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DEVELOPMENT OF BLACK MULBERRY, RED GRAPE AND BLACKCURRANT JUICE

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Abstract

Raw materials from black mulberry, red grapes, and blackcurrants, rich in biologically active components and secondary metabolites (polyphenols), were studied to participate in the development of model prototypes of fruit-based juices.
Based on the analyzed fruit varieties and genotypes shown in our previous publications technologies juices were developed.
Model prototypes of monocomponent, bicomponent and multicomponent juices from the listed raw materials, received by cold pressing, were developed.
We used the planned simplex zitroid plan, based on which they prepared juices based on fruits (black mulberry, red grape, and blackcurrant).
Characterization of the developed prototypes was performed based on the conducted biochemical, sensory, and spectral studies on the day of their receiving.
As a result of the obtained mathematical models for the content of total polyphenols, and antioxidant capacity, estimated by determining the radical scavenging ability (DPPH-test), sensory evaluation, and color parameters, the composition of developed test samples of fruit-based juices was optimized and the following composition was recommended: black mulberry from 60 to 75%, red grapes up to 40% and blackcurrants from 30 to 90%.
The products are rich in bioactive substances and polyphenols with the content of total polyphenols ranging from 130 to 360 mg% and anthocyanins from 180 to 750 mg%.
Different content of biologically active substances determines the different antioxidant activity of products whose values are from 4417 to 5242 µmolTE/100g. It was found that the antioxidant ability of the tested samples was mainly due to the antioxidants acting as electron donors and the synergistic and antagonistic effects of other components in the system as the physical interaction between the phenolic compounds.

Keywords: total polyphenols, color, antioxidant activity, sensory evaluation

1. Introduction

Nowadays, fresh vitamin foods created with or without minimal heat treatment are increasingly in demand. They can keep our bodies toned throughout the day, as they do not lose their valuable vitamin and mineral composition due to prolonged preparation at high temperatures (Ivanov et al., 2004).

Leading positions in modern healthy eating are occupied by fruits as one of the largest suppliers of micronutrients to the human body (Baikova et al., 2004; Yurukov et al., 2004). A trend in modern nutrition is that in the next 5 to 8 years the market will focus more and more on human health, including healthy foods as one of the factors for maintaining good physical and mental condition. (Araújo et al., 2019).

There are data in the literature which indicate that the consumption of fruits with specific chemical composition provides protective mechanisms against cellular damage caused by exposure to high levels of free radicals (Ivanova L.2003). Fruits have various antioxidant compounds (vitamins C and E, anthocyanins, carotenoids, phenolic substances, etc.), for which it is difficult to measure the antioxidant activity of each component (Lugasi et al., 2003; Kalt, W. 2005; Cies Lik, E. 2006; Manach et al., 2004; Muller et al., 2010; Vinson et al., 2001). The values of the antioxidant activity are influenced by the type and variety of the fruit, chemical changes that occurred during ripening, minimal processing before cold storage and thermal processing, storage time and temperature, time and temperature of food processing, additional materials, and others. (Kalkan Y. 2006; Tiwari et al., 2009).

Aim

This work aims to study the possibilities for creating and developing new fruit-based juices with short shelf life by cold pressing, helping to stimulate a healthy diet under national recommendations for reducing the risk of nutritional deficiencies and chronic diseases associated with nutrition.

2. Material and methods

2.1. Raw materials: There were many studies of the raw materials - natural sources of biologically active substances, which directly or as extracts in various combinations allowed the creation of formulas that after well-selected and applied technology were a prerequisite for obtaining functional foods with beneficial effects on human health.

Research and selection of raw materials for the development of new products based on the botanical characteristics of raw materials, nutritional value, the availability of the internal market, and their purpose for industrial processing were done.

Black mulberry, red grapes, and blackcurrants were studied from the group of berries.

- *Mulberry* is extremely rich in vitamins, with the highest content of vitamins A, C, and B2. They are a source of sodium, phosphorus, calcium, magnesium, and potassium. The content of pectin is also high. Mulberry works well on the nervous and cardiovascular systems and is a remedy for anemia, kidney disease, fatigue, and premature greying of hair. It is used to treat urinary incontinence, tinnitus, dizziness, and constipation in the elderly. The content of resveratrol in fruits provides health benefits in reducing the risk of lung cancer, preventing colon and prostate cancer, and slowing aging. (Ercisli et al., 2008). Developments by Temple, N. 2000 in conducting tests for antioxidant activity prove that mulberry fruit contains up to 79 percent more antioxidants than other fruits in this group. Suh et al., 2003 have identified compounds in black mulberries - cyanidin 3-glucoside and cyanidin 3-rutinoside. These anthocyanins are labile compounds and easily susceptible to degradation of factors such as light, pH, and temperature during storage and especially to heat treatment (Cam et al., 2009). Phenolic and other phytochemical antioxidants found in mulberry fruits are bioactive compounds capable of neutralizing free radicals and may play an important role in the prevention of some diseases (Özgen et al., 2009). Mulberry juice improves health, enhances immunity, enriches the blood, tones the liver and kidneys, calms the nerves, and stimulates metabolism. Without the addition of preservatives, mulberry juice remains freshly chilled for 3 months, and in the form of a bottled drink remains fresh at room temperature for 12 months. Chemical composition per 100 g: calories – 42.00 kcal; carbohydrates - 9.80 g; protein - 1.44 g; fat - 0.39 g; water - 87.68 g; dietary fibre - 1.70 g. From the vitamin and mineral composition vitamin A – 1.00 μg; vitamin C -36.40 mg; vitamin E - 0.87 mg; vitamin K - 7.80 μg; vitamin B1 - 0.029 μg; vitamin B2 - 0.101 mg; vitamin B3 - 0.620 mg; vitamin B6 - 0.050 mg; Ca – 39.00 mg; Fe - 1.85 mg; Mg – 18.00 mg; P – 38.00 mg; K – 194.00 mg; Zn - 0.12 mg.
- *Grape*- in its raw state is a very good source of antioxidants. Nutritionists say it helps to reduce the risk of coronary heart disease and cancer. In the form of juice, it cleans the liver and leads to the excretion of more uric acid from the body. Grapes are rich in organic acids such as malic, tartaric,

citric, phosphoric, etc., which stimulate metabolism. The vitamins it contains are the reason it strengthens the nervous system, and the walls of blood vessels, bones, and nails and improves vision. It also has a large amount of potassium, which strengthens the heart muscle and helps remove excess fluids from the body. It is useful to consume grapes for high blood pressure, heart attacks, atherosclerosis, arthritis, gout, kidney stones, or gallbladder. The magnesium, calcium, phosphorus, and iron contained in grapes help in the treatment of rickets and prevent anemia. Grape juice is very useful because besides many sugars and minerals it contains phosphates, proteins, and vitamins P and C. It is recommended to drink half a liter of grape juice three times a day because the organic acids in it have a beneficial effect on appetite and endocrine and external glands. It quenches thirst, improves intestinal peristalsis, and supports the excretory function of the kidneys. Chemical composition per 100 g: calories – 69.00 kcal; carbohydrates - 18.10 g; protein - 0.72 g; fat - 0.16 g; water - 80.54 g; dietary fiber - 0.9 g. Vitamins and mineral composition per 100 g: vitamin A - 3.00 μg; vitamin C - 3.20 mg; vitamin E - 0.19 mg; vitamin K - 14.6 μg; vitamin B1 - 0.069 μg; vitamin B2 - 0.070 mg; vitamin B3 - 0.188 mg; vitamin B6 - 0.086 mg; Ca – 10.00 mg; Fe - 0.36 mg; Mg – 7.00mg; P – 20.00 mg; K – 191.00 mg; Zn - 0.07 mg.

- *Blackcurrant* (black French grape) is a fruit of high biological value. In addition to the high content of sugars, organic acids, dyes, and pectin, essential oils and et all. It is one of the richest in vitamin C and P fruit. The sugar content is from 7.9 to 15.40%. Sugars are mainly fructose and glucose with a slight predominance of fructose. Acids range from 2.5 to 4.2%, with citric and malic acids predominating. Blackcurrant fruits contain 0.85-0.93% pectin. The amount of cellulose is about 4%. Blackcurrant contains anthocyanins, which are natural compounds in the fruit. They are very powerful antioxidants and are the reason for the characteristic color of blackcurrant. Anthocyanins inhibit the enzymes cyclooxygenase 1 and 2 and reduce inflammation as well as the effects of arthritis in the body. Good results have been obtained for the treatment of gastrointestinal, renal, hepatic, and cardiovascular diseases. The average content of tannins and dyes is from 0.33 to 0.93% (Rashid et al., 2018). Blackcurrant is consumed in limited fresh condition, but it is processed into many varied products- high-quality juices, jams, jellies, and liqueurs are obtained from its fruits. The interest in blackcurrants is not very high due to the complexity of harvesting. Blackcurrant fruit juice contains proanthocyanidins, anthocyanins, and polysaccharides and has a stimulating effect. It has been found to have toxic effects against tumor cells and thus reduce the risk of cancer. Recommended for beriberi, anemia, gastritis with low acidity, acute bronchitis, influenza, and angina. It helps destroy viruses, boosts immunity, has a general strengthening effect, and is rich in vitamin C.Chemical composition per 100 g: calories – 63.00 kcal; carbohydrates 15.40%, protein – 1.40 g; fat - 0.41 g; water - 81.96 g. Vitamins and mineral composition per 100 g : -vitamin A - 2.30 μg; vitamin C – 219.00 mg; vitamin E – 1.00 mg; vitamin B1 - 0.04 mg; vitamin B2 - 0.02 mg; vitamin B3 - 0.30 mg; vitamin B6 - 0.07 mg; Ca – 8.00 mg; Fe - 0.2 mg; Mg – 8.00mg; P – 50.00 mg; K – 253.00 mg. The aim of the analysis of the selected raw materials is that when developing new assortments of juice to have high functional characteristics.

2.2. Methods:

2.2.1. Physicochemical and biochemical parameters were determined by the following methods:

- The content of total polyphenols in fruits and juices obtained from them was determined by the method of Singleton and Rossi, (1965) in the following modification: In a measuring tube of 10 ml dose 0.1 ml of sample extract (fruit and/or juice), ~ 7 ml of distilled water, 0.5 ml of Folin - Ciocalteu - reagent (diluted 1: 4 with distilled water) and 1.5 ml of 7.5% (w / v) aqueous sodium carbonate solution. Makeup to the mark with distilled water. After 2 hours at 20 - 25°C, the absorbance of the reaction mixture was measured at 750 nm. Similarly, a blank sample was prepared using distilled water instead of extract. The results obtained are presented as gallic acid equivalents (GAE) per 100 g of extract.

- Determination of antioxidant activity (DPPH-test). The antioxidant activity is determined using the method of Brand – Williams et al., (1995) in the following modification: In a cuvette dosed sequentially 2250 µL solution of DPPH (2.4 mg DPPH in 100 mL of methanol) and 250 µL extract previously diluted with distilled water in a volumetric relation 1:3. An analogous blank sample is prepared using methanol instead of extract. After standing in the dark for 15 min at a temperature of 20-25 °C the absorbance of the reaction mixture is measured at 515 nm. The results are expressed as equivalents of Trolox (TE) per 100 g of extract. All measurements were performed on UV-Vis spectrophotometer model Helios Omega, completed with software VISIONlite (all elements are of Thermo Fisher Scientific, Madison, WI, USA), using cuvettes with an optical path of 1 cm.
- Determination of metal reduction ability (FRAP- test). The procedure was based on the method of Benzie and Strain, (1996) applied with some modifications. FRAP reagent was prepared after mixing 2.5 ml of the solution of TPTZ (10 mmol/L) in hydrochloric acid (40 mmol/L), 2.5 ml of aqueous FeCl3 (20 mmol/L), and 25 ml of acetate buffer (0.3 mol/L), pH 3.6). For the reaction, 2250 µl of FRAP reagents were mixed with 250 µl of extract (diluted with distilled water in a ratio of 1:3, v/v); the absorbance at 593 nm was measured after 4 min of the reaction mixture in a closed cuvette at room temperature in the dark.

2.2.2. Spectral analyses:

- Gardner color determination - with colorimeter „Colorguard 05/CIELab 2000", by BYK-Gardner Inc. USA. The indicators were reported by the system CIE Lab. During the measurement 3 color coordinates were taken: L, a и b;
  - L– color brightness (L=0 – black, L=100 – white)
  - a– positive values of the indicator characterized the amount of red color, and negative - of the green color;
  - b– positive values are characterized by the yellow color and negative values - are the blue color.

Five measurements on each sample were performed. The color coordinates of each sample represented the arithmetic mean of the measured coordinates.

2.2.3. Sensory analysis: Organoleptic evaluation of the obtained fruit-based juices was performed using a scoring system for qualification. The drinks were given to testers to make an organoleptic assessment and they filled in a testing card. Each indicator had a weighting factor respectively:

- appearance – 0,2;
- colour – 0,2;
- consistency – 0,15;
- taste – 0,3;
- smell– 0,15.

A scoring table from 1 to 5 was used (with a step of 0.25), which corresponded to the quality of the product according to the relevant indicator.

The final assessment of the quality of the finished product, based on the total number of points obtained was made by the five-point grading system.

- 4.50 ÷ 5.00 – the end product is very good;
- 4.00 ÷ 4.49 – the end product is good;

- 3.50 ÷ 3.99 the end product needs improvement;
- less than 3.50 – the ready product needs significant improvement.

2.2.4. Mathematical-statistical processing: The results were presented as average values processed by the ANOVA program, with a 95% confidence interval and Exel 2013.

2.3. Approach: The approach is theoretical and experimental and covers the following stages: analysis, experimentation, modeling, and optimization of the recipe composition of beverages.

Plan of the experiment

A simplex centroid plan, suitable for mixtures in which the sum of the variables (X1, X2, X3) is equal to 100%, was used for the preparation of the juices. Simplex lattice plans with seven experiments were used to derive an adequate mathematical model of the studied indicators. Simplex lattices were applicable if the condition $0 < X_i < 1$ was fulfilled for all components (i - serial number of the component). The general form of the equation by which the properties of mixtures can be described as:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

where ßi, ßij и ßijk were the coefficients of the equation and Xi were the components of the mixture.
Table 1 shows the matrix for conducting the experimental work.

Table 1. Matrix for conducting the experiment

№	X1	X2	X3
1	100	0	0
2	0	100	0
3	0	0	100
4	50	50	0
5	0	50	50
6	50	0	50
7	33	33	33

The experimental experiments of the developed samples of juices were conducted in the Department of „Food Technologies“ at the Institute of Food Preservation and Quality – Plovdiv according to the technological scheme presented in Figure 1.
The development of the experimental samples of juices and the study of the physicochemical, biochemical, and sensory qualities were carried out in the laboratories of the IFPQ-Plovdiv.

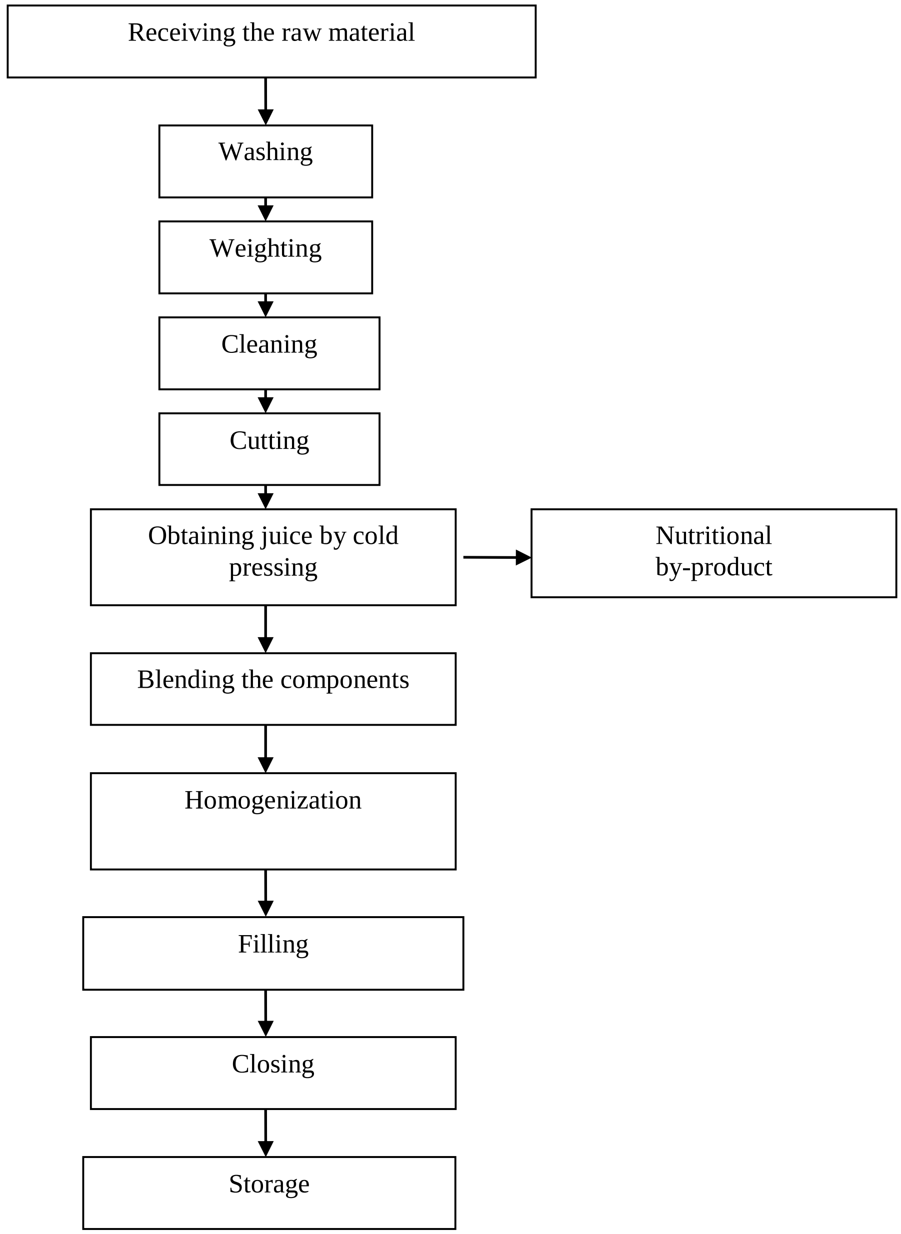

Fig1. Experimental staging

3. Results and discussions

Table 2 are given the final sensory valuation (SV), the content of total polyphenols (TPP), and the radical scavenging ability (DPPH) of the preparation for the juices components composed of the black mulberry, red grape, and black currant.

Table 2. Sensory evaluation, TPP, and DPPH of component compositions of the black mulberry, red grape, and blackcurrant juices

№	Sensory valuation (SV)	TPP (mgGAE/100g)	DPPH (μmolTE/100g.)
1.	4,15	490,00	5275,00
2.	4,55	330,00	3700,00
3.	4,49	240,00	5260,00
4.	4,21	230,00	5242,00
5.	4,35	130,00	4417,00
6.	3,92	180,00	5200,00
7.	4,84	360,00	4250,00

After processing the experimental results, the mathematical models for the sensory valuation, the content of total polyphenols, and radical scavenging capacity of the component compositions for the juice of black mulberry, red grape, and blackcurrant were obtained, as follows:

$$SV = 4{,}15.X1 + 4{,}55.X2 + 4{,}49.X3 - 0{,}56.X1X2 + 0{,}12.X1X3 - 2{,}4.X2X3 + 20{,}49.X1X2X3 \qquad (2)$$

$$TPP = 490.X1 + 330.X2 + 240.X3 - 720.X1X2 - 940.X1X3 - 420.X2X3 + 6420.X1X2X3 \qquad (3)$$

$$DPPH = 5275.X1 + 3700.X2 + 5260.X3 + 3018.X1X2 - 3402.X1X3 + 2880.X2X3 - 20852{,}9.X1X2X3 \qquad (4)$$

The obtained equations described with high accuracy the change of the concentration of the dependent variables at confidence level $P < 0.05$, as $R > 0.9$.

The results of the sensory evaluation of the studied products are graphically presented in Figure 2.

Figure 3 shows the reflection surface of the overall sensory evaluation of the juice component compositions.

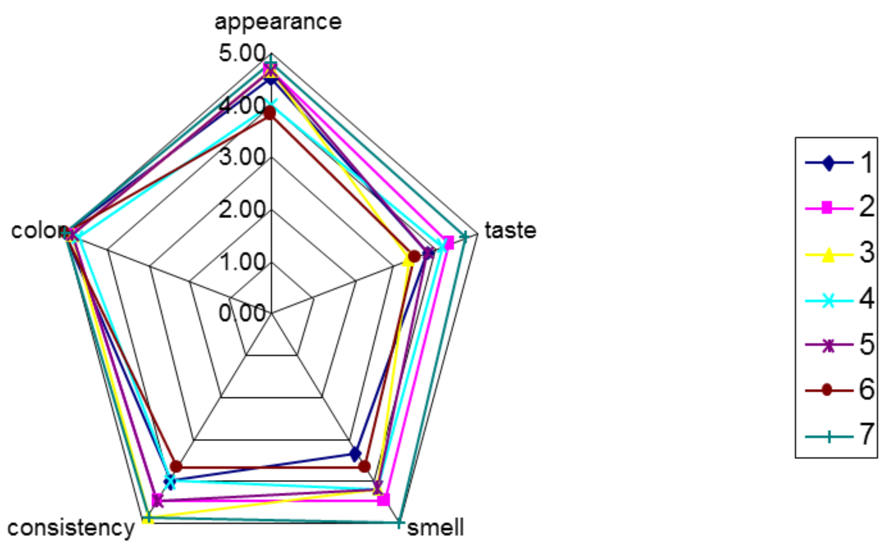

Fig2. Sensory profile of the component formulations of the black mulberry, red grape, and blackcurrant juice.

From the conducted sensory analysis it was found that the juice is best accepted by the tasters in equal amounts of the three selected components: black mulberry, red grape, and black currant- 4.84. The lowest score is the juice from the combination of black mulberry and blackcurrant -3.92.

Figures 3, 4, and 5 present the reflection surfaces for sensory evaluation, total polyphenol content, and radical scavenging capacity of the components of the black mulberry, red grape, and black current juice.

The tested samples of mono-component beverages have high content of total polyphenols in black mulberry drinks 490.00 mgGAE/100g, followed by red grapes 330.00 mgGAE/100g. For all tested samples the lowest quantity content of polyphenols is for a juice of red grapes and blackcurrants 130.00 mgGAE / 100g e.

It was found that the mixing of the component compositions reduces the number of total polyphenols (except for a juice of equal parts of the black mulberry, red grapes, and blackcurrant) The data are statistically distinguishable by differences due to the varied component composition $P<0,05$ (Fig.4).

The radical scavenging ability of the developed model juice in all test samples has high values in comparison with the juice products from 1 to 4. In the first stage of the working program in the study of mulberry varieties, the high values of the radical scavenging ability of 5275.00 µmolTE/100g gave prerequisites for the choice of this component in the development of juices. It was found that with the combination of the chosen component composition an increase in the radical scavenging capacity is achieved only compared to the mono-component red grape drink 3700.00 µmolTE/100g (Fig. 5).

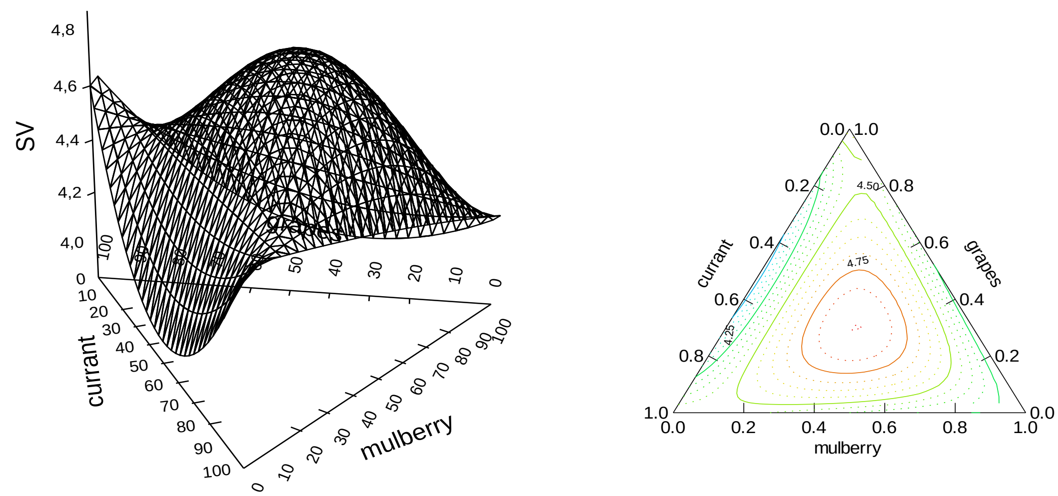

Fig3. Surface reflection of the overall sensory evaluation of the component formulations of the black mulberry, red grape, and blackcurrant juice.

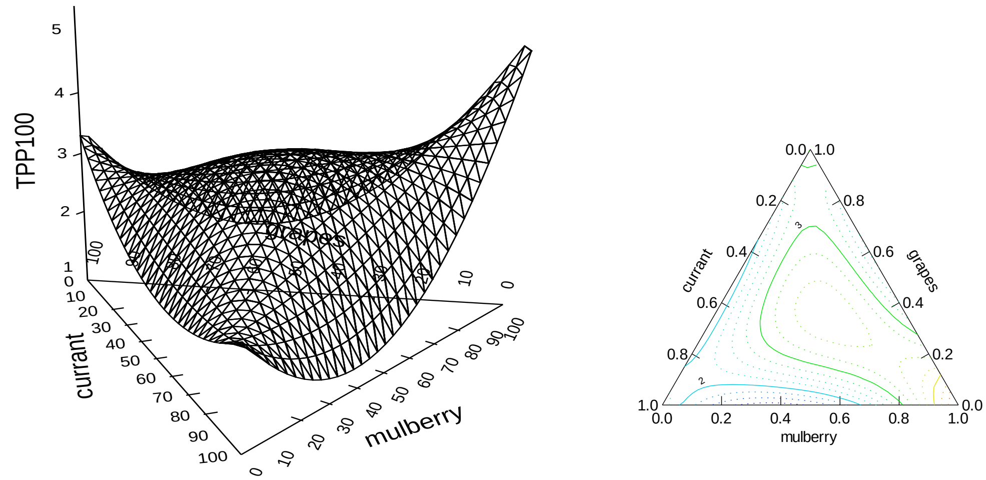

Fig4. Surface reflection of TPP (* 100) of the component formulations of the black mulberry, red grape, and blackcurrant juice.

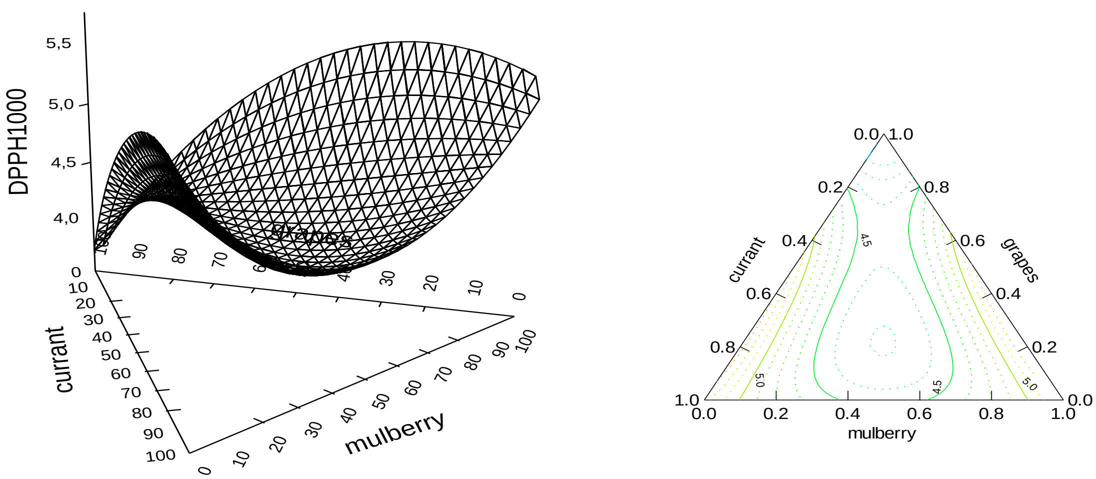

Fig5. Surface reflection of DPPH (* 1000) of the component formulations of the black mulberry, red grape, and blackcurrant juice.

The antioxidant ability of the tested samples was mainly due to the antioxidants acting as electron donors and the synergistic and antagonistic effects of other components in the system. as the physical interaction between the phenolic compounds (Fig 6)

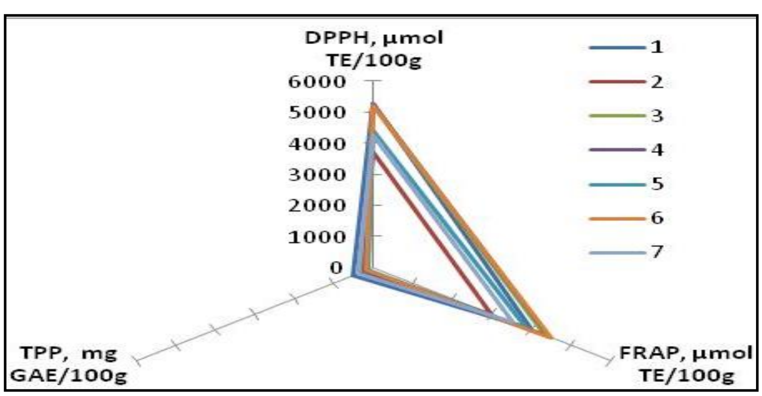

Fig6. Radar chart of the black mulberry, red grape, and blackcurrant juice.

In Table 3 are given the average values of the measured color coordinates – L, a, b.

Table 3. Color coordinates of the black mulberry, red grape, and blackcurrant juice

№	L	a	b
1.	0	2,14	-0,79
2.	22,78	33,09	0,91
3.	-0,44	4,04	-0,09
4.	4,77	18,97	-0,52
5.	12,46	33,27	3,23
6.	-0,25	4,95	-0,09
7.	4,18	21,59	1,49

After processing the results, the following mathematical models for color coordinates were obtained L, a, and b:

$$L = 0{,}0.X1 + 22{,}78.X2 - 0{,}44.X3 - 26{,}48.X1X2 + 50{,}72.X1X3 - 45{,}68.X2X3 - 23{,}88.X1X2X3 \quad (5)$$

$$a = 2{,}14.X1 + 33{,}09.X2 + 4{,}04.X3 + 5{,}42.X1X2 + 120{,}72.X1X3 - 54{,}46.X2X3 + 14{,}46.X1X2X3 \quad (6)$$

$$b = - 0{,}79.X1 + 0{,}91.X2 - 0{,}09.X3 - 2{,}32.X1X2 + 14{,}68.X1X3 - 2{,}0.X2X3 + 8{,}88.X1X2X3 \quad (7)$$

The obtained equations describe the change of color coordinates with good accuracy.
In Figures 7, 8, and 9 the surfaces of reflection of the color coordinates L, a и b are presented.

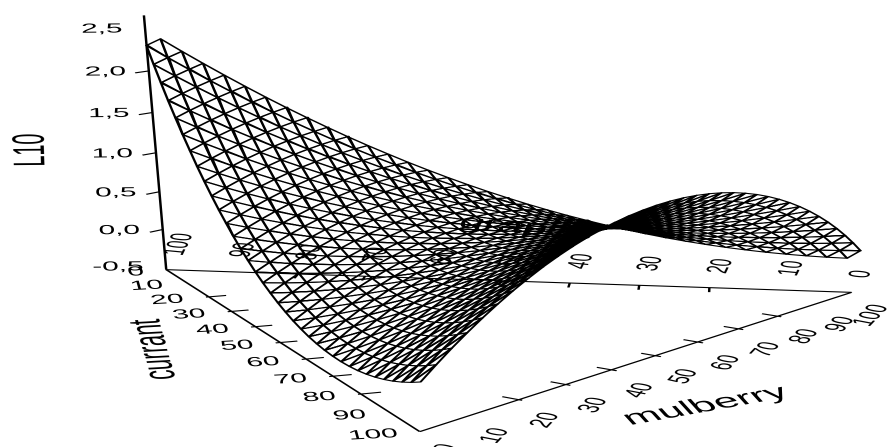

Fig7. Surface reflection of „L" (*10) of the component composition of the black mulberry, red grape, and blackcurrant juice.

The color characteristics of the developed prototypes of the black mulberry, red grapes, and blackcurrant juice show that the brightness was highest in the drink of red grapes 22.78, in the other two monocomponent drinks it was absent. The data were statistically distinguishable; the difference was due to the different component compositions. In the combinations of the selected components, the brightness dominates in the juice of red grapes and blackcurrants at 12.46 and increases only compared to the monocomponent juice of black mulberry and blackcurrant. (Fig.7)

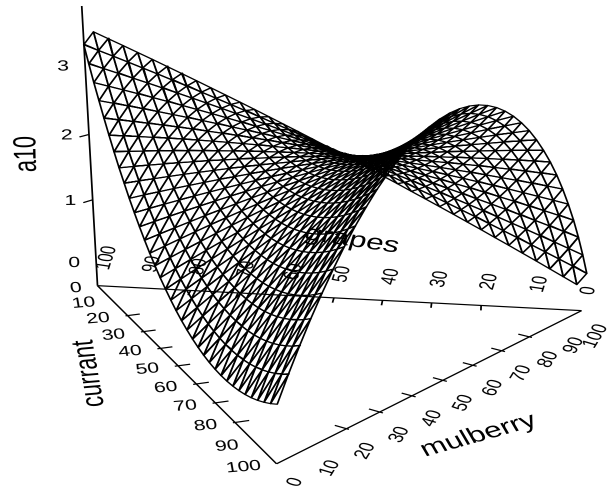

Fig8. Surface reflection of „a" (*10) of the component composition of the black mulberry, red grape, and blackcurrant juice.

The results of the red component of the color show that the highest value was a juice of red grapes -33.09. For all mono-component juice, the lowest value of red color was measured for black mulberry juice -2.14. The data were statistically distinguishable, the difference being due to the different component compositions. From the performed measurements, it was found that in the model prototypes of combinations, the red color increased its quantitative value only compared to the monocomponent juice of black mulberry and red grape, and blackcurrant.

Blue color tone was measured in the mono-component juice of black mulberry - 0.79 and blackcurrant -0.09, as well as their combinations of black mulberry with red grapes -0.52 and black mulberry with blackcurrant -0.09.

For all other developed variants of the experimental samples, the yellow color tone was measured in low values, which had the highest quantitative value in the red grape drink with blackcurrant- 3.23. The data are statistically different; the difference was due to the different component compositions. During the conducted experiment plan it was found that when mixing the components an increase in the quantitative value of the yellow component of the color is achieved (Fig. 9).

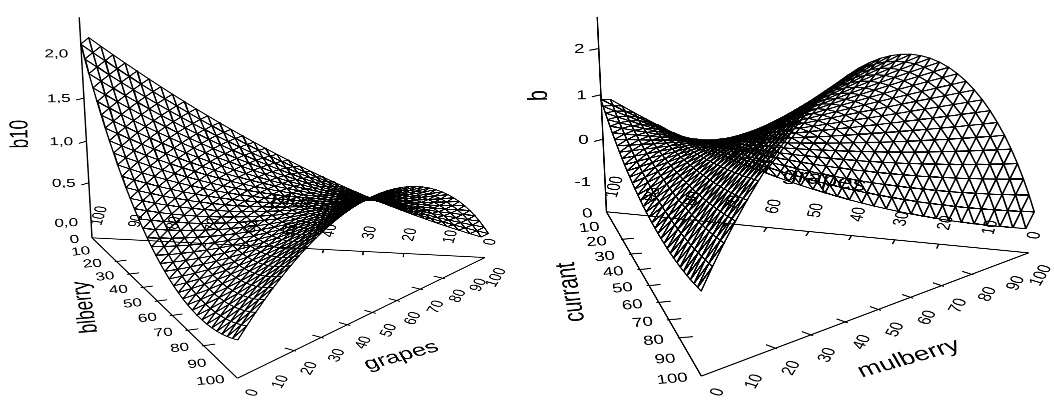

Fig9. Surface reflection of „b" of the component composition of the black mulberry, red grape, and blackcurrant juice

Optimization

To optimize the component compositions of the black mulberry, red grape, and blackcurrant drink, the following are selected as target functions: total sensory evaluation (SV), content of total polyphenols (TPP), and radical scavenging ability (DPPH). The target functions and their limits are given in Table 4.

Table 4. Limits of the target functions for optimizing the composition of the black mulberry, red grape, and blackcurrant juice

Target function	Limits of the target function
Sensory evaluation (SV)	> 4,40
Total polyphenols (TPP)	> 300,00 mgGAE/100g d.b.
Radical scavenging ability (DPPH)	> 4500,00 µmolTE/100g d.b.

The optimal area for the component composition of the juice prepared from black mulberry, red grape, and blackcurrant is locked between points A, B, C, and D (shaded area in Fig. 10) based on which the following composition of the drink can be recommended: black mulberry from 60 to 75%, red grapes up to 40% and blackcurrant from 30 to 90 %.

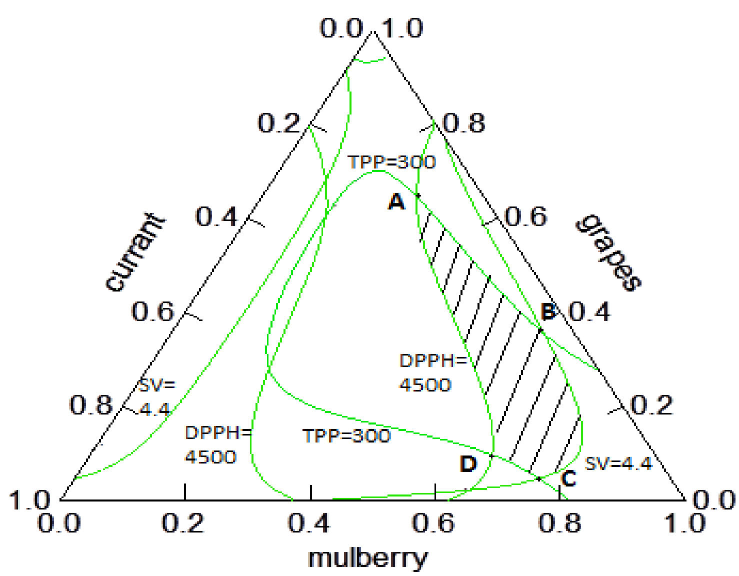

Fig10. Graphic optimization for a component of the black mulberry, red grape, and blackcurrant juice.

4. Conclusions

Raw materials of the black mulberry, red grapes, and blackcurrants rich in biologically active components and secondary metabolites (polyphenols) were studied to participate in the development of model prototypes of fruit-based juice obtained by cold pressing.

Characterization of the created model prototypes was performed based on obtained mathematical models for total polyphenols content, and antioxidant capacity, estimated by determining the radical scavenging ability (DPPH-test), sensory evaluation, and color parameters on the day of their receiving.

As a result of the above indicators, the composition of developed multi-component test samples of fruit-based juice is optimized and the following composition of the juice can be recommended: black mulberry from 60 to 75%, red grapes up to 40%, and blackcurrant from 30 to 90%.

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