

EFFECT OF PROCESSING ON ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITIES OF BILBERRY (*Vaccinium myrtillus* L.) JUICE

Kamila Borowiec^{ORCID}, Dominik Sz wajgier^{ORCID}✉

Department of Biotechnology, Microbiology and Human Nutrition, University of Life Sciences, Skromna 8, 20-704 Lublin, Poland

ABSTRACT

Bilberry fruit is a valuable source of many antioxidant and anticholinesterase agents. Thus, the aim of the present study was to determine the effect of heat treatment combined with enzyme preparations on the antioxidant and anticholinesterase activity of bilberry juices. Each bilberry juice efficiently ‘scavenged’ DPPH and ABTS radicals. The highest ability to remove DPPH radicals was found in pre-heated (80–90°C, 5 min) and non-heated (only 50–55°C, 2 h) juices obtained using Pectinex BE XXL. The pre-heating treatment caused an increase in the anti-acetylcholinesterase activity compared to non-heated juices, with the highest elevation ($p < 0.05$) recorded for juice produced using Panzym BE XXL. Similarly, the heat pretreatment caused an increase in the anti-butyrylcholinesterase activity in bilberry juices. The combined application of pre-heating and enzyme preparations during juice processing resulted in an increase in the antioxidant and anticholinesterase activities of the end juices.

Key words: ABTS, AChE, BChE, DPPH, inhibition, temperature

INTRODUCTION

Polyphenols have been matter of concern for a long time because these compounds act as antioxidants by i.e. scavenging free radicals. This and other pro-health activities of polyphenols are reviewed in many valuable papers [e.g. Santini and Novellino 2014]. It is known that the changes caused by oxidative stress are slowed down due to the antioxidant potential of polyphenolic compounds. For this reason, a diet containing antioxidant-rich food is considered to be a promising approach to strengthen the physiological antioxidant defense system in a human organism to reduce the occurrence of chronic diseases as well as neutralize neurodegenerative cases [e.g. Ávila-Escalante 2020, Di Meo et al. 2020, Nandita et al. 2020].

Interest in bilberry and the conditions of the bilberry juice processing is caused by the pro-health potential of this fruit and efforts are made to preserve

its biological activity. Bilberry fruit exert health promoting properties due to the presence of phenolic compounds. The total phenolics content (TPC) is estimated at 492–624 mg of gallic acid equivalent in 100 g fresh weight (f.w.) [Aaby et al. 2013, Ancillotti et al. 2016, Celik et al. 2018, Colak et al. 2018]. Rouanet et al. [2010] found that TPC in bilberry juice is about 744 nM cm⁻³. The total content of flavonoids is estimated at about 630 mg 100 g⁻¹ f.w. [Ochmian et al. 2009] and the content of monomeric anthocyanins is approx. 550 mg 100 g⁻¹ f.w. of fruit [Ancillotti et al. 2016] and approx. 3270 µg cm⁻³ [Slatnar et al. 2012] or about 599 nmol cm⁻³ of juice [Rouanet et al. 2010]. It was found that the most common bilberry anthocyanins are the glycosides of delphinidin, cyanidin, malvidin, peonidin and petunidin [Ancillotti et al. 2017, Miljković et al. 2018]. The concentration of

✉ dominik.sz wajgier@up.lublin.pl

anthocyanins is 20 times higher in the skin of the fruit than in the pulp [Riihinen et al. 2008]. The dominant sugars connected to aglycons are: arabinose, galactose and glucose. Other interesting classes of compounds are: flavonols and their glycosides (mainly quercetin and myricetin; 40 mg 100 g⁻¹ f.w. [Aaby et al. 2013]), flavan-3-ols, proanthocyanidins, iridoids [Ancillotti et al. 2017, Miljković et al. 2018] and phenolic acids (e.g. chlorogenic, sinapic, *p*-coumaric, approx. 9.5 mg 100 g⁻¹ f.w.) [Ochmian et al. 2009]. The correlation between the high content of total phenolics and elevated antioxidant capacity of bilberry is well documented [Brasanac-Vukanovic et al. 2018]. Prior et al. [1998] showed that the Oxygen Radical Absorbance Capacity of bilberry fruit was 282.3 μM Trolox g⁻¹ dry weight (d.w.) and Talavéra et al. [2006] confirmed that the consumption of bilberry anthocyanin extract has a positive effect on plasma antioxidant capacity, in spite of a low bioavailability. Last but not least, a ferric reducing antioxidant power (FRAP) value was found in bilberries at 0.663 mmol Trolox equivalent (TE) g⁻¹ d.w., corresponding to about 99 μmol TE g⁻¹ f.w. [Ancillotti et al. 2016].

Fruits are consumed fresh or processed into different food products (puree, infusion, liqueur, fresh and pasteurized juices). Consumers show more requirements according to food quality nowadays and consequently more bio-active properties of fruit preserves should be provided by producers. Thus, optimizing the extraction of valuable components from fruit should be considered on an industrial scale. Durazzo et al. [2019] reviewed some examples of advanced process technologies and their results in the antioxidant potential of juices. There are: pectinolytic enzymes, a thermal or nonthermal digestion process, concentration techniques, the effect of ultrasound, pulsed electric field, reverse osmosis, pressing methods and others. This work is focused on two of the above-mentioned aspects, i.e. heat treatment and pectinolytic enzymes. Primarily, a few earlier works have reported negative effect of pasteurization (80°C for 11 sec.) on TPC, especially on the flavonoids content in grapefruit juice [Iguar et al. 2011], and its antioxidant capacity measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [Iguar et al. 2010]. However, Mennah-Govela and Bornhorst [2017] demonstrated no effect of thermal and nonthermal digestion process on TPC and the anti-

oxidant potential measured using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and in a FRAP assay in a fresh-squeezed orange juice. The thermal degradation of anthocyanins belonging to flavonoids has been also discussed in berry fruits, including e.g. blackberries [Wang and Xu 2007]. In addition, Kechinski et al. [2010] showed that the degradation rate for anthocyanins in a blueberry juice increased in direct proportion to the temperature rise (in the range: 40–80°C), and the $t_{1/2}$ values were 8.6 and 5.1 h at 70 and 80°C, respectively. On the other hand, Kowalczewski et al. [2019] showed that elevated temperatures used during a spray-drying process of potato juice resulted in the increased TPC as well as the higher antioxidant activity measured using ABTS. Similar conclusions were also made by Aaby et al. [2013] on the example of the thermal processing of bilberry (*Vaccinium myrtillus* L.) demonstrated that temperature (high but in a short time, namely 80°C for 15 min or 100°C for 4 min) had a significant positive effect on total polyphenols and total monomeric anthocyanins in aqueous extracts from bilberry press residue.

Pectinolytic enzymes are used in industrial juice processing to increase its extraction efficiency. These enzymes affect the cell wall and as a result, juice recovery is increased. At the same time, more efficient extraction of phenolic compounds is observed [Landbo and Meyer 2004, Buchert et al. 2005]. It is also known that the type of enzyme preparation affects the structure of extracted anthocyanins [Koponen et al. 2008a]. High doses of industrial preparations with the activity of glycosidase cause the hydrolysis of anthocyanins to produce appropriate aglycons. The effect of bilberry fruit processing on the phenolics content in end products, including the use of commercial pectinolytic enzymes, is also documented [Koponen et al. 2008a, b]. Considering the above cited reports, it is worth paying attention to Alzheimer's disease (AD) – an example of a neurodegenerative disease involving the mechanisms of inflammation and oxidative stress in the brain. In addition, AD is characterized by extracellular senile plaques formed by amyloid-β (Aβ) and intracellular neurofibrillary tangles in the central nervous system (CNS) [Castellani et al. 2010]. Thus, the inhibition of cholinesterase enzymes (ChE) is only approved therapeutic tool in the treatment of AD. In this context, polyphenols from various fruits, includ-

ing bilberry are valuable cholinesterase inhibitors, as reported earlier in numerous papers [Sz wajgier 2015, Sz wajgier et al. 2018]. It was already demonstrated that bilberry fruit is a considerable source of ChE inhibitors (including derivatives of chlorogenic and benzoic acids) [Borowiec et al. 2014]. In addition, Ramirez et al. [2005] showed that a diet containing lyophilized berries enhanced the working and short-term memory in adult rats. Vepsäläinen et al. [2013] proved that long-term supplementation of the diet with bilberry extract resulted in decreased $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in brains of aged APdE9 mice. Yamakawa et al. [2016] demonstrated that the anthocyanoside extracts from bilberry inhibited the formation of $A\beta$ fibrils and $A\beta$ toxicity towards Neuro2a cells and a diet containing 1% of this extract protected AD mice against cognitive degeneration. The neuroprotective effect of the diet with bilberry ($5.0 \text{ g kg}^{-1} \text{ day}^{-1}$) was also shown in the modified elevated plus-maze test. As a result, the improvement of short- and long-term memory ($p < 0.05$) is suggested in comparison with the control group [Borowiec et al. 2019].

Therefore, the aim of the presented work was to study the effect of combined heat pre-treatment and the use of hydrolytic enzyme preparations at the dosages recommended by the manufacturers, on the pro-health activities of bilberry juice. Basis of the decision about temperature used during the pre-treatment were the recommendation of the producers of enzyme preparation ($50\text{--}55^\circ\text{C}$) and previous reports ($80\text{--}90^\circ\text{C}$) [Aaby et al. 2013]. The tested enzyme preparations were commonly used by the local manufactures of berry juices. Eventually, the impact of the conditions used during bilberry juice processing on its antioxidant activities was determined using two commonly accepted procedures – ABTS and DPPH methods. Anti-ChE activities of the bilberry juices were also examined.

MATERIAL AND METHODS

Preparation of juice. Bilberry fruit in harvest maturity was purchased from Trading Consortium GHL Sp. z o.o. (Lublin, Poland). Bilberry juice was produced using a juice extractor (3 min, Thermomix TM31, Vorwerk, Wuppertal, Germany) according to Figure 1. Crushed fruit (250 g) was preheated at $80\text{--}90^\circ\text{C}$ (5 min) and cooled to $50\text{--}55^\circ\text{C}$ or fruit was heat-

ed to $50\text{--}55^\circ\text{C}$ followed by the addition of an enzyme preparation (0.01% w/w) and maceration ($50\text{--}55^\circ\text{C}$, 2 h) according to the producer's recommendations. The following commercial preparations were used: Panzym BE XXL (E. Begerow GmbH & Co., Langensheim, Germany), Pectinex BE XXL (Novozymes A/S, Kalundborg, Denmark), Rohapect Classic (AB Enzymes GmbH, Darmstadt, Germany), Pektoenzym (Biowin, Łódź, Poland) with pectin lyase activity; Pectinex Ultra Color (Novozymes A/S) with pectin lyase and polygalacturonase activities; Klerzyme 150 (DSM Food Specialties, Hoek van Holland, the Netherlands) with polygalacturonase activity; Rohament CL (AB Enzymes GmbH) with cellulase activity. After maceration, juice was cooled to 23°C , centrifuged (4°C , 13131 g, 30 min) and frozen (-20°C). Control juices (without enzyme preparations) were also produced. All juices were produced in two repeats.

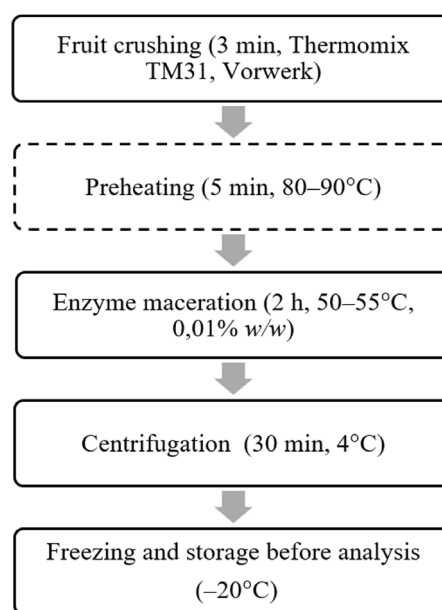


Fig. 1. The preparation of bilberry juice

Antioxidant activity measured using DPPH. The DPPH assay is the most common antioxidant method used for plant extract according to which a potential antioxidant will react with a stable free radical DPPH \cdot (a violet solution) causing discoloration of a free radical. The method of Brand-Williams et al. [1995] was

used with own modifications which were conditioned by adapting the procedure to a 96-well microplate reader. Namely, 0.02 cm³ of bilberry juice (25 mg d.w. mass cm⁻³, so the final dry mass content in the reaction mixture was 0.5 mg) was mixed with 0.08 cm³ of distilled deionized (DDI) water and 0.090 cm³ of DPPH solution in methanol (0.06 mM). Absorbance (A_1) was read at 515 nm for 5 min at intervals of 30 s (20°C, a 96-well microplate reader Tecan Sunrise, Grödig, Austria). Negative blank samples (DDI water in place of bilberry juice) were also run (A_0). Blanks containing juice and DDI water (without DPPH solution; A_2) were considered in the final calculations: $A_0 - (A_1 - A_2)$. Antiradical activity was expressed as Trolox equivalent antioxidant capacity (TEAC value) using Trolox standard solutions in DDI water (0.006–0.623 mM). All samples were examined in four repeats.

Antioxidant activity measured using ABTS. The ABTS assay has been used to determine the antioxidant capacity of food products by the addition of sodium persulfate in order that ABTS is converted to its radical cation (blue). ABTS^{•+} is reactive towards potential antioxidants and during the reaction is converted back to a colourless neutral form. The original method of Miller et al. [1993] modified by Re et al. [1999] was used with minor modifications to adapt the procedure to a 96-well microplate reader. ABTS solution (in DDI water, 7 mM, containing 2.45 mM potassium persulphate) was prepared. After 24 h (at ambient temperature), the absorbance (at 700 nm) was adjusted to 0.70 ± 0.02. Then, 0.01 cm³ of bilberry juice (25 mg d.w. cm⁻³, so the final dry mass content in the reaction mixture was 0.25 mg) was mixed with 0.1 cm³ of DDI water and 0.09 cm³ of ABTS solution. Absorbance (A_1) was read at 700 nm for 5 min at intervals of 30 s (20°C, Tecan, Sunrise). Negative blank samples (bilberry juice replaced by DDI water) were also run (A_0). The absorbance of blanks containing juice and DDI water (without ABTS solution; A_2) was also used in order to calculate the activity using equation: $A_0 - (A_1 - A_2)$. The activity was expressed as a TEAC value, as described above. All samples were examined in four repeats.

Determination of ChEs inhibitory activity. Inhibition of ChEs was evaluated based on the method of Ellman et al. [1961] with minor modifications [Szwajgier and Borowiec 2012] using bilberry juices adjusted to 25 mg dry mass cm⁻³. The inhibition of ChEs by enzyme preparations used in the course of this study was tested in the same manner after diluting in Tris-HCl buffer (100 mM, pH 8.0) to obtain 0.01% w/w. The false-positive effect was determined according to the method of Rhee et al. [2003] with minor modifications, as described previously [Szwajgier and Borowiec 2012]. The inhibitory activity of the samples (studied in eight repeats) was expressed in Es equivalents (μM Es) and calibration curves, as proposed previously [Borowiec et al. 2014].

Statistical analysis. Results were expressed as mean ± standard deviation (SD). An intergroup variation was measured by one-way analysis of variance (ANOVA) followed by post hoc Tukey's HSD test. Statistical significance was considered at $p < 0.05$. The statistical analysis was done using STATISTICA 8.0 software (StatSoft Inc., Tulsa, OK, USA).

RESULTS

The antioxidant and antiradical activity. The results (Tab. 1) confirm the antioxidant activities of bilberry juices tested in the DPPH and ABTS methods (TEAC values were in the range 0.006–0.623 mM). It should be emphasized that the exclusive use of elevated temperature in the control juice resulted in an increase in the TEAC value (TEAC 0.42 ± 0.01 mM vs. 0.35 ± 0.01 mM). Next, comparing the results obtained by the DPPH method for juices with pre-heating (80–90°C), it was found that all TEAC values were at a comparable satisfactory level. However, only for juices with pre-heating and the addition of Pectinex BE XXL or Pektoenzym, is the increase in TEAC value statistically significant ($p < 0.05$). In the variant of juice processing using pre-heating, additional attention should also be paid to the bilberry juices after maceration with the following enzyme preparations: Rohament CL, Rohapect Classic and Pectnax Ultra Color. In the variant without pre-heating, using only the optimal temperature for enzymes (50–55°C), significant ($p < 0.05$) increases in the TEAC values in the DPPH assay were shown in the juices prepared using: Pectinex BE XXL, Rohament CL and Pectinex Ultra Color. In addition, the bilberry juices obtained using Pectinex BE XXL showed the highest abili-

Table 1. The antioxidant and antiradical activity of bilberry juices (mean \pm SD, n = 4)

Bilberry juices		TEAC value (mM Trolox)	
Enzyme preparation (0.01% w/w)	Pre-heating (80–90°C)	DPPH	ABTS
None (control)	+	0.42 \pm 0.01 ^{abc}	0.40 \pm 0.01 ^a
None (control)	–	0.35 \pm 0.01 ^{ab}	0.46 \pm 0.01 ^a
Panzym BE XXL	+	0.43 \pm 0.02 ^{bcd}	0.44 \pm 0.01 ^a
Panzym BE XXL	–	0.42 \pm 0.02 ^{abc}	0.41 \pm 0.00 ^a
Klerzyme 150	+	0.44 \pm 0.04 ^{bcd}	0.39 \pm 0.05 ^a
Klerzyme 150	–	0.44 \pm 0.01 ^{bcd}	0.46 \pm 0.01 ^a
Rohament CL	+	0.48 \pm 0.01 ^{cde}	0.43 \pm 0.01 ^a
Rohament CL	–	0.47 \pm 0.02 ^{cde}	0.42 \pm 0.01 ^a
Rohapect Classic	+	0.48 \pm 0.03 ^{cde}	0.41 \pm 0.01 ^a
Rohapect Classic	–	0.31 \pm 0.04 ^a	0.42 \pm 0.02 ^a
Pectinex BE XXL	+	0.55 \pm 0.01 ^e	0.45 \pm 0.01 ^a
Pectinex BE XXL	–	0.55 \pm 0.00 ^e	0.41 \pm 0.02 ^a
Pectinex Ultra Color	+	0.49 \pm 0.02 ^{cde}	0.42 \pm 0.01 ^a
Pectinex Ultra Color	–	0.50 \pm 0.01 ^{cde}	0.43 \pm 0.01 ^a
Pektoenzym	+	0.53 \pm 0.02 ^{de}	0.46 \pm 0.01 ^a
Pektoenzym	–	0.45 \pm 0.01 ^{bcd}	0.38 \pm 0.02 ^a

In each column, values sharing a common letter do not differ significantly at $p < 0.05$. ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH – 1,1-diphenyl-2-picrylhydrazyl; TEAC – Trolox equivalent antioxidant capacity

ty to remove DPPH radicals, statistically significant ($p < 0.05$) in comparison to the control juices, irrespective of whether the juice was pre-heated or not (TEAC 0.55 \pm 0.01 mM vs. 0.55 \pm 0.00 mM, respectively).

As a result of the analysis of the TEAC values in the performed ABTS method, no significant ($p < 0.05$) differences were found between the control bilberry juices and the bilberry juices obtained in the different temperature variants and with the addition of various enzymatic preparations. Interestingly, there was not even a difference in the TEAC values between the controls.

The anti-ChE activity. The anti-ChE activities of single enzyme preparations used in the study were evaluated (data not shown) and subtracted in calculations to obtain the anti-ChE activities of juices (Fig. 2, 3). It should be noted that no false-positive effect of bilberry juices was observed in this study.

The analysis of the outcomes showed that there were no significant differences between anti-AChE

activity of the juices obtained by the use of a single enzyme preparation, at both temperature ranges (the pre-heating procedure at 80–90°C and 2-hour maceration at 50–55°C). The exception is the juice after maceration with Pektoenzym. In this case, the pre-heat treatment resulted in a significant increase in the anti-AChE activity compared with juice obtained without pre-heating (2.95 \pm 0.29 μ M Es vs. 2.21 \pm 0.28 μ M Es, Fig. 2). However, only the use of Panzym BE XXL during the maceration at both temperature ranges resulted in a significant ($p < 0.05$) increase in the anti-AChE activity (3.34 \pm 0.83 μ M Es and 3.07 \pm 0.10 μ M Es, respectively) versus the activity of the relevant control bilberry juices (2.74 \pm 0.13 μ M Es and 2.92 \pm 0.16 μ M Es, respectively, Fig. 3). Thus, it seems that elevated temperature (80–90°C or 50–55°C, without enzyme preparations) was a single sufficient factor to increase the AChE-inhibitory activity of final juices.

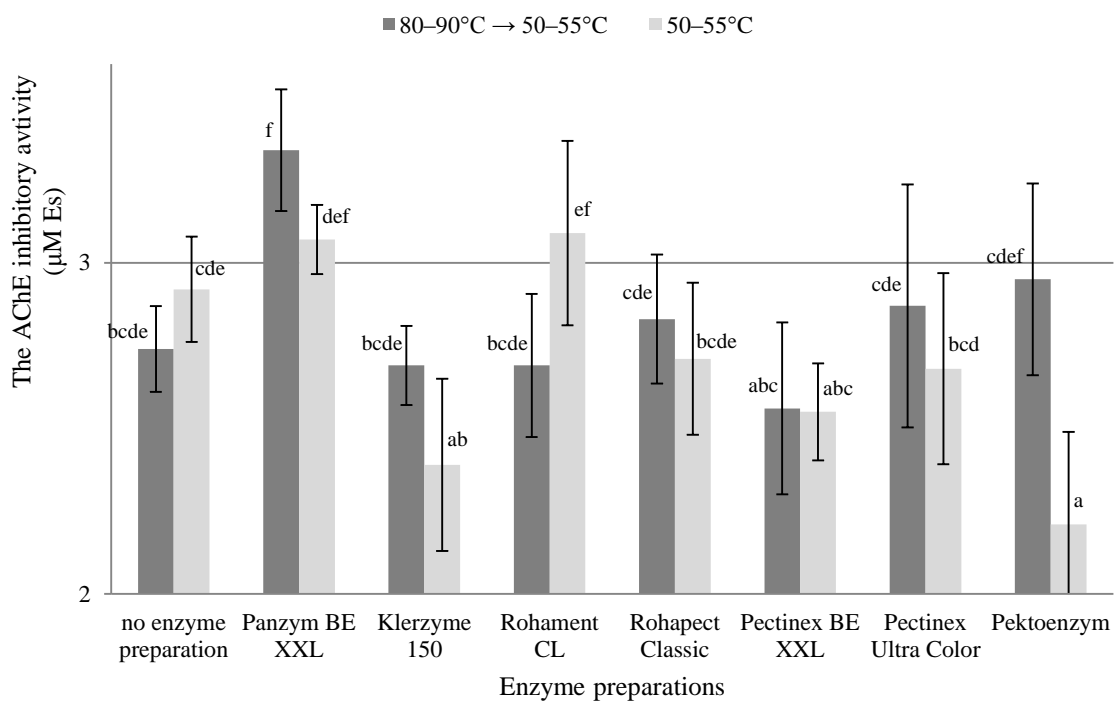


Fig. 2. The inhibition of AChE by bilberry juices prepared with the enzyme preparations (mean \pm SD, n = 8). Different letters denote significant ($p < 0.05$) differences

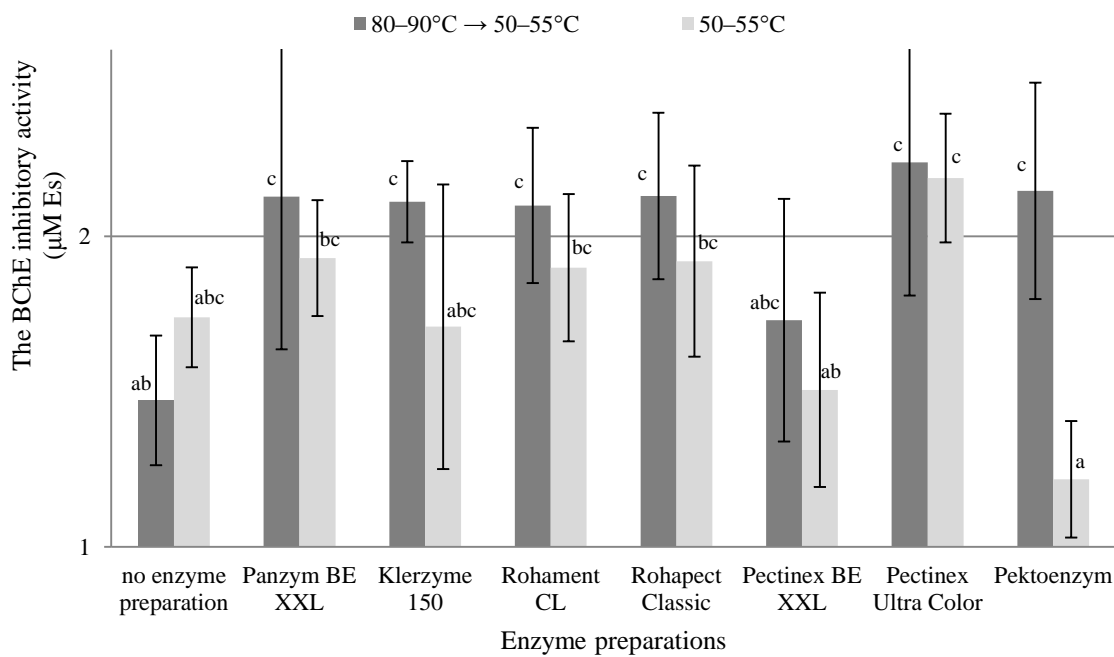


Fig. 3. The inhibition of BChE by bilberry juices prepared with the enzyme preparations (mean \pm SD, n = 8). Different letters denote significant ($p < 0.05$) differences

The inhibition of BChE by 16 experimental juices is shown in Figure 3. A significant ($p < 0.05$) increase in the BChE inhibition is noticeable, in most cases, when the pre-heating treatment was combined with enzyme preparations: Pectinex Ultra Color ($2.24 \pm 0.43 \mu\text{M Es}$), Pektoenzym ($2.15 \pm 0.35 \mu\text{M Es}$), Panzym BE XXL ($2.13 \pm 0.49 \mu\text{M Es}$), Rohapect Classic ($2.13 \pm 0.27 \mu\text{M Es}$), Klerzyme 150 ($2.11 \pm 0.13 \mu\text{M Es}$) and Rohament CL ($2.10 \pm 0.25 \mu\text{M Es}$) versus the relevant control bilberry juice ($1.47 \pm 0.21 \mu\text{M Es}$). In addition, it should be emphasized that there is no significant effect of the pre-heating treatment, applied singly, on the tested anti-BChE activity.

DISCUSSION

The presented work provided important information on the effect of selected technological factors – temperature and enzyme preparation on the pro-health properties of a final berry juice. The most favourable increase in the antioxidant activity determined in the DPPH assay was found for the technological procedures: (i) pre-heating (80–90°C) and the application of Pectinex BE XXL or Pektoenzym (pectin lyases) enzyme preparations; (ii) only maceration (50–55°C) and the application of Pectinex BE XXL or Rohament CL (cellulase) or Pectinex Ultra Color (pectin lyase and polygalacturonase). All obtained juices were characterized by a high anti-oxidative capacity in this assay. Unfortunately, the ABTS method was insufficient to identify the subtle differences resulting from the applied technological procedures. Further, the impact of the studied factors on the anti-ChE activity depends on the enzyme tested as BChE is less selective [Masson et al. 1996]. An increase in the anti-BChE activity was found in the pre-heating variant with almost all preparations (the activities of pectin lyase, polygalacturonase and cellulase) except for Pektoenzym. By contrast, the highest anti-AChE activity was found in the bilberry juice prepared using Panzym BE XXL, regardless of the temperature of the process. Summarising these results, it can be stated that the pre-heating positively affects the antioxidant capacity as well as the anti-BChE activity, while the role of the enzymatic preparations applied (possessing pectin lyase, polygalacturonase or cellulase activities) is peripheral. However, it should be noted that the use of the specific en-

zyme preparation with the pectin lyase activity may be sufficient to increase the antioxidant activity (Pectinex BE XXL) as well as the anti-AChE activity (Panzym BE XXL).

Our conclusions are therefore consistent with previous scientific papers regarding the impact of selected unit processes on the pro-health activity and the content of polyphenolic compounds in products. In fact, there are enzyme preparations such as Pectinex BE Color that are reported to decrease the antioxidant capacity and the total phenolic content in bilberry juice [Sandri et al. 2013]. On the other hand, it was shown that the extraction of polyphenols was improved after the use of commercial pectinases, resulting in the higher antioxidant activity of the products [Koponen et al. 2008a, b, Puupponen-Pimiä et al. 2008]. Our results also confirmed previous findings [Szajdek et al. 2009] that pre-heated juices, obtained by enzymatic maceration (with e.g. Pektopol PT-400, Pectinex BE Colour and Gammapect LC Color) exhibited the highest ability to remove DPPH and hydroxyl radicals, probably due to the high content of total polyphenols.

Pectinolytic preparations cause degradation of the cell wall matrix, thus enhancing the juice yield and an extractability of anthocyanins in fruit juices after enzyme-aided processing [Buchert et al. 2005]. Higher concentrations of individual anthocyanins were observed in suspensions obtained from bilberry skin treated with Pectinex Ultra Color or Panzym BE XXL [Dinkova et al. 2014]. Econase CE, however, produced a dramatic decrease in the total anthocyanin content in the bilberry juice.

As it was mentioned earlier, the content of both total polyphenols and total monomeric anthocyanins in bilberry juices was increasing with temperature and extraction time up to a point (at 80°C for 15 min or at 100°C for 4 min), and then was decreasing [Aaby et al. 2013]. However, higher temperatures and longer extraction time may cause thermal decomposition of polyphenols [Holtung et al. 2011]. Once more, the positive effect can be attributed to the destruction of cell membranes at elevated temperatures [Aaby et al. 2013] and the inactivation of polyphenol oxidase, which have been reported to be responsible for the polyphenol loss in fruit processing [Senica et al. 2016]. In turn, juices obtained by combined treatment of fruit mash (heat pre-treatment and enzymatic mac-

eration) were reported to be the richest source of total phenolics (2304–4418 mg dm⁻³) [Szajdek et al. 2009].

Taking under consideration the above results, the type of enzyme preparation seems to be an important factor during juice processing in light of the discussed quality parameters. The present results are ambiguous, but most enzyme preparations with pectin lyase as well as some with polygalacturonase and cellulase activities had the positive effect on the antioxidant capacity of bilberry juices. Landbo and Meyer [2001] previously reported that pectinases, cellulases, hemicellulases and amylases are suitable for enzymatic treatment and releasing polyphenols. Buchert et al. [2005] demonstrated that the use of enzyme preparations with polygalacturonase activity (Pectinex Ultra SP-L, Pectinex Smash, Pectinex BE 3-L and Biopectinase CCM) positively affected the composition of anthocyanins in the bilberry juice. Some enzyme preparations with exoglycosidase activities can additionally hydrolyse anthocyanins to the corresponding aglycones [Puupponen-Pimiä et al. 2008].

The impact of juice processing on the content of total polyphenolic compounds and anthocyanin as well as the antioxidant capacity of juices is quite well described. But still, not many papers define the role of individual groups of phenolics as ChE inhibitors. Hence the significant value of the results presented in this work, and the next step should be an attempt to identify them. However, the previous work reported on the efficient inhibition of both AChE and BChE exerted by bilberry fruit extract [Borowiec et al. 2014]. The anti-ChE activity of some phenolic compounds (especially phenolic acids) is reported [Szwajgier and Borowiec 2012]. Nevertheless, no information on the direct effect of the processing parameters on the anti-ChE activity of the end bilberry juice is available in databases. The work presented here can be considered the first study on this topic.

CONCLUSIONS

The combined application of pre-heating the bilberry pulp (80–90°C, 5 min) and enzyme preparations (especially pectin lyases such as Pectinex BE XXL and Panzym BE XXL, respectively) during juice processing (50–55°C, 2 h) was the most efficient approach for elevation of the antioxidant and anti-ChE activi-

ties of the end bilberry juice. The pre-heating effect can be attributed to the destruction of cell membranes and the inactivation of polyphenol oxidase at elevated temperatures. Thus, the use of temporary pre-heating seems to be the most significant factor in terms of practice. The pre-heating is not only responsible for the increase of the juice yield, but also in increment of the health-promoting properties of the bilberry juice. In addition, the use of enzyme preparations with pectin lyase activity is worth considering in the bilberry processing. Shortening of the time of the technological process will be an additional benefit of the proposed procedure.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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