Technical paper

Degradation of Polyphenolic Antioxidants in Blueberry Nectar Aseptically Filled in PET

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Received: 31-03-2008

Abstract

One of the conservation processes for blueberry preservation is aseptic filling of blueberry nectar in PET packaging. As virgin PET exhibits a low oxygen barrier, oxygen scavengers can be added to multi-layer bottles that sandwich a barrier plastic between layers of PET or simply mixed with PET to add barrier properties. As polyphenolic antioxidants are known to positively influence human health, stability determination is crucial. This study shows changes in total polyphenol concentrations and total and individual anthocyanin concentrations showing antioxidant activity. A stability scale for individual anthocyanins was made. Since oxygen can destroy the antioxidant ability of polyphenols, various methods of protecting against oxidation were used and characterized by a gradient HPLC-Vis technique. Total polyphenol analyses and antioxidant activity analyses were carried out using spectrophotometric measurements. During 9 months of blueberry nectar storage there was a 3–9.9% decrease in polyphenol concentrations and antioxidant activity decreased by 20.9–24.2%. The biggest drop was seen among anthocyanins, whose concentration decreased by 78–84% of total anthocyanins. Among the quantified anthocyanins, cyanidin 3-glucoside and cyanidin 3-galactoside were the most stable, while malvidin 3-arabinoside was the least stable. The packaging that offered the least protection was the virgin PET bottle without oxygen protection. Antioxidant activity analyses suggest that a three-layer multilayer bottle is the packaging that gives the best protection to the product.

Keywords: Blueberry nectar, antioxidants, barrier PET, anthocyanins, HPLC-VIS

1. Introduction

Blueberry nectar is a rich source of anthocyanins and other phenolic compounds.[1] Anthocyanins are a large group of polypenols in blueberries and also an important factor in food quality. They provide colour to the fruit, which is important for sensory evaluation, and this can change during product storage.[2] Phenolic compounds have an antioxidant activity, which is correlated to decreases in many diseases.[3]

Various data about anthocyanin and polyphenol concentrations can be found in the existing literature. Data vary mostly with different cultivars and product types. Research carried out by[4] reported that blueberries contain 1435.2–8227.3 $mgkg^{-1}$ of total anthocyanins expressed as cyaniding 3-glucoside and 172.5–327.5 $mgkg^{-1}$ of total flavonols expressed as rutin equivalents. Individual anthocyanin and flavonol concentrations were also determined by this research. Similar data are reported by[5] describing 1000–4000 mg/kg^{-1} of total anthocyanins in the Estonian blueberry. Literature data[6] for total anthocyanin content in blueberry juices show smaller values than in fruit (800–2000 mgL^{-1}). There are also greater differences in anthocyanin, procyanidin, flavonol and hydrohxycinnamic acid content in different plant segments.[7] Another relatively new product from blueberries is Blueberry extract, which has a very high polyphenol content and is used in the food industry as a food colorant or high antioxidant food supplement. The concentration in extracts can be up to 9000 mgl^{-1} of total polyphenol content.[8]

The concentration of total polyphenols and anthocyanins decreases during storage and so does the nectar's antioxidant activity.[9] Many publications have reported on

the degradation of polyphenols, anthocyanins, and other antioxidants in different fruits and fruit products.[10] described the stability of total polyphenols and radical scavenging activity in grape concentrate. According to their research, stability is influenced more by pasteurization than by storage.[11] described litchi pericarp anthocyanin changing with different pH and enzymatic activity. Their results show that lower pH stabilizes anthocyanins in litchi. [12] showed anthocyanin degradation curves for blackberry juice. They concluded that blackberry anthocyanins decrease by first order kinetics influenced by temperature. Cherry juices also showed changes in colour and anthocyanin storage.[13] Monitoring of the phenolic content and antioxidant capacity of six industrially filled dark nectars showed minor changes in phenolic content but apparent differences in antioxidant capacity during refrigerated storage.[14] In this study they also described ascorbic acid degradation.

Quality loss can be decreased by proper packaging choice[15]. One of the main reasons for the decomposition of commercially filled products is oxygen, which promotes oxidation reactions. In the bottling industry, one of the most commonly used materials is polyethylene terephtalate (PET). The critical point of the PET bottle is its medium oxygen and carbon dioxide barrier. A passive or active barrier decreases dissolved oxygen concentration in the product. A passive barrier depends on bottle wall thickness, PET crystallinity and an application of ethyl vinyl alcohol (EVOH) or materials such as SiO_x or carbon. An active barrier is formed from an oxidizable polymer. It can be placed in the middle layer of a multilayer or it can be mixed into the base PET.[16]

Oxidation increases the destruction of antioxidants such as phenolic fruit components, carotenoids and vitamins. Antioxidants donate hydrogen to radicals and help to end chain radical reactions.[9] Antioxidant activity depends on the number and arrangement of hydroxyl groups. It is also influenced by conjugated sugars and electron-withdrawing substituents in a ring structure.[17, 18]

The aim of this research was to determine changes in anthocyanin and polyphenol concentrations and antioxidant activity for blueberry nectar during commercial storage. These parameters have been widely investigated by other researchers, but never in PET and rarely after aseptic filling processes.[19, 20, 21] The second aim of this research is to characterize the effects of different PET materials, which vary in their price and ecological acceptability. This has been determined for other fruit products such as orange and apple juice [15, 22] and model solutions[23] but never for blueberry nectar. A stability scale of anthocyanins can be made from individual anthocyanin analyses. This study also characterizes the oxygen protection properties of packaging used and shows the most appropriate ones for product quality. There is a strong correlation between anthocyanin stability and the oxygen protection properties of the packaging.

2. Material and Methods

2. 1 Sample Preparation

Product processing and bottle blowing was performed by Sidel in its Aseptic Test Center (Octaville sur Mer; France).

2. 1. 1 Packaging Materials

Different PET materials were used for this study:
- PET-1: Standard monolayer PET bottle with cap without liner (41 g),
- PET-2: Standard PET bottle with oxygen scavenger with cap without liner (41 g),
- PET-3: Five layer multilayer bottle with cap with liner (36 g).
- PET-4: Three layer multilayer bottle with cap without liner (39 g).

Preforms were bought on the European market and blown on a Sidel blowing machine (SBO Sidel, France).

2. 1. 2 Blueberry Nectar Production

2. 1. 2. 1 Composition

According to the Fructal d.d. recipe for blueberry nectar with a 40% fruit content, wild blueberry (*Vaccinium* angustifolium*)* concentrate, sugar syrup, citric acid and water were mixed in a mixing tank. The product was adjusted to a 12.3% Brix (soluble solid) with sucrose and 0.52% of total acids expressed as citric acid by adding citric acid. The nectar was then mixed again.

2. 1. 2. 2 Processing

The nectar was deaerated to a concentration of 0.2 mg/L of dissolved oxygen and processed by UTH (ultra high temperature) pasteurization at a temperature of 93–97 °C for 30 seconds. After pasteurization the product was stored in a sterile tank.

2. 1. 3 Packaging

Packaging was performed on a monohead filler in an aseptic white room. All operations were carried out under laminar flow. Prior to packaging, bottles and caps were sterilized with peroxiacetic acid and sterile water. Bottles were not sealed with aluminum foil. Prior to product closing, a drop of liquid nitrogen was placed in the product. For each type of packaging 150 bottles were filled.

2. 1. 4 Storage and Sampling

The product was stored in a Fructal d.d. (Slovenia) warehouse in the dark. Temperatures varied from 4 to 20 °C measured four times during the shelf life in order to simulate real circumstances. An analysis of dissolved oxygen was made monthly. Samples for other analyses were

chosen four times during the one-year shelf life period and stored at –80 °C until analyzed. Every measurement was made on one bottle. The RSD for individual anthocyanin and polyphenol analyses was determined from six measurements on the same sample. The standard deviations for DPPH analyses were determined from six measurements on each sample.

2. 2 Methods

2. 2. 1 Dissolved Oxygen

The dissolved oxygen concentration in nectar was measured using Orbisphere Micro O_2 logger 3650. The bottles were opened and the pump tube was immediately inserted into product and pumped over a sensor for 5 min.

2. 2. 1 Determination of Total Polyphenols

This method is based on the oxidation of phenols using the Folin-Ciocalteau reagent (Fluka, Switzerland). This includes a reaction with $H_3PW_{12}O_{40}$ and $H_3PMo_{12}O_{40}$ acids in the alkaline medium. The result of this reaction is a blue complex. The calibration curve was made using gallic acid (Sigma Germany) and all phenols were adjusted to it. The calibration curve was determined in the range from 0.01 mg/mL to 1.5 mg/mL. Spectrophotometer (Beckman DU 640 U.S.A.) measurements were made as for the calibration curve and samples. The absorbance was measured at 765 nm. Both samples and the calibration curve were prepared by adding 2 ml of the sample into 10 mL of Folin-Ciocalteau reagent in a test-tube, which was then left for 5 to 10 min. After that, 8 mL of Na_2CO_3 (Merck Germany) was added. The absorbance was measured after 2 hours against water instead of the sample. The concentration of phenols equal to gallic acid was calculated from calibration curve.[24]

2. 2. 2 HPLC Analysis of Anthocyanins

Quantification of individual anthocyanins was determined by HPLC. We used an HPLC system by TSP (Thermo Separation Products, Germany) with a Gradient pump Gostametric 4100 and Spectra Monitor 3200 LDC UV/VIS detector. The column used was Nova-Pak C 18, 3.9 × 150 mm (Waters). Measurements were made at room temperature. A linear gradient of mobile phases was used. Mobile phase A consisted of acetonitrile (Merck, Germany) and water in a ratio of 5:95 v/v. Mobile phase B consisted of acetonitrile (Merck, Germany) and water in a ratio of 60:40 v/v. Both mobile phases were adjusted to pH 1.3 with perchloric acid (Kemika, Croatia). Mobile phase B was raised from 5% to 10% in 5 min, from 10% to 20% in 20 min, from 20% to 25% in 13 min and from 25% to 100% in 7 min. After the gradient, the system was held steady for 5 min. The flow rate was 1.5 mL/min. The operational wavelength was 530 nm.

Blueberry nectar was analyzed immediately after unfreezing and filtering through 45 μm Millipore (USA) PVDF filters. The volume of injections was 20 μL.

Concentrations were calculated using external standard calibration curves. Each of the five concentrations were measured three times in the range described. Curve equations and statistical data are: cyanidin 3-glucoside (y = 30192x; st. error = 99; R^2 = 0.9999; range: 9.6 mg/L – 95.6 mg/L), delphinidin 3-glucoside (y = 25967x; st. error = 122; R^2 = 0.9999; range: 10.3 mg/L–103 mg/L), peonidin 3-glucoside (y = 45245x; st. error = 85; R^2 = 0.99999; range: 1.3 mg/L – 63.6 mg/L), petunidin 3-glucoside (y = 32540x; st. error = 43; R^2 = 0.99999; range: 2.2 mg/L – 110.4 mg/L) and malvidin 3-glucoside (y = 30600x; st. error = 135; R^2 = 0.9997; range: 2.2 mg/L – 21.6 mg/L).

The standards used were provided by Polyphenol laboratories, Norway. Identification was made using the same five standards and as described.[25] This method has been previously described.[26] The gradient and anthocyanin identification made were modified by the Laboratory for Food Chemistry–National Institute of Chemistry, Slovenia in order to fit the instrumentation used and the specific samples.

2. 2. 3 Measurement of Total Antioxidant Activity Using DPPH Test

The DPPH method is based on free radical scavenging of 1,1-diphenyl-2-picryl-hydrazil (DPPH) by antioxidants and is used to evaluate the antioxidant capacity of food. Free radicals have an absorbance maximum at 515 nm and free radical scavenging reduces absorbance at this wavelength. Samples were stirred for 20 min at 4000 min^{-1}. 50 μL of sample was added to 3 mL of a DPPH methanol (Merck, Germany) solution with a concentration of 10mg DPPH/100ml. DPPH was provided by Aldrich, Switzerland. The absorbance difference was measured after exactly 15 min against deionised water instead of sample. The solutions were prepared fresh each day and stored in darkness.[24]

The results are presented as antioxidant efficiency (% AU), which is calculated by the formula: % AU = 1 – (absorbance of the sample/absorbance of blind sample with deionised water instead of sample)

2. 2. 4 Statistical Analysis

SDs and RSDs were calculated using Microsoft Excel software. The difference between different packaging types and sampling dates were determined using Statgraphics Centurion ver. 15 (StatPoint, Inc.; U.S.A.). For this purpose multivariate analyses were carried out using the GLM (General Linear Models) procedure. Middle values for experimental groups were calculated using LSD (Fisher's least significant difference). They were compared with a 95% confidence level.

3. Results and Discussion

3. 1 Dissolved Oxygen

Figure 2 shows the oxygen concentration evolution in correlation to the type of packaging and date of measurement. The first measurement shows different oxygen concentrations depending on packaging type. The highest concentration of oxygen is found in sample PET-2, followed by PET-1, PET-4 and PET-3. The smallest fall is visible in sample PET-1. The last measurements taken again show an increase in oxygen concentrations in samples PET-1, PET-2 and PET-4.

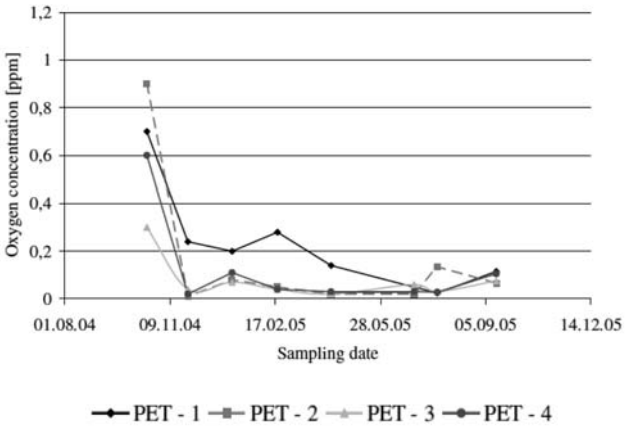

Fig. 2: Oxygen concentration evolution depending on packaging type and date of measurement.

3. 2 Measurement of Total Polyphenol Concentration

In Table 1 total polyphenol concentrations correlating to the time of sample freezing and the type of packaging are shown. Total polyphenol concentrations decrease with time. After nine months the highest value is seen in nectar in PET-2 followed by PET-3, then PET-4 and lastly PET-1. After a year, total polyphenol concentration is the highest in PET-3, followed by PET-4, then PET-2 and lastly PET-1. If we start with December 2004, in nine months, concentrations decrease by 3% in PET-3 up to 9.9% in PET-1.

Table 1: Concentration of total polyphenols blueberry nectar for different samplings using general linear models statistical evaluation

	Total polyphenol concentration[a] [mg/L]						
Sampling date	**PET-1**	**PET-2**	**PET-3**	**PET-4**			
22.12.2004	650	669	681	657	*		
12.2.2005	656	664	649	653	*	*	
1.6.2005	626	668	628	621		*	*
15.9.2005	592	623	625	623			*
	*		*	*			
		*	*	*			

[a] expressed as gallic acid equivalent
RSD for total polyphenol concentration was determined for six measurements RSD = 3%
* denotes a statistically significant difference between experimental groups with a 95% confidence level.

There have not been many studies made describing the correlation between oxygen scavenging packaging materials and dissolved oxygen.[15] described a correlation between oxygen scavenging packaging with different treatments on the dissolved oxygen concentration. They concluded that oxygen scavenging material reduces dissolved oxygen more than packaging without protection, which we also observed. In another study carried out by[27], they describe the influence of oxygen scavenging material in laminated cartons for orange juice. Their research also provides data on oxygen concentration changing during shelf life, where they concluded that dissolved oxygen disappears faster in packaging with oxygen scavenger material. It also stays at a lower level until the oxygen scavenging material is consumed.

PET-1 had no oxygen scavenging material added and it shows slower decreases in oxygen concentration with time than the others. The delayed concentration decrease in PET-2 and PET-4 is connected to the activation of scavenging material in PET. The scavenging polymer in PET-3 starts to work immediately. Oxygen concentration increases after one year shows that invading oxygen flow overcomes antioxidant scavenging potential.

Total polyphenol evolution for blueberry nectar can be correlated to studies of other fruit juice products.[28] detected a 20% decrease in total polyphenol concentration in pomegranate juice stored at 20 °C for 160 days. The juice was packed into transparent and green glass, as well as a laminated carton. No large differences were detected between packaging types. Higher relative decrease (in polyphenols) over a shorter period can be correlated to a different fruit product, a larger volume or higher temperature conditions.[8] were investigating the influence of temperature on decreasing of total polyphenols in blueberry extract. They found out that the storage period has an influence on TPP concentration especially at higher temperatures; after 60 days of storage at 35 °C, only 50% of the initial concentration remained. Similar studies have also been carried out for grape concentrate by[10], for apple juices by[29] and for orange juices by[30].

3. 3 Individual Anthocyanins

Figure 1 shows a typical chromatogram for an individual anthocyanin analysis. In blueberry nectar, the anthocyanidins delphinidin, cyanidin, petunidin, peonidin and

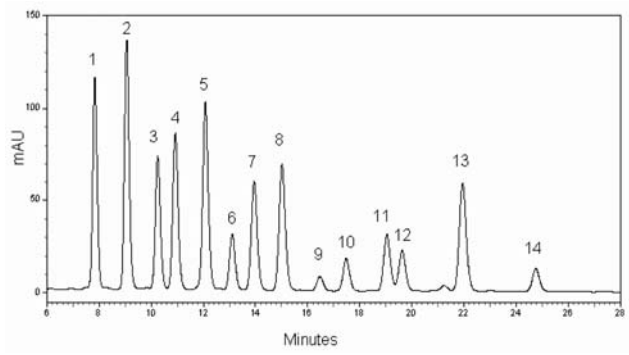

Fig. 1: Typical chromatogram of individual anthocyanins from blueberry nectar.

malvidin are present. They are complexed as glucosides, galactosides and arabinosides.

For individual anthocyanin identification authentic glucoside standards were used. External calibration curves and spiked samples were used for cyanidin 3-glucoside, delphinidin 3-glucoside, petunidin 3-glukoside, peonidin 3-glukoside and malvidin 3-glucoside identification. Other identified anthocyanins were determined according to the research using HPLC – MS identification by [25] as they used similar chromatographic conditions. Between every spiked chromatographic peak there were two peaks that were determined according to the retention time from.[25] Out of 14 identified anthocyanins, twelve were quantified in order to avoid undetected peaks. Individual anthocyanin concentrations and statistically significant differences between the different sampling dates are presented in Tables 2–5.

In order to determine the stability of individual anthocyanins further data analysis was made. The analysis determines the relative change in individual anthocyanins between the first and last measurement. GLM was used to determine groups with different stabilities. The equation describing the normalized relative change x was calculated by the x [%] = $((c_x/\Sigma_{ij}c_{ij})/(c0/\Sigma_{kl}c_{kl}))*100–100$, where c_x means anthocyanin concentration x measured on 1.9.05.

$\Sigma_{ij}c_{ij}$ represents the sum of individual anthocyanins measured on 1.9.05. c_0 describes the anthocyanin concen-

Table 2: Table of individual anthocyanin concentrations in blueberry nectar filled in PET-1 in industrial storage

Anthocyanin concentrations [mg/L]				
	Sampling date			
Anthocyanin identification	22. 12. 2004	12. 2. 2005	1. 6. 2005	15. 9. 2005
Del 3-Gal[a]	49.7	49.5	25.7	7.8
Del 3-Glu [a]	62.3	62.2	33.2	10.6
Cy 3-Gal [b]	30.9	30.1	17.6	6.1
Del 3-Ara [a]	43.4	43.3	20.3	5.2
Cy 3-Glu [b]	48.3	47.1	26.4	9.2
Pet 3-Gal [c]	13.6	13.4	7.1	2.3
Cy 3-Ara [b]	30.0	29.2	15.3	4.5
Pet 3-Glu [c]	33.0	32.6	17.8	6.0
Peo 3-Glu [d]	11.6	11.3	6.3	2.1
Mal 3-Gal [e]	12.2	11.9	6.4	2.1
Mal 3-Glu [e]	33.9	33.0	17.7	5.5
Mal 3-Ara [e]	7.2	7.1	3.0	0.6
	*	*		
			*	
				*

[a] Concentration calculated as Delphinidin 3-Glucoside;
[b] Concentration calculated as Cyanidin 3-Glucoside;
[c] Concentration calculated as Petunidin 3-Glucoside;
[d] Concentration calculated as Peonidin 3-Glucoside;
[e] Concentration calculated as Malvidin 3-Glucoside.
RSD was determined for each individual anthocyanin with six measurements (Del 3-Gal, 1.5%; Del 3-Glu, 1.3%; Cy 3-Gal, 1.0%; Del 3-Ara, 1.9%; Cy 3-Glu, 1.0%; Pet 3-Gal, 1.7%; Cy 3-Ara, 1,1%; Pet 3-Glu, 1.5%; Peo 3-Glu, 1.2%; Mal 3-Gal, 1.4%; Mal 3-Glu, 1.2%; Mal 3-Ara, 2.2%)
* denotes a statistically significant difference between experimental groups with a 95% confidence level.

Table 3: Table of individual anthocyanin concentrations in blueberry nectar filled in PET-2 in industrial storage

Anthocyanin concentrations [mg/L]				
	Sampling date			
Anthocyanin identification	22. 12. 2004	12. 2. 2005	1. 6. 2005	15. 9. 2005
Del 3-Gal[a]	54.9	54.3	37.1	10.5
Del 3-Glu [a]	68.7	68.2	47.3	14.7
Cy 3-Gal [b]	33.4	32.3	22.7	7.8
Del 3-Ara [a]	48.7	48.2	30.8	7.3
Cy 3-Glu [b]	52.0	50.5	34.1	11.9
Pet 3-Gal [c]	14.9	14.7	10.0	3.1
Cy 3-Ara [b]	32.6	31.5	20.3	5.5
Pet 3-Glu [c]	36.2	35.5	24.7	7.6
Peo 3-Glu	12.5	12.1	8.1	2.7
Mal 3-Gal [e]	13.2	12.7	8.5	2.7
Mal 3-Glu [e]	36.8	35.6	23.6	7.2
Mal 3-Ara [e]	8.0	7.7	4.5	0.9
	*	*		
			*	
				*

[a] Concentration calculated as Delphinidin 3-Glucoside;
[b] Concentration calculated as Cyanidin 3-Glucoside;
[c] Concentration calculated as Petunidin 3-Glucoside;
[d] Concentration calculated as Peonidin 3-Glucoside;
[e] Concentration calculated as Malvidin 3-Glucoside.
RSD was determined for each individual anthocyanin with six measurements (Del 3-Gal, 1.5%; Del 3-Glu, 1.3%; Cy 3-Gal, 1.0%; Del 3-Ara, 1.9%; Cy 3-Glu, 1.0%; Pet 3-Gal, 1.7%; Cy 3-Ara, 1,1%; Pet 3-Glu, 1.5%; Peo 3-Glu, 1.2%; Mal 3-Gal, 1.4%; Mal 3-Glu, 1.2%; Mal 3-Ara, 2.2%)
* denotes a statistically significant difference between experimental groups with a 95% confidence level.

Table 4: Table of individual anthocyanin concentrations in blueberry nectar filled in PET-3 in industrial storage

Anthocyanin concentrations [mg/L]					
	Sampling date				
Anthocyanin identification	22. 12. 2004	12. 2. 2005	1. 6. 2005	15. 9. 2005	
Del 3-Gal[a]	52.5	54.0	34.0	11.4	
Del 3-Glu [a]	65.9	67.7	44.0	15.7	
Cy 3-Gal [b]	32.3	32.2	21.4	8.3	
Del 3-Ara [a]	46.3	47.8	28.6	7.9	
Cy 3-Glu [b]	50.5	50.2	33.4	12.7	
Pet 3-Gal [c]	14.4	14.6	9.4	3.1	
Cy 3-Ara [b]	31.5	31.3	19.0	6.1	
Pet 3-Glu [c]	34.9	35.5	23.0	8.3	
Peo 3-Glu	12.1	12.0	7.6	2.8	
Mal 3-Gal [e]	12.8	12.7	7.9	2.8	
Mal 3-Glu [e]	35.6	35.5	22.1	7.8	
Mal 3-Ara [e]	7.7	7.7	4.1	1.1	
	*	*			
			*		
				*	

[a] Concentration calculated as Delphinidin 3-Glucoside;
[b] Concentration calculated as Cyanidin 3-Glucoside;
[c] Concentration calculated as Petunidin 3-Glucoside;
[d] Concentration calculated as Peonidin 3-Glucoside;
[e] Concentration calculated as Malvidin 3-Glucoside.
RSD was determined for each individual anthocyanin with six measurements (Del 3-Gal, 1.5%; Del 3-Glu, 1.3%; Cy 3-Gal, 1.0%; Del 3-Ara, 1.9%; Cy 3-Glu, 1.0%; Pet 3-Gal, 1.7%; Cy 3-Ara, 1,1%; Pet 3-Glu, 1.5%; Peo 3-Glu, 1.2%; Mal 3-Gal, 1.4%; Mal 3-Glu, 1.2%; Mal 3-Ara, 2.2%)
* denotes a statistically significant difference between experimental groups with a 95% confidence level.

Table 5: Table of individual anthocyanin concentrations in blueberry nectar filled in PET-4 in industrial storage

Anthocyanin concentrations [mg/L]					
	Sampling date				
Anthocyanin identification	22. 12. 2004	12. 2. 2005	1. 6. 2005	15. 9. 2005	
Del 3-Gal[a]	54.1	54.6	34.1	10.5	
Del 3-Glu [a]	67.9	68.5	44.2	14.5	
Cy 3-Gal [b]	33.1	32.5	21.4	7.7	
Del 3-Ara [a]	48.1	48.4	28.7	7.2	
Cy 3-Glu [b]	51.7	50.7	33.4	11.9	
Pet 3-Gal [c]	14.9	14.7	9.4	2.8	
Cy 3-Ara [b]	32.4	31.7	19.0	5.6	
Pet 3-Glu [c]	35.9	35.9	23.2	7.7	
Peo 3-Glu	12.5	11.8	7.6	2.6	
Mal 3-Gal [e]	13.1	12.8	8.0	2.6	
Mal 3-Glu [e]	36.6	35.9	22.3	7.2	
Mal 3-Ara [e]	8.0	7.8	4.2	1.0	
	*	*			
			*		
				*	

[a] Concentration calculated as Delphinidin 3-Glucoside;
[b] Concentration calculated as Cyanidin 3-Glucoside;
[c] Concentration calculated as Petunidin 3-Glucoside;
[d] Concentration calculated as Peonidin 3-Glucoside;
[e] Concentration calculated as Malvidin 3-Glucoside.
RSD was determined for each individual anthocyanin with six measurements (Del 3-Gal, 1.5%; Del 3-Glu, 1.3%; Cy 3-Gal, 1.0%; Del 3-Ara, 1.9%; Cy 3-Glu, 1.0%; Pet 3-Gal, 1.7%; Cy 3-Ara, 1,1%; Pet 3-Glu, 1.5%; Peo 3-Glu, 1.2%; Mal 3-Gal, 1.4%; Mal 3-Glu, 1.2%; Mal 3-Ara, 2.2%)
* denotes a statistically significant difference between experimental groups with a 95% confidence level.

tration x measured on 1.12.04 and $\Sigma_{kl}c_{kl}$ represents the sum of individual anthocyanins measured on 1.12.04. The results are presented in Table 6.

Table 6: Normalized differences between relative anthocyanin concentrations between the 1.12.04 and 1.9.05 sampling with GLM statistical evaluation

	Normalized relative difference x [%]							
Anthocyanin identification	PET-1	PET-2	PET-3	PET-4				
Mal 3-Ara	–52.3	–44.4	–33.9	–35.8	*			
Del 3-Ara	–27.6	–24.6	–23.4	–25.3		*		
Cy 3-Ara	–9.0	–15.0	–13.0	–13.2			*	
Del 3-Gal	–6.0	–3.8	–2.4	–3.3				*
Mal 3-Glu	–2.2	–2.7	–2.0	–1.4				*
Pet 3-Gal	3.1	4.7	–2.8	–4.5				*
Mal 3-Gal	1.9	2.4	–1.4	–0.2				*
Del 3-Glu	2.8	6.7	6.8	6.6				*
Peo 3-Glu	8.4	6.4	4.5	4.5				*
Pet 3-Glu	9.9	5.5	7.2	7.6				*
Cy 3-Glu	14.9	14.4	12.7	14.6				*
Cy 3-Gal	18.7	17.4	14.7	16.0				*

* denotes a statistically significant difference between experimental groups with a 95% confidence level.

The statistical evaluation shows that out of the anthocyanins analyzed some anthocyanins are more stable than others. Among the anthocyanins quantified in blueberry nectar, cyanidin 3- glucoside and cyanidin 3- galactoside were the most stable and malvidin 3- arabinoside the least stable. The proposed stability scale conforms to that in the study by.[31]

3. 4 Total Anthocyanins

Table 7 presents the relative total anthocyanin concentrations measured in samples collected at different time periods and with different packaging types. Most anthocyanins were still present after nine month in PET-2 and after a year in nectar stored in PET-3. The minimum amount of anthocyanins are present in PET-4 nectar during its total shelf life.

Total anthocyanin level decreases over the nine month period starting in December 2004 by 78–84%. Total anthocyanin level decreases more with time than with packaging type where it varies by a maximum of 12%.

A similar stability study was made by[8] on blueberry extract. They determined that after 60 days of storage there is only 50% of the initial anthocyanin concentration left in the product. They also identified eight individual anthocyanins and showed that temperature has a substantial

Table 7: Table of total anthocyanin concentrations in blueberry nectar filled in different PET packaging in industrial storage

	Sum of individual anthocyanin concentration [mg/L]				
Sampling date	**PET-1**	**PET-2**	**PET-3**	**PET-4**	
22.12.2004	364.7	398.7	384.0	395.4	*
12.2.2005	358.7	390.0	388.2	392.0	*
1.6.2005	191.1	262.8	246.6	247.5	*
15.9.2005	60.5 *	79.7 *	85.7 *	79.2 *	*

Total anthocyanin concentration was calculated as the sum of individual anthocyanins.
* denotes a statistically significant difference between experimental groups with a 95% confidence level.

influence on anthocyanin stability, as after 60 days of storage at 35 °C there were almost no identified anthocyanins left to be quantified. Similar studies have been carried out by [13] for sour cherry juice, where they correlated degradation curves to first order kinetics in order to determine the most stable anthocyanin. Total anthocyanin degradation curves in correlation to different storage temperatures were also made for blackberry juices and concentrates by[1,28] also noticed that packaging type has an influence on total anthocyanin content, noting that glass packaging protects anthocyanins in pomegranate juice better than laminated cartons. Another study describing the degradation kinetics of total anthocyanin content in various fruit juices was made by[32]. Their research describes the influence of peroxide concentration and temperature on anthocyanin degradation rate. They conclude that anthocyanins from strawberries degrade at the highest rate in comparison to pomegranate anthocyanins and sour cherry anthocyanins.

3. 5 Measurement of Total Antioxidant Activity Using DPPH Test

In Table 8, the absolute antioxidant activities evolution is shown. Total antioxidant activity decreases by 20.9% to 24.2% of the initial value in nine months, depending on the packaging type. The greatest difference is visible after a one year storage period. After a year, nectar in PET-3 and PET-4 show the greatest antioxidant capacity while PET-1 the smallest.

Although measurements of antioxidant activity are quite popular when describing the potential benefits of fruit products, different conclusions have been drawn from different research, depending on the various storage temperatures and times and various packaging.[14] found a significant decrease in antioxidant activity by up to 62% in 29 days in six dark juices in refrigerated storage. Similar results were obtained for orange juice by[30]. They also concluded that temperature, in addition to long storage times, plays a role in the decrease in antioxidant capacity.

On the other hand[10] did not find any change in antioxidant capacity during 10 months of grape concentrate storage at 5 °C. Similar results were reported by[33] for strawberry juice filled in glass or PET stored at 8 °C for 11 weeks.

Previous works thus confirm that besides fruit type[34] there are other influences on antioxidant capacity, especially oxygen availability and temperature. Because of this antioxidant capacity can be a useful tool for packaging and product quality determination.

From the results it can be seen that using different antioxidant analyses yields different decreases in the measured parameter. On one hand approximately 80% of total anthocyanins are destroyed during storage time, but the total polyphenol concentration decreases by only 3–9%. The total polyphenols measurement includes anthocyanins in addition to phenolic acids, hydroxycinnamic acids, flavan-3-ols and other hydrophilic compounds like acids, sugar, and proteins. The reason for this is as reported by[35,36] , i.e. that anthocyanins undergo a process of polymerization during storing. In this case, anthocyanins cannot be detected in total or individual anthocyanin measurements but they can be still detected in the measurement of total polyphenols. The antioxidant capacity measurement partially overcomes the polymerization problem since it is more specific and does not count phenols without antioxidant properties, while including the polymerization effect. The measure of antioxidant capacity can give a fair overview of product oxidation.

Table 8: Antioxidant activity measured by DPPH method in blueberry nectar for different samplings using a GLM statistical evaluation

	Antioxidant activity [%]				
Sampling date	**PET-1**	**PET-2**	**PET-3**	**PET-4**	
22.12.2004	36.36 ± 0.00	36.24 ± 0.05	36.63 ± 0.06	36.98 ± 0.03	*
12.2.2005	35.51 ± 0.07	36.29 ± 0.03	36.43 ± 0.04	36.46 ± 0.05	*
1.6.2005	30.65 ± 0.04	32.45 ± 0.10	32.16 ± 0.07	32.92 ± 0.04	*
15.9.2005	27.65 ± 0.08 *	28.60 ± 0.04 *	28.99 ± 0.05 *	29.10 ± 0.03 *	*

* denotes a statistically significant difference between experimental groups with a 95% confidence level.

From the presented results, a comparison between different packaging types and different storage times can be made. Anthocyanin analyses and antioxidant analyses show that PET-1 material without any oxygen scavenger additive shows a statistically significant difference from the others. Similar results were shown by[15] for orange juice. On the other hand, analyses of total polyphenols do not show a clear trend, which could be explained by small relative concentration changes. While comparing different barriers, PET statistical analysis of antioxidant capacity shows statistically significant differences leading to the conclusion that three layer multilayer PET-4 offers better protection than the other two. No difference could be determined between PET-2 and PET-3 although they were made by different technological procedures. The reason for this is most probably their different mass and wall thickness.

4. Conclusions

From the presented results, there is a decrease in the observed concentrations in all forms of packaging. The relative decrease depends on the kind of analyses carried out. Over nine months, the concentration of total polyphenols decreases by 3–9.9%, the concentration of antioxidant activity by 21–24%, and the total anthocyanin concentration by 78–84%. These differences are due to the different substances group analyses cover. Individual anthocyanins degrade at different rates. Among the anthocyanins quantified in blueberry nectar, cyanidin 3-glucoside and cyanidin 3-galactoside was the most stable and malvidin 3-arabinoside the least stable. The differences in each method are connected to packaging type. Oxygen scavenging bottles protect the product better than PET-only bottles. Among the different oxygen barrier bottles, the three layer multilayer gives best protection to the product out of the bottles tested.

5. Acknowledgments

The authors would like to thank the Ministry of Higher Education, Science and Technology, Slovenia for financial support, Fructal d.d. for the samples analysed and the National Institute of Chemistry, Slovenia, Laboratory for Food Chemistry staff for food analysis help.

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Povzetek

Eden izmed novejših tehnoloških postopkov polnjenja borovničevega nektarja je aseptično polnjenje v PET embalažo. Ker PET omogoča proizvodu razmeroma majhno zaščito pred kisikom, je mogoče dodati lovilce kisika, ki so lahko razporejeni v srednjo plast telesa ali pa so vmešani čez celoten material. Preučevanje stabilnosti polifenolnih antioksidantov je pomembno, ker je znano da imajo pozitiven vpliv na človekovo zdravje, ki se skladiščenjem proizvoda zmanjšuje. Ta raziskava prikazuje spremembe koncentracije skupnih polifenolov, skupnih in posameznih antocianov in spremembe v antioksidativni učinkovitosti borovničevega nektarja tekom skladiščenja v različnih vrstah PET embalaže. Iz analiz posameznih antocianov je bilo mogoče določiti lestvico stabilnosti posameznega antociana. Ker je oksidacija z kisikom eden pomembnejših mehanizmov razpada antocianov so bile za raziskavo uporabljene PET embalaže, ki so različne zaščite pred kisikom. Za določitev vsebnosti antocianov je bila uporabljena gradientna tekočinska kromatografija visoke ločljivosti z detekcijo v vidnem delu spektra. Za določitev skupnih polifenolov in antioksidativne učinkovitosti so se uporabile spektrofotometrične metode. Tekom 9 mesecev skladiščenja se je koncentracija skupnih polifenolov zmanjšala od 3 % do 9.9 %. Antioksidativna učinkovitost se je zmanjšala za od 20.9 % do 24.2 %. Največji padec koncentracije je bilo zaslediti pri določevanju antocianov. Njihova koncentracija se je znižala od 78 % do 84 %. Izmed analiziranih antocianov sta bila najbolj stabilna cianidin 3-glukozid in cianidin 3-galaktozid. najmanj stabilen pa je bil malvidin 3-arabinozid. Embalaža, ki omogoča borovničevemu nektarju najslabšo zaščito je embalaža, ki nima dodanih lovilcev kisika. Glede na analize antioksidativne učinkovitosti ščiti proizvode pred oksidacijo najbolje troslojna večslojna embalaža.