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Influence of copigmentation on the stability of spray dried anthocyanins from blackberry

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ABSTRACT

Anthocyanins or anthocyanin rich extracts from several fruits are frequently applied as food colorants. One major drawback of these natural pigments is their poor stability and the rapid loss of coloring properties. Spray drying of anthocyanin extracts is a suitable approach for encapsulating and stabilizing them. In this study the effects of added copigments (rutin and ferulic acid) on the storage stability of spray-dried anthocyanins from blackberry were investigated. Degradation of anthocyanins during storage under light and different temperatures showed first order kinetics. Light had a higher impact on anthocyanin stability than elevated temperatures. The addition of copigments led to significantly lower anthocyanin losses compared to samples without copigments and to increased half-life values of the obtained powders. This stabilizing effect may be attributed to the antioxidative properties of the copigments and the prevention of the hydration of the anthocyanins. The observed color changes did not correlate with the anthocyanin loss. Degradation of anthocyanin might led to the formation of colored derivatives and polymers during storage.

1. Introduction

The adjustment of food color to retain, or reconstitute, natural color intensity or to create new hues is common practice in the food industry. Application of synthetic dyes in foods has faced tremendous objections in the last years after studies have demonstrated severe adverse biological effects (McCann et al., 2007) and awareness of consumers for healthy and natural food has grown concomitantly. As a consequence, natural colorants and coloring foodstuffs have increasingly been used to create appealing colors (Dufosse, 2006). Among the natural pigments applied in the food industry, anthocyanins have gained a lot of attention. Besides the broad range of colors induced by anthocyanins, their antioxidative capacity and potential health benefits rendered them attractive compounds for the coloring of different foodstuffs (Wrolstad, 2004). Anthocyanins from various sources are applied as food colorants including extracts or concentrates from black carrot (Kammerer, 2005), black currant (Bakowska-Barczak & Kolodziejczyk, 2011), and blackberries (Francis & Markakis, 1989). The stability of the anthocyanins and their color in the concentrated form as a food ingredient as well as in the final product depends on numerous factors. Besides exogenous parameters like temperature, light, and oxygen, especially the pH and the presence of copigments are the most determining factors of anthocyanin stability (Markakis, 1982; Stintzing & Carle, 2004). An acidic environment is necessary to form the colored flavylium cation, which induces the red color and has been shown to be the most stabile form of the anthocyanins (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Other compounds may form non-covalent complexes with the anthocyanins by stacking of the π-electrons. This copigmentation prevents the hydration of the flavylium moiety and thus stabilizes the red color of the anthocyanin. Depending on the compounds which form the copigmentation complexes, this effect predominantly results in bathochromic and hyperchromic shifts of the absorption maximum (Boulton, 2001). Flavonols like rutin and cinnamic acids such as ferulic acid are potent copigments (Asen, Stewart, & Norris, 1972).

To obtain anthocyanins from the plant materials as a stable and usable food ingredient, several processing steps are necessary which expose anthocyanins to detrimental conditions. These steps include extraction, concentration, and drying. The latter can be conducted by simple thermal treatment, freeze drying, or spray

Abbreviations: cy-3-glc, cyanidin-3-*O*-glucoside; cy-3-dioxlglc, cyanidin-3-*O*-dioxalylglucoside; cy-3-malglc, cyanidin-3-*O*-malonylglucoside; cy-3-xyl, cyanidin-3-*O*-xyloside; cy-3-rut, cyanidin-3-*O*-rutinoside.

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drying. Especially spray drying has been demonstrated to be a fast and gentle method for the drying of anthocyanin extracts (Robert & Fredes, 2015). The application of various polymeric compounds like polysaccharides encapsulates the anthocyanins during the drying process. By embedding the anthocyanins into a polymeric matrix, they are less exposed to oxygen and thus exhibit a greater stability and a longer shelf-life (Robert & Fredes, 2015). Other processing parameters of the spray drier like inlet temperature and feed velocity can be adjusted to optimize product retention. Besides spray drying, numerous techniques for encapsulation of natural pigments have been studied and are reviewed extensively (Cavalcanti, Santos, & Meireles, 2011; Robert & Fredes, 2015). Spray drying was applied in numerous studies to encapsulate and stabilize anthocyanins from various sources like black carrot, pomegranate, blueberry, and blackberry, and also for stabilizing isolated anthocyanins (Idham, Muhamad, & Sarmidi, 2012; Osorio et al., 2010). These studies focused on the optimization of the spray drying parameters to enhance anthocyanin retention. The aim of the present study was to investigate the potential of copigmentation to enhance the storage stability of a spray dried anthocyanin powder obtained from a blackberry (*Rubus fruticosus* agg.) extract. Blackberries were chosen because of their high concentration of anthocyanins and the simple anthocyanin profile.

2. Material and methods

2.1. Sample material

The blackberry concentrate was supplied by Rudolf Wild GmbH & Co. KG (Eppelheim, Germany). It was concentrated to 69° Brix.

2.2. Chemicals and standards

Ultrapure water was obtained from a Synergy purification system (Millipore, Molsheim, France). Acetonitrile (HPLC grade), formic acid and acetic acid (both $\geq$ 98%) were from VWR (Mannheim, Germany). Methanol (HPLC grade) was from Th. Geyer (Renningen, Germany). Malvidin 3-*O*-glucoside ($\geq$87%) was obtained from Phytoplan (Heidelberg, Germany), ferulic acid was from Fluka Analytical (Steinheim, Germany), and rutin was obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Regenerated cellulose filters (Chromafil RC-20/15 MS) were supplied by Macherey-Nagel (Düren, Germany).

2.3. Isolation of anthocyanins

Anthocyanins were isolated according to the procedure described by Juadjur and Winterhalter (2012). Blackberry concentrate was diluted (1:2) with water and was subjected to a glass column (100 cm × 10 cm) filled with XAD7 HP (Sigma Aldrich, Munich, Germany) with a flow rate of 10 mL/min. After washing with water with approximately 5 bed volumes to remove sugars and other polar compounds, anthocyanins and other polyphenols were eluted with methanol/acetic acid (19:1 mL/mL). Lyophilization of this fraction yielded sample XAD7. The remaining eluate from the column was loaded on the membrane adsorber Sartobind S IEX 150 mL (Sartorius Stedim Biotech, Göttingen, Germany) with a flow rate of 80 mL/min using a peristaltic pump. After washing the membrane with 2 L of methanol/acetic acid (19:1 mL/mL) to remove any non-anthocyanin compounds, anthocyanins were eluted with 0.5 mol/L NaCl in aqueous methanol (50mL/100 mL) acidified with approximately 1 mL/100 mL acetic acid. The eluate was concentrated under vacuum to remove the methanol and diluted with water. To remove the salt, the above-mentioned adsorption on XAD7 HP was repeated. The eluate was lyophilized and stored at −20 °C until further use as anthocyanin fraction in samples AC, AC + R, AC + F and AC-red. The composition of these feed solutions for spray drying are shown in Table 1.

2.4. Spray drying of anthocyanins

The anthocyanin fraction and the XAD7 extract were spray dried using a lab-scale drier B-290 (Büchi, Essen, Germany). The method followed the reported method of Ferrari, Marconi Germer, Alvim, and de Aguirre (2013) with modification according to preliminary tests. The inlet temperature was set at 150 °C, the air-flow rate was approximately 470 L/h, and the aspirator was set at 100% (approximately 35 m^3/h). The resulting outlet temperature was held constant at 90 °C by adjusting the flow rate (approximately 5 mL/min). The applied coating material was maltodextrin 19 (DE-value 18–20). All components were dissolved in 100 mL ultrapure water and treated in an ultrasound bath for 20 min and were subsequently filtered through a 595 Whatman paper filter (GE Healthcare, Amersham Place, UK). The content of the anthocyanin fraction in the feed solutions was consistently set at 1 g and a molar ratio of anthocyanin to copigment of 1:1 was established. The amount of maltodextrin varied accordingly to obtain a final dry matter of 6 g/100 g of the feed solution. To assess the influence of the amount of coating material, a sample with reduced maltodextrin (AC-red) content was also prepared. All samples were spray died in duplicate and were pooled for storage test after evaluation of composition.

2.5. Storage stability of the spray dried anthocyanins

An amount of 500 mg of each spray-dried sample was spread in closed petri dishes and subjected to the storage conditions for 98 days. Samples were stored at 4 °C and 35 °C with and without light exposure provided by an UV lamp (25 W, approximately 5000 lux) in a temperature controlled cabinet. Samples were taken every second day in the first week and once every week thereafter. The samples were dissolved in water/acetonitrile/formic acid (80:15:5, mL/mL/mL) with a final concentration of 0.5 mg/mL and stored at −80 °C until analysis. First order reaction rate constants (*k*) and half-lives ($t_{1/2}$) were calculated using the following equations:

Table 1
Composition of the feed solution.

Sample	Anthocyanins or XAD7 extract (g)	Copigment (g)	Maltodextrin (g)
Pure anthocyanins (AC)	1		5
Anthocyanins plus ferulic acid (AC + F)	1	0.43	4.57
Anthocyanins plus rutin (AC + R)	1	1.48	3.52
Anthocyanins with reduced maltodextrin (AC-red)	1		3.52
XAD7 extract (XAD7)	2.66[a]		3.34

[a] 2.66 g XAD7 extract contains 1 g anthocyanins.