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CHANGE OF ANTHOCYANINS CONTENT DURING RASPBERRY EXTRACTION

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Abstract: Change in anthocyanins content under different conditions of extraction, enzymatic maceration and heat treatment of two raspberry cultivars (Villamette and Meeker) was determined**.** Exsperiments were carried out on a laboratory hydraulic extractor. During extraction commercial operating conditions were emulated using a "Bucher" extractor (pressure 180-200 bar, 1-3 pulp shakings). A pectin preparation Klerzyme®120, manufactured by DSM, France, was used for maceration and depectinization, because it is specific for "sour fruits" with pH below 3.2. At a single - stage maceration, it was applied in the amount of 200-400 g/t, for 1-2 hours at 50^{o}C. In a two-stage maceration and depectinization, the enzyme was added as follows: in the first stage 100-200 g/t for 0.5 – 1 hour at 20^{o}C and in the second stage 4-8 g/hl for 1-2 hours at 20^{0}C. Thermal breaks of raspberry pulp were performed at raised temperatures for 2 and 5 min in order to examine the effects of raised temperatures on anthocyanins extraction. The application of heat-enzymatic treatments of the pulp prior to extraction proved to be suitable in both raspberry cultivars. Apart from well-known degrading effects of heat on anthocyanins, the juice obtained by extraction, after enzymatic maceration, has higher anthocyanins content by 30% than the juice obtained without enzymatic maceration. Juice pasteurization, in each stage of processing, produced markedly negative effect on anthocyanins content therefore their content decreases considerably. The forms of anthocyanins that are lost most are those uncolored. In batches subjected to a two -stage enzymatic maceration, despite initial high anthocyanins content, the content of total anthocyanins is reduced after the second pasteurization to the approximate value as that in batches subjected to a single-stage enzymatic maceration.

Key words: anthocyanins, raspberry, extraction, pectin preparation.

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Introduction

Red raspberry (*Rubus idaeus* L.) is extensively grown in Serbia and Montenegro. Recently, due to a considerably increasing demands, the areas under this fruit crop are being expanded and raspberry plantations account for 10,000 h of arable areas. The annual production of raw raspberry fruit amounts to approx. 50,000 tons. Of this quantity, approx. 75% is exported, while 23% is purchased in this country, so that approx. 98% of the total yield is sold, which presents a perfect market value [2,3]. These goals could not have been achieved, had the construction of many new cold storage plants not been in line with increased primary production over the past few years. Thus, it was possible to store, quickly freeze and prepare raw raspberries for export. In Arilje a unique monument to raspberry fruit has been erected, and in Valjevo a business-tourist event called "Raspberry Day" is traditionally held every year sponsored by the company "Srbijanka", Valjevo.

Raspberry ranks 23rd among fruit crops produced in the world. It is mainly grown in the northern hemisphere, Europe being the greatest producer. North America comes next. Raspberry originates from Asia Minor and England was the first to start growing it in 1548. In the present-day Serbia and Montenegro it has been growing for nearly two millennia [2]. Serbia and Montenegro, Poland, Chile, USA, Hungary and Russia are the greatest world producers of raspberry. Serbia and Montenegro are the greatest exporters of raspberry, while developed countries such as Germany, France, Japan, England and Austria are the greatest importers [5]. The volume of raspberry production in the world is still lagging behind the demands. Raspberry is one of our most important berrylike fruits. In its production it falls behind plum, apple, sour cherry and pear fruit crops. Serbia and Montenegro exploit only partially their favorable conditions for producing raspberry: natural resources, less frequent virus diseases attack and sufficient labor force in raspberry growing areas.

Raspberry is a very tasty fruit and can be used in diet in its raw state or processed into juice, soft drinks, jelly products, stewed fruit etc. It is increasingly used by confectionary industry for fillings, by dairy industry for creams in fruit yogurts and ice creams. Raspberry has a very pleasant aroma so it is used very much in alcoholic drinks and wine production.

Raspberry reproduces easily. It bears fruit in its first year after planting and reaches its maximum in the third year. It is fully ripe in June and July when few fruit crop species are offered on the market. Two-yielding cultivars produce the second, though less high, yield in September and October. The risk of unfavorable natural conditions is lower than in other fruit crops. The costs of raspberry growing are high because of manual harvesting and necessity for pesticides application. Over the past years manual harvesting has been replaced by mechanical, but not for table use.

The cultivars Villamette and Meeker are of utmost economic importance in our country. The fruit of cultivar Villamette is large (approx. 4g), firm to very firm, dark red, sweet-sour, very tasty, aromatic. It is easily harvested. The fruit bears freezing well, shipping and handling. Frozen Villamette fruit is the most important fruit crop export product. Meeker fruit is large and of even size (significant for Rolend), firm and bright red. The fruit is easily manually harvested and bears handling well. Its yielding capacity is excellent. Meeker fruit contains higher contents of soluble dry matter and acids than Villamette [2,5]. Due to lower anthocyanins content (to 0.1 %), it is less suited to processing into juice. Meeker is a good parent for developing new cultivars by hybridization. In its economic importance Meeker is expected to replace Villamette to some extent.

In Rubus sp. anthocyanins were identified and characterized as early as 1964 by Harborn and Hall [4]. Those authors examined variations of anthocyanins in raspberry on a large scale and found four anthocyanins: cyanidin-3-glucosilruthinoside, cyanidin-3-glucoside, cyanidin-3-ruthinoside and cyanidin-3-sophoroside. Many authors confirmed their investigations but new anthnocyanins were also found in raspberry. In 1975 Barritt and Torre identified and characterized cyanidin-3,5-diglucoside in cultivar Villamette. The same authors have confirmed later on those five anthocyanins (cyanidines), but they have found two new cyanidines as well as four pelargonidines, present in small amounts [4]. The dominant anthocyanin in raspberry is cyanidin-3-glucoside [2,6]. Besides it, cyanidin-3-sophoroside is always present too, while others occur sporadically. The total amount of anthocyanins in raspberry reaches 0.2 %.

Raw materials containing water-soluble pigments such as anthocyanins are particularly suitable for the production of juices (especially clear ones). Raspberry belongs to those materials. Anthocyanins are found in vacuoles of plant cells, as a neutral to slightly acid water solution. They are responsible for many red, purple, blue hues in flowers, fruits and other parts of various plants. They are found most in skin or near-to-skin cells, their biological function being to attract other living organisms that will set seed free and thus enable plant reproduction. In some cherry, plum, grape fruits anthocyanins are found only in skin, but not in other part of fruit or they are present only in small amounts.

The mechanism of coloration in plants is very complex. The phenomenon called co-pigmentation can explain why the color of isolated anthocyanins can vary considerably depending on the presence of other substances. The color of anthocyanin itself can not provide full explanation for high variations in colors in the examples as follows: 1- the same anthocyanin can have different color in different plants, 2- different anthocyanins can manifest the same color in different plants, 3- anthocyanins can disappear in water solution when pH is identical with that in a plant they give color to. It is evident that there develops intensive interaction between anthocyanins and various organic substances, whereby

various compounds with polysaccharides, polypeptides, phenol substances, metals and other substances are formed, all exerting influence on plant color.

Anthocyanins belong to a widely spread group of substances, present in plant tissues, called flavonoids. In their chemical composition flavonoids are compounds having the structure C_6C_3-C_6. i.e. they have two built in benzole nuclei linked by a three-carbon bond condensed via oxygen into an intermediary ring.

Anthocyanins are glucosidized polyhydroxy and polymethoxy derivatives of 2-phenilbenzopyrolium salts. At high temperature in acid medium anthocyanins are decomposed into their essential components - anthocyanidins and a sugar molecule – monose. About 20 anthocyanidins are well - known, 6 being important of that number (Fig. 1). They can not be found in free state in nature. They are much less soluble in water than anthocyanins. The greater the number of hydroxyl groups in a side chain, the bluer the hue. Methoxylation usually takes place on C_3' and C_5' atoms. Unlike other flavonoids, all anthocyanidins absorb light in a visible part of a spectrum, however, they differ in their absorption capacity maximum (500 – 535 nm). They are unstable in the light and they decompose in alkali.

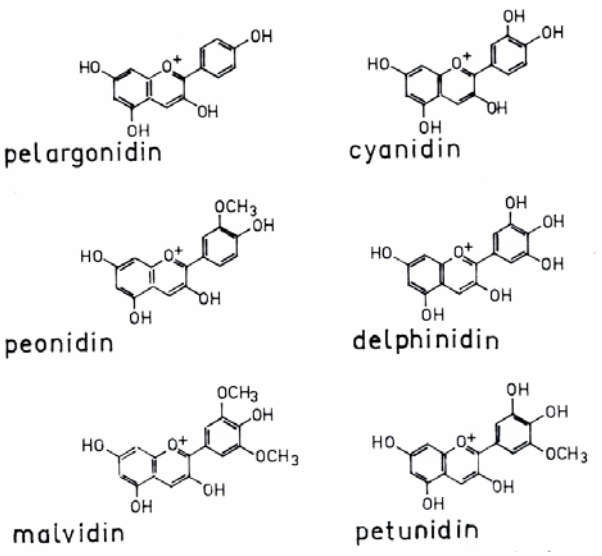

Fig. 1. - The most important anthocyanidins. Their names are derived from plant species they have been isolated from [4].

The basic structure of all anthocyanidins is 3, 5, 7, 4′ tetrahydroxyflavilium cation. Glycosylation of anthocyanidins takes place most frequently only on C_3 – atom, so anthocyanins are most often in the form of monoglucosides.

Diglucosides are less frequently on C_3 and C_5 atoms, and triglucosides are very rarely on C_3, C_5 and C_7 atoms. Glycosylation is most frequent with glucose, but less frequent with galactose, xylose, arabinose, ramnose and other sugars. Sugars are often esterified on C_4 or C_6 atoms with some organic acids (p-cumaric, caffeic, ferulic or sinapine acid, but less often with p-hydroxybenzoic, malonic and acetic acid).

The amount of total anthocyanins in fruits depends on many factors such as: species and cultivar, ripening conditions and ripeness, applied agricultural practices, geographical situation etc. In fruit processing, the color of extracted juice depends on anthocyanins extraction from the skin, amount of acids present, particularly on heat treatment of raw fruit and juice, degree of water dilution, enzymatic presence etc. Hence, this paper deals with changes in anthocyanins content during extraction, enzymatic maceration and heat treatment of raspberry. Juice extraction from very sour red fruit (raspberry) is a very delicate process because natural juice color and aroma have to be preserved. Crushed raw material is subjected to heat treatment (thermal break) so that as much juice as possible can be extracted. The amount of heat-extracted anthocyanins is higher than that of heat-degraded molecules. Any further heating or keeping at raised temperatures results in color decrease i.e. its degradation [10]. Also, the choice of right preparation for maceration and depectinization is of great importance. Pectin preparation of enzimes used for maceration and depectinization provide full decomposition of pectins but also the release and stability of colored compounds [1].

Anthocyanins are very reactive compounds. In water solutions they undergo structural changes followed by changes in color. This is the result of high reactivity of aglucon share and great affinity for H^+, OH^- and H_2O. Esterified sugars and methoxyl groups are important for anthocyanins' characteristics but they are not reactive in general. To better understand the role of anthocyanins in plant pigmentation, it is necessary to study their structural transformation in acid water solution at room temperature because those are physical conditions similar to natural. Thus, better insight is gained into their chemical and biochemical interaction with other substances found in foodstuffs. This can be useful for preserving fruit's natural color during processing and storage.

One of the important and examined characteristics of anthocyanins, as colored substances, is that the color of compound is changed with the change of pH value as follows: from red in acid medium (pH<6) over colorless to slightly violet in weakly acid medium (6<pH<7), to blue in neutral or alkaline medium (pH>7). In cyanidin-3-glucoside (dominant anthocyanin in raspberry), for instance, those transformations in color are the result of structural changes (Fig. 2). The structure of cyaniding-3-glucoside (I) exists in acid medium in the form of favilium cation and shows red color. In weakly acid medium the structure is changed into colorless form of pseudo base or carbinol base (II) and it is in equilibrium with anhydro base (IIIa and IIIb), where water molecule is lost, and

unstable form develops that absorbs light of 538 nm wavelength. In alkaline medium phenol groups are ionized whereby phenates or phenolates are formed, more stable than undissociated bases (IVa and IVb), and blue color develops. Lastly, at pH=12 blue changes into green or yellow, which indicates the occurrence of chalcone (IV).

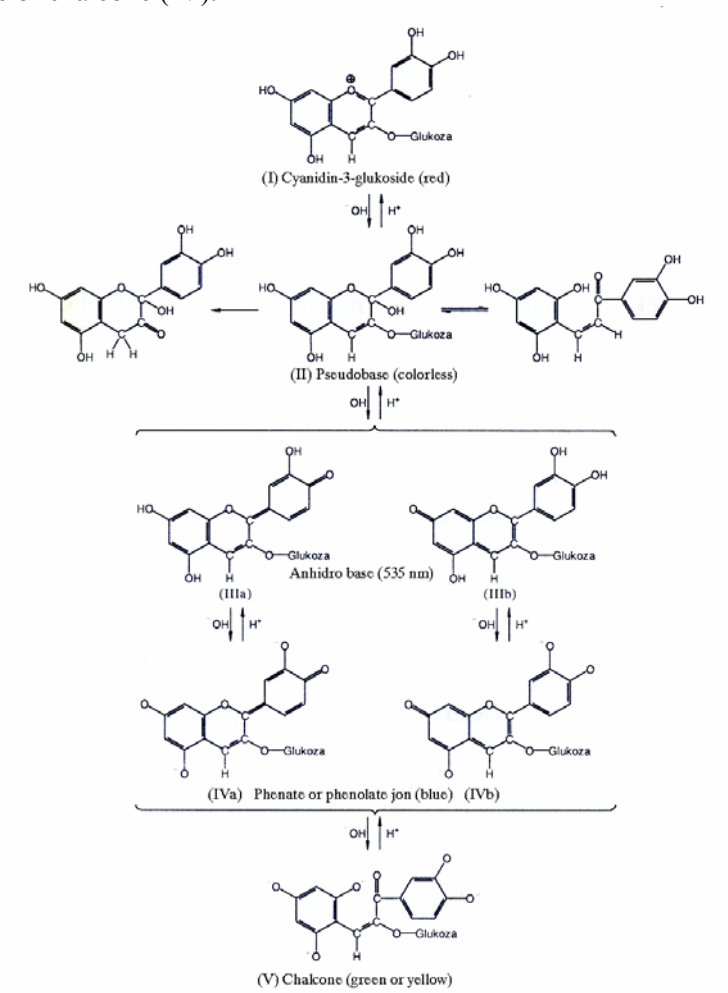

Fig 2. - Change of color and structure of cyanidine-3-glucoside in the function of reactive medium [15].

In alkaline medium, with pH above 8, anthocyanins are decomposed by the opening of heterocyclic ring and oxidation. When acid medium is reverted only a small number of molecules can revert to their previous form, thus a small portion of red color recurs.

SO_2 is often used for decoloration in commercial processing of fruits with dominat anthocyanin color. Loss of color due to fruit treatment with the said agent can be reversible or irreversible. Reversible decoloration of anthocyanin is explained by the formation of colorless anthocyanin-SO_2 complex [4]. The binding of SO_2 takes place on the fourth C atom. At pH=1 the developed complex dissociates into flavilium form and free SO_2 [13].

Sulphur dioxide acts on the fourth C atom of anthocyanin flavilium form, but only if a big molecule, for example, of phenyl group had not already substituted this position. Otherwise, SO_2 loses decoloration capacity, which is explained by the formation of dimer via the fourth C atom, and guides primer of anthocyanin that contains fructose and is resistant to this agent. If the concentration of a bisulphite ion is high, anthocyanins can be decolored in acid medium too, when pH<1.

Polymerized anthocyanins, unlike susceptible ionized (flavilium) forms, are considerably more resistant to decoloration. This fact is of importance for determining the share of polymeric anthocyanins in juice color [12].

Material and Methods

In all experiments we used raspberry frozen for 60 days and defrosted spontaneously at room temperature. In experiments 1 - 12 raspberry cultivar Villamette was used, while cultivar Meeker was used in experiment 13. The reason for giving priority to cultivar Villamette in our experiments is its wider distribution and higher anthocyanins content than in cultivar Meeker, which is desirable for raspberry juice and concentrate [2,3,5]. In experiments 8 - 13, intermediate samples were taken after the first enzymatic maceration and extraction. Also, samples were taken after the second enzymatic maceration. All juice samples were frozen prior to further analyses.

Experiments were carried out on a laboratory hydraulic extractor manufactured by “Ivo Lola Ribar” – Belgrade. Commercial operating conditions were emulated during extraction by using “Bucher” extractor, only shaking being done manually. Between each shaking raspberry pomace was subjected to pressure of 180-200 bar for 10-15 min. The number of shakings represents the number needed for obtaining maximum yield at given working pressures of 180 bar.

By using brochures of a leading world firm in the sphere of enzyme production (DSM, Lille – France), we carried out the experiments that can be grouped into three modes of extraction i.e. enzymatic maceration [1]:

- Extraction without enzyme application:

Experiment 1.- Defrosting of cultivar Villamette (1 kg), heating and crushing at 50^{o}C, extraction (50^{o}C, with 3 shakings), juice frezeeing,

Experiment 1a – Defrosting of cultivar Villamette (1 kg), heating and crushing at 50°C for 60 min so that natural enzymes can act, thermal break at 90°C for 2 min, cooling at 50°C, extraction (50°C, 3 shakings), juice freezing,

Experiment 2 – Defrosting of cultivar Villamette (1 kg), heating and crushing at 50°C, thermal break at 90°C for 2 min, cooling at 50°C, extraction (50°C, 3 shakings), pasteurization (90°C, 2 min), cooling at 20 °C, juice freezing.

- Extraction in a single-stage enzymatic maceration with a pectin preparation KLERZYME ® 120, manufactured by ″DSM″ France - Operations common to experiments 3 - 7 are: Defrosting of cultivar Villamette (1 kg), heating and crushing (50°C), thermal break (90°C, 2 or 5 min), cooling (50°C), enzymatic maceration (0.2 – 0.4 g/kg enzymes, 60 – 120 min, 50°C), extraction (50°C, 1 shaking), pasteurization (90°C, 2 min), cooling (20°C), sample freezing.

Experiment 3 – thermal break (90°C, 2 min), enzymatic maceration (0.2 g/kg enzymes, 60 min., 50°C),

Experiment 4 – thermal break (90°C, 2 min), enzymatic maceration (0.2 g/kg enzymes, 120 min, 50°C),

Experiment 5 – thermal break (90°C, 2 min), enzymatic maceration (0.4 g/kg enzymes 60 min, 50°C),

Experiment 6 – thermal break (90°C, 2 min), enzymatic maceration (0.4 g/kg enzymes 120 min, 50°C),

Experiment 7 – thermal break (90°C, 5 min), enzymatic maceration (0.2 g/kg enzymes 60 min, 50°C),

- Extraction in a two-stage enzymatic maceration with a pectin preparation KLERZYME ® 120, manufactured by ″DSM″ France – Operations common to experiments 8 - 13 are: Defrosting of cultivar Villamette or Meeker (1 kg), heating and crushing (50°C), thermal break (90°C, 2 or 5 min), cooling (20°C), I-enzymatic maceration (0.1 – 0.2 g/kg enzymes, 30 – 60 min, 20°C), extraction (20°C, 1 shaking), pasteurization (90 °C, 2 min), cooling (20°C), sample freezing, II-enzymatic maceration (8 g/hl, 120 min, 20 °C), pasteurization (90°C, 2 min), cooling (20°C), sample freezing.

Experiment 8 - Villamette, thermal break (90°C, 2 min), I-enzymatic maceration (0.1 g/kg, 30 min, 20°C),

Experiment 9 – Villamette, thermal break (90°C, 2 min), I-enzymatic maceration (0.1 g/kg, 60 min, 20°C),

Experiment 10 - Villamette, thermal break (90°C, 2 min), I-enzymatic maceration (0.2 g/kg, 30 min, 20°C),

Experiment 11 - Villamette, thermal break (90°C, 2 min), I-enzymatic maceration (0.2 g/kg, 60 min, 20°C),

Experiment 12 - Villamette, thermal break (90°C, 5 min.), I-enzymatic maceration (0.1 g/kg, 60 min, 20°C)

Experiment 13 - Meeker, thermal break (90°C, 5 min), I-enzymatic maceration (0.1 g/kg, 60 min, 20°C)

Fruit extraction [1] from a very red fruit (raspberry) is a very delicate process because natural juice color and aroma have to be preserved. Hence, the choice of the right preparation to be used for maceration and depectinization is of great importance. Klerzyme®120 is a pectin preparation used for maceration and depectinization of "sour fruit" with pH below 3.2. It is an ideal enzymic preparation since it is active at low pH values and high concentrations of polyphenolic substances in raspberry. No matter whether it is used for maceration or depectinization, it enables full decomposition of pectin substances as well as the release and stability of colored substances. Klerzyme®120 is a pectin preparation obtained from the moulds Aspergillus niger. Pectinase and chemicellulase stable at low pH values are its ingredients. It is active at temperature range from 10 - 60°C, its maximum activity being from 45 - 50°C and at pH 2 - 6. If it is added in a single stage, only at maceration, it is used in the amount of 200 – 400 g/t for 1 - 2 hours at 50°C. If the process of enzyme addition is conducted in two stages, then in the first stage at maceration 100 – 200 g/t is added during 0.5 - 1 hour at 20°C, while in the second stage at depectinization 4 – 8 g/hl is added during 1 - 2 hours at 20°C.

Thermal break of crushed raspberry fruits at 90°C for 2 and 5 min was carried out to examine the effects of raised temperatures on yield at extraction and anthocyanins content. That was the reason for carrying out experiments 7 and 12 in the manner identical with that in the most productive batches for single-stage and two-stage enzymatic maceration, respectively. However, only thermal break was longer and lasted 5 instead of 2 min. It should be added that thermal break inactivates natural enzymes and causes partial reduction of the initial number of microorganisms.

In experiments with two-stage enzymatic maceration, time and enzyme concentration were varied in the first stage of enzymatic maceration, while the second stage of it was identical in all experiments. Experiment 13, with cultivar Meeker, was carried out in conditions identical with those for the most productive experiment with cultivar Villamette (Experiment 12).

Change of color can be monitored by using various methods. The following analytical methods were applied in this work: 1- Determination of total anthocyanins by the method of Niketić-Hrazdina[14], 2- Determination of total anthocyanins and some forms of anthocyanins by Somers-Evans method[12], 3- Determination of color angle by Tanner-Brunner method[13].

Results and Discussion

The content of anthocyanins for different experiments is presented in Tab. 1. Experiments from 1 to 12 were carried out with cultivar Villamette and experiment 13 with cultivar Meeker. Notations with indexes 1 and 2 are for experiments in a two-stage enzymatic maceration: 1 – after the first stage, 2 – after the second stage of enzymatic maceration. Notation for experiment 14 designates raspberry juice produced from raspberry concentrate 3 years old, diluted to 10% soluble dry matter.

Two raspberry cultivars, Villamette and Meeker, differ considerably in their chemical composition. Meeker contains slightly higher percent of soluble dry matter (11.9%) than Villamette (9,8 %). Meeker acidity, expressed as citric acid, amounts to 2.85 %, compared with Villamette that contains 2.9 % acids. Visually, the color of Meeker is considerably lighter than that of Villamette, which indicates considerably lower anthocyanins content [5] (Table 1). Meeker has slightly larger fruit than Villamette.

T a b. 1. - Content of total anthocyanins and some of their forms as determined by methods of Niketić-Hrazdina and Somers-Evens

No of batch	Niketić method Total antthocyanins (g/l)		Somers-Evans method			
	Cyanidin 3 glucoside	Cyanidin 3.5 diglucoside	Anthocyanins total (g/l)	Colored forms (%)	Uncolored forms (%)	Polymer forms (%)
1	0.760	0.883	0.796	32.27	65.04	2.69
1a	0.768	0.892			64.12	
2	0.663	0.770			68.26	
3	0.628	0.729			65.24	
4	0.799	0.928			63.74	
5	0.793	0.921			66.5	
6	0.717	0.831			68.43	
7	0.912	1.059	1.020	27.15	70.36	2.49
8_1	0.742	0.860			71.67	
8_2	0.721	0.823			68.23	
9_1	0.823	0.956			64.75	
9_2	0.756	0.877			64.35	
10_1	0.708	0.821			69.3	
10_2	0.643	0.747			64.78	
11_1	0.774	0.898			65.84	
11_2	-	-			-	
12_1	1.299	1.508			68.19	
12_2	0.828	0.961	0.912	30.28	66.95	2.77
13_1	0.900	1.045			76.94	
13_2	0.588	0.682	0.656	42.28	55.04	2.68
14	0.174	0.201	0.072	17.59	18.59	63.82

We should bear in mind that all experiments were carried out with frozen raspberry fruit because, according to literature data [9], the total coloration percent decreases by approx. 25% in the freezing and defrosting processes.

When heat-treated, juice loses its color for good, which was confirmed by the inrease of colorless chalcones content [4]. Therefore, it is necessary to determine the optimum temperature regime for raspberry thermal break, maceration, depectinization and especially pasteurization of extracted juice.

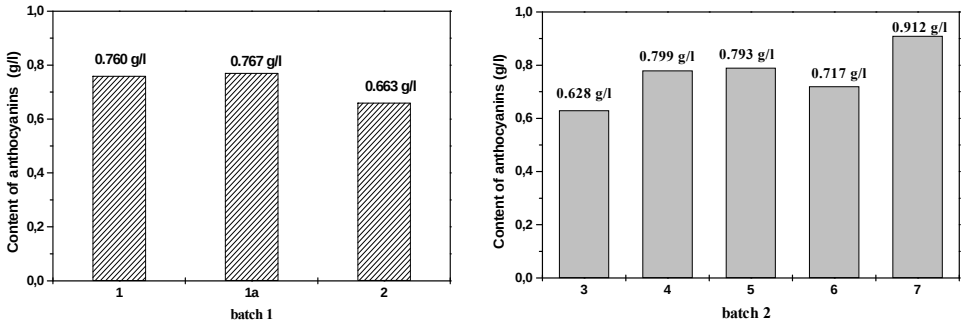

Fig. 3. - Content of anthocyanins in enzymatic non-treated and single-stage enzymatic treated samples as determined by Niketić-Hrazdina method

Fig. 3. shows anthocyanins content in experiments where enzymatic treatment was not applied prior to extraction (Experiments 1, 1a, 2) and in experiments where a single-stage enzymatic maceration was carried out followed by extraction (Experiments 3 - 7). It is evident that there is not difference in anthocyanins content between Experiments 1 and 1a, while in Experiment 2 the content is slightly lower. The reason for this is subsequent pasteurization i.e. negative effects produced by raised temperature. In experiments with batches subjected to single-stage enzymatic maceration two kinds of effects are interwoven: positive effects of enzymes action on anthocyanins extraction and negative effects of pasteurization after juice extraction. In Experiment 7 it is noticeable that anthocyanins content is increased as the outcome of longer thermal break (5 min) compared with other experiments (2 min). In the case of a single-stage enzymatic maceration concentration of enzymes and duration of their action are of significance. Experiments 4 and 5 show the best results: Experiment 4 with enzymes concentration of 0.2 g/kg for 120 min and Experiment 5 with enzymes concentration twice as much than 0.4 g/kg but for 60 min.

Fig. 4 shows the content of anthocyanins in two-stage enzymatic treated samples. We should bear in mind that in two-stage enzymatic maceration extraction and enzymatic maceration were performed at 20 ^{o}C, while in a single-stage enzymatic maceration and non-enzymatic-treated samples at 50 ^{o}C. Four types of effects can be noticed: 1 – in all experiments drop in anthocyanins content after pasteurization (experiments with notations 2 have lower values than experiments with notations 1), 2 – positive effects of longer duration of thermal

break in Experiments 12 and 13 (5 min) compared with other experiments (2 min), 3 – lower content of anthocyanins in cultivar Meeker (Experiment 13) than in Villamette (Experiment 12), 4 – effects of variations in enzymes concentration and duration of enzymes action was not found. This indicates that it is sufficient to apply lower enzymes concentrations and shortest time.

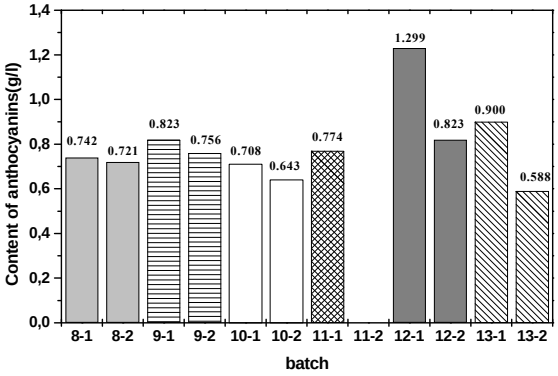

Fig. 4. - Content of anthocyanins in two-stage enzymatic treated samples as determined by Niketić-Hrazdina method

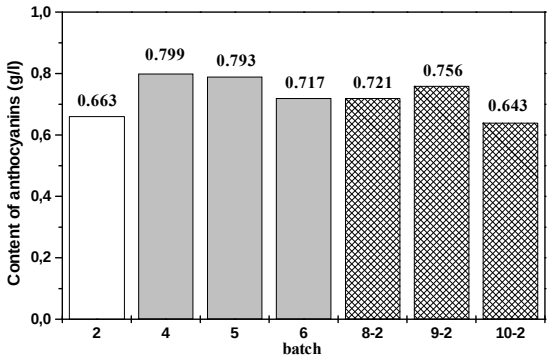

Fig. 5. - Content of anthocyanins in non-enzymatic-treated samples (Experiment 2), single-stage (Experiments 4,5,6) and two-stage (Experiments 8,9,10) enzymatic treated samples (after Niketić-Hrazdina method)

Juice samples obtained by extraction, after enzymatic maceration, contain more anthocyanins - by 30% - compared with juices obtained without enzymatic maceration. Comparative analysis of batches subjected and not subjected to enzymatic maceration (Fig. 5) leads to the conclusion that, concerning the final anthocyanins content, the best results are still produced by experiments with a single-stage enzymatic maceration (Experiments 4, 5, 6). Although in experiments with two-stage enzymatic maceration, higher anthocyanins content is

achieved after the first stage of enzymatic maceration (probably due to lower enzymatic maceration and extraction temperatures as well as to better drainage properties because of lower pectin decomposition), after the second stage of enzymatic maceration and pasteurization anthocyanins content drops even below the first stage of enzymatic maceration. This indicates that a single-stage enzymatic maceration is better as for total anthocyanins content. However, final decision-making about commercial application of some procedure involves consideration about yield during extraction process too.

The data obtained for the share of polymer forms in the color of fresh juices at pH=1 reaches 3%, which is in agreement with literature data [12] (Fig. 6). The share of ionized forms that comprise colored and uncolored forms amounts to 97 %. The ratio of colored and uncolored forms depends on raspberry cultivar too. Colored forms are those considered to show absorption at 513 nm at juice pH, while uncolored ones do not show color at juice pH but only with acidification at pH=1. Meeker contains 55% of uncolored and 42% of colored forms. Villamette contains even more uncolored forms 65 - 70%, compared to 27 - 32% colored. Raspberry juice 3 years old contains expectedly much more polymer forms – 64% and the same ratio of colored and uncolored forms, 18% each.

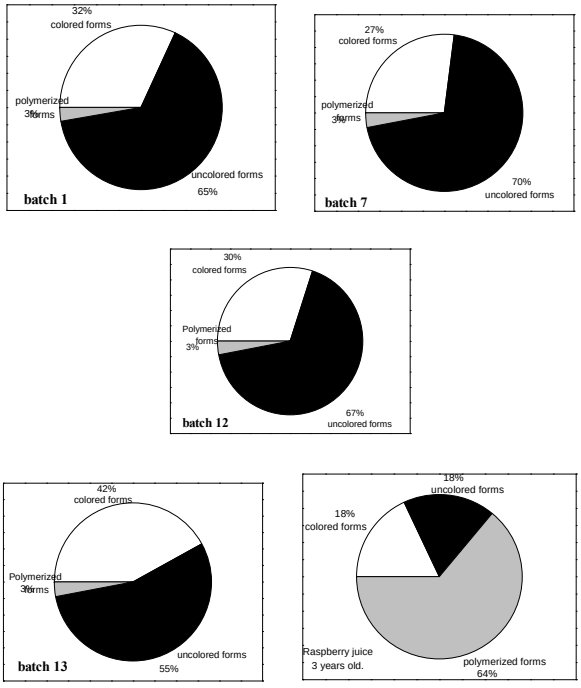

Fig. 6. - Share of some forms of anthocyanins as determined after Somers-Evans

The share of polymer forms in juice color, at juice pH, is given in Fig. 7. Juice 3 years old shows high share of polymer forms, while in fresh juices their share reaches 8.39 %.

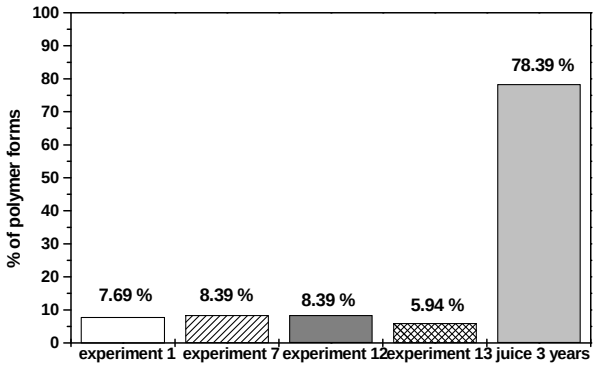

Fig. 7. - Share of polymer anthocyanin forms with juice pH as determined after Somers-Evansu in different experiments and in juice 3 years old

Pasteurization process has crucial effects on anthocyanins content decrease. It can be seen from Tab. 2. where data for total anthocyanins content are presented in g/l.

T a b. 2. - Change of total anthocyanin content during the second pasteurization

Experiment	Anthocyanins content prior to pasterization g/l	Anthocyanins content after pasterization g/l	Losses %
8	0.742	0.721	2.83
9	0.823	0.756	8.14
10	0.708	0.643	9.18
12	1.299	0.828	36.26
13	0.900	0.588	34.67

In cultivar Villamette, percent of loss during juice pasteurization (after Niketić-Hrazdina method) ranges from 2.83 % (Experiment 8) to 36.26 % (Experiment 12). In Experiment 13 (Meeker) loss is also high and amounts to 34.67 %. The reasons are in content, structure and type of anthocyanins. From the abovesaid it can be concluded that batches 12 and 13 have highest losses but also have highest anthocyanins content prior to pasteurization. This indicates that longer thermal breaks extract anthocyanins which are the most unstable and become lost the most during pasteurization.

If we take into account only decrease in total anthocyanins content after pasteurization and not the share of some anthocyanins in the total content, we could draw wrong conclusion: juice color loss is proportionate to total

anthocyanins content loss. Drastic visual loss of juice color was not registered owing to the fact that the highest percent of lost anthocyanins are colorless forms becoming lost most intensively during pasteurization. The rest of anthocyanins exist in juice predominantly in colored (flavilium) form. Colored forms are lost either by decomposing or participating in polymer pigment formation. In fresh juice the amount of polymer anthocyanins is low, however, during processing and storage their amount rises considerably. The loss of colorless forms of anthocyanins during pasteurization is presented in Tab. 3.

Batch 13 has highest loss of anthocyanins colorless forms – 53.26%. This proves that colorless anthocyanins forms in cultivar Meeker are very susceptible to pasteurization during processing. In cultivar Villamette the share of colorless forms is approximately identical with that prior to pasteurization, but the loss of anthocyanins colorless forms is proportionate to their total content. Thus, batch 12 has highest decrease of colorless forms – 37.42%, but it also has highest content of anthocyanins colorless forms prior to pasteurization, whereas batch 8 has lowest decrease of colorless anthocyanins forms – 7.49%.

T a b. 3. - Change of anthocyanins colorless forms during pasteurization

Experiment	Content of anthocyanins prior to pasterization g/l	Content of anthocyanins after pasterization g/l	Losses %
8	0.532	0.492	7.49
9	0.533	0.384	27.89
10	0.491	0.417	15.10
12	0.886	0.554	37.42
13	0.692	0.324	53.26

C o n c l u s i o n

Color is one of the most important parameters for fruit juice quality. Color is expected to be attractive and stable during both processing and storage. The presence of anthocyanins in juice is primarily the cultivar property but is largely dependent upon technological maturity and applied technological method of processing. The term color involves great complexity. Consequently, in selecting the most suitable manner for raspberry processing, concerning coloration, it is of importance to select the procedure for extraction, heat treatment and enzymatic maceration that influences the content of total anthocyanins in juice to the highest extent.

It is necessary to distinguish between the term total anthocyanins content and the term juice color. A great number of anthocyanins in fresh juice, at juice pH, exist in uncolored ionized forms – approx. 2/3, while 1/3 comprises juice color. Pasteurization of fresh juice produces markedly negative effects, so total

anthocyanins content decreases considerably. Uncolored anthocyanins forms become lost most, visual change of color being not that great. Since heat treatment has crucial influence on anthocyanins content decrease, pasteurization has to be performed very carefully.

The amount of polymer pigments in fresh raspberry juice is low and amounts to 3%.

Pulp thermal break must be done prior to enzymatic maceration. A 5-min thermal break yields higher anthocyanins content in extracted juice.

Juice samples produced by extraction contain after enzymatic maceration by 30% more anthocyanins than juices obtained without enzymatic maceration. Although in experiments with two-stage enzymatic maceration higher anthocyanins content is achieved after the first stage of enzymatic maceration, after the second stage of enzymatic maceration and pasteurization the level of anthocyanins content drops even below that of a single-stage enzymatic maceration. This indicates that for total anthocyanins content a single-stage enzymatic maceration produces best results.

REFERENCES

1. Fruit Processing Ingredients, Solutions to Optimise Fruit Processing Worlwide, DSM Lille - France, Brochures, catalogues, leaflets.
2. Mišić, P. (1998.): Malina, Zajednica za voće i povrće, Beograd.
3. Veličković M. (2000): Jagodasto voće, Zajednica za voće i povrće, Beograd.
4. Markakis, P. (1982): Anthocyanins as Food Colors, Food Science and Technology, series of Monographs, Academic Press, USA.
5. Deuel, Charlotte L. (1996): Strawberries and Raspberries, Processing Fruits: Science and Technology, Major Processed Products, Vol. 2, Technomic Publishing co.inc., Lancaster, USA.
6. Niketić Gordana (1988.): Tehnologija voća i povrća, Poljoprivredni fakultet, Naučna knjiga, Beograd.
7. Janda, LJ. (1970.): Opšta tehnološka svojstva nekih sorti maline, Tehnologija voća i povrća, Vol. 5, 5 – 13, Poljoprivredni fakultet, Beograd.
8. Zarić, J., Savić, M., Uljarević M. (1987.): Uticaj uslova ekstrakcije i koncentrisanja na stabilnost antocijana, Tehnologija voća i povrća, Vol. 20, 19 – 25, Poljoprivredni fakultet, Beograd.
9. Ćirić, D., Vujačić, B., Baradić, Ž. (1974.): Promena boje jagode za vreme prerade u sok i koncentrat, Tehnologija voća i povrća, Vol. 9, 25 – 34, Poljoprivredni fakultet, Beograd.
10. Bukvić, B., Zlatković, B., Vukosavljević, P. (1998.): Parametri kvaliteta koncentrisanog soka višnje i faktori uticaja, III Jugoslovenski simpozijum prehrambene tehnologije, Tehnologija voća i povrća, poljoprivredni fakultet, Beograd, 37 – 44.
11. Fuleki, T., Francis, J. (1968.): Quantitative Methods for Anthocyanins – Extraction and Determination of Total Anthocyanin in Cranberries, Journal of Food Science, Vol 33, 72 – 77.
12. Somers, T.C., Evans, E.M. (1977.): Spectral Evaluation of Young Red Wines, Anthocyanin Equlibria, Total Phenolic, free and Molecular SO_2, "Chemical age", J.Sci.Fd. Agric., 28, 279.

13. Tanner, H., Brunner, H.R. (1976.): Getranke-Analytik, Verlag Heller Chemie-und Verwaltungsgesellschaft mbH D-7170 Schwabisch Hall, Unterlimpurgerstrabe 101.
14. Niketić-Aleksić, G., Hrazdina, G.: Quantitative Analysis of the Anthocyanin Content in Grape juices and Wines, Lebensm.-Wis.U.Technol., Vol 5, No. 5.
15. Milić, B., Djilas, S., Brunet, Jasna, Sakač, Marijana (2000.): Biljni polifenoli, Tehnološki fakultet - Novi Sad.
16. Pravilnik o metodama uzimanja uzoraka i vršenja hemijskih i fizičkih analiza radi kontrole kvaliteta proizvoda od voća i povrća, Službeni list SFRJ br 29/83.
17. Vračar, Lj. (2001.): Priručnik za kontrolu kvaliteta svežeg i preradjenog voća, Tehnološki fakultet - Novi Sad.
18. Bukvić, B.(1988.): Prilog poznavanju bojenih materija u grožđu i soku nekih sorti vinove loze tipa bojadisera, Doktorska disertacija, Poljoprivredni fakultet, Beograd.
19. Rommel, Angelika, Wrolstad, R. (1993.): Influence of Acid and Base Hydrolysis on the Phenolic Composition of Red Raspberry Juice, J.Agric.Food Chem., 41, 1237-1241.
20. Rommel, Angelika, Wrolstad, R. (1993.): Composition Of Flavonols in Red Juice As Influenced by Cultivar, Processing, and Enviromental Factors, J.Agric.Food Chem., 41, 1941-1950.
21. Janković, M., Bukvić, B., Mašović, S., Vukosavljević, P. (2002.): Promena kvaliteta pri liofilizaciji maline, 10. Jugoslovenski kongres nutricionista, Zbornik izvoda radova, Jugoslovensko društvo za ishranu, Beograd.
22. Jović, S., Bukvić, B. (1994.): Characteristics of Colored Substances in Red Wine Considering Their Age, Review of Reasearch Work at the Faculty of Agriculture, Vol. 39, No 1, Belgrade.

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PROMENA SADRŽAJA ANTOCIJANA PRI CEDJENJU MALINE

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Rezime

U radu je odredjivana promena sadržaja antocijana pri različitim uslovima cedjenja, enzimiranja i toplotnog tretiranja dve sorte maline (vilamet i miker)**.** Pri cedjenju su imitirani industrijski radni uslovi sa "Bucher" cednice (pritisak 180-200 bar, 1-3 rastresanja kljuka). Za maceraciju i depektinizaciju korišćen je pektinski preparat Klerzyme®120, proizvodjača DSM – Francuska, koji je

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specifičan za "kiselo voće" sa pH ispod 3,2. Pri maceraciji u jednom stepenu primenjen je u količini od 200 – 400 g/t, u toku 1 do 2 sata na 50^{o}C. Pri dvostepenoj maceraciji i depektinizaciji, dodavan je enzim: u prvoj fazi 100 – 200 g/t u toku 0,5 do 1 sata na 20^{o}C, a u drugoj fazi dodavan je 4 – 8 g/hl u toku 1 do 2 sata na 20^{o}C. Blanširanja kljuka maline su izvedena na povišenim temperaturama u trajanju od 2 i 5 minuta radi ispitivanja uticaja povišenih temperatura na ekstrakciju antocijana. Primena odgovarajućeg toplotno-enzimskog tretmana kljuka pre cedjenja, kod obe sorte maline, se pokazala prihvatljivom. Sok dobijen cedjenjem, posle enzimiranja, sadrži oko 30% više antocijana u odnosu na sok dobijen bez enzimiranja. Kod serija sa dvostepenim enzimiranjem, i pored velikog početnog sadržaja antocijana, posle druge pasterizacije dolazi do smanjenja na približnu vrednost sadržaja ukupnih antocijana kao kod serija sa jednostepenim enzimiranjem.Pasterizacija soka, u svakoj fazi prerade, dala je izrazito negativan efekat na količinu antocijana, tako da se njihov sadržaj znatno smanjuje. Najviše se gube neobojene forme antocijana.

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