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Matrix and Preservation Technology Dependent Stability and Bioaccessibility of Strawberry Anthocyanins during Storage †

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Abstract: Anthocyanins, often associated with health benefits, readily degrade during processing and storage, and anthocyanin-matrix-interactions affect their stability and bioaccessibility. Our study investigated how anthocyanins in strawberry puree were affected by preservation technologies and addition of relatively protein-rich kale juice. Strawberry-kale-mix (M) was compared to strawberry-water-mix (S), untreated and treated thermally, by pulsed electric fields (PEF) and high pressure (HPP). Anthocyanin stability and bioaccessibility after in-vitro digestion was evaluated during refrigerated storage. The degradation of the strawberry anthocyanins during storage followed 1st-order-kinetics ($c = c_0 \cdot e^{-kt}$). The degradation rate constant k varied depending on juice system, preservation technology and anthocyanin structure. Generally, k was at least doubled for M compared to S. The untreated sample showed the highest k, followed by thermal, PEF and HPP. Both likely resulted from high enzyme activity measured in M and untreated samples. Relative gastric bioaccessibility of anthocyanins was higher for M (1.27–1.29) compared to S (1.10–1.14), indicating interactions of anthocyanins with the kale matrix. Additionally, relative gastric bioaccessibility increased during storage, possibly resulting from anthocyanin polymerization during storage, followed by their decomposition during digestion. Intestinal bioaccessibility was overall low with 20–30% compared to the initial bioaccessible fraction due to instability at neutral pH. This research shows evidence that processing and formulation strongly affect stability and gastric bioaccessibility of anthocyanins during storage.

Keywords: anthocyanins; bioaccessibility; matrix interactions; pulsed electric fields (PEF); high pressure processing (HPP); thermal processing

1. Introduction

Strawberries are a major source of anthocyanins in human nutrition [1]. Anthocyanins are reported to exhibit health-promoting properties, reducing the risk for cardiovascular diseases, cancer and diabetes, by their capacity to scavenge free radicals and indirectly inhibit cell proliferation [2]. However, due to their chemical structure, anthocyanins are unstable and readily degrade and undergo oxidation or polymerization reactions during processing and storage [3–6].

One approach for enhancing anthocyanin retention and stability during processing and storage is related to the effects of preservation technologies working on a different principle than heat, such as pulsed electric fields (PEF) and high pressure processing (HPP) [7–9]. Another approach to alter anthocyanin stability is the formulation with other matrices to create more complex products such as smoothies with potential health effects, as anthocyanins are likely to interact with other food components. In addition, the claimed health-effects of anthocyanins might also require their stability, availability and absorption during gastrointestinal digestion. Such properties can be influenced by the processing technology, matrix properties and interactions, and storage conditions [10].

The aim of the current study was to investigate the stability of anthocyanins in strawberry puree during refrigerated storage, as influenced by preservation technologies including PEF, HPP and thermal treatment, and by formulation with a complex matrix being relatively protein-rich kale juice. Furthermore, the bioaccessibility was assessed before and after storage for a more comprehensive understanding of the potential health effects of such complex multicomponent juice systems.

2. Materials & Methods

2.1. Preparation of Juice Formulations, Processing Conditions and Storage

Strawberry puree and kale juice were mixed (1:1 wt). Appropriate control without the interfering matrix of strawberry-water was prepared. The pH effect on the anthocyanin stabilitywas eliminated by adjusting the strawberry-water to the pH of the strawberry-kale-mix (4.0 ± 0.1) using NaOH.

The thermal treatment, serving as a reference, was applied at 72 °C in a tube-in-tube heat exchanger at a flowrate of 35 L/h, resulting in a holding time of 1 min. The PEF treatment was conducted after pre-heating to 35 °C at an electric field strength of 11.7 kV/cm and an energy input of 120 kJ/kg. The HPP treatment was conducted at 600 MPa for 1 min at room temperature.

For the shelf life tests, the samples were stored at 4 °C for 42 days. The samples were directly frozen at −40 °C until further analyses. Analyzed samples are untreated (control), thermal, PEF and HPP treated strawberry-water-system (S) and strawberry-kale-mix (M).

2.2. Anthocyanin Quantification via HPLC-DAD

Anthocyanins originating from strawberry were analyzed as previously described [11], with slight modifications. Analyses were performed on a Nexera-i LC-2040C 3D Plus (Shimadzu Corporation, Kyoto, Japan) equipped with a UV/Vis detector and a 3 µm Luna® column C18(2) (4,6 mm × 250 mm) (Phenomenex, Torrance, CA, USA). To determine anthocyanins in the aqueous phase, samples were centrifuged at 15,000× *g* for 30 min at 4 °C, filtered using a 0.45 µm before injection.

2.3. In-Vitro Digestion and Relative Bioaccessibility Determination of Anthocyanins

Semi-dynamic in-vitro digestion was conducted on a dual auto titration unit (Titrando 902, Metrohm, Herisau, Switzerland), with a double wall reactor based on the harmonized protocol of Minekus et al. (2014), as previously described [13]. Samples were taken after gastric and intestinal digestion. The *in-vitro* digestion was done directly after processing (day 0) and after storage (day 42).

The bioaccessibility was assessed as described in Stübler et al. (2020) with slight modifications using Amicon® filter with a 10 kDa membrane. After the filtration step, samples were diluted 10-fold, filtered using a 0.45 µm membrane filter and injected in the HPLC-DAD (2.2).

3. Results & Discussion

3.1. Anthocyanin Degradation Kinetics during Storage

The major anthocyanins from strawberry detected in strawberry-containing samples are pelargonidin-3O-glucoside (PG), accounting for 80% of the total anthocyanin content, followed by pelargonidin-3O-malonylglucoside (PMG) with 12%, cyanidin-3O-glucoside (CG) with 5.4%, and pelargonidin-3O-rutinoside (PR) and 5-pyrano-pelargonidin-3O-glucoside (PPG), each with 1.3%.

The kinetics of these major strawberry anthocyanins in the aqueous phase in strawberry-water and strawberry-kale-mix, followed a first order degradation model, as in Equation (1) (c—relative concentration, c_0—relative concentration at time 0, k—degradation constant, t—time in days) and are displayed in Figure 1 and degradation constant in Table 1. The fitting of the model was appropriate with the coefficient of determination (R^2) of 0.9 and higher for all samples.

$$c = c_0 \cdot e^{-kt} \quad (1)$$

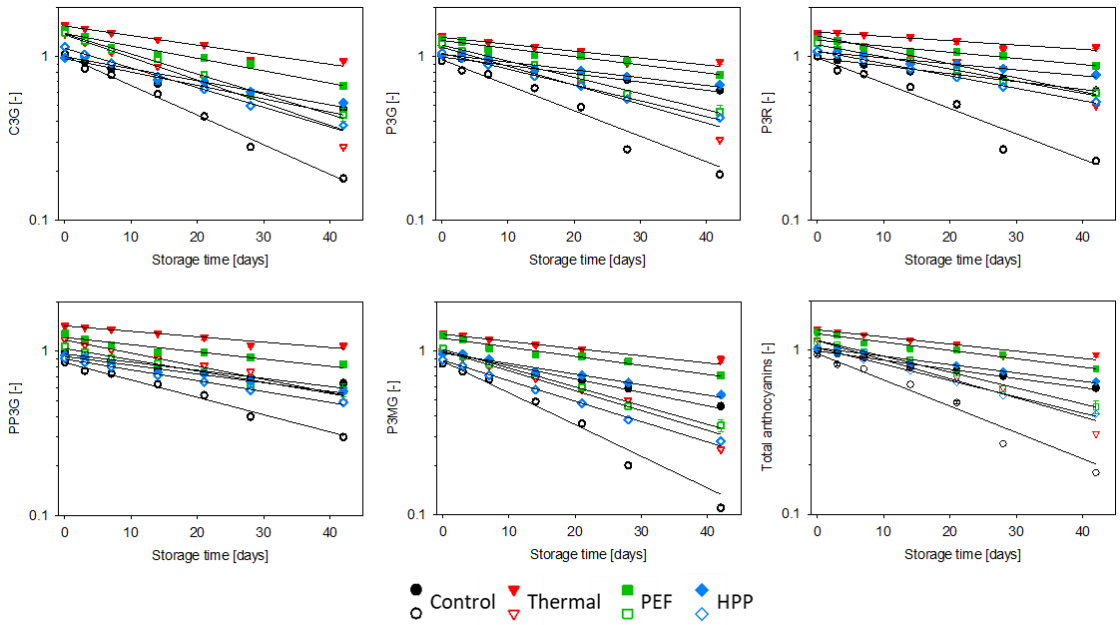

Figure 1. Kinetic modeling for relative anthocyanin content in the aqueous phase measured via HPLC-DAD during refrigerated storage at 4 °C for strawberry-kale-mix (empty) and strawberry-water (filled) (n = 3).

Table 1. Degradation rate constant k ($\times 10^{-2}$) of major strawberry anthocyanins during storage fitting to 1st order kinetic models (n = 3). Capital letters indicate significant differences per row (anthocyanin) and small letters indicate significant differences per column (sample formulation and processing) ($p < 0.05$).

		Cyanidin-3O-Glucoside	Pelargonidin-3O-Glucoside	Pelargonidin-3O-Rutinoside	5-Pyrano-Pelargonidin-3O-Glcuoside	Pelargonidin-3O-Malonyl-Glucoside	Total Anthocyanins
Strawberry-kale-mix	Control	4.40 ± 0.36 ^{a,B}	3.64 ± 0.16 ^{a,C}	3.56 ± 0.04 ^{a,C}	2.45 ± 0.14 ^{a,D}	4.47 ± 0.18 ^{a,B}	3.71 ± 0.09 ^{a}
	Thermal	3.18 ± 0.02 ^{b,A}	2.67 ± 0.01 ^{b,B,C}	1.96 ± 0.02 ^{b,D}	1.86 ± 0.03 ^{b,D}	2.81 ± 0.01 ^{b,B}	2.69 ± 0.01 ^{b}
	PEF	2.83 ± 0.21 ^{b,A}	2.42 ± 0.27 ^{b,c,A}	1.71 ± 0.21 ^{c,B}	1.57 ± 0.24 ^{b,c,B}	2.64 ± 0.19 ^{b,A}	2.22 ± 0.27 ^{c}
	HPP	2.77 ± 0.06 ^{b,B}	2.21 ± 0.04 ^{c,C}	1.75 ± 0.03 ^{b,c,D}	1.51 ± 0.05 ^{c,d,E}	2.86 ± 0.03 ^{b,B}	2.29 ± 0.04 ^{c}
Strawberry-water	Control	1.89 ± 0.05 ^{c,B}	1.17 ± 0.02 ^{d,C}	1.10 ± 0.02 ^{d,C}	1.14 ± 0.05 ^{e,C}	1.87 ± 0.03 ^{c,B}	1.28 ± 0.03 ^{d}
	Thermal	1.35 ± 0.03 ^{d,A}	0.98 ± 0.06 ^{d,B}	0.61 ± 0.06 ^{f,C}	0.76 ± 0.02 ^{f,C}	1.03 ± 0.06 ^{e,B}	1.00 ± 0.06 ^{d}
	PEF	1.75 ± 0.04 ^{c,d,A}	1.13 ± 0.03 ^{d,C,D}	0.86 ± 0.03 ^{d,e,E}	1.01 ± 0.05 ^{e,f,D}	1.30 ± 0.04 ^{d,e,B}	1.18 ± 0.03 ^{d}
	HPP	1.71 ± 0.06 ^{c,d,A}	1.11 ± 0.05 ^{d,C,D}	0.86 ± 0.04 ^{e,D}	1.22 ± 0.18 ^{d,e,B,C}	1.47 ± 0.04 ^{d,A,B}	1.18 ± 0.04 ^{d}

When interpreting the kinetics and the degradation constant k, differences between juice systems, processing technologies and the type of anthocyanin were observed. The degradation

constant was significantly higher for the strawberry-kale-mix compared to the strawberry-water-mix. This difference between the formulations might be, at least partially, caused by higher activity of oxidative enzymes in the strawberry-kale-mix compared to the strawberry-water-mix [14]. In addition, all investigated preservation technologies slowed down the degradation of anthocyanins in the aqueous phase, while the untreated samples exhibited the highest degradation rate during storage. In the strawberry-water-mix, the thermal treatment resulted in the slowest degradation along with the storage, followed by PEF and HPP. While in the strawberry-kale-mix, the degradation was faster for thermal compared to PEF and HPP, that are in a comparable range. Literature reports lower degradation rate constants during storage for thermally treated juice systems, while HPP resulted in comparable or slightly lower degradation rate constants compared to the untreated juices [15,16] as observed for the strawberry-water-mix.

As already mentioned, the degradation kinetics can be influenced by the anthocyanin structure. PMG exhibited a slightly decreased stability over storage compared to PG, except for the thermally treated samples. Previously, the malonyl moiety was reported to decrease the stability [17]. CG exhibited an increased degradation constant compared to PG, suggested to be a result of increased reactivity of CG due to the additional hydroxyl-group at the B-ring [18]. PPG exhibited overall the highest stability, which might be caused by two interfering effects—firstly, the high stability of pyranoanthocyanins in general and secondly, the storage of the juice leading to the formation of newly formed PPG from monomeric PG and low molecular compounds [19–21].

3.2. Relative Bioaccessibility (BA) of Anthocyanins before and after Storage

Bioaccessibility is defined as the fraction being released and made available for absorption in the gastrointestinal tract [22]. Some studies suggest the absorption of anthocyanins already during gastric digestion. Thus, it will be referred to relative gastric and intestinal bioaccessibility as compared to the bioaccessible fraction of the respective sample at the beginning of in-vitro digestion (Table 2).

Table 2. Bioaccessibility (compared to initial content of respective sample) of total anthocyanins after processing and after storage. Different letters indicate significant differences per column and bioaccessibility (G-gastric, I-intestinal) ($p < 0.05$, $n = 4$).

Anthocyanin Content			After Processing	After Storage
Strawberry-water	Control	G	1.11 ± 0.02 ^{b}	-
		I	0.27 ± 0.01 ^{c,d}	-
	Thermal	G	1.10 ± 0.02 ^{b}	1.15 ± 0.01 ^{c}
		I	0.19 ± 0.01 ^{e}	0.14 ± 0.00 ^{d}
	PEF	G	1.13 ± 0.02 ^{b}	1.21 ± 0.02 ^{c}
		I	0.30 ± 0.01 ^{a,b}	0.24 ± 0.00 ^{b}
	HPP	G	1.14 ± 0.01 ^{b}	1.30 ± 0.01 ^{c}
		I	0.24 ± 0.01 ^{d}	0.20 ± 0.00 ^{b,c}
Strawberry-kale-mix	Control	G	1.27 ± 0.02 ^{a}	-
		I	0.28 ± 0.01 ^{b,c}	-
	Thermal	G	1.25 ± 0.01 ^{a}	2.17 ± 0.18 ^{a}
		I	0.19 ± 0.01 ^{e}	0.19 ± 0.03 ^{c}
	PEF	G	1.27 ± 0.03 ^{a}	2.17 ± 0.04 ^{a}
		I	0.32 ± 0.01 ^{a}	0.40 ± 0.02 ^{a}
	HPP	G	1.29 ± 0.02 ^{a}	1.81 ± 0.03 ^{b}
		I	0.26 ± 0.01 ^{c,d}	0.20 ± 0.02 ^{b,c}

An increase in anthocyanins after gastric digestion was observed for all investigated samples, followed by a strongly pronounced decrease during the intestinal digestion. This was previously reported as the pH decrease during gastric digestion leads to augmented presence of the flavylium cation of anthocyanins, which is the most stable and detectable form [23]. During the intestinal digestion at mild alkaline conditions, anthocyanins are considered as highly unstable [24,25].

For the differently formulated and processed mixes, the thermally and PEF treated strawberry-kale-mix exhibited the highest relative gastric BA, followed by the untreated and HPP treated strawberry-kale-mix. The strawberry-water samples, regardless of processing, exhibited the lowest relative BA compared to the initial content. Additionally, the relative gastric BA was significantly increase after storage, which might be a result of polymerization occurring during storage, followed by degradation and release of these anthocyanins during gastric digestion. As the samples assessed immediately after processing exhibit higher concentrations of monomeric anthocyanins compared to the samples after storage, and thus the bioaccetablessibility did not show a similarly pronounced increase of gastric bioaccessibility in samples after processing compared to the samples after storage.

However, the relative intestinal BA was regardless of formulation, processing technology and storage in a range of 20–30 % and thus can be assessed as comparably low as previously reported [26]. The alkaline digestion conditions show to be the limiting factor for intestinal bioaccessibility and to a lesser extent other effects such as matrix (level of bioencapsulation), processing and storage [27].

4. Conclusions

This study investigated the stability and relative bioaccessibility of anthocyanins in strawberry puree during refrigerated storage as influenced by processing and formulation with kale juice. It reports evidence that processing, formulation and anthocyanin structure strongly affect both stability and gastric bioaccessibility of anthocyanins during storage. Thus, when aiming at the development of new products with improved nutritional values derived from anthocyanins, matrix interactions, as well as stability with different processing technologies, should be considered.

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