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Characterization of biscuits enriched with black currant and jostaberry powder

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Sažetak

U ovom radu, istraživan je utjecaj dodatka praha bobičastog voća (crni ribiz i josta) u tijesto za kekse kako bi se procijenilo unapređenje nutritivne vrijednosti i antioksidacijska aktivnost te teksturalna i senzorska svojstva proizvoda. Zahtjev tržišta za funkcionalnim pekarskim proizvodima kao što su keksi jest u porastu. Dobiveni rezultati su pokazali da dodatak bobičastog voća rezultira povećanjem udjela ukupnih fenola i prehrambenih vlakana te povećanjem antioksidacijske aktivnosti keksa. Također, rezultati istraživanja su pokazali da dodatkom bobičastog voća u prahu se smanjuje udio brzo probavljive glukoze i brzo probavljivog škroba. Keksi obogaćeni bobičastim voćem bolje su senzorski ocijenjeni. Keksi koji su imali i čokolodni preljev pokazali su bolja antioksidacijska i senzorska svojstva. Dodatak bobičastog voća utjecao je na čvrstoću i lomljivost keksa.
Ključne riječi: *crni ribiz, josta, prah,, keks, senzorska svojstva, probavljivost škroba*
Summary

The aim of this research was to characterize the improvement in nutritional value, antioxidant activity as well as to evaluate textural and sensory properties of enriched biscuits. In this work black currant and jostaberry powder were used as ingredients for biscuits production since consumer demands for functional bakery products, like biscuits, are increasing. Obtained results showed that addition of berries to biscuits resulted in increase of total polyphenol and crude fibre content as well as antioxidant activity. Addition of berry powder resulted in decrease of rapidly available glucose and rapidly digestible starch. All examined antioxidant and sensory properties were improved when a chocolate coat was formed on the biscuits. Fracturabillity and hardness were affected by berry powder addition.

Keywords: *black currant, jostaberry, powder, biscuit, sensory properties, starch digestibility*

Abbreviations

GAE- gallic acid equivalents
AAE - ascorbic acid equivalent
FG - free glucose
RAG - rapidly available glucose
RDS - rapidly digestible starch
SDS - slowly digestible starch
RS - resistant starch
TS - total starch

Introduction

Dietary habits and physical activity are notably known to have a direct effect on the prevalence of chronic diseases. Dietary guidelines recommend an increase in fruit intake as a part of healthy diet. Interest in the chemical composition of berry species has been intensified lately due to the strengthened awareness of their potential health benefits, as they are rich sources of micronutrients, fibre and numerous phytochemicals which reduce the oxidative damage of human cells that can lead to cancer, heart disease and other degenerative and non-infectious diseases (Bazzano et al., 2003; Hope Smith et al., 2004). Berries belonging to the *Ribes genus* represent abundant source of phytochemicals: anthocyanins, phenolics, organic acids (Moyer et al., 2002; Lugasi et al., 2011). *Ribes nigrum* cultivars have almost the highest ascorbic acid content (100 – 250 mg/100g) among fruits but their consumption as fresh fruits is limited because of the characteristic aftertaste (Viskelis et al., 2012). Jostaberry (*Ribes nidigrolaria*) is the result of a complex breeding between Ribes species, especially *R. nigrum* and *R. uva-crispa*. It contains the phytochemical profile of parent species (anthocyanins as delphinidin 3 glucoside, delphinidin 3 rutinoside, cyaniding 3 glucoside, cyanidin 3 rutinoside) without the tart aftertaste. Black currant is used for jams, juices, jellies and beverages, while jostaberry is consumed fresh or used for flavouring juices and jams and processing into beverages and wines (Lim et al., 2012). Bakery products are consumed daily in large quantities and they provide a convenient medium for delivering phytochemicals and other healthy compounds to consumers (Ktenioudaki et al., 2012). Biscuits belong to one of the most popular bakery products because they are sold as ready-to-eat foodstuffs, have affordable prices, good nutritional quality, are available in different tastes, and have prolonged shelf-life (Ajila et al., 2008). The physical and sensory characteristics of commercial manufactured biscuits vary depending on the particular functional ingredients in products formulation. There are several reports on usage of fruit and vegetable powders in biscuit formulations such as ginger powder (Balestra et al., 2011), mango peel powder (Ajila et al., 2008), amaranth leaf powder (Singh et al., 2009), guduchi leaf powder (Sharma et al., 2013) and grape skin powder as well (Mildner-Szkudlarz and Bajerska, 2013). Incorpo-

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ration of these powders affected nutritional composition (protein, minerals, fibre, phenolics) of biscuits, their organoleptic properties and starch digestibility. Namely, the digestibility of starch in different food products varies. Starch fractions, depending on the kinetics as well as extent of starch's digestion process, could be classified in: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992).RDS is the starch fraction that causes a rapid increase of plasma glucose and insulin levels after ingestion. Starch that is degraded completely, but more slowly by human digestive enzymes in the small intestine (SDS) is the most desirable form of dietary starch. It has health benefits, due to the slow release of glucose resulting in reduced postprandial glucose and insulin response. Resistant or undigested starch has been defined as the fraction of starch that escapes digestion in the small intestine and is considered as a prebiotic since it is a good substrate for the beneficial intestinal microflora responsible for the gastrointestinal health. Incorporation of berry powder rich in phenolic compounds might both influence shelf-life and improve nutritional value, sensory properties as well as antioxidant activity (Aksoylu et al., 2015). To the best of our knowledge this is the first work which performs these berries as biscuit ingredients.

The aim of this work was to produce and enrich biscuits with blackcurrant and jostaberry powders with promising antioxidant attributes and to evaluate mentioned products from the consumer's acceptance point of view in terms of appearance, taste and texture.

Material and methods

Black currant (*Ribes nigrum L.*) was purchased in a local supermarket and jostaberry (*Ribes x nidigrolaria*) was obtained from a local grower. Jostaberries and black currants were freeze-dried using LYOVAC GT2 freeze-drier (Germany). The dried berries were ground to powder using a household grinder and were stored at ambient temperature until biscuits preparation.

The ingredients for control biscuits were: wheat flour 100 g (48.5%); butter 60 g (29 %); powdered sugar 40 g (19.4%); sour cream (12%) fat 5 g (2.4%); salt 0.3 g (0.15%); vanilla extract 0.5 g (0.24%); ginger powder 0.5 g (0.24%). In other samples certain amount of the main raw material (wheat flour) was diminished on account of berry powders to obtain enriched biscuits. Namely, 10 % of the flour was replaced by berry powder (4.85%). Dough was prepared by mixing all the ingredients, rolled out (thickness 5 mm) and shaped as round biscuits (3 cm). The biscuits were baked in oven for 12 minutes at 180^{o}C. Half of the biscuits were dipped into melted dark chocolate coverture (60 % cocoa) and then were dried on a gitter. After cooling, the biscuits were packaged into plastic boxes and than stored at ambient temperature for further analysis.

Moisture and ash content was estimated according to the Hungarian standards (MSZ 6369-3). Protein content was determined by the Kjeldahl method (MSZ EN ISO 20483) and fat content according to method for bakery products (MSZ 20501-1:2007/4). Crude fibre content of biscuits was measured using FIBRETEC 2010 & M6 (Foss, Sweden). The method is based on subsequent steps of chemical treatments to solubilize ''non-fibre'' components and final determination of residue obtained.

In vitro starch digestibility of the examined samples was determined according to the procedure of Englyst et al. (1992). The method is based on measurement of glucose, released from a test food during timed incubation with digestive enzymes under standardized conditions, and allow evaluation the amount of FG, RAG, RDS, SDS, RS and TS. Enzymes used for in vitro starch hydrolysis were: pancreatin (P7547), amyloglucosidase (A7095), invertase (I4504) while heat stable α-amylase (A3306) was used for the total starch determination. All enzymes were obtained from Sigma (St. Luis, USA).

Total polyphenol content was determined spectrophotometrically at 725 nm using the modified Folin–Ciocalteu method (Vattem and Shetty, 2003) and expressed as gallic acid equivalents (GAE) in mg/100 g biscuits. All the measurements were performed in triplicates on a Pye Unicam 8700 Series UV/Vis spectrophotometer (Pye Unicam, England). Before extraction, biscuits were ground to powder. One g powder was extracted with 20 ml extraction solution, containing 50:50 ethanol/distilled water and 0.5 ml cc. hydrochloric acid, mixed and incubated overnight at room temperature in darkness. The supernatant was filtered through Macherey-Nagel filter paper (MN 640w) and used for determination of total phenols, antioxidant capacity and radical scavenging activity of the biscuits.

Ferric reducing capacity was determined by the modified method of Kampfenkel et al. (1995). Briefly, to 2 ml extract, 6 ml absolute ethanol and 1 ml α,α'- dipyridyl solution and 1 ml of iron (III) chloride (0.2% w/v) were added, mixed and after 2 min the absorption was measured at 520 nm. Ferric reducing capacity was expressed as ascorbic acid equivalent (AAE) mg/100 g biscuits.

Radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Bondet et al., 1997). Absorbance was measured at 517 nm. Radical scavenging activity was expressed by the equation:

$$\text{Radical scavenging activitiy (\%)} = (A_{control} - A_{sample})/A_{control}) \times 100 \quad (2)$$

Biscuit surface colour was measured using a spectrocolorimeter Model CM-3500d, (Konica Minolta, Japan). Results were expressed via SpectraMagic NX software. The C.I.E. color coordinates (X, Y, Z) and the L*, a*, b* components of the CIELAB space were recorded. L* parameter indicates the intensity of light or darkness. The parameter a* takes positive values for reddish colours and negative values for the greenish ones, whereas b* takes positive values for yellowish colours and negative values for the bluish colours.These numerical values were converted into "chroma" (C) and "hue angle" (h) color functions using the following equations:

$$C = (a^{*2} + b^{*2})^{1/2} \quad (3)$$

$$h=\tan^{-1}(b^*/a^*) \quad (4)$$

Colour was evaluated both in control biscuits and in biscuits made with berries. Three replicates were used for each type. Two readings were taken at two different positions of each biscuit. The total colour difference (ΔE) between control and berry-added biscuits was calculated by the formula:

$$\Delta E= ((\Delta L)^2+(\Delta a)^2+(\Delta b)^2)^{1/2} \quad (5)$$

***Table 1**. Nutritional characteristics of berry powder enriched biscuits*

Type of biscuit	Moisture	Fat	Protein	Ash	Crude fibre
g/100 g of original sample					
Control	6.85±0.05 a	25.20±0.25 a	5.74±0.33 a	0.79±0.01 a	0.57±0.03 a
With black currant	5.78±0.05 b	21.40±1.40 b	6.01±0.20 a	1.03±0.01 bc	1.0±0.02 b
With josta berry	7.76±0.06 c	24.43±0.44 a	7.47±1.07 b	1.22±0.08 c	0.86±0.04 c
Control with chocolate coat	5.54±0.04 d	27.99±0.10 c	5.45±0.27 a	1.03±0.01 bc	-*
With black currant and chocolate coat	4.21±0.04 e	25.98±0.05 a	5.80±0.15 a	1.10±0.05 c	-*
With josta berry and chocolate coat	4.21±0.09 e	26.23±0.65 ac	6.36±0.05 ab	1.11±0.05 c	-*

*Different letters in columns indicate significant differences. P<0.001 for moisture and crude fibre content; p<0.05 for fat and protein content. * not determined*

The texture of the biscuits was measured instrumentally using a HD.Plus Texture Analyzer (Stable Micro System, Godalming, UK) using a load cell of 5.0 kg and 2 mm cylinder probe (P/2). Penetration test was conducted. The test parameters were: pre-test speed, 1.0 mm/s; test speed, 0.5 mm/s; post-test speed, 10.0 mm/s; distance, 5 mm; and trigger force, 50 g. Fracturability and hardness values were measured in each test. The hardness was determined from the maximum peak force (N), fracturability was determined from the distance (mm) at which the biscuit breaks. Hardness and fracturability of biscuits were calculated using the TE 32 Texture Exponent programme.

Descriptive sensory analysis was carried out by a trained sensory panel (14 persons; 8 female and 6 male). Four parameters were examined: colour, odour, taste and texture. Ratio of positive and negative properties was determined in fresh and stored biscuits. Based on the description of biscuits 5 person (female) from the sensory panel were asked to make an evaluation for odour and taste acceptance using a 10 point hedonic scale where 1 was the lowest score and 10 the highest. The trained panellists were provided with half biscuits on a white plate and instructed to cleanse their palate with cold, tap water before tasting each sample.

Total germ count was determined by the most probable number (MPN) method and mould count was determined by plate count method on malt extract medium (peptone, 10 g; glucose, 20 g; malt extract, 20 g; agar-agar, 30 g; in 1000 ml distilled water).

Data were analyzed by one-way variance analysis (ANOVA) followed by Tukey's test. Statistical analysis of total phenols and antioxidant activities was carried out by using SPSS 19, and analysis of color, texture and fibre content by Statistica for Windows (STATISTICA version 12, StatSoft). Differences between samples at the 5 % (P<0.05) level were considered significant.

Results and discussion

In this work, black currant and jostaberry powder were used as potential sources of functional ingredients. The most common dietary fibre source used to enrich bakery products are wheat, oat, barley and rice. In the last decade, fruit and vegetable fibres have been used as sources of fibre for bakery products (orange, sugar beet, peach, mango, potato, and apple) (Kteniqudaki and Gallagher, 2012). Obtained results showed that incorporation of black currant and jostaberry powders in formulation significantly increased fibre content and ash content of biscuits due to the fibre and mineral content present in the berries. Jostaberry influenced the protein content significantly. The moisture content of biscuits enriched with berry powder was significantly (P<0.001) lower after storage than of those of freshly prepared. The chocolate coat significantly increased fat content of control samples but addition of berries decreased the fat amount to the level of control biscuits (Table 1).

Addition of berry powder affected starch digestibility of examined biscuits (Table 2). Obtained results showed significant differences regarding the contents of rapidly available glucose, different starch fractions and total starch. There are significant changes in RAG and RDS vs. control indicating reduced starch digestibility.

At the same time, there was an increase in SDS content (Table 2). Several factors can explain the differences in quantities of determined starch fractions. These results can be explained by reduced gelatinization degree resulting from addition of the berry powder during preparation as indicated and the RS increase. Also, lower digestibility of biscuits enriched with berry powder can be attributed to the higher fibre content. Furthermore, studies have shown that berry phenolic compounds inhibit digestive processes involved in starch breakdown and slow or modulate nutrient release from food at low level (Grussu et al., 2011; Boath et al., 2012). Since lower RAG and RDS content, and higher SDS content, are directly correlated with glycemic response (Vujić et al., 2014), addition

***Table 2.** In vitro digestibility*

Sample	FG	RAG	**RDS**	**SDS**	**RS**	**TS**
g/100g dry matter						
Control	8.48 ± 0.03[a]	46.00 ± 0.16[a]	33.77 ± 0.12[a]	3.83 ± 0.58[a]	2.02 ± 0.23[a]	39.62 ± 0.46[a]
With black currant	8.96 ± 0.10[a]	41.06 ± 0.61[b]	28.89 ± 0.64[b]	6.59 ± 0.09[b]	2.48 ± 0.02[b]	37.95 ± 0.30[b]
With jostaberry	8.75 ± 0.04[a]	41.44 ± 0.12[b]	29.42 ± 0.14[b]	4.61 ± 0.46[c]	3.30 ± 0.54[c]	37.34 ± 0.87[b]

FG – free glucose; RAG – rapidly available glucose; RDS - rapidly digestible starch; SDS – slowly available starch; resistant starch; RS- resistant starch; TS - total starch. Different letters indicate significant differences.

Table 3. *Antioxidant characteristics of biscuits*

	Radical scavenging activity (%)		Ferric reducing capacity (mg AAE/100g biscuits)	
Type of biscuit	Fresh biscuits	1 month storage	Fresh biscuits	1 month storage
Control	38.13 ± 0.18^{Aa}	35.53 ± 7.06^{Ba}	25.11 ± 1.43^{Aa}	5.33 ± 3.50^{Ba}
With black currant	51.99 ± 1.38^{Ab}	60.48 ± 9.16^{Ab}	54.10 ± 0.13^{Ab}	41.09 ± 2.90^{Bb}
With josta berry	47.08 ± 3.26^{Ac}	49.55 ± 7.19^{Abc}	71.90 ± 1.91^{Ac}	51.32 ± 7.21^{Bc}
Control with chocolate coat	78.35 ± 0.49^{Ad}	35.71 ± 0.54^{Bac}	65.74 ± 0.19^{Ac}	56.19 ± 1.73^{Bd}
With black currant and chocolate coat	86.19 ± 0.14^{Ad}	55.51 ± 1.50^{Bb}	67.66 ± 0.38^{Ac}	50.03 ± 0.89^{Bd}
With josta berry and chocolate coat	65.69 ± 0.72^{Ae}	53.75 ± 0.45^{Bb}	100.37 ± 6.14^{Ad}	79.41 ± 1.85^{Be}

Different capital letters in rows indicate significant differences.
Different lower case letters in columns indicate significant differences.

of berry extracts seems to represent a convenient choice for lowering glycemic index of the final product.

The antioxidant and radical scavenging properties of berry polyphenols have been studied extensively (Manganaris et al., 2014). In order to enhance the bioactive profile of biscuits, black currant and jostaberry powder were added to biscuit formulation. Obtained results showed that the highest concentration of phenols was found in biscuits with blackcurrants (164 mg GAE/100 g). Addition of black currant powder and/or covering with dark chocolate significantly ($P<0.001$) increased the amount of phenolics compared to the control (Fig 1).

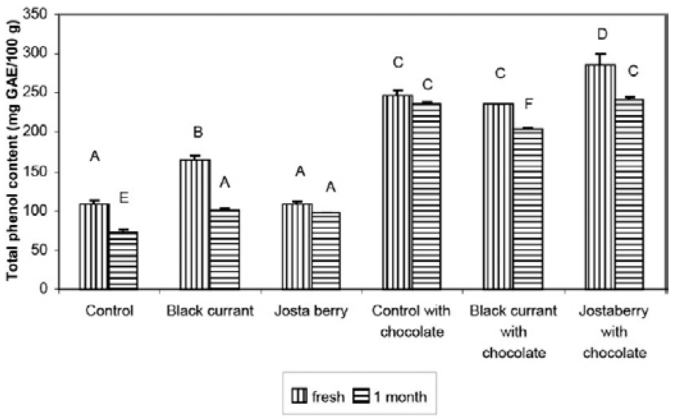

Figure 1. *Total phenol content of berry powder enriched fresh and stored (1 month) biscuits. Different letters indicate significant differences.*

After 1 month of storage, a significant decrease in the phenol content of all biscuits was observed. The highest antioxidant capacity was determined in the biscuits with jostaberries (71,90 mg AAE/100g), while the lowest value was in the control biscuits (25,11 mg AAE/100g). Higher antioxidant capacity, from 66 mg AAE/100 g to 100 mg AAE/100 g was found in biscuits with chocolate coatings. Addition of berry powders and/or covering with dark chocolate significantly ($P<0.001$) increased the antioxidant capacity vs. control. After storage, a significant decrease in reducing capacity was observed (Table 3). However, decrease was less pronounced in enriched biscuits. After storage, a significant decrease ($P<0.05$) in radical scavenging activity was determined in the biscuits with chocolate coatings, but not in radical scavenging activity of biscuits with berry powders (Table 3).

Colour is one of the main factors influencing consumers choice and preferences regarding biscuit acceptability (Chakraborty et al., 2010).

Differences in perceivable colour can be classified as very distinct ($\Delta E>3$), distinct ($1.5<\Delta E<3$) and small difference ($1.5<\Delta E$); Adekunte et al. 2010). Total colour changes were very distinct for all examined samples. The colour of biscuits was influenced by the content of berry powder as well as by the Maillard and caramelization reactions that occur during the baking process. L^* values decreased with berry powder addition which means that biscuits became darker. The a^* value, an indicator of redness, was higher for biscuits with berry addition. The highest value of b^* was observed in control biscuits. Colour intensity (C value) and hue angle (h) decreased with berry addition (Table 4).

Addition of berry powders to biscuit formulation affected the textural properties of biscuits. Biscuits became harder with addition of berry powders (Table 5). It could be result of starch composition, starch-protein interactions, higher proportion of protein than control which resulted in harder structure (Gallagher et al., 2005; Tiwari et al., 2011, Brnčić et al., 2011). The least fracturable were the control biscuits.

Table 5. *Hardness and fracturability of biscuits*

Type of biscuit	**Hardness (N)**	**Fracturability (mm)**
Control	10.511 ± 0.289^{a}	0.705 ± 0.156^{a}
With black currant	$12.586 \pm 0,487^{b}$	1.050 ± 0.127^{b}
With josta berry	18.575 ± 0.607^{c}	1.089 ± 0.141^{b}

All data are the mean ±SD of three replicates.
Different letters indicate significant differences.

In the descriptive session, the pink-purple colour of berry enriched biscuits without chocolate was acceptable but with chocolate coat it was unpleasant. Odour was characterised with the harmonic mixture of sweet, vanilla and fruit aromas. The

Table 4. *Colour parametrs of biscuits*

Type of biscuit	**L***	**a***	**b***	**C**	**h**
Control	75.64±1.15	1.49±0.08	26.46±	26.50±0.26	86.76±0.22
With black currant	35.62±1.17	10.28±1.46	1.59±0.41	10.51±0.73	3.63±1.97
With jostaberry	42.59±1.34	14.51±0.59	5.30±0.7	15.47±0.39	20.08±3.09

All data are the mean ±SD of three replicates.

sensory panel reported that jostaberry enriched biscuits have a little bit foreign aftertaste while biscuits with black currant have a harmonic sweet, fruity and spicy taste. There were some negative opinions about consistency (e.g. tackiness). The general opinion was that the taste and odour of all types of the tested biscuits were highly positive. 10 point hedonic scale ranged from 7.60 to 9.20 for biscuits with berry powder (Figure 2).

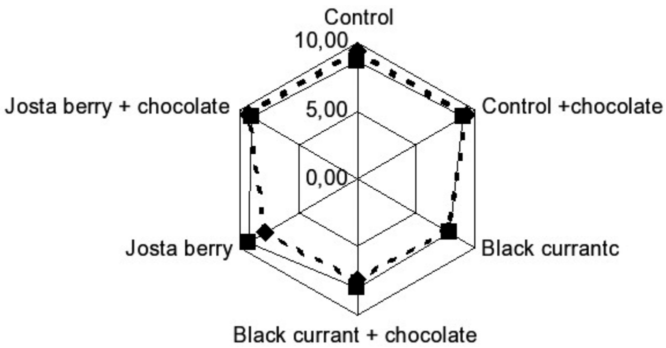

***Figure 2.** Sensory evaluations of the investigated biscuits using a 10 point hedonic scale.*

All types of fresh and stored biscuits had a very low total germ count (<10 to 6.3x10[1]cfu/g). No moulds were found in berry enriched biscuits while control biscuits before and after storage had 1.0-5.3x10[1] cfu/g mould count. It seems that berry powders had also antimicrobial activity.

Conclusions

Biscuits with josta berries or blackcurrants represent good sources of antioxidants and fibres. Berry addition improved the nutritional quality of the biscuits with significantly higher amounts of slowly digestible starch and resistant starch. Sensory analysis showed that the taste and odour of all types of biscuits were highly positive. Hardness and overall mechanical properties were higher compared to the control sample. Microbiological analysis confirmed that examined biscuits are microbiologically safe even after four weeks of storage at room temperature.

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