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Optical characteristics and antioxidant activity of lingonberry (*Vaccinium vitis-idaea*) fruit juice

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This research is focused on juices of wild berries and more specifically on lingonberry juice. The kinetics of color parameters of the samples with inulin or lactulose during the storage is investigated. The content of anthocyanins, phenolic content, antioxidant activity and fluorescence spectra has been measured. The dependences between chemical parameters, color characteristics and ratio between intensity of emission and intensity of excitation for exciting wavelength 275 nm have been found. The dependence between antioxidant activity and total phenolic content also exists. Excitation in the UV region is suitable for distinguishing the phenolic content and antioxidant compounds. The most suitable wavelengths found to be 265 nm and 275 nm.

Keywords: Lingonberry (*Vaccinium vitis-idaea*), Antioxidant activity, Total phenolic content, Fluorescence in UV region**,** fruit juice

INTRODUCTION

Fruit juices contain a large amount of vitamins and minerals and are often recommended by nutritionists and medics for their healing effect. They contain alkali-acid equilibrium of cells, influence nutrition of organs, change positively or negatively the reactivity of the body.

Recently an increased interest has been demonstrated towards wild blueberries, blackberries and raspberries. The fruit of cranberry exhibits antiseptic, anti-putrefactive and anti-inflammatory effects. They contain bioactive compounds such as phenolic compounds [1-3], anthocyanins, omega-3 fatty acids, vitamins [4]. These compounds have nutritional and medical application – they stimulate the immune system, modulate hormone metabolism and possess antibacterial and antiviral action [5-7]. In numerous studies authors reported that the foods from lingonberry can reduce the incidence of cancer, cataracts, macular degeneration and cardiovascular disease [8,9]. They are also used in case of avitaminoses, scurvy, or as generally strengthening beverages to improve appetite. Extracts of these berries are used with nephrolithiasis, pielitis, cystitis, joint rheumatism and gout.

The aim of the present research is to demonstrate the capability of fluorescence spectroscopy for quality detection of some biologically active substances and antioxidants in fruit juice from lingonberry with additional substances such as lactulose or inulin, and to investigate the changes in the color characteristics during the storage period.

MATERIALS AND METHODS

Samples and technologies

The investigation used wild fruits of *Vaccinium vitis-idaea* L. harvested in 2015 in the region of Velingrad. The juice from lingonberry was obtained by using a technological scheme. The pasteurized juices with added lactulose or inulin were investigated.

The samples were produced by crushing and squeezing of fruits after which the juice was filtered and poured in glass bottles with metal caps. The samples were enriched with lactulose at concentrations of 0.5 ml, 1 ml and 1.5 ml and with inulin at concentrations of 1g, 2g, 3g, respectively.

Content of polyphenols and anthocyanins

0.5 - 1 ml of fresh plant material was triturated with quartz sand and 2-5 ml of 70% methanol in a mortar, quantitatively transferred to a flask with a reflux refrigerator. It was extracted at 700ºC three times for 20 min each. Anthocyanin pigment concentration, expressed as cyanidin-3-glucose equivalent, was calculated. The contents of biologically active substances such as anthocyanin and phenolic components in the juice samples were measured spectrophotometrically.

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Ferric reducing antioxidant power (FRAP) assay was used for determining the antioxidant activity. The reaction was started by mixing 3.0 ml of FRAP reagent with 0.1 ml of the investigated juice. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm.

Fluorescence spectra measurements

The sources used to measure the fluorescence spectra were 245 nm, 265 nm, 275 nm and 295 nm light-emitting diodes (LEDs). A fiber optic spectrometer (AvaSpec-2038, Avantes) with sensitivity in the (200-1100) nm range and a resolution of about 8 nm was used to measure the fluorescence spectra. The lingonberry juices were placed in a cuvette 10 mm × 10 mm and illuminated by LEDs.

Color measuring

The color characteristics in the CIELab colorimetric system were determined by the spectrophotometer Konica Minolta CR-400 / 410. They were determined by measuring the reflectance spectrum in a cuvette of 8 mm length. The color parameters a, b and the brightness L of the tested samples were measured. Parameter chroma C was defined as follows:

$$C = \sqrt{a^2 + b^2} \qquad (1)$$

All measurements were carried out at room temperature and the average value was taken from 3 measurements.

RESULTS

The juice from lingonberry with different concentrations of lactulose and inulin was investigated for total content of anthocyanins and polyphenolic compounds. The results are presented in Table 1. By using FRAP method the data for antioxidant activities were obtained (Table 1).

Table 1. Chemical characteristics of juices from lingonberry

Sample	Anthocyanins, mg/L	Total polyphenolic content, mg GAE/L	Antioxidant activity, mMTE/L (FRAP method)
Juice+Lactulose 0.5 ml	161. 65	2607. 95	21554. 68
Juice+Lactulose 1 ml	131. 59	2002. 31	16175.56
Juice+Lactulose 1.5 ml	121. 23	1966. 47	15857.26
Inulin, 1 g	137. 9328	1763. 399	14053.61
Inulin, 2 g	140. 6046	1759. 815	14021.79
Inulin, 3 g	134. 76	1806. 403	14435.56

The juice from lingonberry with inulin has a lower phenolic content than that obtained from lingonberry with lactulose. The fluorescence signal is too weak. For this reason, the fluorescence spectra were obtained only for the juices from lingonberry with lactulose. There is a connection between the investigated chemical parameters (Table 1) and the fluorescence peaks in the visible region. The fluorescence spectra for excitation wavelengths 245 nm, 265 nm, 275 nm and 295 nm are presented on Figure 1. Color characteristics in CIE Lab colorimetric system were measured at the first, the 5th, and the 10th day (Figures 2 and 3) from the production of the juices. There is a correlation between some color parameters and storage time. The dependences between chemical parameters, color characteristics and ratio between intensity of emission and intensity of excitation for exciting wavelength 275 nm were found. There is also dependence between antioxidant activity and total phenolic content. The existing dependences and their correlation coefficients are presented in the discussion.

DISCUSSION

The research is focused on juices of wild berries and more specifically on cranberry juice.

The fruits of cranberry have very good nutritional and gustatory qualities, rich in vitamin C. They are widely used in traditional medicine. In the present study we established relations between the optical characteristics and the contents of biologically active substances, the influence of additions such as inulin and lactulose on the kinetics of color indicators, as well as the benefits for healthy nutrition.

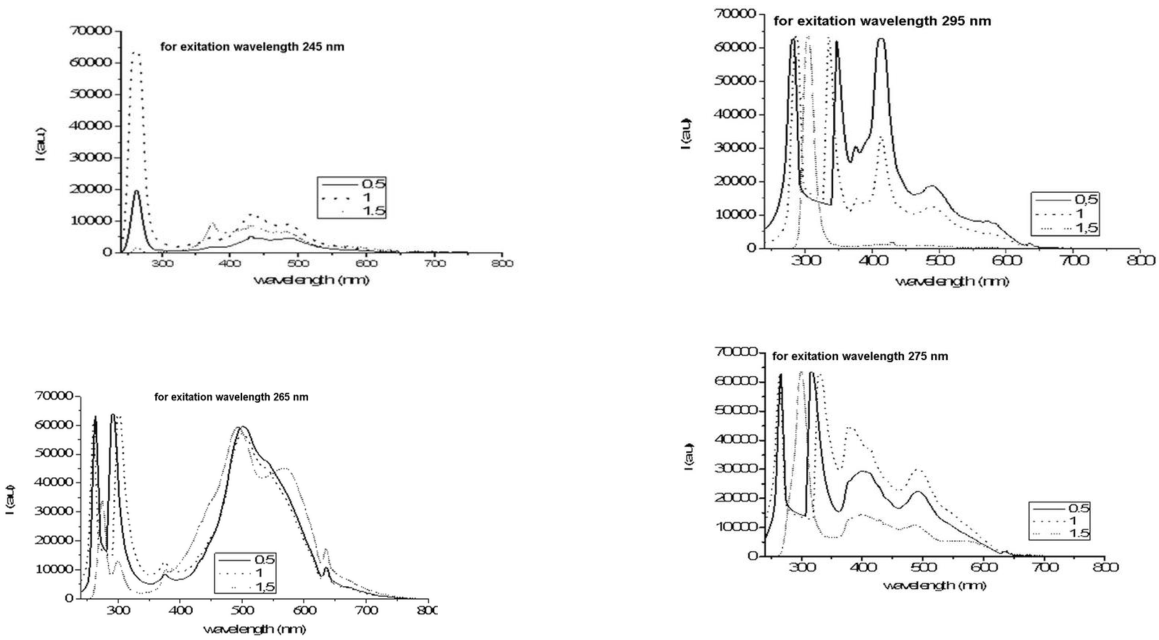


Figure 1. Fluorescence spectra of juices from lingonberry with different concentrations of lactulose

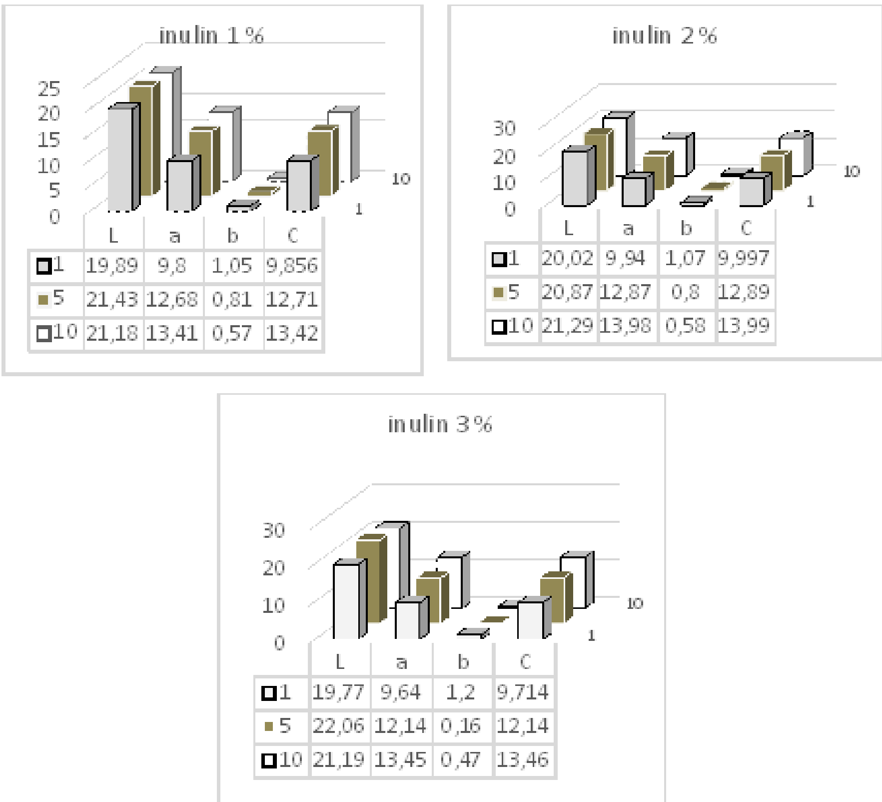
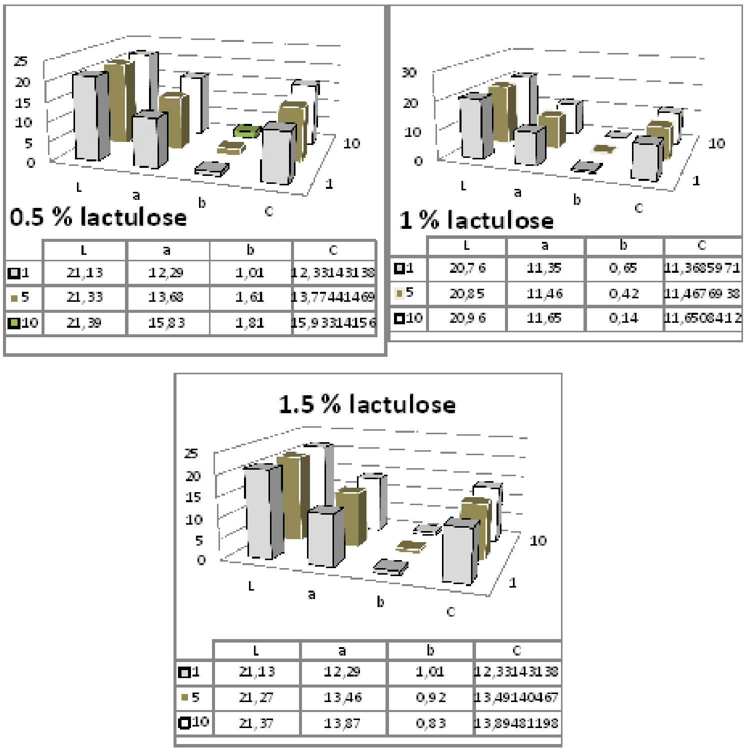


Figure 2. Kinetics of color parameters during the storage time of juice from lingonberry with inulin



0.5 % lactulose

	L	a	b	C
1	21,13	12,29	1,01	12,33143138
5	21,33	13,68	1,61	13,77441469
10	21,39	15,83	1,81	15,93314156

1 % lactulose

	L	a	b	C
1	20,76	11,35	0,65	11,3685971
5	20,85	11,46	0,42	11,4676938
10	20,96	11,65	0,14	11,6508412

1.5 % lactulose

	L	a	b	C
1	21,13	12,29	1,01	12,33143138
5	21,27	13,46	0,92	13,49140467
10	21,37	13,87	0,83	13,89481198

Figure 3. Kinetics of color parameters during the storage time of juice from lingonberry with lactulose

The inulin and lactulose added are often used as substitutions of sweeteners. Lactulose is a disaccharide formed by monosaccharides fructose and galactose connected through a glycosidic bond. Inulin is a reserve polysaccharide, accumulated in underground roots (roots, rhizomes, tubers) of plants such as elecampane, ground apple, dahlia, dandelion, etc. It has been proven that inulin has a number of effects beneficial for the body such as:

- Reduces toxic metabolites;
- Has anti-constipation effect;
- Reduces liver cholesterol and triglycerides;
- Reduces blood pressure;
- Normalizes blood sugar level;
- Improves lipid metabolism in patients with diabetes;
- Improves absorption of minerals in the organism.

Both the brightness L and the value of the red color component "a" increase in the lingonberry juices with concentration of inulin between 1 g and 2 g. Slight decrease was observed for the indicated components if the concentration of inulin increases up to 3g. No similar tendency was observed for samples with lactulose.

For inulin concentrations between 1 g and 2 g an increase of lightness and of the red component of the investigated samples was observed. An increase of the concentration to 3 g leads to a weak reduction of the indicated components. No similar tendency was observed for samples with lactulose.

For all concentrations of inulin and lactulose in cranberry juice an increase of lightness and chroma was observed from the first to the tenth day. For the same period a reduction of the yellow and an increase of the red component was observed.

The latter fact can be explained with the change in the quantity of anthocyanins. Probably, the course of chemical reactions leads to an increase in their contents during storage. The addition of even higher quantity of lactulose leads to their reduction (Table 1), while that of inulin leads to weak changes of the contents of anthocyanins.

An increase in brightness and chroma for all concentrations of inulin and lactulose in the lingonberry juice from the first to the 10th day was observed. The yellow color component decreased and the red component increased during the same period. The latter fact can be explained with the change in the quantity of the anthocyanins. The possibility of chemical reactions to take place led to an increase in their content during juices storage. The addition of a quantity of lactulose lead to a decrease in the anthocyanins (Table 1), and the addition of inulin lead to slight changes in the content of anthocyanins.

The interest to anthocyanins is not dictated by their application as natural colorants alone [10], but by their potential for the development of pharmaceutical products. They exhibit a strong Pvitamine activity, reduce the fragility and the permeability of the capillaries [11]. They are characterized by a strong anti-inflamatory, hypotonic and collagen stabilizing action [12]. The intake of anthocyanins leads to a normalization of the capillary permeability and improvement of eye sight. They play an important role in the cross-linking of collagen by inhibiting the enzymatic digestion during inflammatory processes [13].

Anthocyanins are applied successfully in the prophylaxis and treatment of glaucoma and other eye diseases [14]. In model systems anthocyanin extracts exhibit cardio-protective effect [15] and inhibit the growth of cancer cells. Some of the therapeutic effects of anthocyanin are attributed to their antioxidant properties [16].

It is seen from Table 1 that cranberry with inulin possesses a lower phenol content and a weaker antioxidant activity. This explains the considerably weaker fluorescence signal of samples containing lactulose. Therefore, in the present study we have studied and commented only fluorescence spectra from cranberries with lactulose.

It is evident from Figure 1 that the emission intensity has the lowest value for excitation wavelength of 245 nm. The fluorescence spectra are clearly distinguishable for 275 nm and the best ratio between excitation and emission intensities is for 265 nm excitation. The excitation/emission ranges in the fluorescence spectra can be connected with the following phenolic components and flavonoids:

- Chlorogenic acid: (245-250 nm)/ (430-440 nm). Similar results have been obtained by Mazina *et al*. [17] for apple juice;
- Caffeic acid: (230-350 nm)/ (405-470 nm) [18];
- Vanillic acid: (295-300 nm)/ (305 -355 nm);
- Tannins: (230-315 nm)/ (345-405 nm);
- Catechin and epicatechin: (275-280 nm)/ (320-335 nm) [19].

The fluorescence spectroscopy is an advanced technique for the rapid screening of lingonberry juices for total phenolic contents. A dependence was found between the ratio of intensity of fluorescence and intensity of excitation for wavelength 275 nm and total phenolic content. A positive correlation was obtained between phenols content and antioxidant activity in accordance with literature data for apple juice [20].

Linear dependences exist between:

- Total phenolic content (TPC) and anthocyanins (A): $TPC=-132.66+16.83\times A$ with correlation coefficient $R^2 = 0.96$;
- Antioxidant activity (AA) and total phenolic content (TPC): $AA=8.88\times TPC-1609.2$, correlation coefficient $R^2 = 1$;
- Total phenolic content and the ratio $I_{emission}/I_{exitation}$ for exciting wavelength 275 nm: $TPC=1750+262.20\times I_{emission}/I_{excitation}$, correlation coefficient $R^2 = 0.99$;
- Lightness of the samples and the ratio $I_{emission}/I_{exitation}$ for exciting wavelength 275 nm: $L=-20.31+0.99\times I_{emission}/I_{excitation}$, correlation coefficient $R^2 = 0.937$.

CONCLUSIONS

From the obtained results it can be concluded that:

Front–face fluorescence spectroscopy gives the possibility of qualitative detection of phenolic components in lingonberry juice, which are important for nutrition hygiene. The proposed technique includes fast and cheap methods, without using chemical reagents.

The phenolic content and the antioxidant activity of the juice from lingonberry with lactulose are higher than those of the juice from lingonberry with inulin.

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ОПТИЧНИ ХАРАКТЕРИСТИКИ И АНТИОКСИДАНТНА АКТИВНОСТ НА СОК ОТ ЧЕРВЕНА БОРОВИНКА (*Vaccinium vitis-idaea*)

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Постъпила на коригирана на

(Резюме)

Изследването е съсредоточено върху сокове от горски плодове, по-специално върху сок от червена боровинка (*Vaccinium vitis-idaea*). Изследвана е кинетиката на цветните параметри на пробите с добавен инулин или лактулоза по време на съхранението. Измерено е съдържанието на антоцианини, фенолното съдържание, антиоксидантната активност и флуоресцентните спектри. Открити са зависимостите между физико-химичните параметри, цветните характеристики и съотношението между интензитета на излъчване и интензитета при дължина на вълната на възбуждащата светлина (275 nm). Установена е зависимост между антиоксидантната активност и общото фенолно съдържание. Възбуждането в областта на ултравиолетовите лъчи е подходящо за качествено откриване на феноли и на антиоксиданти. Най-подходящите дължини на вълните са 265 nm и 275 nm.