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Effect of Time and Storage Temperature on Anthocyanin Decay and Antioxidant Activity in Wild Blueberry (*Vaccinium angustifolium*) Powder

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ABSTRACT: This study evaluated the effects of storage on total and single anthocyanin (ACN) content, and total antioxidant activity (TAA) of freeze-dried wild blueberry (WB) powder maintained at 25, 42, 60, and 80 °C for 49 days. Storage reduced single and total ACN content at all of the temperatures; it was slower at 25 °C (−3% after 2 weeks), whereas it was faster at 60 °C (−60%) and at 80 °C (−85%) after 3 days. The values of half-life time ($t_{1/2}$) were found to be 139, 39, and 12 days at 25, 42, and 60 °C, respectively, utilizing the Arrhenius equation. No significant effects were detected on TAA by temperature increase. In conclusion, this study provides important information on the stability of WB powder at 25 °C; this is interesting scientific research for the food industry.

KEYWORDS: *wild blueberry powder, anthocyanins, total antioxidant activity, storage temperature*

INTRODUCTION

In recent years, several studies documented the beneficial effects of berries (i.e., cranberries, raspberries, and blueberries) on human health. Wild blueberries (*Vaccinium angustifolium*) have been reported to have a protective effect against chronic diseases, especially cardiovascular disease;[1−3] this has generally been attributed to their polyphenol content, anthocyanins (ACNs) in particular. These compounds are responsible for the blue and purple color of the berries and for these reasons are also used as natural food colorants in the food industry.[4] However, ACNs are labile in nature and susceptible to deterioration during processing and storage.[5] Blueberries are often quick-frozen at very low temperatures (−80 °C) for long-term preservation with minimal effects on quality.[6,7] The majority of berries, including blueberries, are consumed as processed foods, that is, juices, purees, jams, syrups, jellies, and various ready-to drink beverages to ensure extension of shelf life and consumption independent of the growing season.[8−11] Another common system to preserve blueberries is through the freeze-drying process, which has several advantages for the food industry such as reduction of storage space, size, and cost. Moreover, the freeze-drying process permits the standardization content of nutrient and phytochemicals useful for human health.

Several mechanisms of degradation during processing and storage have been documented. In freezing and cold storage, the retention of ACNs depends on the rate of freezing, temperature, the presence/absence of oxygen, and the food matrix.[5,12] Studies verified the stability or at least a slight increase in ACN content in berries/blueberries during cold storage[13,14] or storage in high-oxygen atmospheres.[15] On the contrary, a reduction was observed for extruded products such as cereal blueberry-rich products[16] and for thermally processed foods such as juices,[8,9,17] jams,[10,18] and purées.[11]

Anthocyanin degradation is high when these products are treated at higher temperature (up to 121 °C) and then refrigerated.[19,20] Concerning dry storage, the major parameters determining the stability of ACNs are water content, water activity (a_w), temperature, presence/absence of oxygen, light, and relative humidity.[11,19]

However, no data are available concerning the effect of storage on ACN content in freeze-dried wild blueberry powder. This is very important because the food industry uses the freeze-dried products as ingredients in many food formulations, such as jams, jellies, sauces, purées, toppings, syrups, juices, bakery, and dairy products. In addition, this information is extremely important for scientists that use freeze-dried products (frequently stored at room temperature for several months before using) as a feed ingredient in animal and human studies.

Moreover, in past studies, the ACN concentration was commonly quantified as total ACNs, and no information was reported on the fate of the single compounds contained in the blueberries.[8−11,17−20]

For these reasons, the objective of this study was to investigate, for the first time, the degradation kinetics of single ACNs contained in freeze-dried wild blueberry (WB) powder samples stored at different temperatures (25, 42, 60, and 80 °C) for 49 days. Total ACN content and total antioxidant activity (TAA) were investigated as well, under the above conditions.

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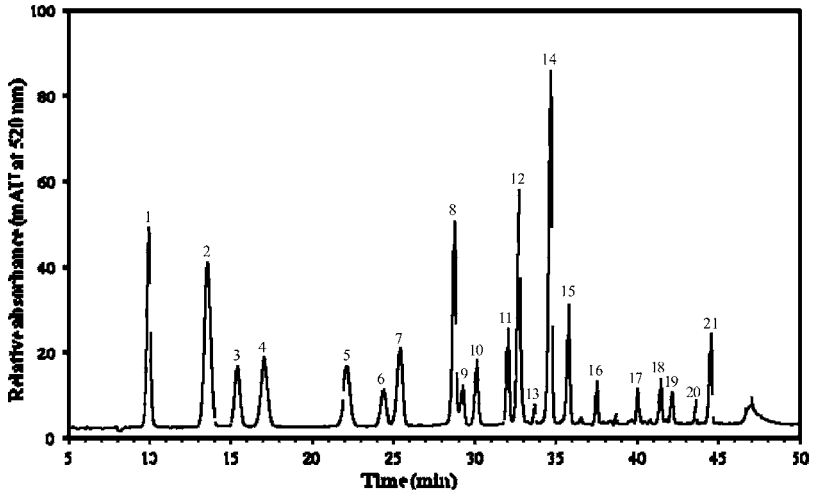

Figure 1. HPLC profile of the individual ACNs in WB (*Vaccinium angustifolium*) powder detected at 520 nm. Peaks: (1) Dp-gal, (2) Dp-glc, (3) Cy-gal, (4) Dp-ara, (5) Cy-glc, (6) Pt-gal, (7) Cy-ara, (8) Pt-glc, (9) Pe-gal, (10) Pt-ara, (11) Pe-glc, (12) Mv-gal, (13) Pe-ara, (14) Mv-glc, (15) Mv-ara, (16) Dp-glc-Ac, (17) Cy-glc-Ac, (18) Pt-glc-Ac, (19) Mv-gal-Ac, (20) Pe-glc-Ac, and (21) Mv-glc-Ac. HPLC, high-performance liquid chromatography; ACNs, anthocyanins, WB, wild blueberry.

MATERIALS AND METHODS

Chemicals and Materials. Standard of cyanidin (Cy)-, delphinidin (Dp)-, petunidin (Pt)-, peonidin (Pe)-, and malvidin (Mv)-3-*O*-glucoside (glc) and Cy-, Pt-, Pe-, and Mv-3-*O*-galactoside (gal) were purchased from Polyphenols Laboratory (Sandes, Norway). Potassium chloride, hydrochloric acid, methanol, acetonitrile, phosphoric acid, and trifluoroacetic acid (TFA) were from Merck (Darmstadt, Germany). 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid (Trolox) were purchased from Sigma (St. Louis, MO, USA). Water was obtained from a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Freeze-dried WB powder was provided by FutureCeuticals Co. (Momence, IL, USA) and maintained at 4 °C for 2 months before the analysis.

Sample Preparation. Wild blueberry powder was stored at −80 °C until analysis. Sixty samples of 1 g each were placed in zip-lock plastic bags used for commercial products, sealed under vacuum, and stored in the dark at four controlled temperatures (25, 42, 60, and 80 °C).

Degradation Studies. The thermal degradation of ACNs as well as the TAA of the WB powder was investigated at 25, 42, 60, and 80 °C for 49 days. Two samples of WB powder (1 g each) were taken, based on the accelerated shelf life testing method at appropriate time intervals (3−4 days) for analyses. They were rapidly cooled, and ACN extraction was performed for the determination of total and single ACN concentration and TAA. All analyses were done in duplicate.

Extraction of ACNs from WB Powder. Anthocyanin extraction was performed as follows: 50 mg of WB powder was dissolved in 5 mL of methanol acidified with 1% TFA and sonicated for 10 min. The ultrasonic treatment permits the optimal ACN extraction (95.6 ± 1.1%; mean ± SD) from the WB powder. The suspension was centrifuged at 3000*g* for 15 min, the supernatant was recovered, and the volume was adjusted to 10 mL by methanol acidified with 1% TFA.

Determination of Total Anthocyanins. The total content of ACNs was determined spectrophotometrically (Perkin-Elmer Lambda 20, Waltham, MA, USA) as described by Lee et al.[21] Briefly, two aliquots of the extracted ACNs were diluted 1:10 in 0.025 M KCl at pH 1 and in 0.4 M CH_3COONa at pH 4.5. The absorbance was measured twice for each sample and buffer at the following wavelengths: 520 and 700 nm. The absorbance, *A*, was calculated as follows:

$$A = (A_{520\text{nm}} - A_{700\text{nm}})\ \text{at pH 1} - (A_{520\text{nm}} - A_{700\text{nm}})\ \text{at pH 4.5}$$

The total ACN content was calculated as

$$\text{mg ACNs}/100\ \text{g} = A \times \varepsilon^{-1} \times \text{MW}^{*}W/V \times \text{DF}$$

where ε is the Cy-glc molar extinction coefficient (26900 mol L^{-1} cm^{-1}), MW is the molecular weight (449.2 Da), *W* is the sample weight, *V* is the volume (mL), and DF is the dilution factor.

Determination of Single ACNs. The liquid chromatography (LC) system was an Alliance model 2695 (Waters, Milford, MA, USA) equipped with a photodiode array detector (model 2998, Waters). The separation was carried out by a C_{18} Kinetex column (150 × 4.6 mm, 2.6 μm, Phenomenex, Torrence, CA, USA) maintained at 45 °C. The flow rate was 1.7 mL min^{-1}, and the eluents were (A) 1% H_3PO_4 and (B) acetonitrile/water (35:65, v/v). The elution gradient was linear as follows: 0−15 min 14% B; 15−25 min from 14 to 20% B; 25−35 min from 20 to 32% B; 35−45 min from 32 to 50% B; 45−48 min from 50 to 90% B; 90% for 3 min. Chromatographic data were acquired from 200 to 700 nm and integrated at 520 nm.

Calibration curves ranged from 2 to 50 μg mL^{-1}; the working solution was obtained by diluting the stock solution (1 mg mL^{-1} in methanol acidified with 0.1% TFA) with 0.1% TFA. Each analysis was carried out in duplicate.

The concentrations of the five ACNs not commercially available (Dp-gal, Dp-ara, Cy-ara, Mv-ara, and Pt-ara) were estimated using the calibration curve equation of the same anthocyanidin with different glycosylation. The acetylated ACNs were determined by the Cy-glc curve, and the resulting data were corrected by their corresponding molecular weight ratios. The identification of single ACNs was confirmed by LC-ESI/MS according to a method previously published.[22]

Determination of TAA. The TAA was determined by the Trolox equivalent antioxidant capacity (TEAC) assay as described by Pellegrini et al.[23]

Degradation Kinetic Studies. The thermal degradation of ACNs was performed according to the method reported by Kechinski et al.[24] Degradation is a temperature-dependent process, as described by the Arrhenius equation:

$$k = k_0 \times e^{-E_a/RT}$$

k_0 is the frequency factor (per min), E_a the activation energy (J mol^{-1}), *R* the universal gas constant (8.314 J mol^{-1} K^{-1}), and *T* the absolute temperature (K).

The coefficient Q_{10} expresses ACN degradation when the temperature is increased to 10 °C, and it is calculated as follows:

$$Q_{10} = (k_{at,T_2}/k_{at,T_1})^{(10/(T_2-T_1))}$$

Statistical Analysis. Statistical analysis was performed by means of Statistica software (Statsoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) with type of treatment as the dependent factor was used to evaluate the variations of ACNs and TAA. One-way ANOVA was performed to determine the variation among the samples stored at different temperatures. Differences between means were evaluated by the least significant difference (LSD) test. Differences were considered to be significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

This is the first study that focuses on ACN degradation in a freeze-dried WB powder and its shelf life. The ACN profile of the WB powder before the storage treatment is reported in Figure 1. The HPLC method used allowed for the separation of 21 ACNs, 15 glycosylated anthocyanidins and 6 acetylated forms, the identities of which were confirmed by LC-MS and MS/MS as previously reported.[22] The mean relative standard deviation (RSD) was 6.1% for concentrations from 0.5 to 20 μg mL^{-1}. The main ACNs detected in the WB powder were Pt-glc, Mv-glc, Mv-gal, Dp-glc, and Dp-gal; these five compounds represented about 35% of the total amount of ACNs.

The decay of total ACNs evaluated at four different temperatures (25, 42, 60, and 80 °C) is reported in Figure 2.

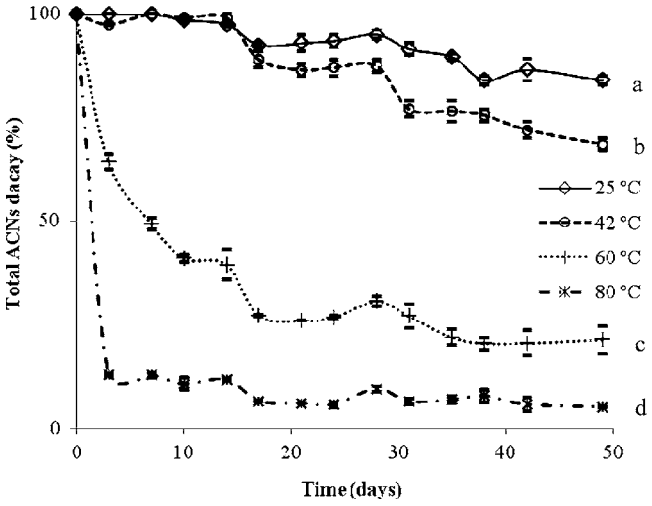

Figure 2. Decay (%) of total ACNs in the WB powder stored at (a) 25 °C, (b) 42 °C, (c) 60 °C, and (d) 80 °C. Curves with different letters are significantly different at $P \leq 0.05$. ACN (−), anthocyanin; ACN_Ac (○), acetylated anthocyanin.

In general, a significant difference ($P \leq 0.0001$) on ACN content was detected for each temperature studied. Predictably, time and degree of ACN decay were dependent on temperature. In fact, we observed that the ACN decay occurred slowly up to 3% at day 14 at 25 and 42 °C, whereas it was faster, achieving about 60 and 85% decay, at day 3 at 60 and 80 °C, respectively.

The quantification of the single ACNs allowed for the calculation of the decay slope (mean ± SD) in the WB powder (Figure 3). The reduction in ACN content at 80 °C was >90% after 3 days only; thus, the data of single ACNs at this temperature were not used to evaluate their degradation rate. The slopes calculated at 25, 42, and 60 °C showed that the degradation rate followed first-order kinetics. This trend was in accordance with that observed by several researchers on

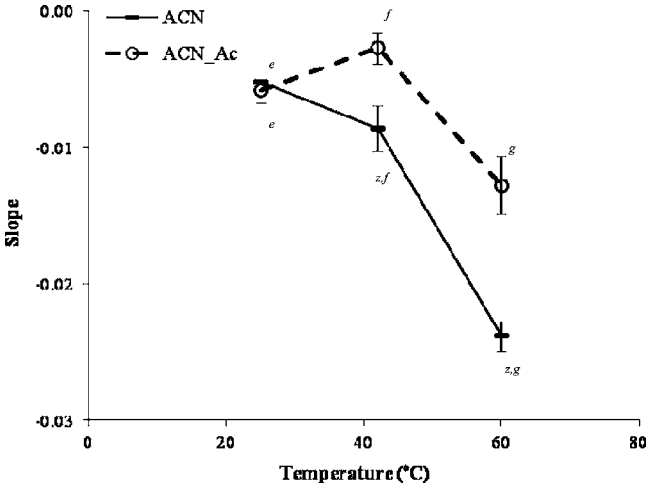

Figure 3. Effect of temperature on slope (mean ± SD) for glycosylated and acetylated ACN degradation in WB powder stored at 25, 42, or 60 °C. *z*, data between curves (ACN vs ACN_Ac) at 42 and 60 °C are significantly different at $P \leq 0.05$; *e*, *f*, *g*, data between points (25, 42, and 60 °C) of the same curves are significantly different at $P \leq 0.05$. ACN (−), anthocyanin; ACN_Ac (○), acetylated anthocyanin.

different juices, such as blood orange, blackberry, and blueberry juices, and red wine.[24−27] Each compound displayed its own specific decay, related to the sugar binding and the storage temperature. Moreover, it seems that the ACNs bound to glucose exhibited a faster degradation rate than those bound to galactose (data not shown). For all of the ACNs, the correlation indices (R^2) were >0.90, demonstrating a direct correlation between ACN concentration decrease and storage time. Good correlation indices were also found for the acetylated forms (R^2 > 0.81), which seems more stable that the correspondent glycosides.

The linear regression approach allows also for the calculation of the reaction rate constant (k). A direct relationship between k values and temperature was found (Figure 4), confirming the major effect of temperature on ACN degradation.

The values of E_a and half-life of total and single ACNs are reported in Table 1. The value of E_a for the total ACNs was about 58 kJ mol^{-1}. This is lower than that reported by Kechinski et al.,[24] who found a value of about 80 kJ mol^{-1} in blueberry juice. The difference may be due to the different type of tested product, suggesting that ACNs contained in the WB powder are more susceptible to temperature than that in the juice.

This could be attributable to a matrix effect and/or a different pH (pH 4 or lower in case of juice) that maintains ACN stability. With regard to single ACNs, as already observed from the slope values (Table 1), the ACNs linked to galactose such as Cy-gal, Mv-gal, Pt-gal, and Pe-gal have values of E_a >70 kJ mol^{-1}. This implies that in the WB powder the galactosylated ACNs are more heat-stable. These data are in accordance with those reported by Scibsz et al.,[28] who hypothesized a possible protective effect of galactose compared to glucose.

Contrarily to the data reported for blueberry juice, delphinidin glycosides were not the compounds decaying faster with increased temperature.[19] Indeed, in our product the most temperature labile compounds were Pt-glc (E_a = 18.14 kJ mol^{-1}) and Cy-ara (E_a = 38.98 kJ mol^{-1}) as reported in Table 1. The possible relationship between their chemical structure,

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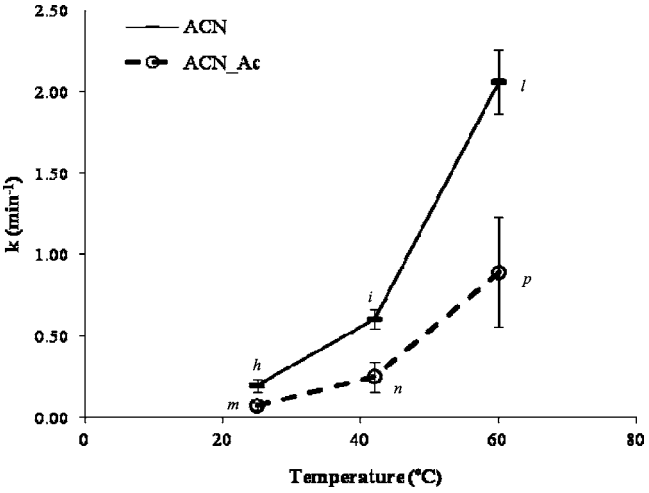

Figure 4. Effect of temperature on reaction rate constant (k) slope (mean ± SD) for single ACN and ACN_Ac degradation in WB powder stored at 25, 42, and 60 °C. *h*, *i*, *l*, *m*, *n*, *p*, data between curves (ACN vs ACN_Ac) and within temperatures (25, 42, and 60 °C) of the same curves are significantly different at $P \leq 0.05$. ACN (−), anthocyanin; ACN_Ac (○), acetylated anthocyanin.

Table 1. Activation Energy (E_a) and Half-life ($t_{1/2}$) of Total and Individual ACNs of the WB Powder Stored at 25, 42, and 60 °C[a]

		$t_{1/2}$ (days)		
compound	E_a (kJ mol^{-1})	25 °C	42 °C	60 °C
total ACNs	58.26	139	39	12
individual ACNs				
Dp-gal	57.82	212	60	18
Dp-glc	45.44	131	49	19
Dp-ara	64.85	256	62	16
Cy-gal	72.17	460	95	21
Cy-glc	55.72	234	69	22
Cy-ara	38.98	117	49	21
Mv-gal	73.54	608	122	27
Mv-glc	55.81	162	48	15
Mv-ara	65.29	261	63	16
Pt-gal	69.81	374	81	19
Pt-glc	18.14	86	58	40
Pt-ara	51.40	611	199	69
Pe-gal	84.15	549	87	15
acetylated ACNs				
Dp-glc-Ac	62.00	625	161	45
Cy-glc-Ac	84.28	1948	310	54
Mv-gal-Ac	27.07	936	519	296
Mv-glc-Ac	62.15	295	76	21
Pt-glc-Ac	51.29	542	177	61
Pe-glc-Ac	nd	nd	nd	nd

[a]ACNs, anthocyanins; Ac, acetylated; nd, not detectable; WB, wild blueberry.

such as the number of hydroxyl groups or the glycosylation degree or the acylated form, and heat stability was studied by several researchers.[29−31] Unfortunately, the data reported in the literature are often contradictory.[5] For example, Trost et al.[32] reported that ACN stability in a blueberry−aronia nectar stored for over 207 days at 30 °C was higher for Cy- and Pe-glycosides and lower for Pt-, Mv-, and Dp-glycosides. In regard to conjugated sugars, the ranking order was glucoside > galactoside > arabinoside from the most to the least stable.[32] The greater stability of ACNs bound to glucose and galactose compared to arabinose was proposed to be due to steric hindrance, which is larger for the hexose sugars. On the contrary, Ichiyangi et al.[33] documented that the ranking order was arabinoside > galactoside > glucoside from the most to the least stable.

From our observations, the relative amount of a single ACN did not affect its heat stability. Indeed, Pe-gal is one of the compounds present in lower amount in the WB powder but with the highest E_a (84.15 kJ mol^{-1}) (Table 1). Among the acetylated forms, Pe-glc-Ac is the most heat sensitive (E_a = 7.76 kJ mol^{-1}), whereas Cy-glc-Ac is the compound most heat resistant (E_a = 84.28 kJ mol^{-1}).

In addition to the degradation rate, the half-life time ($t_{1/2}$) was calculated by the Arrhenius equation for the single and total ACNs in relation to the investigated temperatures (Table 1). The $t_{1/2}$ values obtained for the total ACNs decay were 139, 39, 12, and 4 days at 25, 42, 60, and 80 °C (data not shown), respectively. Large differences in the $t_{1/2}$ value existed among the single compounds stored at the same temperature (Table 1), as well as at different temperatures. The $t_{1/2}$ value ranged from 86 to 611 days at 25 °C, from 48 to 199 days at 42 °C, and from 15 to 69 days at 60 °C. Thus, storage at room temperature (25 °C) can induce important losses of some ACNs such as Pt-glc, Cy-ara, and Dp-glc even though for most of them the $t_{1/2}$ value is much higher than 150 days (Table 1). Moreover, the acetylated forms were generally more resistant than only glycosylated compounds for all of the temperatures considered. Additionally, our study found considerable changes for the different acetylated ACNs, which had $t_{1/2}$ values from a few days to 1948, 519, and 296 days at 25, 42, and 60 °C, respectively (Table 1).

The Q_{10} values for the total and single ACNs at the temperatures investigated are presented in Table 2. The Q_{10} values for total ACN and for each ACN decreased as temperature increased. In particular, for the single ACNs the highest values were observed for the low temperatures (25−35 °C), whereas low Q_{10} values were observed for the high temperatures (42−52 and 60−70 °C). This may be attributed to a molecular change, such as ACN polymerization, that occurs at the high temperatures and may decrease the rate of ACN degradation.[24,34] Moreover, because most of the Q_{10} values were about 2.0, the increase of temperature by 10 °C approximately doubled the decay rate (Table 2). In contrast to our results, Kechinski et al.[24] observed a higher Q_{10} value (4.27 at the range from 40 to 50 °C) in highbush blueberry juice, probably due to the high content of water in juice with respect to the powder.

The values of activation energy (E_a), half-life ($t_{1/2}$), and Q_{10} were calculated for TAA of the WB powder stored at different temperatures (Table 3). The TAA showed values of E_a (52.31 kJ mol^{-1}) and Q_{10} comparable to those obtained for the total ACNs, whereas the value of $t_{1/2}$ was higher, ranging from 130 days at 60 °C to 1200 days at 25 °C.

Additionally, the Arrhenius equation was used to predict the shelf life of total ACNs when stored at 4 °C. Under these experimental conditions, the half-life time for total ACNs is up to 829 days and for TAA, more than 10 years.

The logarithmic reduction kinetics of total ACNs (A) and TAA (B) of the WB powder stored at different temperatures are reported in Figure 5. The TAA and the content of ACNs decreased with increasing temperature, but the reduction of the

Table 2. Q_{10} Values for the Total and Individual ACNs of the WB Powder Stored at Different Temperatures[a]

	Q_{10}		
compound	25−35 °C	42−52 °C	60−70 °C
total ACNs	2.15	1.98	1.85
individual ACNs			
Dp-gal	2.14	1.97	1.84
Dp-glc	1.82	1.71	1.61
Dp-ara	2.34	2.15	1.98
Cy-gal	2.58	2.34	2.14
Cy-glc	2.08	1.93	1.80
Cy-ara	1.69	1.60	1.52
Mv-gal	2.63	2.38	2.17
Mv-glc	2.08	1.93	1.80
Mv-ara	2.36	2.16	1.99
Pt-gal	2.50	2.27	2.09
Pt-glc	1.27	1.24	1.21
Pt-ara	1.96	1.83	1.72
Pe-gal	3.02	2.69	2.43
acetylated ACNs			
Dp-glc-Ac	2.26	2.07	1.92
Cy-glc-Ac	3.02	2.70	2.43
Mv-gal-Ac	1.43	1.38	1.33
Mv-glc-Ac	1.11	1.10	1.09
Pt-glc-Ac	1.96	1.83	1.72
Pe-glc-Ac	nd	nd	nd

[a]ACNs, anthocyanins; Ac, acetylated; nd, not detectable; WB, wild blueberry.

Table 3. Activation Energy (E_a), Half-life ($t_{1/2}$), and Q_{10} Values of Total Antioxidant Activity (TAA) of WB Powder Stored at 25, 42, and 60 °C[a]

	$t_{1/2}$ (days)			Q_{10}		
E_a (kJ mol^{-1})	25 °C	42 °C	60 °C	25−35 °C	42−52 °C	60−70 °C
52.31	1212	387	131	1.99	1.85	1.74

[a]WB, wild blueberry.

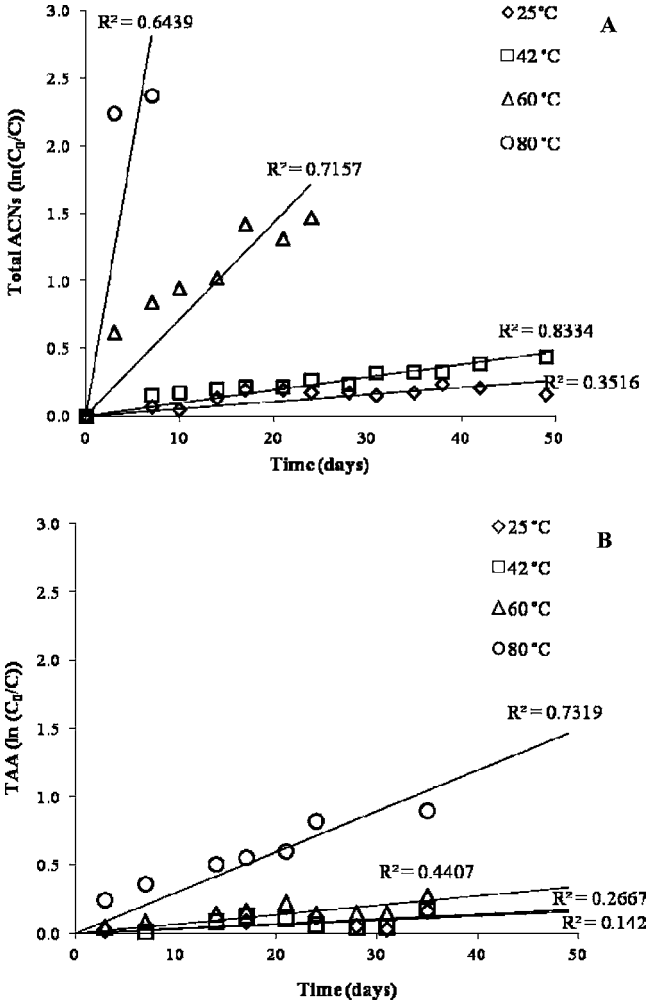

Figure 5. Logarithmic reduction kinetics of total ACNs and TAA in WB powder stored at 25, 42, 60, and 80 °C. ACNs, anthocyanins; TAA, total antioxidant activity; WB, wild blueberry.

TAA does not seem directly correlated to that of the ACNs. Indeed, no significant difference (P = 0.89) was observed in TAA values at 25 and 42 °C. Moreover, the logarithmic decrease of TAA at 80 and 60 °C (1.5 and 0.3) was lower in comparison to the logarithmic reduction of total ACN content (2.5 and 1.5). This result is not surprising because it has been reported that at high temperatures (i.e., 60 and 80 °C) Maillard and caramelization reactions occur and the generated products show an increase of TAA.[35] These reactions can also occur in the presence of hexoses and in the absence of the aminic group.[35]

The reduction of total ACNs and the maintenance of antiradical activity have been described for several processed blueberry products.[11,36−38] This is probably due to the formation of antioxidant polymers, such as low molecular weight procyanidins, or the formation of degradation products of ACNs or phenolic acids, which show antioxidant activity as well.[5,38−40]

In our study, the initial TAA value of the WB powder was 58.5 mmol Trolox equiv TE/100 g DW of product, similar to the data (52.9 mmol Trolox equiv TE/100 g DW) obtained from fresh wild blueberry by Kalt et al.[8] These results further confirm the importance of the freeze-drying process to preserve TAA. In fact, after storage for 50 days, the TAA was 48.7, 49, 41.9, and 22.5 equiv TE/100 g of product, stored at 25, 42, 60, and 80 °C, respectively.

In summary, the degradation of ACNs in freeze-dried WB powder followed first-order kinetics; thus, its storage at room temperature (25 °C) reduced ACN content less in comparison to other temperatures. The decrease of single ACN monomers may be attributed to the formation of ACN polymers through a mechanism that is not well understood. The TAA of the WB powder was almost unchanged after storage at 42 °C for 50 days, suggesting that other compounds (e.g., fiber, polymers, Maillard reaction products) affect its antioxidant power. The use of this freeze-dried WB powder for food ingredients may be important because the content of ACNs and the TAA are maintained longer, up to 130 days at 25 °C, in comparison to other blueberry products. At the same time, storage of WB powder would maintain the stability of ACNs. This could be very important for scientists that use WB powder as a feed ingredient in animal and human studies and for the food industry.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ACN(s), anthocyanin(s); Dp-gal, delphinidin-galactoside; Dp-glc, delphinidin-glucoside; Dp-ara, delphinidin-arabinoside; Cy-gal, cyanidin-galctoside; Cy-glc, cyanidin-glucoside; Cy-ara, cyanidin-arabinoside; Pt-gal, petunidin-galactoside; Pt-glc, petunidin-glucoside; Pt-ara, petunidin-arabinoside; Pe-gal, peonidin-galctoside; Pe-glc, peonidin-glucoside; Pe-ara, peonidin-arabinoside; Mv-gal, malvidin-galctoside; Mv-glc, malvidin-glucoside; Mv-ara, malvidin-arabinoside; Dp-glc-Ac, acetylated delphinidin-glucoside; Cy-glc-Ac, acetylated cyanidin-glucoside; Pt-glc-Ac, acetylated petunidin-glucoside; Pe-glc-Ac, acetylated peonidin-glucoside; Mv-gal-Ac, acetylated malvidin-galctoside; Mv-glc-Ac, acetylated malvidin-glucoside; DW, dry weight; TAA, total antioxidant activity; TEAC, Trolox equivalent antioxidant capacity; ASLT, accelerated shelf life testing; LC-ESI/MS, liquid chromatography coupled with electrospray ionization and mass spectrometry; LSD, least significant difference; RSD, relative standard deviation; a_w, water activity; E_a, activation energy; $t_{1/2}$, half-life time; nd, not detectable

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