found that the stimulator treatment for sweet cherry trees (and other stone fruit), black (white, red) currant and gooseberry bushes the solution with the concentration of 600 mg l^{-1} is used respectively. The stimulator can also be combined with mineral fertilizers, traditionally used in agriculture. Technologies for the application of hydrolysates – protein growth stimulators and plant development for berry, fruit and green crops, as well as meadow grasses have been developed.

The terms and concentrations of the stimulator have been determined. The stimulation effect on the properties of plant products, including the processes of refrigeration and subsequent cold storage, is established. In the conditions of the North-

West region of Russia, the fruit and berry raw materials treated ripen 10 – 14 days faster. Semimature black currant berries intended for further refrigeration treatment, have a higher content of phenolic compounds comparing with the one registered in the control group: oxicoric and phenol carboxylic acids by 21.6%, flavonols – 9.6%, anthocyanins – 35.9% and other essential substances: vitamin C – 12.4%, monosaccharides – 15.5%, sucrose – 20.0%, sugar index – 60.1%, with an increase in yield by 20–25%, and the timing maturing reduced in 6 – 10 days. It is important in terms of industrial production of berry crops in the North-West region of Russia. The stimulator treatment with the concentrations makes it possible to obtain raw materials with a high content of phenolic compounds. The further usage of such raw materials, that have an increased content of phenolic compounds, can be recommended for the creation various products for functional nutrition.

The use and creation of new technologies of quick-frozen berries is challenging. The products based on sol-gel matrices are also relevant to light, cosmetic, medical and other industries. The work prospects further development of new confection involving a sol-gel matrix. The product promises to be sustainable during cold storage and in high-temperature baking.

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