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Short communication

Stability of anthocyanins in high pressure homogenisation

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ABSTRACT

Foods containing anthocyanins have shown significant health benefits in various studies. However, outside of their natural environment they are extremely unstable. Encapsulating them in aqueous emulsion droplets under adapted conditions could improve anthocyanin stability. Producing emulsion droplets, however, requires mechanical shear stresses, resulting in increased temperatures known to be critical to anthocyanin stability. Aqueous anthocyanin-rich bilberry extract solutions were therefore exposed to defined thermal, as well as combined thermal and mechanical, stresses, as typically occur during emulsification processes. At pH 3.5, anthocyanin degradation could be detected, especially for thermal stressing, showing the temperature–time–load as the main parameter. Anthocyanins were, however, found to be stable against mechanical stresses. To limit thermal degradation during emulsification, processing time at elevated temperature has to be limited. It was shown, that these conditions can be realised in a high pressure homogenisation process, followed by fast cooling in iced water.

1. Introduction

Foods containing anthocyanins, in different experimental systems (*in vitro*, animal studies, clinical trials) show high antioxidant, anti-carcinogenic and immune-modulating effects (He & Giusti, 2010; Kay, Kroon, & Cassidy, 2009; Middleton, 1998; Wang & Stoner, 2008). Especially, the European bilberry (*Vaccinium myrtillus*) contains high amounts of anthocyanins (Kalt, McDonald, Ricker, & Lu, 1999). However, without adapted protective measures, it is not possible to stabilize anthocyanins after being isolated from their original surroundings, the plant cells. Particularly, thermal stresses, pH-values above four and the presence of oxygen or specific enzymes have been reported to accelerate the degradation of anthocyanins (Brouillard, 1982; Hubbermann, 2005; Queiroz, Oliveira, Pinho, & Ferreira, 2009; Shenoy, 1993). Thus incorporating isolated anthocyanins into food, their storage and their passage through the human digestive system usually leads to a fast degradation (Hager, Howard, & Prior, 2008; Kirca, Ozkan, & Cemeroglu, 2007). This might be a reason why only small amounts of anthocyanins are traceable in the human bloodstream after the consumption of an anthocyanin-rich food (He & Giusti, 2010; Koli et al., 2010; Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Therefore, studies of anthocyanin bioavailability and bioactivity still remain a challenging task.

In the study being reported here, we encapsulated these molecules in submicron-sized aqueous droplets surrounded by a dense monolayer of emulsifiers and triglycerides, serving as 'nano-containers', to stabilize them for use in food systems and bioactivity studies (Frank, Köhler, & Schuchmann, accepted for publication). Within the aqueous droplets, the micro-climate (chemical composition or pH value) was adapted to provide optimal conditions for anthocyanin stability. As oil droplets have been reported to survive stomach conditions (Nik, Wright, & Corredig, 2011; Ribeiro, Schuchmann, Engel, Briviba, & Walz, 2009; Yin, Kobayashi, & Nakajima, 2008) and thus are a perfect shell for anthocyanin protection in gastric passage, the triglyceride phase – containing the aqueous, anthocyanin loaded droplets – may then be emulsified again in a second aqueous phase (W_2), being adapted to food or gastro-intestinal conditions (see Fig. 1).

In order to incorporate the bioactive molecules into the inner aqueous droplets of submicron size, they have to be mixed with the aqueous phase first and then survive an emulsification step of high energy input (Ribeiro et al., 2009). This imposes mechanical and thermal stresses on the bioactive molecules. Therefore, the degradation of anthocyanin molecules in aqueous phases was investigated by exposing aqueous anthocyanin-rich bilberry extract solutions to defined mechanical or thermal stresses, comparable to those found in emulsification processes.

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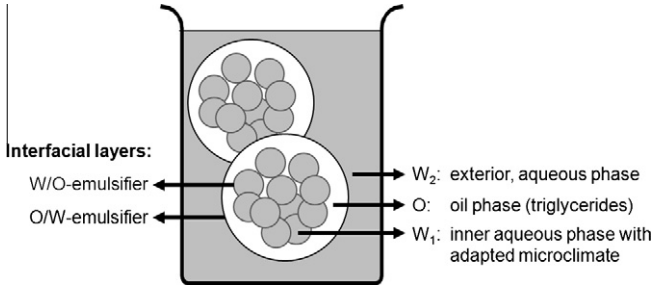

Fig. 1. Principle scheme of a multiple emulsion: encapsulation of anthocyanins in submicron-sized aqueous droplets (W_1) of adapted microclimate (e.g. pH buffered), using stomach-stable shells, based on triglycerides and interfacial layers.

2. Materials and methods

2.1. Materials

Bilberry extract (product name: 600761 Bilberry Extract 25%) was provided by Kaden Biochemical GmbH, Germany. As stated by the manufacturer, the raw material for the 600761 Bilberry Extract is the pomace of the European bilberry. The content of anthocyanin aglycones is adjusted to 25% (w/w) whereas the total anthocyanin content of the extract is approximately 37% (w/w). Hydrochloric acid (37%, pure, CAS No. 7647-01-0), and cyanidin-3-glucoside (Kuromaninchlorid Rotichrom® HPLC, CAS No. 7084-24-4) were purchased from Carl Roth, Germany.

2.2. Methods

For the preparation of the aqueous anthocyanin solutions, the anthocyanin-rich bilberry extract was dissolved in distilled water, stirring constantly for 30 min, via a magnetic stirrer at room temperature. Insoluble solid extract particles were separated by filtration (Sartorius Stedim biotech Germany, Filter Discs, Grade 388). For the heat-stability tests, bilberry extract solutions, with concentrations of c_{BE} = 250 μg/ml, were heated to 333, 343 and 363 K, these being temperatures typically found in emulsification processes. The short residence time of emulsifying machines (30 ms < $t_{process}$ < 5 s, depending on the emulsifying device) were realised by heating the aqueous bilberry extract solutions in micro-heat-exchangers (produced by the Institute for Micro Process Engineering, KIT, $t_{residence}$ < 1 s, heating rate $\Delta T/\Delta t \sim$850 K/s). Longer heating times (up to 120 min (7200 s)) were realised by storing the samples in hot cabinets tempered to the adequate temperatures. The anthocyanin concentration was analysed every 30 min (1800 s).

Emulsification always imposes mechanical stresses, which also induce thermal stresses to the product. Therefore, the anthocyanin stability was also investigated under combined thermal and mechanical stresses. For this, bilberry extract solutions of 250 and 1000 μg/ml were homogenised in a high pressure homogenizer (MF-110EH-30, Microfluidics®, USA) at pressure differences Δp between 300 and 1500 bar. Under these process conditions, high shear rates, as well as cavitation (Baldyga, Makowski, Orciuch, Sauter, & Schuchmann, 2009), act on the droplets and, thus, also on the bioactive molecules. After the high pressure treatment the samples were cooled immediately to 298 K by putting them into iced water.

The anthocyanin concentration was analysed by a spectrophotometer according to the method described by Bonerz, Würth, Patz, and Dietrich, (2006). For this, hydrochloric acid (diluted to 5% (v/v)) was added to either cyanidin-3-glucoside-solutions of defined concentrations for calibration or the samples, respectively. Cyanidin-3-glucoside was chosen for calibration as it is a major component of the bilberry extract used here. Adding the hydrochloric acid shifted the equilibrium of the anthocyanin chromophores to the most stable form, the flavylium cation, whose characteristic absorption wavelength is between 500 and 520 nm (depending on the glycosylation of the anthocyanin type). The absorption was measured at 520 nm, before and after treatment. The anthocyanin concentration c of the samples was quantified, based on cyanidin-3-glucoside equivalents, by using the pre-determined calibration line. Herewith the relative anthocyanin concentration of the samples was determined according to Eq. (1).

$$a = \frac{c(t)}{c(t_0)}, \tag{1}$$

where $c(t_0)$ is the concentration before and $c(t)$ the concentration after thermal or/and mechanical treatment.

The time dependency of the degradation was described by a first order kinetics (Eq. (2)) while an Arrhenius equation was applied in describing the temperature dependency (Eq. (3)), (Kirca et al., 2007; Wang & Xu, 2007):

$$c(t) = c(t_0) \cdot e^{-k(T)\cdot t} \tag{2}$$

$$k(T) = A \cdot e^{\frac{-E_A}{R\cdot T}} \tag{3}$$

$k(T)$ = temperature T dependent degradation constant [s^{-1}]; A = Arrhenius factor [s^{-1}]; E_A = activation energy [J/mol]; R = universal gas constant [J/(K mol)].

Standard deviations, given in the results, followed from three repetitions of each experiment and three for each analysis, respectively.

3. Results

Fig. 2 shows results for the thermal degradation of anthocyanins in bilberry extract solutions at pH 3.5 for temperatures between 333 and 363 K. Treatment time ranged from less than 1 s to 120 min (7200 s). The relative anthocyanin concentration, a, is reduced with increasing time and increasing temperature. Using an exponential fit (according to Eq. (2)) results in degradation rate constants, k, as shown in Table 1. Based on Eq. (3), an activation energy of 34 kJ/mol and an Arrhenius factor A of 10.4 s^{-1} were determined.

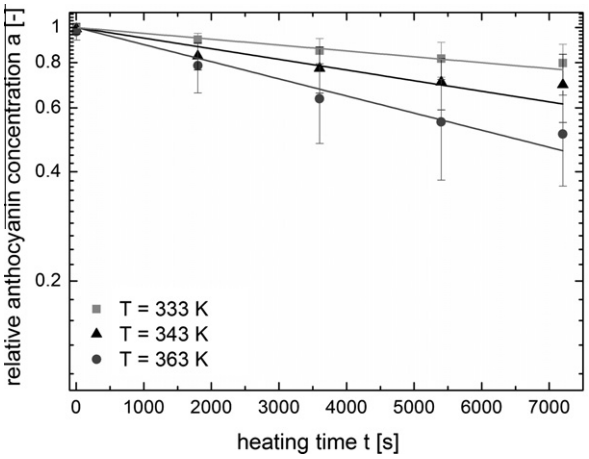

Fig. 2. Influence of thermal treatment on anthocyanin degradation: decrease of the relative anthocyanin concentration $a(t)$ in bilberry extract solutions ($c_{BE}(t = 0)$ = 250 μg/ml) at 333 K (■), 343 K (▲) and 363 K (●). pH (298 K) 3.5. The full lines represent the applied first order-models.