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ORIGINAL PAPER

The Influence of Selected Osmotic Dehydration and Pretreatment Parameters on Dry Matter and Polyphenol Content in Highbush Blueberry (*Vaccinium corymbosum* L.) Fruits

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Abstract The paper presents an assessment of the influence of selected highbush blueberry pretreatment methods and parameters on the process of osmotic dehydration conducted in 65 °Brix sucrose solution for 5 to 240 min at 30–70 °C. The pretreatment methods used included: fruit immersion in boiling water (15 s) and in 0.5 % NaOH solution (15 s at 95 °C), exposure to ultrasound at atmospheric pressure (vibration frequency of 35±5 kHz, 500 W, for 15 min.) and at low pressure (0.92 kgcm^{-1}), and enzymatic processing; pectinase (enzyme activity of 46,000 PGU/mL; 0.6 mL/90 g of fruits; 30 min at approx. 22 °C) and lipase (enzyme activity of 750 PGU/mL; 0.7 mL/90 g of fruits; 30 min at approx. 22 °C) were used. Dehydration was also conducted in the presence of pectinolytic enzymes. The dehydrated material was analyzed in terms of the content of dry matter, total polyphenols, and particular polyphenols using high performance liquid chromatography. It was observed that dehydration was much more intensive at 60 and 70 °C, but such temperatures led to substantial losses of phenolic compounds (by 15–30 % after 2-h dehydration) and unfavorable changes in the texture of the final product. A promising method of pretreatment is fruit immersion in solutions containing pectinolytic and lipolytic enzymes, which increase dry matter content by 26 % (after 1 h of dehydration at 30 °C) with a low loss of phenolic compounds (4 %). Among the identified anthocyanins, the greatest retention during dehydration at various temperatures was displayed by petunidin-3-galactoside (over 80 % after 1 h of dehydration) and petunidin-3-glucoside (over 78 %).

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Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) fruits have a unique aromatic taste and a wealth of valuable nutrients (Mazza 2005; Skupień 2006; Ochmian et al. 2009). The nutritional qualities of the berries are attributed to the presence of phenolic compounds (Joseph et al. 2005; Yi et al. 2006; Zafra-Stone et al. 2007; Krikorian et al. 2010). Albeit not essential for human life, flavonoids may act as a health-improving factor, if consumed over a long period of time. These compounds exhibit antioxidant activity, thus being supportive to the natural defenses of the human body and decreasing the risk of developing diseases of civilization (Pietta 2000; Heim et al. 2002; Petti and Scully 2009). Clinical studies confirm that the biological activity of natural antioxidants is higher than that of corresponding pharmacological supplements (Wang et al. 1996; Manach et al. 2004; Pokorný 2007; Perron and Brumaghim 2009). Highbush blueberries are a rich source of anthocyanins and contain monoglycosides (glucosides, galactosides, and arabinosides) of delphinidin, cyanidin, petunidin, peonidin, and malvidin, as well as their acyl derivatives (Gao and Mazza 1994; Kalt et al. 1999; Wu and Prior 2005; Krupa and Tomala 2007; Barnes et al. 2009). The most abundant phenolic acid is chlorogenic acid (Cho et al. 2004; Taruscio et al. 2004; Wang et al. 2008); other acids include hydroxybenzoic acids (gentisic, gallic, protocatechuic, and salicylic acids) and hydroxycinnamic acids (m-coumaric, o-coumaric, and p-coumaric) occurring in the form of esters or glycosides (Zadernowski et al. 2005). Polyphenols characteristic of the highbush blueberry include flavonols, represented mostly by quercetin and its derivatives (Cho et al.

2005, 2004; Zheng and Wang 2003). The presence of proanthocyanidins has also been detected (Gu et al. 2003; 2004).

Due to the seasonality of highbush blueberry fruits, fresh berries are available only for a few months a year. One of the methods of extending the postharvest life of blueberries is osmotic dehydration, which also makes it possible to modify the composition of the raw material. The process consists in immersing raw material of cellular structure in a hypertonic solution (Behsnilian and Spiess 2006). During the process, the water present in the tissues is removed to the solution and mass is transferred between the solution and tissue components. Results obtained by Saurel et al. (1994a) indicate that the gradient of osmotic pressure created between the osmotic solution and the vacuolar sap of the fresh material subjected to dehydration is the major driving force of the process at low temperatures and short processing times (under 50 °C and up to 30 min for apples). Water and substances from the sap are transported through the semipermeable cell membrane of the biological material. The state of the membrane may change from partial to full permeability, which depends on the process conditions (Torreggiani and Bertolo 2001). According to Saurel et al. (1994a), at higher temperatures and long process times transfers are controlled by diffusion phenomena. Thus, inadequate dehydration parameters may lead to unfavorable changes in the dehydrated material, including the loss of semipermeability of cell membranes and substantial losses of valuable nutrients (Chiralt and Talens 2005; Falade and Igbeka 2007) as well as high sugar impregnation, which increases the caloric value of the product. During dehydration of frozen fruits, where penetration of the fruit tissue by osmotic substance is more intensive as the structure of cellular material is more damaged (Ohnihisi et al. 2003), the dehydration principle is also based on an overall diffusion mechanism (Saurel et al. 1994b).

A factor that hampers mass transfer in the process of osmotic dehydration is the fruit epidermis. Due to its low permeability, it constitutes a barrier to the osmotic solution, water, and substances dissolved in vacuolar sap. Consequently, in many cases, it is necessary to pretreat the raw material. The various pretreatment methods proposed in the literature include: ultrasound, lower hydrostatic pressure, steaming, immersion in alkaline or salt solutions, and exposure to a high-intensity electric field. Rodrigues et al. (2009) used ultrasound in the experiments on sapota fruits. Samples were peeled, cut into slices, immersed in water, and subjected to ultrasonic waves for 10–30 min. Fernandes et al. (2009) evaluated the effect of ultrasound on pineapple tissue (near triangular shaped samples were pretreated under the above-mentioned conditions). Ultrasound was also tested on banana, genipap, jambo, melon, papaya, and pinha (Fernandes and Rodrigues 2008). In the experiments by Mújica-Paz et al. (2003), vacuum was used at the first stage of osmotic dehydration (10 min) of mango, apple, and melon. Vacuum was also used by Bórquez et al. (2010). Defrozen raspberries were osmo-dehydrated initially at low pressure (for 8 min) and then at ambient pressure (4 h). Grabowski et al. (2007) performed a chemical pretreatment. Cranberries were dipped into 0.5–2.0 % NaOH solution for 3 min at 20 °C; 3 % sodium oleate and ethyl oleate were also tested. Additionally, different thermal pretreatments (3 min at 100 °C) were evaluated. High-intensity electric field (0.2–1.6 kV/cm) was applied by Rastogi et al. (1999) before osmotic dehydration of carrots. Most of the abovementioned pretreatments led to the increase in the mass transfer during osmotic dehydration, albeit in different degrees.

The objective of this work was to examine the influence of the temperature and duration of the dehydration process as well as the methods of pretreatment on polyphenol content in highbush blueberry fruits.

Materials and Methods

Materials

This study examined frozen highbush blueberry fruits of the cultivar "Bluecrop" (in the consumer maturity phase; pH=3.47; dry matter content was 19.3 g/100 g; average mass of fresh fruit 1.9 g±0.3 g) harvested in late July and early August 2010 from a plantation located in Konstantynów Łódzki, Poland. The fruits were stored at −18 °C.

Chemicals and Standards

Methanol, acetone, and formic acid were purchased from J.T. Baker (Witko, Poland) Ultrapure water (Millipore System) was used to prepare all solutions. Commercial standards of delphinidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside were purchased from Extrasynthèse (Genay, France). Folin-Ciocalteu's phenol reagent was obtained from POCH s.a. (Gliwice, Poland) and (−)-epikatechin standard was purchased from Sigma Aldrich (Steinheim, Germany). The enzymes PectinexYeld Mash, Palatase 750-L were provided by Novozymes (Bagsvaerd, Denmark), and Rapidase C-80 Max was supplied by DSM Food Specialties—Beverage Ingredients (Delft, Netherlands).

Osmotic Dehydration Without Pretreatment

The frozen fruits were dehydrated in 65 °Brix sucrose solution. Samples of 13.5±1.0 g (approx. six berries) were weighed into plastic containers with screw-on lids. The solution was added to the containers immediately before the experiment, and then the sucrose solution was heated up to the dehydration temperature. The weight ratio of the raw material to the solution was 1:4. The process of osmotic dehydration (OD) was conducted

for 5, 15, 30, 60, 90, 120, 180, and 240 min at 30, 40, 50, 60, and 70 °C under atmospheric pressure (the average time required to reach the desired temperature was 60 min following the dipping of frozen fruits). Every experiment was done in duplicate. Continuous shaking was applied throughout osmotic dehydration (200 cycles/min). After the set time of dehydration, the fruits were separated from the osmotic solution by means of a sieve. They were subsequently immersed in water and dried with filter paper. The dehydrated material was analyzed in terms of the content of dry matter, total polyphenols, and individual polyphenols using high performance liquid chromatography (HPLC).

Pretreatment of Fruits Prior to Osmotic Dehydration

Immersion in Boiling Water

Samples of 13.5±1.0 g of the fruits (kept at approx. 22 °C for 15 min after taking out from a freezer) were placed on sieves and immersed in boiling water for 15 s. Subsequently, the fruits were cooled down by immersion in cold water.

Immersion in Hot NaOH solution

Samples of 13.5±1.0 g of the fruits (kept at approx. 22 °C for 15 min after taking out from a freezer) were placed on sieves and immersed in 0.5 % solution of NaOH for 15 s at 95 °C. Subsequently, the fruits were cooled down by immersion in cold water.

Ultrasound Treatment

Plastic containers with 13.5±1.0 g of sample material: fruits (kept at approx. 22 °C for 15 min after taking out from a freezer) and solution at a ratio of 1:4 were placed in an ultrasonic cleaner (Inter Sonic; 2,000 mL, vibration frequency of 35±5 kHz, 500 W) for 15 min.

Ultrasound and Low Pressure Treatment

Samples of 13.5±1.0 g of the fruits (kept at approx. 22 °C for 15 min after taking out from a freezer) and osmotic solution (at a ratio of 1:4) were placed in 150 mL vacuum flasks. Subsequently, the flasks were sealed tight and connected to a vacuum pump (0.92 $kg\,cm^{-1}$). After 15 min, the flasks were disconnected from the vacuum pump and exposed to ultrasound (Inter Sonic; 2,000 mL, vibration frequency of 35±5 kHz, 500 W) for 15 min.

Treatment with Pectinolytic and Lipolytic Enzymes

First, 90±1 g of the fruits (kept at approx. 22 °C for 15 min after taking out from a freezer) was weighed into a 600 mL beaker, after which 360 mL of water and 0.6 mL of Pectinex Yield Mash preparation (enzyme activity of 46,000 PGU/mL) were added, and the beaker was left to stand for 30 min at approx. 22 °C (pH amounted to 4.2±0.2). The fruits were then separated from the solution and washed with water three times. Subsequently, 13.5±1 g fruit samples were weighed into containers with screw-on lids and 65 % sucrose solution was added.

The same procedure was used for the lipolytic preparation Palatase 750-L (0.7 mL, enzyme activity of 750 PGU/mL). Prior to the addition of lipase, the water (with fruits) was brought to pH6.5±0.5 with 0.1 M NaOH.

Furthermore, in another variant, the fruits were first subjected to treatment with lipolytic enzymes and then pectinolytic enzymes, in accordance with the above procedures.

Osmotic Dehydration Following Pretreatment

Osmotic dehydration was carried out at 30 °C. The other conditions of the process as well as the steps taken after its completion were as specified in point Osmotic Dehydration Without Pretreatment.

Osmotic Dehydration in the Presence of Pectinolytic Enzymes

Samples of 13.5±1.0 of raw material (kept at approx. 22 °C for 15 min after taking out from a freezer) were placed on a sieve and immersed in boiling water for 15 s. Subsequently, they were placed in plastic containers with screw-on lids, to which 65 ° Brix sucrose solution was added (at a fruit to syrup ratio of 1:4 *w*/*w*). The sucrose solution had been brought to pH3.5±0.5 prior to addition. A pectinolytic enzyme was added to every container. The following enzymatic preparations were used: Pectinex Yield Mash (Novozymes) and Rapidase C-80 Max (DSM); 0.1 mL of the preparations was added per 60 g of the osmotic solution. The remaining dehydration conditions were as in Osmotic Dehydration Without Pretreatment.

Phenolic Extraction

After dehydration, each sample (including all fruits from a container) was ground under liquid nitrogen with a grinder (A11B, IKA, Germany). Subsequently, 2.0±0.5 g of a sample was weighed and extracted five times over 15 min with a solution containing MeOH, H_2O and HCOOH (50:48:2) by decanting the supernatant to 25 mL volumetric flasks. The flasks were filled to volume with the extraction solution.

Determination of Total Polyphenol Content

First, 0.5 mL of the extract obtained as specified in Phenolic Extraction, 0.25 mL of Folin–Ciocalteu reagent, and 2.5 mL of 20 % Na_2CO_3 were placed in 25 mL volumetric flasks. Then, the flasks were filled to the mark with distilled water, and the

contents were mixed and incubated at room temperature for 1 h. The absorbance of the solutions was measured at a wavelength of 720 nm. Total polyphenol content was expressed as (−)-epicatechin equivalents (Singleton and Rossi1965).

Determination of Anthocyanin Content with the HPLC Method

Chromatographic analysis was performed using a Knauer HPLC chromatograph with Phenomenex Gemini 5u C18 110A columns [150×4.60 mm] with a Phenomenex Security Guard Cartridge system [4×3.0 mm] and a DAD detector at 40 °C and a flow rate of 1 $ml\,min^{-1}$; phase A: H_2O/HCOOH (9:1, *v*/*v*) and phase B: ACN/H_2O/HCOOH (95:4:1, *v*/*v*). The gradient program was as follows: 0–0.6 min, 12 % (*v*/*v*) B; 0.6–16 min, 12–30 % (*v*/*v*) B; 16–20.5 min 30–100 % (*v*/*v*) B; 20.5–22 min, 100 % (*v*/*v*) B; 22–25 min, 100–12 % (*v*/*v*) B, 25–35 min, 12 % (*v*/*v*) B. The injection volume was 20 μL. Data were collected using the Eurochrom 2000 software (Knauer, Berlin, Germany). Separation was performed on extract obtained as specified in Phenolic Extraction.

The total phenolics were measured by the method described by Singleton and Rossi 1965 with some modification. Standards obtained from Extrasynthèse (Geny, France) and Sigma-Aldrich, UV–vis data, and LC-MS data, as well as literature data (Gao and Mazza 1994; Kalt et al. 1999; Häkkinen and Törrönen 2000; Wu and Prior 2005; Castrejón et al. 2008; Lohachoompol et al. 2008; You et al. 2011), were used for the identification of anthocyanins. Quantitative results of the determinations are given as cyanidin-3-glucoside equivalents.

LC-ESI-MS/MS Analysis

The frozen fruits were extracted as specified in Phenolic Extraction. Then, the samples were separated using a Knauer System (Determination of Anthocyanin Content with the HPLC Method) equipped with fraction collector FOXY R1 (Teledyne ISCO Lincoln, NE, USA). Peaks were collected from ten repeated HPLC separations of extract. The obtained samples were diluted (1:3) with distilled water. The samples were passed through the SPE columns (STRATA X, Phenomenex, UK) that were pre-conditioned with 1 mL 100 % MeOH and 1 mL H_2O. The retained compounds were eluted using 1 mL of 100 % MeOH. These solutions were then subjected to LC-ESI-MS/MS (LTQ VETOS, Thermo Scientific, Waltham, MA, USA). The samples were directly injected into MS detector. Analyses utilized the positive ion mode. The source parameters were as follows: ion spray voltage, 3.00 kV; capillary temperature, 325 °C; and sheath gas and auxiliary gas, 30 and 10 units/min, respectively. To generate MS/MS data, the precursor ions were by helium gas collision in the ion trap by optimizing the collision energy in order to obtain an intensity of the precursor ion close to 10 % of the relative scale of the spectrum.

Determination of Dry Matter Content

A weighing bottle with a glass rod and 5±1 g of sand was placed in a dryer at 105±2 °C for 1 h until a constant weight was obtained. Subsequently, the bottle was cooled down in a desiccator and weighed. Then, 2±0.5 g of sample material (after grinding, see "Phenolic Extraction") was weighed into the bottle, mixed with the sand, and weighed. After weighing, the bottle containing the sample material was dried to constant weight in a vacuum dryer (90.0 kPa) at 60±2 °C for 10 h. Finally, the bottle was cooled down in a desiccator and weighed.

Calculation of Osmotic Parameters

In order to calculate water loss (WL) and solids gain (SG), the following formulas were used (Matuska et al. 2006):

$$WL = [m_0(1-s_0)-m_k(1-s_k)]/m_0s_0 \quad [g\ H_2O/g\ initial\ dry\ matter]$$
$$SG = (m_ks_k - m_0s_0)/m_0s_0 \quad [g\ dry\ matter/g\ initial\ dry\ matter]$$

- m_0 weight of sample before osmotic dehydration [grams]
- m_k weight of sample after osmotic dehydration [grams]
- s_0 solids content before osmotic dehydration [grams of dry matter per gram]
- s_k solids content after osmotic dehydration [grams of dry matter per gram]

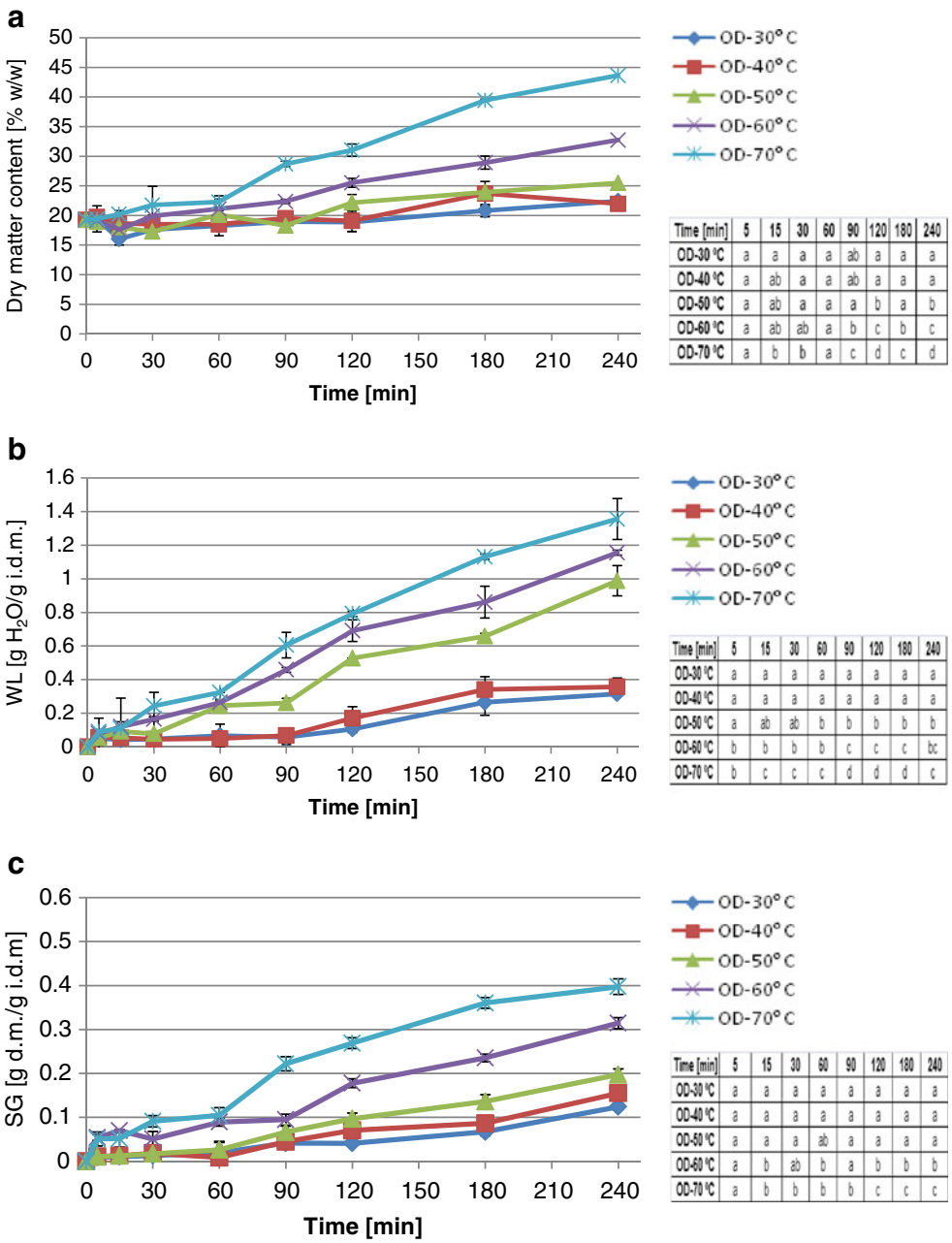
Statistical Analysis

ANOVA analysis was conducted using STATISTICA software to evaluate differences between treatments (by Duncan's test).

Results and Discussion

Changes of Dry Matter Content During Fruit Dehydration Without Pretreatment

Changes of dry matter content, water loss, and solids gain in highbush blueberries subjected to dehydration are presented in Fig. 1. The sucrose solution to fruit ratio was 4 to 1, which, according to literature data, ensures appropriate dehydration conditions by protecting the osmotic solution against excessive dilution with water (Rastogi et al. 2002). The presented results show that dehydration at 30 to 50 °C did not proceed very



Time [min]	5	15	30	60	90	120	180	240
OD-30 °C	a	a	a	a	ab	a	a	a
OD-40 °C	a	ab	a	a	ab	a	a	a
OD-50 °C	a	ab	a	a	a	b	a	b
OD-60 °C	a	ab	ab	a	b	c	b	c
OD-70 °C	a	b	b	a	c	d	c	d

Time [min]	5	15	30	60	90	120	180	240
OD-30 °C	a	a	a	a	a	a	a	a
OD-40 °C	a	a	a	a	a	a	a	a
OD-50 °C	a	ab	ab	b	b	b	b	b
OD-60 °C	b	b	b	b	c	c	c	bc
OD-70 °C	b	c	c	c	d	d	d	c

Time [min]	5	15	30	60	90	120	180	240
OD-30 °C	a	a	a	a	a	a	a	a
OD-40 °C	a	a	a	a	a	a	a	a
OD-50 °C	a	a	a	ab	a	a	a	a
OD-60 °C	a	b	ab	b	a	b	b	b
OD-70 °C	a	b	b	b	b	c	c	c

Fig. 1 Changes in dry matter content (**a**), water loss (**b**), and solids gain (**c**) in highbush blueberry fruits during osmotic dehydration (OD) in 65 °Brix sucrose solution at 1:4 fruit to syrup ratio at different temperatures (30, 40, 50, 60, and 70 °C). In the tables attached, the same letter in different cells within a given column indicates a lack of statistical differences ($\alpha = 0.05$) between the results at particular processing times

effectively. After 180 min, the increase in dry matter was 7.8, 22.7, and 24.0 % at 30, 40, and 50 °C, respectively. Water loss amounted to 0.27 and 0.34 g H_2O/g i.d.m. at 30 and 40 °C, respectively (Fig. 1b). At 50 °C, higher WL (0.66 g H_2O/g i.d.m.) was observed; however, it did not yet translate into a significantly higher dry matter content. Solids gain was at a low level, amounting to 0.07–0.14 g d.m./g i.d.m. (Fig. 1c). At 60 and 70 °C, dehydration proceeded faster, but it also took a relatively long time to obtain a highly dehydrated product. At 60 °C, dry matter content increased by 32 % after 120 min, while at 70 °C by about 61 %. Water loss amounted to 0.69 g H_2O/g i.d.m. and 0.79 g H_2O/g i.d.m., respectively. After 3 h of processing, dry matter content significantly increased (by 49.7 % at 60 °C and twofold at 70 °C). At 60 °C, WL and SG reached a level of 0.86 g H_2O/g i.d.m. and 0.24 g d.m./g i.d.m., respectively, while at 70 °C a level 1.13 g H_2O/g i.d.m. and 0.36 g d.m./g i.d.m., respectively. Much lower values for blueberry dehydration were observed by Nsonzi and Ramaswamy (1998). In their experiments, an increase in dry matter content after 180 min at 40, 50, and 60 °C was 3, 6, and 9.5 %, respectively. The use of high temperatures for accelerating the dehydration process entailed unfavorable changes in the processed material. As early as after 60 min, the fruits started to soften and acquired unfavorable texture. Furthermore, thermal processing may lead to the loss of semipermeability of cell

membranes and accelerate the chemical reactions occurring inside the dehydrated material (Lewicki et al. 1998).

The presented results show that osmotic dehydration at 30–50 °C is time consuming and does not deliver an adequate degree of dehydration. Therefore, further research focused on finding a pretreatment method that would ensure better mass transfer.

Changes in Dry Matter Content in Pretreated Fruits

Figure 2a shows the influence of treating the material with hot 0.5 % solution of NaOH on changes in dry matter content during osmotic dehydration. In comparison to the control, which is material dehydrated without pretreatment, no sizeable changes in dry matter content were observed, although both some transfer of water from the tissue (Fig. 2b) and migration of osmotic agent to the tissue (Fig. 2c) were observed. The above pretreatment methods were designed to disturb the external waxy layer protecting the fruits against water loss (Skurtys et al. 2011), but they did not bring the expected results, namely, a more intensive mass transfer. The literature provides an example of pretreating cranberries with 0.5 M NaOH solution in the process of osmo-convective drying (Grabowski et al. 2007).

Interesting pretreatment methods include the use of ultrasound and low pressure. The action of acoustic waves (ultrasounds) on plant tissue leads to a series of contractions and removal of water, resulting in an effect similar to squeezing a sponge. This treatment method induces the formation of microscopic channels and improves the capillary flow of osmotic solution to intercellular spaces in the material subjected to dehydration (Fernandes et al. 2009). Furthermore, it has been shown that the process of osmotic dehydration with sonification facilitates water diffusion during convective drying. Similar effects were reported by Fernandes and Rodrigues (2011) in the process of drying pineapples, Malay apples, and soapberries.

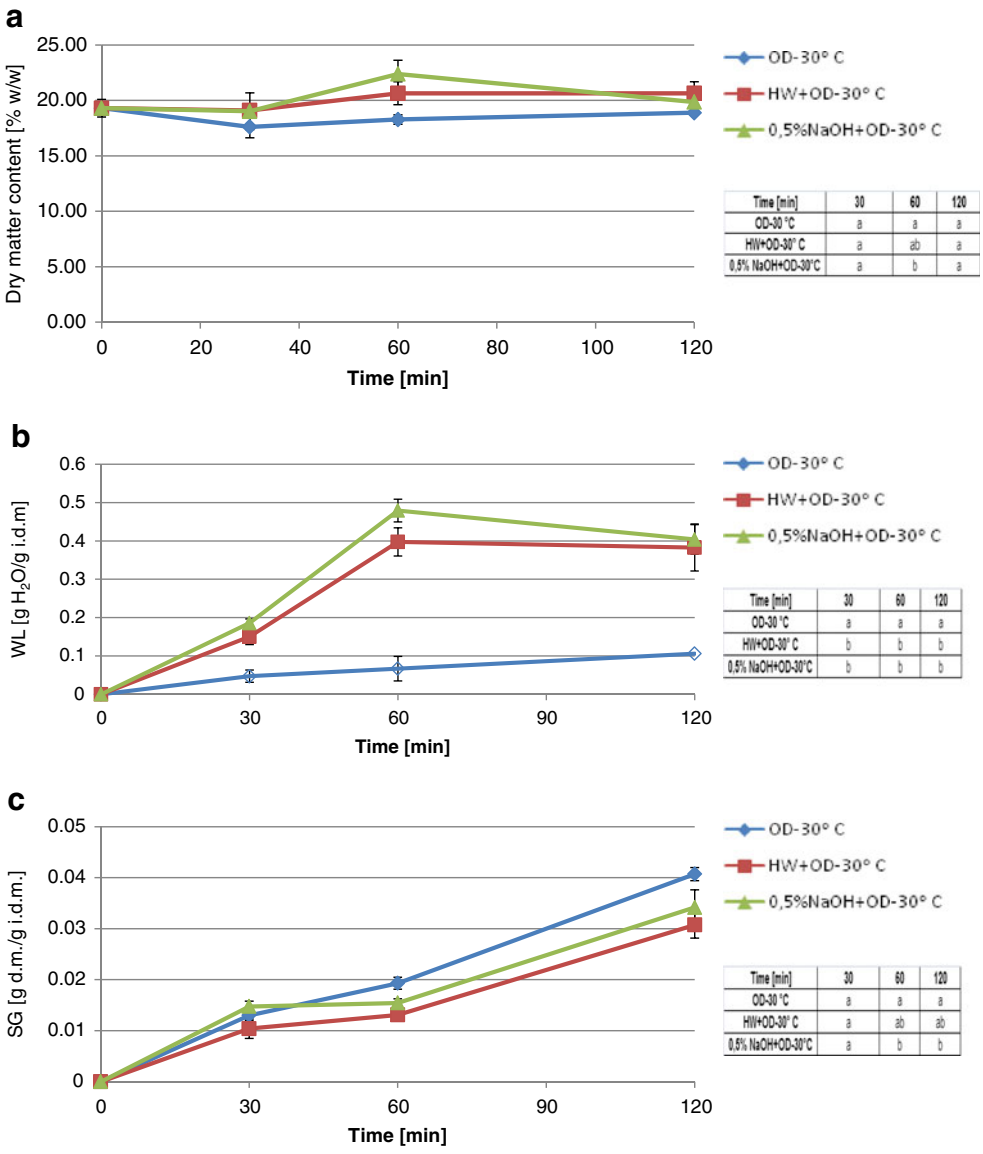


Fig. 2 Changes in dry matter content (**a**), water loss (**b**), and solids gain (**c**) in highbush blueberry fruits during osmotic dehydration at 30 °C: without pretreatment (OD-30 °C); preceded by pretreatment in hot water (HW+OD-30 °C); preceded by pretreatment in hot water and in hot NaOH at 100 °C (HW+NaOH+OD-30 °C). In the tables attached, the same letter in different cells within a given column indicates a lack of statistical differences ($\alpha = 0.05$) between the results at particular processing times

Literature data also confirm that dehydration under low pressure proceeds faster as compared to samples dehydrated under atmospheric pressure. Low pressure pretreatment makes it possible to remove the gas present in the pores of the raw material, which leads to an increased surface of mass transfer and facilitates further dehydration under atmospheric pressure (Rastogi et al. 2002). This method decreases the time of dehydration, thus reducing energy consumption in further technological processes (Deng and Zhao 2008; Janowicz et al. 2008).

In our experiments, the action of ultrasound had no significant effect on dry matter content of blueberries. Ultrasound combined with low pressure only moderately improved osmotic dehydration (Fig. 3a). After 60 and 120 min dry matter content increased by 10 and 13 %, respectively. In both cases, some water migration was observed; the WL level was about 0.6 g H_2O/g i.d.m. after 2 h (Fig. 3b). Solids gain was very low and amounted to approx. 0.04 g d.m./g i.d.m. In the case of using vacuum, WL and SG were more intensive in the first phase of the process as compared to dehydration performed under atmospheric pressure.

From the point of view of increasing dry matter content, a good method of dehydration is using solutions containing pectinolytic enzymes (Fig. 4). After 1 h of dehydration in the presence of enzymatic preparations Pectinex Yield Mash and Rapidase, dry matter content increased by 40.9 and 56.2 %, respectively. After 120 min, dry matter content nearly doubled.

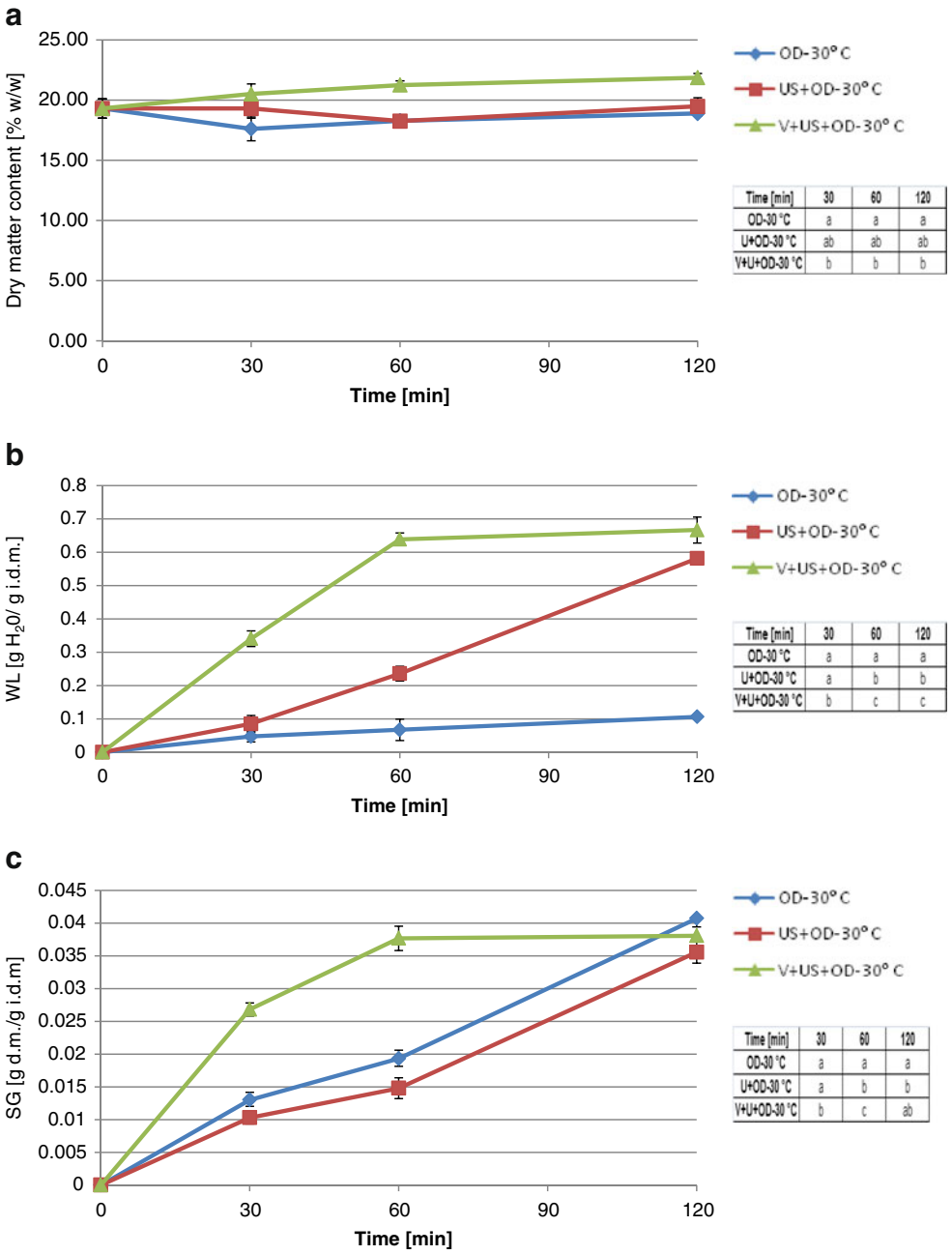


Fig. 3 Changes in dry matter content (**a**), water loss (**b**), and solids gain (**c**) in highbush blueberry fruits during osmotic dehydration at 30 °C: without pretreatment (OD-30 °C); preceded by pretreatment with ultrasound (US+OD-30 °C); preceded by pretreatment with ultrasound and under low pressure (US+V+OD-30 °C). In the tables attached, the same letter in different cells within a given column indicates a lack of statistical differences ($\alpha = 0.05$) between the results at particular processing times

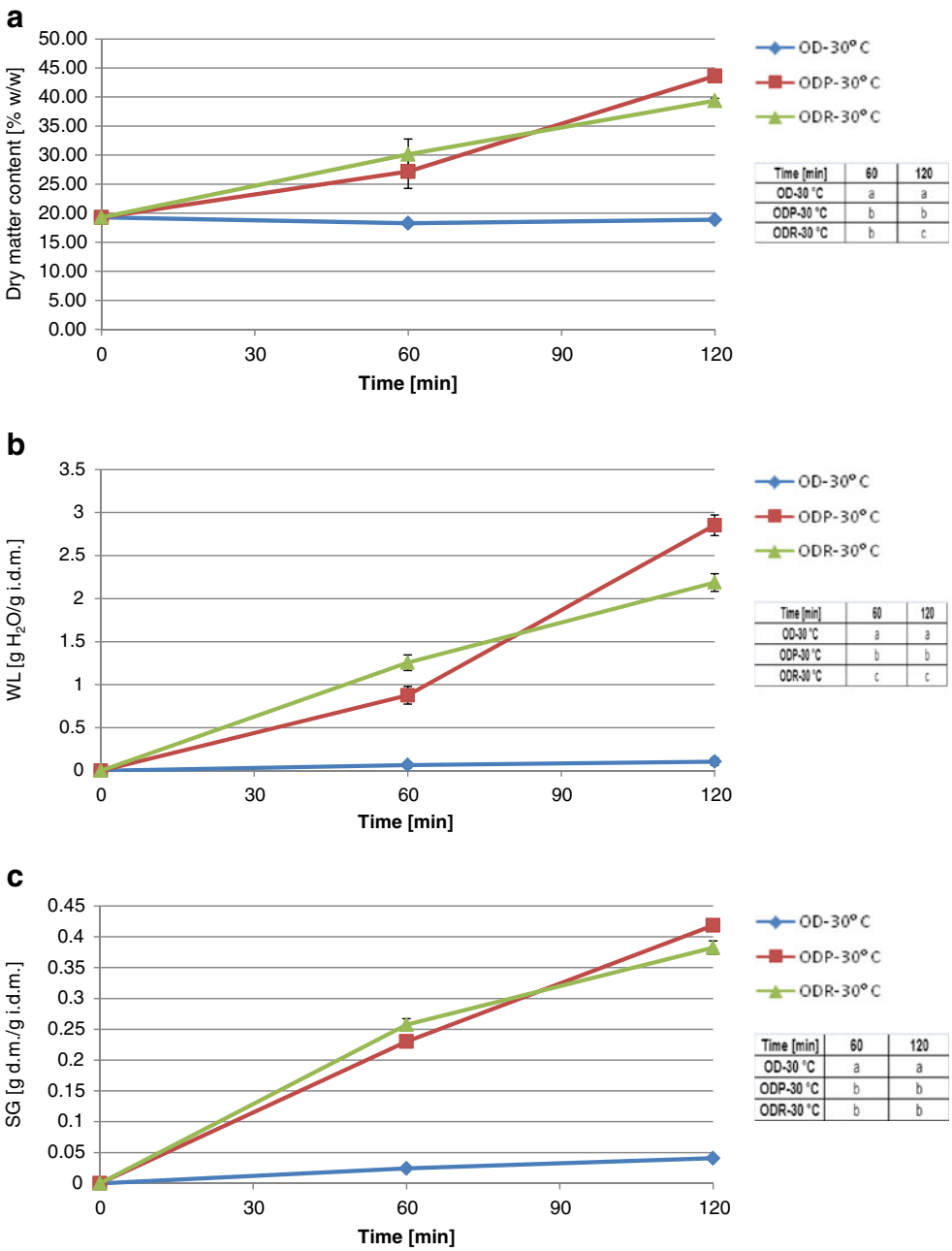


Fig. 4 Changes in dry matter content (**a**), water loss (**b**), and solids gain (**c**) in highbush blueberry fruits during osmotic dehydration at 30 °C (OD-30 °C); in the presence of Pectinex Yield Mash enzyme (ODP-30 °C); and in the presence of Rapidase C-80 Max enzyme (ODR-30 °C). In the tables attached, the same letter in different cells within a given column indicates a lack of statistical differences ($\alpha = 0.05$) between the results at particular processing times

In both cases, considerable water loss was observed. After 2 h of processing, the value amounted to 2.19–2.85 g H_2O/g i.d.m. Solids gain reached a level of 0.38–0.42 g d.m./g i.d.m. However, fruits dehydrated under these conditions were characterized by an unacceptable appearance and texture. The presence of enzymes led to hydrolysis of pectins in the raw material, resulting in the loss of firmness. Osmotic dehydration with solutions containing pectinolytic enzymes was also performed by Grabowski et al. (2007) on cranberries. However, in his case, the kinetics of the process was unsatisfactory, as after 10 h the decrease in water content was 200 g/100 g of dry matter.

Figure 5a shows that the most promising pretreatment method among those tested is the application of lipolytic and pectinolytic enzymes. In this case, the increase in dry matter content after 1 h of dehydration was 26.1 %. The level of water loss (Fig. 5b) was higher than that of solids gain (Fig. 5c). At the same time, both parameters were the highest for this method of pretreatment among the tested ones. When only pectinase or lipase was used, water loss was lower by 40 and 47 %, respectively, and solids gain was lower by 73 and 76 %, respectively, after 1 h of dehydration. The results show that the epidermis of the fruits was disturbed, which facilitated mass transfer. The epidermis of highbush blueberries consists of waxes, cutin, and pectins. A wax layer is present on the fruit surface, visible as a white bloom (Skurtys et al. 2011). The use of lipase catalyzes the

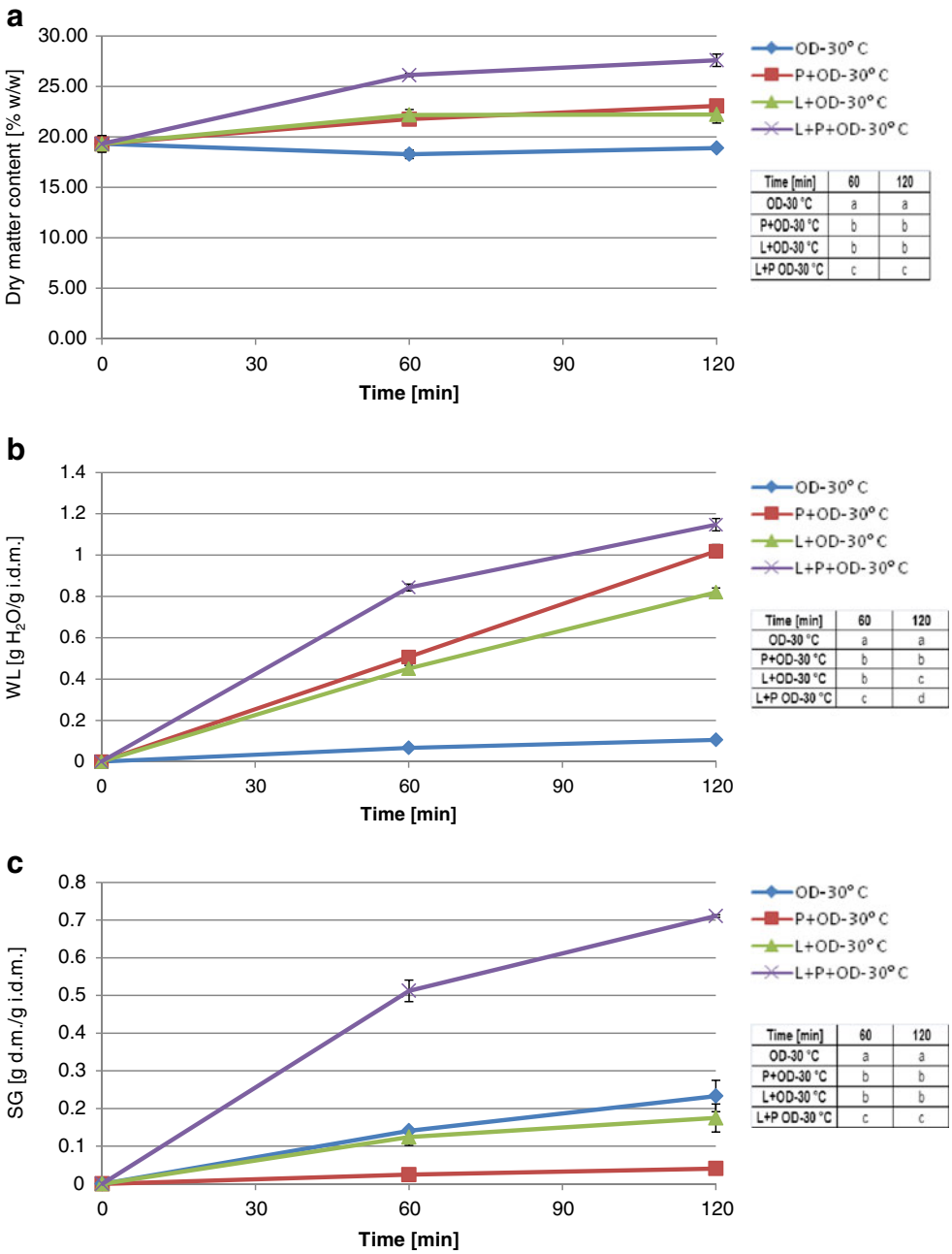


Fig. 5 Changes in dry matter content (**a**), water loss (**b**), and solids gain (**c**) in highbush blueberry fruits during osmotic dehydration at 30 °C: without pretreatment (OD-30 °C); preceded by pretreatment by immersion in a water bath containing: pectinolytic enzyme (P+OD-30 °C); lipolytic enzyme (L+OD-30 °C); pectinolytic enzyme and following a water bath containing lipolytic enzyme (P +L+OD-30 °C). In the tables attached, the same letter in different cells within a given column indicates a lack of statistical differences ($\alpha = 0.05$) between the results at particular processing times

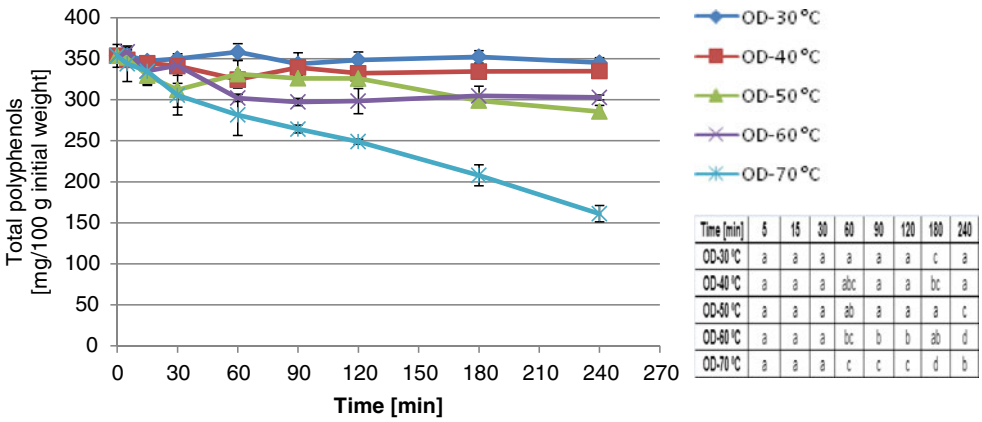


Fig. 6 Changes in total polyphenol content in highbush blueberry fruits during osmotic dehydration (OD) in 65 °Brix sucrose solution (without pretreatment) at different temperatures (30, 40, 50, 60, and 70 °C). In the tables attached, the same letter in different cells within a given column indicates a lack of statistical differences ($\alpha = 0.05$) between the results at particular processing times

Table 1 Total polyphenols in the whole amount of fruits subjected to processing (before dehydration), in the whole amount of processed fruits (after dehydration), and in the whole amount of syrup after dehydration

Before dehydration		After dehydration		Fruits+syrup [mg]
Time [min]	Fruits [mg]	Fruits [mg]	Syrup [mg]	
5	49.53±0.38	49.61±1.30	nd	49.61±1.30
15	49.20±0.47	48.31±1.35	nd	48.31±1.35
30	49.88±0.35	49.37±1.23	nd	49.37±1.23
60	48.04±0.47	48.67±0.93	nd	48.67±0.93
90	49.13±0.85	48.73±1.10	nd	48.73±1.10
120	49.06±0.47	48.38±1.81	0.11±0.02	48.49±1.84
180	48.60±0.05	48.43±0.98	0.13±0.05	48.55±0.93
240	49.26±0.81	48.11±1.64	0.27±0.01	48.38±1.50
OD-40 °C				
5	48.01±1.08	47.36±1.73	nd	47.36±1.73
15	47.60±1.43	46.40±2.19	1.54±0.10	47.93±2.29
30	50.43±2.92	48.64±1.86	1.26±0.11	49.89±1.97
60	51.65±0.49	47.44±3.31	0.92±0.10	48.36±3.21
90	51.56±0.51	49.49±0.92	1.18±0.57	50.67±0.34
120	50.51±2.18	47.46±1.70	2.07±0.29	49.52±1.99
180	52.00±0.61	49.22±3.69	1.61±0.34	50.83±4.03
240	49.07±3.52	46.51±3.75	1.76±0.01	48.27±3.76
OD-50 °C				
5	49.98±0.05	49.71±1.68	nd	49.71±1.68
15	48.63±1.05	45.31±2.33	nd	45.31±2.33
30	48.56±0.68	42.88±4.77	3.30±0.84	46.18±3.93
60	48.26±0.37	45.22±1.96	2.19±0.50	47.40±0.46
90	47.74±1.30	44.05±2.22	2.86±0.70	46.91±3.92
120	48.11±0.50	44.35±0.78	2.03±0.53	46.38±0.24
180	48.41±1.47	40.92±0.31	6.57±1.42	47.48±1.73
240	48.79±0.28	39.42±0.81	6.76±1.05	46.19±2.24
OD-60 °C				
5	47.81±0.90	48.47±1.66	nd	48.47±1.66
15	48.62±0.47	46.11±2.89	nd	46.11±2.89
30	49.13±0.98	47.51±2.60	2.57±0.37	50.07±2.97
60	48.32±0.29	41.28±0.83	2.53±1.15	43.81±1.98
90	48.23±0.24	38.72±1.80	3.97±0.44	44.52±1.23
120	49.62±0.79	41.86±1.47	5.61±0.34	47.47±1.12
180	48.98±0.48	42.24±2.04	7.72±0.02	49.96±2.06
240	49.28±0.40	42.21±0.08	8.61±0.11	50.81±0.19
OD-70 °C				
5	48.33±0.56	47.01±2.43	1.29±0.42	48.30±2.85
15	47.76±0.52	45.21±2.69	2.39±0.04	47.61±2.73
30	46.90±0.59	40.55±2.44	4.90±0.33	45.44±2.11
60	47.68±0.48	37.95±3.00	8.23±0.04	46.19±2.96
90	47.05±1.39	35.17±0.42	13.05±0.66	48.23±0.23
120	47.09±0.03	33.16±0.39	13.92±2.12	47.08±1.73
180	47.14±0.45	27.73±1.96	21.14±1.84	48.87±0.13
240	48.05±0.38	21.90±1.17	26.28±1.11	48.19±2.28

Process conditions: 65 °Brix sucrose solution at 1:4 fruit to syrup ratio, without pretreatment, temperature: 30–70 °C

nd not detected

cleavage of ester bonds in the waxes, facilitating the migration of water from the material. Given the other results presented in Fig. 5, preliminary immersion of the fruits in a bath with lipolytic enzymes alone is not so effective.

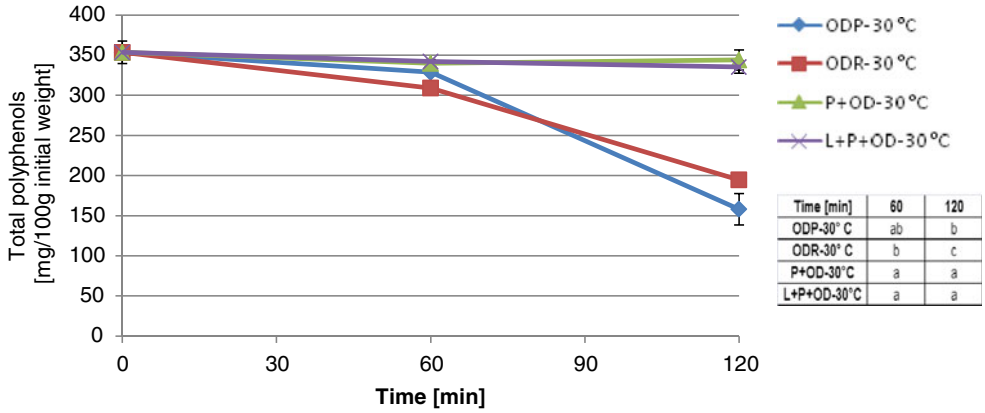


Fig. 7 Changes in total polyphenol content in osmotically dehydrated fruits: *a* during dehydration in the presence of pectinolytic enzymes: Pectinex Yield Mash (ODP-30 °C), Rapidase C-80 Max (ODR-30 °C), *b* during dehydration preceded by pretreatment with pectinolytic enzyme (P+OD-30 °C); c) during dehydration preceded by pretreatment with lipolytic and pectinolytic enzymes (P+L+OD-30 °C). In the tables attached, the same letter in different cells within a given column indicates a lack of statistical differences ($\alpha = 0.05$) between the results at particular processing times

Additional hydrolysis of pectins improves the conditions of mass transfer, especially that it facilitates the diffusion of osmotic agent to the fruits, as can be seen from data on solids gain (Fig. 5c). It needs to be remembered that dehydration of fruits pretreated with enzymatic solutions is not accompanied by any unfavorable changes in shape or texture, which shows that apparently cell membranes are not damaged.

The Influence of Pretreatment and Osmotic Dehydration on Polyphenol Content in Blueberries

The present experiments showed that the dehydration process affected total polyphenol content in highbush blueberry fruits (Fig. 6) and that it largely depended on the process temperature. After 1 h of dehydration, the greatest loss in polyphenol content (20.4 %) was observed at the highest temperature (70 °C), while a temperature of 60 °C led to a loss of 14.6 %. The smallest losses (↓5 %) were observed at 30 °C. Polyphenol content decreased also with time of dehydration. Thus, data show that the intensification of dehydration through increasing temperature will occur at the cost of polyphenol content. This can be explained by higher migration of phenolic compounds to the dehydrating solution as a result of higher temperature (an increase in temperature leads to a rise in the diffusion flow rate, and high temperature also hampers the selectivity of cell membranes; Lewicki et al. 1998). The fact that migration played a substantial role in decreasing polyphenol content is confirmed by data presented in Table 1, which

Table 2 Total polyphenols in the whole amount of fruits subjected to processing (before dehydration), in the whole amount of processed fruits (after dehydration), and in the whole amount of syrup after dehydration

Before dehydration		After dehydration		Fruits+syrup [mg]
Time [min]	Fruits [mg]	Fruits [mg]	Syrup [mg]	
60	54.02±0.46	47.23±1.64	2.71±0.25	49.95±1.89
120	54.84±0.91	30.20±0.22	24.13±0.75	54.33±0.96
ODR-30 °C				
60	53.36±0.34	49.62±1.95	3.02±0.85	52.64±1.11
120	54.30±0.17	24.26±2.60	29.12±0.50	53.38±3.11
P+OD-30 °C				
60	53.12±0.45	51.06±0.40	1.53±0.24	52.59±0.16
120	52.03±0.38	50.62±0.56	2.30±0.25	52.92±0.80
L+P+OD-30 °C				
60	53.18±0.38	51.40±0.60	1.95±0.43	53.35±0.17
120	53.74±0.58	50.62±0.51	3.20±0.39	53.82±0.91

Process conditions: 65 °Brix sucrose solution at 1:4 fruit to syrup ratio, at 30 °C, pretreatment with pectinolytic enzyme (P+OD-30 °C) or with lipolytic and pectinolytic enzymes (P+L+OD-30 °C), or without pretreatment but with dehydration in the presence of pectinolytic enzymes [Pectinex Yield Mash (ODP-30 °C) or Rapidase C-80 Max (ODR-30 °C)]

Table 3 Identification of anthocyanins in blueberry fruits using mass spectroscopy

Peak	Anthocyanin	t_R (min)	$[M]^+$ (*m/z*)	MS/MS (*m/z*)
1	Delphinidin-3-O-galactoside	3.7	465	303
2	Delphinidin-3-O-glucoside	4.1	465	303
3	Cyanidin-3-O-galactoside,	4.8	449	287
4	Cyanidin-3-O-glucoside	5.3	449	287
5	Petunidin-3-O-galactoside,	5.8	479	287
6	Petunidin-3-O-glucoside	6.5	479	317
7	Petunidin-3-O-arabinoside	7.5	449	317
	Peonidin-3-O-galactoside		463	301
8	Peonidin-3-O-glucoside,	8.2	463	301
9	Malvidin-3-O-galactoside	8,6	493	331
10	Malvidin-3-O-glucoside	9.5	493	331
11	Malvidin-3-O-arabinoside	10.6	463	331
12	Malvidin+pentose	14.1	463	331
13	Malvidin+acetoyl+hexose (I)	14.8	535	331
14	Malvidin+acetoyl+hexose (II)	15.5	535	331
15	Malvidin+acetoyl+hexose (III)	18.5	535	331

t_R retention time

shows the balance of total polyphenols in fruits and syrups before and after dehydration. As can be seen, after dehydration syrups contained some amounts of polyphenolic substances, especially at higher temperatures; 13.8, 17.5, and 54.7 % of total polyphenols present in blueberries before dehydration migrated to syrups after 4 h of processing at 50, 60, and 70 °C, respectively. Results obtained by Devic et al. (2010), who osmotically dehydrated apple cubes, also confirm that the retention of phenolic compounds drops with increasing temperature. Polyphenols may also migrate (under special circumstances) into the fruits. The use of grape seed extract as an osmotic solution in the process of dehydrating apples and bananas led to the infusion of phenolic compounds into the dehydrated material (Różek et al. 2010).

What is interesting is that a comparison of total polyphenols in the whole system (fruits+syrup) before and after dehydration indicates that, even at higher temperatures, blueberry polyphenols were characterized by a relatively high stability (Table 1). After 4 h of dehydration at 30–70 °C, the loss of polyphenols caused by degradation was 5.3 % at the most.

The most substantial drop in total polyphenol content was observed during osmotic dehydration with pectinolytic enzymes (Fig. 7). After 120 min, the content of phenolic compounds nearly halved. This can be explained by the enzymatic degradation of pectin in cell walls, which led to increased transfer of vacuolar sap to the osmotic solution, also causing substantial polyphenol migration.

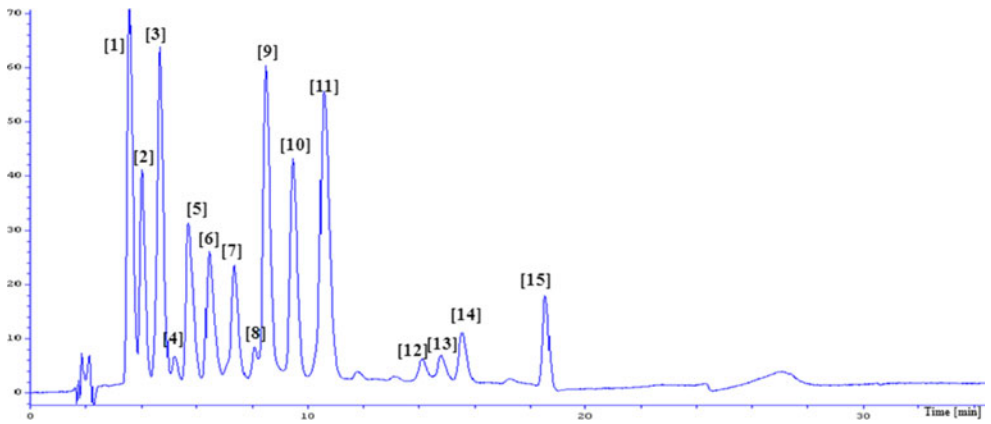


Fig. 8 HPLC chromatogram of blueberry fruits with detection at 520 nm. Compounds: *[1]* delphinidin-3-galactoside, *[2]* delphinidin-3-glucoside, *[3]* cyanidin-3-galactoside, delphinidin-3-arabinoside, *[4]* cyanidin-3-glucoside, *[5]* petunidin-3-galactoside, cyanidin-3-arabinoside, *[6]* petunidin-3-glucoside, *[7]* peonidin-3-galactoside and petunidin-3-arabinoside, *[8]* peonidin-3-glucoside, malvidin-3-galactoside, *[9]* malvidin-3-galactoside, *[10]* malvidin-3-glucoside, *[11]* malvidin-3-arabinoside, *[12]* malvidin-3-O-pentoside, *[13]* malvidin+acetoyl+hexose (I), *[14]* malvidin+acetoyl+hexose (II), *[15]* malvidin+acetoyl+hexose (III)

Table 4 The content of selected anthocyanins in highbush blueberry fruits after osmotic dehydration (OD) at different temperatures (30, 40, 50, 60, and70 °C) in 65 °Brix sucrose solution for 1 h without pretreatment or pretreated by immersion in a water bath containing lipase (30 min) and pectinase (30 min)—dehydration at 30 °C in 65 °Brix sucrose solution (P+L+OD-30 °C)

Compound	Fresh [mg/100 g i.w.]	OD-30 °C [mg/100 g i.w.]	OD-40 °C [mg/100 g i. w.]	OD-50 °C [mg/100 g i.w.]	OD-60 °C [mg/100 g i. w.]	OD-70 °C [mg/100 g i.w.]	L+P+OD-30 °C [mg/100 g i. w.]
Delphinidin-3-galactoside	15.71±1.31	9.29±0.18	10.22±1.30	7.83±1.31	8.94±0.43	8.94±1.20	10.04±0.49
		(59.1)	(65.0)	(49.8)	(56.9)	(56.9)	(63.9)
Delphinidin-3-glucoside	8.52±0.37	6.65±0.13	7.30±1.05	6.87±1.79	6.83±0.11	6.62±0.83	7.13±0.70
		(78.0)	(85.8)	(80.7)	(80.2)	(77.6)	(83.7)
Cyanidin-3-galactoside	17.43±1.12	12.42±0.24	12.61±1.51	10.97±2.10	11.27±0.46	11.87±1.11	12.38±0.25
		(71.3)	(72.4)	(63.0)	(64.7)	(68.1)	(71.1)
Cyanidin-3-glucoside	1.44±0.11	1.26±0.02	1.28±0.20	1.21±0.29	1.29±0.04	0.86±0.04	1.14±0.07
		(87.3)	(88.6)	(83.9)	(89.6)	(59.5)	(79.0)
Petunidin-3-galactoside	7.78±0.10	7.46±0.14	7.06±0.56	6.50±0.80	6.68±0.27	6.24±0.19	7.21±0.24
		(95.9)	(90.7)	(83.6)	(85.9)	(80.2)	(92.7)
Petunidin-3-glucoside	6.47±0.03	6.48±0.12	5.92±0.59	5.76±1.01	6.11±0.28	5.09±0.68	6.25±0.57
		(100.1)	(91.5)	(89.0)	(94.4)	(78.7)	(96.6)
Peonidin-3-glucoside	1.48±0.02	1.26±0.02	0.87±0.09	0.78±0.22	0.74±0.05	0.90±0.17	1.08±0.02
		(84.9)	(59.1)	(53.0)	(49.7)	(60.7)	(72.77)
Malvidin-3-galactoside	19.23±1.07	13.99±0.27	13.59±0.81	12.47±1.79	15.14±0.73	12.73±2.46	14.12±1.73
		(72.7)	(70.65)	(64.84)	(78.7)	(66.2)	(73.4)
Malvidin-3-glucoside	13.62±0.72	10.02±0.19	10.88±0.30	10.11±1.54	11.63±0.08	10.26±1.25	10.72±1.12
		(73.5)	(79.9)	(74.2)	(85.3)	(75.4)	(78.7)
Malvidin-3-O-arabinoside	20.87±0.41	16.42±0.31	15.25±0.16	14.96±2.23	17.44±0.67	14.73±2.31	16.95±0.62
		78.69	(73.1)	(71.7)	(83.5)	(70.6)	(81.2)
Malvidin+acetoyl+hexose (III)	4.68±0.33	3.16±0.06	3.09±0.13	3.10±0.59	3.65±0.52	2.82±0.17	3.37±0.26
		(67.6)	66.24	(66.3)	(78.2)	(60.4)	(72.1)

In brackets, the anthocyanin retention [%] relative to the fresh material

i.w. initial weight

Table 5 Amounts of selected polyphenols: in the whole amount of fruits subjected to processing (before dehydration), in the whole amount of processed fruits (after dehydration), and in the whole amount of syrup after dehydration

Before dehydration		After dehydration		Fruits+syrup [mg]
Time [min]	Fruits [mg]	Fruits [mg]	Syrup [mg]	
Delphinidin-3-O-galactoside				
OD-30 °C	1.72±0.01	1.64±0.08	nd	1.71±0.12
OD-40 °C	1.79±0.02	1.67±0.17	nd	1.67±0.17
OD-50 °C	1.67±0.01	1.43±0.02	0.20±0.00	1.64±0.02
OD-60 °C	1.68±0.01	1.40±0.20	0.20±0.00	1.61±0.20
OD-70 °C	1.65±0.02	1.21±0.17	0.35±0.00	1.56±0.22
L+P-OD-30 °C	1.84±0.01	1.51±0.06	0.22±0.00	1.73±0.07
Cyanidin-3-O-galactoside				
OD-30 °C	2.06±0.02	1.77±0.03	0.17±0.01	1.94±0.04
OD-40 °C	2.14±0.02	1.81±0.05	0.15±0.00	1.96±0.04
OD-50 °C	1.80±0.02	1.50±0.00	0.25±0.00	1.74±0.00
OD-60 °C	2.01±0.01	1.54±0.00	0.31±0.00	1.85±0.01
OD-70 °C	1.98±0.02	1.61±0.05	0.79±0.00	2.00±0.51
L+P-OD-30 °C	2.21±0.02	1.91±0.05	0.31±0.00	2.07±0.17
Petunidin-3-*O*-galactoside				
OD-30 °C	1.09±0.01	0.99±0.02	0.03±0.00	1.02±0.02
OD-40 °C	1.14±0.01	1.09±0.03	0.03±0.00	1.12±0.03
OD-50 °C	1.06±0.01	0.78±0.00	0.06±0.00	0.84±0.00
OD-60 °C	1.06±0.01	0.92±0.00	0.07±0.00	0.99±0.00
OD-70 °C	1.05±0.01	0.84±0.03	0.34±0.01	1.01±0.21
L+P-OD-30 °C	1.17±0.01	1.05±0.03	0.09±0.00	1.09±0.04
Malvidin-3-*O*-arabinoside				
OD-30 °C	2.25±0.02	2.15±0.03	nd	2.15±0.03
OD-40 °C	2.35±0.02	2.39±0.07	nd	2.39±0.07
OD-50 °C	2.20±0.02	2.03±0.02	0.22±0.01	2.25±0.03
OD-60 °C	2.20±0.01	1.97±0.03	0.25±0.01	2.22±0.02
OD-70 °C	2.17±0.02	1.77±0.03	0.39±0.03	2.16±0.06
L+P-OD-30 °C	2.42±0.02	2.12±0.05	0.21±0.03	2.33±0.03

Process conditions: 65 °Brix sucrose solution at 1:4 fruit to syrup ratio, without pretreatment, 1 h of dehydration at 30 –70 °C or pretreatment with lipolytic and pectinolytic enzymes (P+L +OD-30 °C), 1 h of dehydration at 30 °C

nd not detected

The best method of blueberry pretreatment prior to dehydration seems to be the use of pectinolytic and lipolytic preparations. This method is characterized both by low losses of polyphenolic compounds (resulting mainly from migration which follows from the balance of polyphenols presented in Table 2) as compared to fresh material (5.1 % after 1 h of dehydration; Fig. 7) and by a substantial increase in dry matter content after dehydration (26.1 % after 1 h of dehydration, Fig. 5a).

Considering the above results, pretreatment with pectinolytic and lipolytic enzymes may have practical implications, as it enables the intensification of the dehydration–impregnation process without inducing substantial losses in phenolic compounds while preserving the acceptable shape of the fruits (no softening of the fruits was observed). The considerable increase in solids gain after using both enzymes increases possibilities for the fortification of osmo-dried products with desirable substances (for example oligosaccharides; Matusek et al. 2008) from a hypertonic solution.

An important class of polyphenolic compounds in highbush blueberry fruits are anthocyanins (Gu et al. 2003, 2004; Wu and Prior 2005). According to literature data, in highbush blueberry fruits, anthocyanins are found mostly in the skin, while the pulp contains only small or trace amounts of these compounds (Riihinen et al. 2008). Furthermore, the content of particular anthocyanins in highbush blueberry fruits varies substantially, depending on the cultivar and place of cultivation (Connor et al. 2002; Łata et al. 2005; Lohachoompol et al. 2008). Due to the large range of monoglycosides and the fact that they may undergo acylation, 14 different forms of anthocyanins have been identified in highbush blueberry fruits (Lohachoompol et al. 2008). Cho et al. (2004) reported the following distribution of particular monomers: delphinidin (27–40 %), malvidin (22–33 %), petunidin (19–26 %), cyanidin (6–14 %), peonidin (1–5 %), and acylated forms (9 %). The MS experiments conducted in this study (Table 3) indicated the presence of 16 anthocyanins (the HPLC profile shown in

Fig. 8 presents 15 peaks of anthocyanins; one of them corresponds to two compounds). The most abundant ones included monoglycosides of malvidin (58.4 mg/100 g), delphinidin (24.2 mg/100 g), and cyanidin (18.8 mg/100 g) (Table 4). The retention of particular anthocyanins depended on osmotic dehydration conditions. The average levels of particular anthocyanins in the dehydrated material (after 1 h) amounted to at least 50 % as compared to fresh material. The lowest levels of anthocyanin compounds were observed in fruits dehydrated at 70 °C, with the retention ranging from 57 to 80 %. Irrespective of the applied osmotic dehydration temperature, the retention of the following compounds was the highest: petunidin-3-galactoside (minimum 80 % retention at 1 h) and petunidin-3-glucoside (minimum 78 %). The study also examined changes in particular polyphenols during osmotic dehydration following fruit pretreatment with lipolytic and pectinolytic enzymes. The experiments showed that anthocyanin retention was at least 64 % (after 1 h of dehydration). The highest anthocyanin retention was found for petunidin-3-glucoside (95.4 %) and petunidin-3-galactoside (92.3 %). The balance of some selected anthocyanins (occurring in considerable amounts in blueberries) shown in Table 5 indicates that migration to a hypertonic solution rather than degradation is the major cause of decreasing the anthocyanin levels in the fruits processed under the tested conditions.

Conclusions

The factors influencing osmotic dehydration of highbush blueberry fruits include time and temperature, as well as the fruit pretreatment method. At 30–50 °C, dehydration is not very effective, while the application of higher temperatures leads to substantial losses of phenolic compounds in the dehydrated material (30 % after 2 h of dehydration at 70 °C). Initial immersion of fruits in pectinolytic and lipolytic enzymes leads to a greater increase of dry matter content (26.1 %) with a phenolic retention of 96 % and with the retention of individual anthocyanins amounting to at least 64 % after 1 h of dehydration at 30 °C.

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