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EFFECT OF FREEZING AND ENZYMATIC TREATMENT ON THE OUTPUT OF JUICE AND EXTRACTION OF PHENOLIC COMPOUNDS IN THE PRESSING OF LINGONBERRY FRUIT

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K e y w o r d s: lingonberry, total phenolic, anthocyanins, juice.

A b s t r a c t

This paper presents the therapeutic properties of lingonberry and discusses all of the groups of phenolic compounds and anthocyanins. It describes the effect of selected technological operations: freezing and enzymatic maceration of pulp on the content of phenolic compounds in the fruit and juice obtained from them. The antioxidative activity of phenolic compounds present in fruit and juice was also determined.

The study has shown that productivity of pressing juice from pulp obtained from frozen fruit following enzymatic treatment was higher than for pressing the pulp from cold-stored fruit.

The content of phenolic compounds in the juice obtained from lingonberry fruit decreased compared to the fruit. The amount of phenolic compounds which remained in the pulp after pressing ranged from 16.9% to 25.4%, and that of anthocyanins – from 37.2% to 46.1%. The highest content of phenolic compounds was found in juice obtained from the pulp of frozen fruit. All of the juices under study showed high antioxidative activity.

WPŁYW MROŻENIA I OBRÓBKI ENZYMATYCZNEJ NA WYDAJNOŚĆ SOKU I WYDOBYCIE ZWIĄZKÓW FENOLOWYCH PODCZAS TŁOCZENIA OWOCÓW BORÓWKI BRUSZNICY

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S ł o w a k l u c z o w e: borówka brusznica, związki fenolowe, antocyjany.

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Abstrakt

W pracy przedstawiono właściwości lecznicze borówki brusznicy oraz omówiono wszystkie grupy związków fenolowych i antocyjanów. Opisano wpływ wybranych zabiegów technologicznych: mrożenia i enzymatycznej maceracji miazgi na zawartość związków fenolowych w owocach i sokach z nich otrzymanych. Określono aktywność przeciwutleniającą związków fenolowych występujących w owocach i sokach.

Na podstawie przeprowadzonych badań stwierdzono, iż wydajność tłoczenia soku z miazgi otrzymanej z owoców mrożonych i po obróbce enzymatycznej była wyższa niż w przypadku tłoczenia miazgi otrzymanej z owoców przechowywanych w chłodni.

W otrzymanych z borówki brusznicy sokach nastąpiło zmniejszenie zawartości związków fenolowych w porównaniu do ich ilości w owocach. Ilość związków fenolowych pozostająca w miazdze po tłoczeniu wynosiła 16,9–25,4%, a antocyjanów 37,2–46,1%. Najwyższa zawartość związków fenolowych występowała w soku uzyskanym z miazgi owoców mrożonych. Wszystkie badane soki wykazywały wysoką aktywność przeciwutleniającą.

Introduction

In recent years, the tradition of obtaining juice from forest fruit, such as bilberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea* L.) and cranberry (*Vaccinium oxycoccus* L.) has been returning. Forest fruits are processed by small fruit processing facilities. Raw fruits obtained in the process are usually a component of multi-fruit juices, known as one-day juices or low-pasteurisation juice. Their presence in the recipe enriches the final product in valuable bioactive substances. Obtaining raw juice rich in bioactive substances is difficult because most of these substances, mainly phenolic compounds, are found in the skin cells or in cells directly underneath it. These cells are surrounded by much thicker cell walls which are much more difficult to break than fruit pulp cells. Therefore, the process of fruit pressing is frequently preceded by mechanical or enzymatic pre-treatment. These processes allow juice to be obtained with an increased content of bioactive substances.

The aim of the study was to establish to what extent freezing fruit followed by defrosting and enzymatic treatment of the pulp affect the output and quality of the juice. The study was conducted with a view to obtaining juice with improved therapeutic properties.

The study was conducted to determine the pressing productivity, the amount of dry matter and extract and the content of selected groups of polyphenols in the juices.

Materials and Methods

Lingonberry fruit picked in forests in the north of Poland (near Łeba) were used as the study material. A batch of fruit of 5 kg was divided into two parts. One part was stored under refrigerated conditions at 6–8°C and the other was frozen and stored at -18°C. Both batches were vacuum-packed in plastic packaging. After storing the fruit for a month, juice was obtained from them. The organisation of the experiment involved production of juice in three options:

Juice was obtained from frozen fruit in the following manner: 2 kg of frozen fruit were defrosted and ground in a food processor (Thermomix TM 31 Varoma Vorwerk) and the pulp was pressed in a laboratory press (Profil Press, Germany) at the pressure of 250 bar, thus yielding raw juice. The juice was filtered through a laboratory filter (Filter 20x20 · 14K STL pump RVR).

Juice from cold-stored fruit was obtained in the following manner: 3 kg of fruit was ground in a food processor. The pulp was divided into two parts. One part was pressed on a laboratory press and the obtained juice served as the control juice. The other part was treated enzymatically (maceration).

An enzymatic preparation (Pectinex BE XXL by Novozymes) was used; before being applied it was diluted 10 times with distilled water.

Maceration of 1.5 kg of the pulp was carried out with 0.35 ml of 10% of the enzyme solution; the whole solution was mixed thoroughly and incubated at 50°C for 2 hours. After the incubation, the pulp was pressed on a laboratory press (250 bar). The juice was filtered through a plate filter. The characteristics of the enzymatic preparation Pectinex BE XXL manufactured by Novozymes were: activity 30000 UPTE · ml^{-1}; main and secondary activity: pectin lyase; optimum pH: 3.0–5.0; optimum temperature: 50–60°C (recommendations from Novozymes).

Methods

The following were determined in the fruit and in juices: dry matter by weighing according to PN-90:A-75101:03, total phenolic compounds as equivalent of gallic acid according to AOAC, (1974) and SHAHIDI and NACZK (1995), anthocyanins as cyanidin-3-glycoside according to Wrolstad (AOAC 1974), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity μM $DPPH^{\cdot}$ by 1 mg of phenolic compounds according to BRAND-WILLIAMSI et. al. (1995), MOURE et. al. (2001). For easier comparison of the results in this experiment with the results cited in the literature, the results were given per unit mass of juice and fruit on the dry weight basis.

Statistical analysis

Obtained results of researches were statistically analyzed using the Statistica 10.0 PL (StatSoft, Kraków, Poland) program, (ANOVA) with Duncana test of $p \leq 0.05$ significance level was used.

Discussion of Results

The study found that cold-stored lingonberry fruit contained 82.70 ± 5.89% of cell juice with 13.83 ± 0.15% extract. A similar content of juice (82.10 ± 6.50%) and extract (13.35 ± 0.22%) was found in frozen fruit (Table 1). Similarly slight differences were found in the content of individual groups of phenolic compounds (Table 2).

Table 1

The water conten and the extract content in fruit lingonberry

Fruits	Water content [%]	Extract content [%]
Stored in refrigeration	82.70 ± 5.89^{a}	13.83 ± 0.15^{a}
Frozen	82.10 ± 6.50^{a}	13.35 ± 0.22^{b}

Table 2

The total amountof phenolic compounds, anthocyanins in fruit lingonberry stored in refrigeration, and frozen

Fruits	Total phenolic compounds		Anthocyanins	
	fresh matter mg 100 g^{-1}	dry matter mg 100 g^{-1}	fresh matter mg 100 g^{-1}	dry matter mg 100 g^{-1}
Stored in refrigeration	698.55 ± 42.20^{a}	4037.86 ± 320.00^{a}	95.30 ± 5.35^{a}	550.86 ± 18.15^{a}
Frozen	705.10 ± 52.60^{a}	3939.10 ± 88.00^{a}	89.69 ± 7.60^{a}	501.06 ± 30.02^{a}

a, b – mean values indicated by the same letter do not differ significantly ($p \leq 0.05$)

It was established that the total content of phenolic compounds in cold-stored lingonberry fruit was 698.55 ± 42.20 mg · 100 g^{-1} of fruit (4037.86 ± 320.00 mg · 100 g^{-1} d.m.), and in frozen fruit – 705.10 ± ± 52.60 mg · 100 g^{-1} of fruit (3939.10 ± 88.00 mg · 100 g^{-1} d.m.). Similarly, a small difference was observed for anthocyanins, whose content in cold-stored fruit was 95.30 ± 5.35 mg · 100 g^{-1} of fruit (550.86 ± ± 18.15 mg · 100 g^{-1} d.m.), and in frozen fruit – 89.69 ± 7.60 mg · 100 g^{-1} of fruit (501.06 ± 30.02 mg · 100 g^{-1}) (Table 2).

Pressing cold-stored fruit yielded 71.0 ± 2.5% of juice containing 13.65 ± 0.95% of dry matter and 10.85 ± 0.15% of extract. The content of phenolic compounds was 489.23 ± 40.83 mg · 100 g^{-1} of juice (3584.10 ± ± 383.32 mg · 100 g^{-1} d.m.) and anthocyanin contents were 65.70 ± ± 3.22 mg · 100 g^{-1} of juice (481.32 ± 30.24 mg · 100 g^{-1} d.m.) – Table 3.

Table 3

The amount of dry matter and extract the juice obtained from the lingonberry

Product	Yield [%]	Dry matter [%]	Extract content [%]
The juice from the pulp of the fruit stored in refrigeration	71.0 ± 2.5^a	13.65 ± 0.95^a	10.85 ± 0.15^a
The juice from the pulp of the fruit frozen	75.2 ± 0.9^b	15.44 ± 1.50^a	10.99 ± 0.10^a
The juice from the pulp after enzymatic maceration	77.1 ± 1.9^b	14.86 ± 1.00^a	9.83 ± 0.15^b

a, b – mean values indicated by the same letter do not differ significantly ($p \leq 0.05$)

The data gathered in these assays showed that 50% of the total amount of phenolic compounds and 49% of the anthocyanins present in the fruit were extracted by pressing the cold-stored fruit (Figure 1).

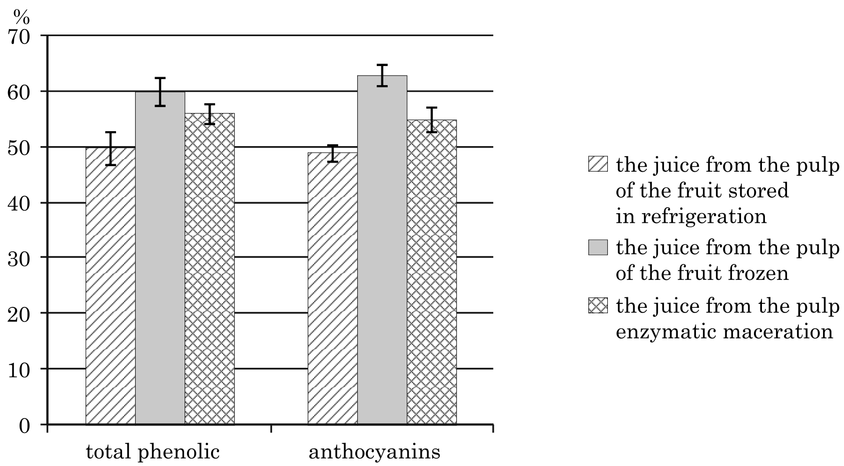

Fig. 1. The percent extraction of total phenolic compounds and anthocyanins to juice

Pressing the fruit pulp from frozen fruit yielded 75.2 ± 0.9% of juice. The juice contains 15.44 ± 1.50% of dry matter and 10.99 ± 0.10% of extract (Table 3). The juice contained the largest amounts of total phenolic compounds of 559.89 ± 44.98 mg · 100 g^{-1} of juice (3625.23 ±

± 393.19 mg · 100 g^{-1} d.m.) and 74.48 ± 2.74 mg anthocyanins in 100 g of juice (482.38 ± 23.98 mg · 100 g^{-1} d.m.) – Table 4. Pressing frozen fruit resulted in the extraction of approx. 60% of polyphenols and approx. 63% of anthocyanins present in the fruit (Figure 1). It was established by applying different methods of fruit preparation for pressing that the highest yield (77.1 ± 1.9%) was achieved by pressing pulp following its enzymatic maceration. The juice contained 14.86 ± 1.00% of dry matter and 9.83 ± 0.15% of extract (Table 3). The content of phenolic compounds was 502.58 ± 40.38 mg in 100 g of juice (3382.10 ± 371.91 mg · 100 g^{-1} d.m.), and that of anthocyanins 68.54 ± 1.13 mg in 100 g of juice (461.24 ± ± 10.43 mg · 100 g^{-1} d.m.). Owing to enzymatic pre-treatment, approx. 56% of phenolic and 55% of anthocyanins were extracted in the juice (Figure 1).

Table 4

The total amount of phenolic compounds, anthocyanins in juices

Product	Total phenolic compounds converted on gallic acid		Anthocyanins converted into content as cyanidin-3-glycoside	
	fresh matter mg 100 g^{-1}	dry matter mg 100 g^{-1}	fresh matter mg 100 g^{-1}	dry matter mg 100 g^{-1}
The juice from the pulp of the fruit stored in refrigeration	489.23 ± 40.83^{a}	3584.10 ± 383.32^{a}	65.70 ± 3.22^{a}	481.32 ± 30.24^{a}
The juice from the pulp of the fruit frozen	559.89 ± 44.98^{b}	3625.23 ± 393.19^{b}	74.48 ± 2.74^{b}	482.38 ± 23.98^{b}
The juice from the pulp enzymatic maceration	502.58 ± 40.38^{b}	3382.10 ± 371.91^{ab}	68.54 ± 1.13^{a}	461.24 ± 10.43^{ab}

a, b – mean values indicated by the same letter do not differ significantly ($p \leq 0.05$)

The higher productivity of fruit pressing was consistent with the findings of other studies, whose authors showed that enzymatic maceration of pulp increased the productivity of pressing and effectiveness of compounds to juice (SZAJDEK et al. 2006, OSZMIAŃSKI 2007a, OSZMIAŃSKI 2007b, KALISZ 2008, NADULSKI and WAWRYNIUK 2009).

All of the juices contained less phenolic compounds and anthocyanins compared to the lingonberry fruit stored under refrigerated conditions, from which they were obtained. This was probably caused by insufficient release of the compound from a hard skin in which their content is the highest (OSZMIAŃSKI 2007a, OSZMIAŃSKI 2007b, KALISZ 2008, NADULSKI, ZAWIŚLAK 2013).

The highest antioxidative activity was manifested by juice obtained from cold-stored fruit (6.63 ± 0.64 µM DPPH$^{\cdot}$ was scavenged by 1 mg of phenolic compounds), which was 19.16% more than the activity of the raw material.

Lower antioxidative activity was observed in the juice obtained from pulp after enzymatic maceration (6.09 ± 0.54 μM $DPPH^{\cdot}$ was scavenged by 1 mg of phenolic compounds, which was 8.1% lower than for the juice obtained from cold-stored fruit (control) and the lowest was the juice from pulp obtained from frozen juice – 5.94 ± 0.12 μM $DPPH^{\cdot}$ scavenged by 1 mg of phenolic compounds (10.4% lower than the control juice) – Table 5.

Table 5

The antioxidant activity of phenolic compounds present in the studied juice

	The juice from the pulp of the fruits stored in refrigeration	The juice from the pulp of frozen fruit	The juice from the pulp after enzymatic maceration
	μM $DPPH^{\cdot}$ scavenged by 1 mg of phenolic compounds		
Juice	6.63 ± 0.64	5.94 ± 0.52	6.09 ± 0.54

Conclusions

The productivity of pressing juice from pulp obtained from frozen fruit following enzymatic treatment was higher than for pressing the pulp from cold-stored fruit. The lower productivity may be caused by the fact that cell walls in fresh or cold-stored fruit remain elastic and difficult to crush mechanically. This has been reported in many publications which describe the pre-treatment of the raw material (ZADERNOWSKI and OSZMIAŃSKI 1994). The low extent to which cells were broken resulted in a low content of phenolic compounds, especially anthocyanins, in the juices compared with the content in cold-stored fruit. In effect, the remainder after pressing cold-stored fruit contained approx. 50% phenolic compounds and 51% anthocyanins, the remainder after pressing frozen fruit contained 40% phenolic compounds and approx. 38% anthocyanins and the remainder after pressing pulp following enzymatic pre-treatment contained 44% of the total phenolic compounds and approx. 45% of anthocyanins. These differences indicate the great diversity of the degradation extent of the cellular walls. The highest antioxidative activity was observed in the juice obtained from cold-stored fruit following treatment.

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