Effect of Processing and Storage on the Content of Selected Antioxidants and Quality Parameters in Convection and Freeze-dried Bilberry (*Vaccinium myrtillus* L.)

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Abstract**—** *Fresh and dried by convection and lyophilisation bilberries (Vaccinium myrtillus L.) were examined for content of water, vitamin C, vitamin E, total anthocyanins, total polyphenols and level of antioxidant activity against the DPPH radical. Both products were analyzed immediately after drying and after 4, 8 and 12 months of storage at 2 ± 1 ºC and 20 ± 2 ºC. Fresh fruit had a dry matter content of 14.74%, 100 g of which contained 208 mg vitamin C; 36.4 mg vitamin E; 2064 mg anthocyanins; and 5000 mg polyphenols, with antioxidant activity of 1750 µM Trolox eq/1 g dry matter. After 12 months' storage, retention rates in products dried by convection and sublimation were respectively: vitamin C 38–47% and 49–53%; vitamin E 73–77% and 76–82%; anthocyanins 47–54% and 55–59%; polyphenols 68–74% and 72–74%; and antioxidant activity 63–70% and 73–77%, the lower value referring to storage at room temperature and the higher to chilled storage. There were no effect of storage temperature on rehydration of both products. Storage led to a significant increase in the proportion of red and yellow colors in both types of dried product compared to the raw material and product after drying.*

Keywords**— *antioxidants, bilberry, convection drying, freeze-drying, storage, quality.***

I. INTRODUCTION

Fruits are recognized as an excellent source of antioxidants in the human diet. These substances form a large group, which comprises polyphenols (including anthocyanins), vitamins C and E and β-carotene [1, 2]. Many studies have shown a correlation between the consumption of antioxidants and decreased risk of cardiovascular disease and some cancer types [3, 4]. In view of the seasonal availability of fresh fruits, there is a need to find relatively inexpensive methods of preservation that will result in a product with a similar nutritive value to that of the raw material.

Although dried fruits have long been a part of the human diet, there is little in the literature on the levels of antioxidant compounds they contain, especially in the case of wild fruits Borowska *et al*. [5]. One such species known for its high levels of antioxidants is bilberry (*Vaccinium myrtillus* L.) Prior *et al*. [6] a low-growing shrub found in forest undergrowth in temperate regions whose berries have traditionally been consumed directly as well as processed for later use. Dried bilberries are also used in herbal medicine for their anti-diarrhoeal and anti-inflammatory properties, and their ability to reduce the permeability of blood vessels, improve night vision and increase the adaptive capacity of the eye to darkness [7].

Convection drying (by air circulation) is more widely used in industrial processing than freeze-drying due the high costs of the latter, both in terms of equipment and the process itself. Although convection drying is a cheaper process, the resulting product is less abundant in nutritive compounds and more difficult to rehydrate owing to the higher drying temperature and intensive aeration of the material, among other factors [8]. Apart from the drying method applied, the quality of the dried product may also be affected by the conditions and length of storage, two factors which have received little attention in the literature.

The aim of this paper was therefore to compare convection dried and freeze-dried bilberries in terms of the antioxidant content in each product and the extent to which antioxidant levels are affected by the conditions and length of storage.

II. MATERIAL AND METHOD

2.1 Material

The experimental material consisted of whole and sound wild forest bilberries of uniform size gathered at consumption maturity. The fruits were washed and dried in circulating air. Representative samples were then taken to determine the level of the selected indicators in the raw material. The remaining fruits were divided into two batches, one each for convection and freeze-drying. For convection drying, electric dryers designed for drying fruits, vegetables and mushrooms (Zorpot

Zalmet, Poland) were used. The process was carried out at 60 ºC for 10 hours to a moisture content of about 10%. For freeze-drying, bilberries were first frozen at -40 ^{o}C in a Feutron 3626-51 climatic chamber (ILKA Feutron, Germany). Next, sublimation was performed using a Gamma 1-16 LSC freeze dryer (Christ, Germany). The process was conducted under the following parameters: initial temperature of the frozen raw material: -30^{o}C; condenser temperature: -52^{o}C, shelf temperature: +20^{o}C; duration of secondary drying: 6 hours; shelf temperature: +30^{o}C. The overall time required to achieve a water content of less than 3% using this method was 36 hours. Immediately after drying, the berries in each separate type of dried product (convection and freeze-dried) were thoroughly mixed, placed in hermetic plastic containers, left for 7 days to allow for any equilibration of humidity, and mixed once more. Next, the containers were opened in conditions of low humidity in order to collect samples for analysis of indicators of chemical composition and to determine rehydration ability at the stage described in this work as "immediately after drying". The remaining dried product was then packed in twist off jars, divided into two groups and stored without exposure to light. One group was placed in chilled storage (2 ± 1^{o}C) and the other stored at room temperature (20 ± 2^{o}C).

2.2 Chemical analysis

The selected indicators were determined in the raw material, in products immediately after drying and after 4, 8 and 12 months of storage. Water content was established by the oven method [9], vitamin C and E content using high performance liquid chromatography (HPLC) [10, 11]. Anthocyanins were quantified according to the procedure described by Plessi et al. [12], the results being calculated using a cyanidin-3-glu equivalent [13]. Polyphenols were determined by the Folin-Ciocalteu spectrophotometric method [14], while antioxidant activity was measured by means of the DPPH (2,2-diphenyl-1-picryhydrazyl) assay [15]. Immediately after production and after 12-month storage, dried products were also examined for water absorption ability [16] as well as for color by an instrumental method with a Minolta CM-3500d spectroscope setting L*a*b* parameters. Analyses were made in four replications. The results were statistically evaluated using single-factor analysis of variance. Standard deviation and correlation coefficients were calculated for the results obtained.

III. RESULTS AND DISCUSSION

Antioxidant levels in fresh fruits, including bilberries, have been broadly discussed in the literature [6, 17-20]. However, there are few works concerned exclusively with preserved products, including dried fruits [5, 8]. Vitamin C, regarded as a fundamental antioxidant in fruits [21], is susceptible to degradation by high pH, increased temperatures, exposure to light and the presence of oxygen, enzymes and such metals as iron and copper [22]. It has been observed that good L-ascorbic acid retention during technological treatment is accompanied by similar retention of other nutritive compounds [21]. The level of vitamin C may therefore be an indicator of the degradation of other biologically active substances.

Fresh bilberry contained 208 mg vitamin C/100 g dry matter (30.7 mg/100 g fresh matter) (Table 1). The values reported by Borowska *et al*. [5] were 28% and 50% lower respectively, which may be explained either by the raw material being harvested in a different location or differences in the freshness of the material.

Drying caused significant vitamin C loss in both convection and freeze dried berries: 35% and 25% respectively. This confirms the findings of Asami *et al*. [23], who noted that in strawberry and American cultivars of blackberry, freeze-drying resulted in better L-ascorbic acid retention than other drying methods, this being attributed to lack of oxygen and lower temperature. Borowska *et al*. [5] recorded vitamin C losses of as much as 80% in convection dried bilberries, however, Ramesh *et al*. [24] reduced vitamin C loss by 13% through using neutral gas instead of air in the convection drying of paprika.

Vitamin C content fell steadily throughout the 12 month period of storage at both storage temperatures, although at every stage of evaluation, the freeze-dried product contained significantly more vitamin C than convection dried berries. In addition, vitamin C levels were higher in products stored at the lower temperature. After 12 months of storage, vitamin C retention compared with the raw material was 38–47% in the convection dried product and 49–53% in the freeze-dried product; and 58–73% and 65–71% respectively compared with the product immediately after drying (the two values refer to the higher and lower storage temperature respectively).

Vitamin E, which comprises a number of tocopherol- and tocotrienol-derived compounds, is subject to degradation from exposure to oxygen and UV radiation and the presence of iron [25]. Vitamin E content in fresh bilberries was 36.40 mg/100 g dry matter, (5.37 mg/100 g FM) (Table 1). According to Chun *et al*. [26] the edible part of the bilberry had 1.05 mg total tocopherols per 100 g, while Piironen *et al*. [27] found that berry fruits contained 0.56–4.14 mg α-tocopherol per 100 g FM. The drying process caused significant though not large losses in vitamin E content compared with the raw material: 13% in the convection dried product and 8% in the freeze-dried product. Karatas and Kamışlı [28] examined vitamin content in apricots after microwave and radiation drying, and showed that the shorter exposure to high temperature in microwave drying resulted in better vitamin E retention. Daood *et al*. [29] comparing natural drying of paprika under ambient conditions with forced-air dehydration, showed that the former method led to higher losses of α-tocopherol. Vitamin E loss after 12 months' storage was significant; levels in convection and freeze dried products were 73–77% and 76–82% respectively of those found in the raw material and 83–88% and 83–89% of those in the product immediately after drying (the two values refer to higher and lower storage temperature respectively). Chun *et al*. [26] found that after industrial drying, peaches, pears, and plums contained respectively 75%, 76% and 48% of the vitamin E levels in fresh fruits, although there is no information concerning the conditions and length of storage. Daood *et al*. [29] who examined convection dried and comminuted paprika, observed falls in α-tocopherol content of 70%, 90% and 100% in products stored for 30, 60 and 90 days respectively.

Fruits owe their color to the presence of anthocyanin and carotenoid pigments and rarely to chlorophyll pigments [30]. Fresh bilberries contained 2064 mg anthocyanins per 100 g dry matter (304.2 mg/100 g FM) (Table 1), which compares with the findings of Borowska and Szajdek [17] (214.7 mg/100 g FM); Prior *et al*. [6] (299.6 mg/100 g FM); and Lätti *et al*. [17] (411.0 mg/100 g FM). Drying resulted in significant decreases in anthocyanin content of 32% and 20% in convection and freeze-dried products respectively compared with the raw material. This confirms the findings of Mejia-Meza *et al*. [31] who determined anthocyanin content in highbush blueberry after convection, sublimation, and vacuum-microwave drying to a water content of 3–5%. Their results showed that anthocyanin retention was highest in freeze-dried and lowest in convection dried products. Other studies involving the convection drying of highbush blueberry reported anthocyanin losses of up to 69% and 41%, the latter reducing the drying time by half [32, 33]. Storage of dried products led to further losses in anthocyanins. At every stage of the experiment, anthocyanin levels were statistically higher in freeze-dried than in convection dried products, assuming that both products were stored at an identical temperature, as well as being higher in chilled products than in those stored at room temperature. After 12 months of storage, anthocyanin retention in convection and freeze-dried products compared with the raw material was 47–54% and 55–59% respectively, and 70–79% and 69–74% compared with the product immediately after drying (the two values refer to storage at 20 ± 2 ºC and 2 ± 1 ºC respectively). The stability of anthocyanins depends on their structure, capacity for copigmentation, ability to bond sugar molecules, and acylation with acids [34, 35]. A combination of higher temperatures and aeration creates a medium conducive to the activity of enzymes present in fruits, which in turn leads to the partial loss of anthocyanins [31]

Polyphenols, which include anthocyanins among their main components, form one of the principal groups of plant secondary metabolites. Berry fruits are characterized by high polyphenol content [36]. In fresh bilberry total polyphenols amounted to 5000 mg/100 g dry matter (737 mg/100 g FM), although considerably lower levels of 525 mg/100 g and 429 mg/100 g FM were reported by Prior *et al*. [6] and Wolfe *et al*. [20] respectively. Convection and freeze-drying caused moderate though still significant reductions in polyphenol content of 23% and 20% respectively compared with the raw material. Borowska *et al*. [5] reported losses in convection dried bilberries of 50%, over twice the level found in the present study. Further slight losses in total polyphenols were observed throughout the 12-month storage period, becoming significant only after 8 months. The effect of both the drying method and lower temperature was not always proved statistically. After 12 months' storage, polyphenol retention in convection and freeze-dried products was 68–74% and 72–74% respectively compared with the raw material, and 88–95% and 90–92% compared with the product immediately after drying (the two values refer to storage at 20 ± 2 ºC and 2 ± 1 ºC respectively).

The level of antioxidant activity depends on the fruit species, cultivation conditions, length of storage and method of measurement [1, 6, 37]. Antioxidant activity in fresh bilberries was 1750 µM Trolox eq/1 g dry matter (258 µM Trolox eq/1 g FM). Katsube *et al*. [18] using an identical method, reported a value of 288 µM Trolox eq/g, while Prior *et al*. [6], who applied the oxygen radical absorbance capacity (ORAC) method, recorded 44.6 µM Trolox eq/g in an extract of bilberries. Convection and freeze-drying caused slight (10% and 6% respectively) reductions in antioxidant activity; product storage, however, led to larger losses, becoming significant after only 4 months. Antioxidant activity was higher in freeze-dried than in convection-dried products at all stages of the experiment. The lower storage temperature was found to have a beneficial effect, although the difference was not always proved statistically. After 12 months of storage, antioxidant activity in convection and freeze-dried products was lower by 63–70%, and by 73–77% compared to the raw material, and by 71–78% and by 77–82% compared to the product immediately after drying (the two values refer to storage at 20 ± 2 ºC and 2 ± 1 ºC respectively).

The content of vitamin C, polyphenols, and anthocyanins in fresh berry fruits was positively correlated with the level of antioxidant activity [1, 37, 38]. In comparison with other fruit species, extracts of bilberry had the highest amounts of polyphenols and anthocyanins [18, 39, 40]. Hence, Wang *et al*. [2] reported that vitamin C did not account for more than 15%

of total antioxidant activity, with an even lower proportion found by Prior *et al*. [6], depending on the fruit species. Our results showed that for dried bilberry products stored for 12 months, the correlation coefficients calculated between antioxidant activity and polyphenols, anthocyanins, and vitamin C were 0.838, 0.927 and 0.938 respectively, regardless of the drying method or storage temperature.

The water content in the raw material affects the yield of the dried product, its quality and tendency to mould. Fresh bilberries contained 85.26 g water per 100 g. Convection drying removed 98.3% of water, resulting in a product with a water content of 9.13 g/100g immediately after production. In freeze-drying the respective values were 99.5% and 2.67 g/100g. Therefore, both products conformed to the methodical assumptions of this research with water contents after 12-month storage of 9.51–9.79 g in the convection dried product and 2.86–3.10 g/100 g in the freeze-dried product.

Good rehydration properties are an essential characteristic of quality in dried products [41]. Krokida and Maroulis [42] found that when apples, bananas, carrots and potatoes were dried using five different methods, freeze-drying resulted in the highest porosity and natural drying in the lowest, which agrees with our findings. Immediately after drying, 100 g of convection dried bilberries absorbed 210 cm^3 of water, while freeze-dried berries absorbed 38% more (Table 2). This tendency remained unchanged after 12 months of storage, although absorption power decreased by 3–8% and 8–13% in convection and freeze-dried products respectively (the two values refer to storage at 20 ± 2 ºC and 2 ± 1 ºC respectively).

Color is a crucial factor determining the sensory attractiveness of fruits. Changes in color may indicate deterioration in the quality of a product due to processing and storage. Color is determined by the presence of natural pigments and the degree of their decomposition as well as the interactions and degradation of other components in fruit, which occur, for example, during the process of enzymatic and non-enzymatic browning [43, 44]. In the present work, the color of the raw material and dried products was determined according to the CIE (L*a*b*) system (Table 3). The drying process caused a slight, insignificant reduction in the L* value (L* - lightness). Convection drying resulted in a 66% increase in a* value (the proportion of red color) compared with the raw material. After freeze drying, however, the difference in this value was insignificant. As a result, the proportion of red color in the convection dried product was 47% higher than in freeze-dried berries. Conversely, the b* value (the proportion of yellow color) determined in the convection dried product was identical with the raw material, while in the freeze-dried product it was 78% higher, although this difference was insignificant.

IV. TABLES

TABLE 1
EFFECT OF DRYING METHODS AND STORAGE TEMPERATURE ON THE NUTRIENTS CONTENT IN THE DRIED FRUIT OF BILBERRY.

Object		Vitamin C [mg/100 g DM]		Vitamin E [mg/100 g DM]		Anthocyanins [mg/100 g DM]		Total polyphenol [mg/100 g DM]		Antioxidant activity [µM Trolox/1g DM]	
Raw material		**208 ± 7**		**36.4 ± 1.2**		**2064 ± 36**		**5000 ± 83**		**1750 ± 60**	
Dried fruits, time and temperature of storage											
[months]	**[ºC]**	**CD**	**FD**	**CD**	**FD**	**CD**	**FD**	**CD**	**FD**	**CD**	**FD**
0	-	135 ±7	156 ±6	31.8 ±0.6	33.4 ±1.4	1399 ±40	1649 ±52	3867 ±98	3993 ±82	1568 ±43	1643 ±66
4	2±1	122 ±5	136 ±7	28.8 ±0.9	30.0 ±0.8	1310 ±30	1511 ±44	3805 ±70	3881 ±60	1444 ±39	1533 ±49
	20±2	109 ±3	127 ±5	27.2 ±0.9	29.2 ±0.7	1183 ±38	1418 ±49	3734 ±93	3802 ±72	1365 ±27	1494 ±40
8	2±1	111 ±7	127 ±5	27.2 ±0.8	27.3 ±0.9	1222 ±35	1354 ±38	3763 ±46	3800 ±68	1305 ±44	1440 ±34
	20±2	92 ±4	117 ±4	26.7 ±0.7	26.4 ±0.9	1083 ±33	1247 ±36	3548 ±58	3719 ±69	1216 ±32	1389 ±53
12	2±1	98 ±5	110 ±4	28.1 ±0.1	29.8 ±0.3	1108 ±24	1213 ±27	3687 ±95	3692 ±71	1226 ±36	1348 ±35
	20±2	78 ±4	102 ±3	26.4 ±0.1	27.8 ±0.5	974 ±23	1130 ±34	3406 ±53	3588 ±54	1109 ±12	1273 ±57
LSD (α= 0.05)		7.6		1.12		51.6		101.9		56.8	

CD – conventional drying, FD - freeze-drying

TABLE 2
THE ABILITY OF WATER ABSORPTION BY DRIED BILBERRY IMMEDIATELY AFTER DRYING AND AFTER 12 MONTHS OF STORAGE, ML/100 G DRIED FRUITS.

Dried material after storage time, [months]	Storage temperature [ºC]	Conventional drying	Freeze-drying
0	-	210±12	290±8
12	2±1	203±13	268±10
	20±2	195±10	253±10
LSD (α=0.05)		15.4	

TABLE 3
EFFECT OF DRYING METHOD AND STORAGE TEMPERATURE ON CHANGES OF COLOR PARAMETERS L*a*b* IN THE DRIED FRUIT OF BILBERRY.

Object		L*		a*		b*	
Raw material		36.84±1.04		0.88±0.11		0.10±0.04	
Dried fruits, time and temperature of storage							
[months]	[ºC]	CD	FD	CD	FD	CD	FD
0		36.57±1.50	36.26±1.40	1.46±0.19	0.99±0.19	0.10±0.03	0.18±0.08
12	2 ± 1	35,61±1.41	35.88±1.36	1.51±0.20	1.54±0.24	0.29±0.04	0.44±0.08
	20 ± 2	36.29±1.63	36.15±1.57	1.84±0.24	1.76±0.18	0.35±0.09	0.49±0.10
LSD (α = 0.05)		1.848		0.255		0.093	

CD – conventional drying, FD - freeze-drying

V. CONCLUSION

Retention rates in dried products stored for 12 months were about 50% for vitamin C and anthocyanins and over 70% for vitamin E, total polyphenols, and antioxidant activity against the DPPH radical. Retention in freeze-dried products was superior to that in convection-dried products for all indicators except total polyphenols in products kept in chilled storage. In addition, retention rates were significantly higher at the lower storage temperature. Average losses of vitamin C, anthocyanins and total polyphenols were higher during drying than over the 12-month storage period, while for vitamin E and antioxidant activity the losses were lower: slightly in the case of the former, and distinctly so for the latter. There were no effect of storage temperature on rehydration of both products.

There were no significant differences in L* value between convection and freeze-dried products, either immediately after drying or throughout the storage period. 12-month storage led to a significant increase in the proportion of red and yellow colors in both types of dried product compared to the raw material; however, compared with the product immediately after drying, the differences found were significant in most cases except for the convection dried product kept in chilled storage.

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