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Preparation and Comparative Release Characteristics of Three Anthocyanin Encapsulation Systems

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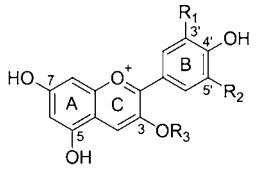
ABSTRACT: Bilberries (*Vaccinium myrtillus* L.) and their major polyphenolic constituents, anthocyanins, have preventive activities *inter alia* against colon cancer and inflammatory bowel diseases. However, anthocyanins are sensitive to environmental conditions; thus their bioavailability in the gastrointestinal tract is an important determinant of their *in vivo* activity. In the study reported here, the potential benefits of encapsulating an anthocyanin rich bilberry extract (BE) on anthocyanin stability were investigated. Nonencapsulated BE and three different BE loaded microcapsule systems were incubated in simulated gastric fluid (SGF) and fed state simulated intestinal fluid (FeSSIF). After exposure to these media, released anthocyanins were identified and quantified by HPLC with UV/Vis detection. Although a rapid release of anthocyanins was observed within the first 20 min, encapsulation of anthocyanins doubled the amount of available anthocyanins after 150 min of incubation. These results illustrate the ability of encapsulation to inhibit early degradation of anthocyanins in the intestinal system.

KEYWORDS: *anthocyanins, bilberry extract, encapsulation, Vaccinium myrtillus L., gastrointestinal tract*

INTRODUCTION

Anthocyanins (see Figure 1 for basic structures) are sugar conjugates of a class of flavonoids, anthocyanidins, that are



R_1	R_2	Anthocyanidin
OH	H	Cyanidin
OH	OH	Delphinidin
OH	OCH_3	Petunidin
OCH_3	H	Peonidin
OCH_3	OCH_3	Malvidin

R_3 = glucopyranoside or galactopyranoside or arabinofuranoside

Figure 1. Chemical structures of the most common anthocyanins found in bilberry.

secondary metabolites of numerous plants.[1−3] In contrast to other flavonoids, anthocyanidins are flavylium cations that vary in numbers and positions of hydroxyl and methoxyl groups on the B-ring.[4−6] Usually anthocyanins consist of anthocyanidins linked at positions 5 and 7, or (less frequently) positions 3′ and 5′, to sugar moieties such as glucose, galactose, arabinose, rhamnose, or xylose.[1] Anthocyanins are water-soluble pigments that are widely distributed in the plant kingdom, and they are especially prevalent in flowers, fruits, and vegetables,[7,8] where they confer bright colors such as orange, blue, and red.[5,9] The color and stability of anthocyanins are pH-dependent; in acidic media (pH ∼ 2) anthocyanins are stable, red-colored flavylium cations, while loss of protons following an increase to pH 6−8 results in conversion to an unstable, blue quinoidal base,[10,11] accompanied by a reduction in bioavailability.[4,12]

In recent decades there has been intense interest in anthocyanins, prompted by increasing evidence that their consumption has beneficial health effects.[13] Numerous *in vitro* and *in vivo* studies have shown that anthocyanin-rich berries provide strong antioxidants,[14−17] inhibit the growth of tumor cells, especially in the colon,[18−20] induce apoptosis,[21,22] and possess anticarcinogenic properties.[23,24] Partly for these reasons, human diets often contain substantial amounts of anthocyanins. For example, the daily intake of anthocyanins averages about 200 mg/person in the U.S.[25] Among dietary sources of anthocyanins, bilberry (*Vaccinium myrtillus* L.), containing 15 different anthocyanins,[6,26,27] is one of the richest sources.

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Table 1. Overview of the Investigated Capsule Systems

no.	system	type	excipients	particle size (μm)	pH
1	PA	core−shell	pectin amide,[a] $CaCl_2$, glycerine	1800 ± 200	3 (stock solution with BE) 1 (cross-linking solution with $CaCl_2$)
2	WPI	matrix	whey protein isolate,	180	1.5 (before encapsulation)
3	SL	coated matrix	pectin amide,[b] citric acid maltodextrin, schellac	250−500	2 (before spray drying)

[a]Degree of esterification, 31.9%; degree of amidation, 21.1%. [b]Degree of esterification, 29%; degree of amidation, 21%.

Clearly, for dietary anthocyanins to have any pharmacological effects, the food sources or supplements must be sufficiently rich initially. However, even then, poor bioavailability and the pH-dependent degradation of anthocyanins after oral consumption may prevent sufficient concentrations of bioactive anthocyanins reaching target sites. For example, in a study by González-Barrio et al.[28] after ten healthy human volunteers with an ileostomy each consumed 300 g of raspberries, ileal, urine, and plasma contents of anthocyanins, relative to initial levels, were reportedly 40%, 0.1%, and subdetectable, respectively. Encapsulation of these substances might provide a robust means to increase concentrations of bioactive anthocyanins in the small intestine and thus boost their beneficial effects, e.g. inhibition of the growth of tumor cells.[29,30]

The possibilities and potential benefits of encapsulating anthocyanins have not yet been widely examined by food technologists. However, Fang and Bhandari reviewed polyphenol encapsulation techniques and considered spray- and freeze-drying processes for encapsulating anthocyanins using maltodextrin as wall material.[31] In addition, Cavalcanti and coworkers[32] have reviewed spray-drying processes and several other possible techniques, including spray cooling, extrusion coating, fluidized bed coating, liposome entrapment, and simple or complex coacervation for anthocyanin encapsulation. Excipients used for encapsulating various substances have included maltodextrin, β-cyclodextrin, pullulan, glucan gel, curdlan, sodium alginate, and pectin. In addition, Oehme et al.[33] have used pectin and shellac to encapsulate anthocyanins.

The ultimate objectives of the study reported here were to develop and characterize encapsulation techniques capable of increasing the concentrations of anthocyanins reaching and passing through the small intestine for release in the colon. For this purpose, capsules had to be enteric-coated or water insoluble and ideally require degradation by colonic bacterial flora or enzymes to avoid early release in the upper parts of the GIT. Several materials with such properties were selected as capsule matrices, based on the following considerations. Enteric coating of food materials is restricted to some degree because several common pharmaceutical excipients, e.g. poly(methacrylic acid) (Eudragit), are not approved for food use. However, shellac is a potential biopolymeric alternative with gastric-resistant properties, which has established applications (described in major pharmacopoeias) and has been registered for use in food (E 904).[34,35] Due to the shift in pH from acidic to close to neutral or mildly alkaline during passage from the stomach to the intestine, the shellac coating dissolves and the concentration of released anthocyanins (or other carried substance) increases. Calcium pectinate is a gel formed by ionotropic cross-linking of pectin by calcium ions. This excipient is insoluble in water but is fermented by the bacterial flora of the colon.[36] Thus, it is appropriate for colon targeting.[37,38] Whey protein isolate (WPI), a byproduct of dairy processing, is a water-soluble complex protein mixture that forms stable, insoluble but biodegradable hydrogels under various conditions, e.g. after alkaline or acidic treatment followed by heating or in the presence of calcium ions. In this study gels were generated by heating an acidified (pH ∼2) WPI-solution. These hydrogels are promising matrices for the microencapsulation of anthocyanin-rich bilberry extracts because the acidic environment stabilizes incorporated anthocyanins and they are released time-dependently in aqueous environments.[39]

The aim of the present study was to examine the differences in stability between nonencapsulated and encapsulated BE anthocyanins, and consequent release of the encapsulated anthocyanins. Three types of encapsulation systems were tested, designated PA (system 1, based on polysaccharide pectin amide), SL (system 3, based on polysaccharide pectin amide with an additional shellac coating), and WPI (system 2, based on whey proteins). The release and stability of the BEs were assayed *in vitro* according to dissolution tests for enteric-coated solid dosage forms described in the European Pharmacopoeia (Ph. Eur. 6.8) and the United States Pharmacopoeia (USP 32). *In vivo*, gastric conditions, e.g. the pH and pepsin concentration, are strongly dependent on the highly variable effects of ingested food.[40] Thus, to standardize incubation conditions, both nonencapsulated BE and BE-loaded capsule systems were incubated in simulated gastric fluid (SGF) at pH 1.2 (without enzymes) to mimic gastric conditions and fed state simulated intestinal fluid (FeSSIF) to simulate intestinal conditions after food ingestion. However, such simulated media must always be recognized as approximations of *in vivo* conditions, which vary widely in terms of factors such as pH, concentrations of bile salts and phospholipids, and mineral composition. In the following text, we present the results obtained and discuss the implications of data obtained for all of the tested systems.

MATERIALS AND METHODS

Chemicals. Amidated pectins (AU-L 027/09 and CU 025) were obtained from Herbstreith & Fox KG (Neuenbuerg, Germany), and maltodextrin (C*Dry 01915, DE 18.5) was obtained from Cargill Deutschland GmbH (Krefeld, Germany). SSB Aquagold was donated, as a source of shellac, by Stroever GmbH & Co. KG (Bremen, Germany). Hydroxypropylmethylcellulose (Pharmacoat 606) was donated by Shin-Etsu (Shin-Etsu Chemical Co., Ltd., Japan). Whey protein isolate (WPI BiPro) with a protein content of 94% (w/w) was obtained from Davisco Foods International Inc. (Le Sueur, MN, USA). Porcine pancreatin and bile extract were supplied by Sigma Aldrich (Steinheim, Germany). Phospholipon 90 G was kindly gifted by Phospholipid (Cologne, Germany). Anthocyanin standards were obtained from three sources: delphinidin-2,5-*O*-diglucopyranoside (internal standard, IS) and cy-3-glc were from Extrasynthése (Lyon, France); cy-3-gal, cy-3-ara, peo-3-glc, peo-3-gal, peo-3-ara, del-3-glc, pet-3-glc, and mal-3-glc were from Polyphenols (Sandes, Norway); del-3-gal, del-3-ara, pet-3-gal, pet-3-ara, mal-3-gal, and mal-3-ara were kindly provided by Michael Kraus (University of Wuerzburg, Germany).[41] All other chemicals and solvents used for analytical purposes were of analytical grade.

Bilberry Extract (BE). Dry BE (600761 Bilberry Extract 25%) was obtained from Symrise GmbH & Co. KG, Holzminden, Germany). This product is made from European bilberry (*Vaccinium myrtillus* L.) pomace by extraction with acidified methanol, filtration, evaporation, and lyophilization (with a residual methanol content of 43 ppm, according to the supplier's certificate of analysis). It was stored dry and cold at −24 °C. The BE also contains other polyphenols, tannins, carbohydrates, and fibers. Basic characterization of the anthocyanin profile was performed by Symrise.

Microcapsule Systems. The microcapsules tested in this study were composed of different excipients and produced by varying techniques, as summarized in Table 1. Their structures are schematically depicted, and their shapes, as seen under a light microscope, are shown in Figure 2.

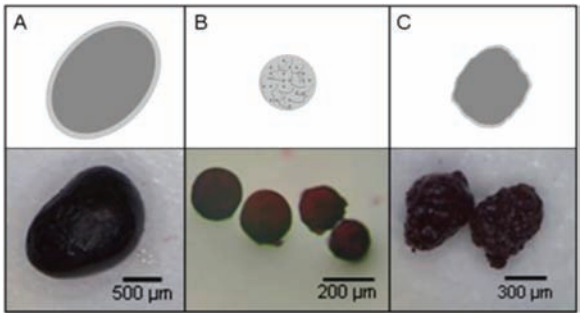

Figure 2. Schematic diagrams and native shapes (as seen under a light microscope) of the investigated capsule systems: A, system 1—PA; B, system 2—WPI; C, system 3—SL. The surfaces and shapes of PA and WPI capsules are uniform and even, while those of SL capsules are rough and uneven.

Generation of Microcapsule System 1—PA. Low methoxy pectin can form gels with an egg-box configuration in the presence of many divalent cations, and the introduction of amide groups tends to make the pectin less hydrophilic, increasing its tendency to form gels. Thus, more rigid and resistant gels can be prepared by using an amidated low methoxy pectin.[42,43] In this study, liquid-filled calcium pectinate capsules were prepared by extrusion from an aqueous solution of ∼1% (w/w) amidated pectin (Pectin amid AU-L 027/09), using a simple one-step process,[44,45] as follows. First, calcium chloride (2% w/w, for cross-linking) was dissolved in double-distilled water, and then 20% (w/w) BE was added. After centrifugation, the supernatant was diluted with a similar volume of anhydrous glycerol. Thus, the resulting cross-linking solution contained 50% (v/v) glycerol, 1% (w/w) calcium chloride, and 10% (w/w) bilberry extract. The pH was adjusted to 3 using concentrated hydrochloric acid.

In the capsule production process, first the cross-linking solution was added dropwise, using a high-precision metering syringe, into a cylindrical glass containing the amidated pectin polymer solution. A capsule membrane formed instantly around each droplet as soon as the two liquids came into contact.[46] The capsules were then transferred into a 2% (w/w) calcium chloride solution at pH 1 containing 20% (v/v) glycerol for 3.5 min at room temperature to stabilize the gel membranes, complete the polymerization process, and provide stable ambient conditions for the encapsulated anthocyanins. The capsules were then filtered and stored in sunflower oil for transportation and to prevent anthocyanin leakage.

The encapsulation efficiency of this procedure, defined as the amount of anthocyanins in the prepared capsules divided by the amount of anthocyanins in the cross-linking solution, is very high, about 99%. This is due to the rapid diffusion of the calcium cations, relative to that of the large polymer molecules, which leads to the rapid formation of intermolecular cross-links between the divalent calcium ions and the negatively charged carboxyl groups of the amidated pectin molecules, resulting in the formation of a network[46] and growth of a gel membrane along the flux direction of the calcium ions.[47]

The diameters, core volumes, shell volumes, shell thickness, and core−shell ratios of the calcium pectinate capsules produced were determined by magnetic resonance imaging, using a Chemagnetics Infinity Plus 600 spectrometer (Bruker, Karlsruhe, Germany),[48] and they were found to be 1.8 ± 0.2 mm, 2.31 mm^3, 0.74 mm^3, 80 μm, and 3.1, respectively.

Generation of Microcapsule System 2—WPI. A stock solution with a protein content of 30% (w/w) was prepared by dissolving WPI powder at 4 °C in distilled water for 12 h, and then BE was slowly added and dissolved in it, and subsequently diluted hydrochloric acid was added to obtain a protein content of 20% (w/w) and a BE content of 5% (w/w). Finally, the pH was adjusted to 1.5 with diluted hydrochloric acid. The generated BE-WPI-solution was centrifuged at 5000*g* for 2 min to remove insoluble fractions.

BE was microencapsulated using the emulsification/heat gelation method, as follows. The BE-WPI supernatant was poured into sunflower oil in a 1000 mL beaker with four standard steel baffles at 25 °C to prepare an emulsion. The mixture was warmed to 50 °C while gently stirring at 1000 rpm with an RW 20 DZM device with a blade impeller (IKA-Werke GmbH & Co. KG, Staufen, Germany), resulting in dispersion of the aqueous phase in ca. 15% of the total volume. This was followed immediately by heating the generated W/O-emulsion to 80 °C within 6 min, holding this temperature for 10 min to promote gelling and then cooling to 20 °C. The resulting microcapsule suspension was centrifuged at 1000*g* for 2 min, and the oil-supernatant was discarded.

During the encapsulation process, 7.4% of the investigated anthocyanins in the nonencapsulated core solution (anthocyanin-containing WPI solution) were degraded, and the final encapsulation efficiency (ratio of encapsulated to nonencapsulated core material) was 95.6%. The sizes of the microcapsules were determined using a Coulter LS 230 laser diffraction analyzer (Beckman-Coulter, Krefeld, Germany), and their mean diameter was found to be ca. 180 μm.

Generation of Microcapsule System 3—SL. The SL-type microcapsules were prepared as follows. A 30% (w/w) feed solution was obtained by diluting a solution containing 65% maltodextrin as the main wall-forming compound, 13% (w/w) citric acid, and 2% (w/w) pectin (Pectin amid CU 025) with tap water. BE was added, to 20% (w/w), the mixture was agitated for 2 min with a hand blender, and the pH was adjusted to 2.

The mixture was then spray-dried using a Mobile Minor laboratory-scale spray dryer with 1−7 kg/h water evaporative capacity (Niro A/S, Denmark) equipped with a rotating disk for atomization. The selected inlet air temperature, outlet air temperature, and disk rotation rate were 70 °C, 160 °C, and ca. 23,000 rpm, respectively. Prior to the coating process, the matrix capsules were granulated with ethanolic shellac solution (10%, w/v) as binder in a granulating pan. Granule sizes ranged between 250 and 500 μm. The coating process was performed in a Mini Glatt laboratory-scale fluidized-bed coater with a Wurster insert (Glatt GmbH, Binzen, Germany). The coating solution contained 9% (w/w) shellac (film-forming polymer), 2% (w/w) glycerol (plasticizer), and 4% (w/w) HPMC (pore former) in distilled water. For 100 g granulated microcapsules, 25 g of polymer was used. Operating conditions were as follows: inlet air temperature, 80 °C; product temperature, 50 °C; process air pressure, 0.35 bar; spray rate, 1 g/min; atomizing air pressure, 1.45 bar; spray nozzle diameter, 0.5 mm. The coated microcapsules were dried for 30 min at 40 °C in a drying oven and finally classified by sieve analysis. Resulting particles exhibit diameters ranging from 250 to 500 μm. The encapsulation efficiency of this encapsulation system was not determined.

Release Media. The stomach milieu was simulated using SGF pH 1.2 (USP 32) without enzymes. The small intestine simulating media (FeSSIF) was Sørensen phosphate buffer pH 6.8 (53.4% potassium dihydrogen phosphate 1/15 M and 46.6% disodium hydrogen phosphate dihydrate 1/15 M) supplemented with 15 mM bile salts, 3.75 mM phospholipids, 150 mM sodium chloride, 5 mM calcium chloride, and 450 U/mL pancreatin as enzyme source.[49] The bile acid content of the heterogenic porcine bile extract used was assayed using the enzymatic Ecoline S^+ test (Diagnostic Systems, Holzheim, Germany) and adjusted as appropriate prior to its addition.

***In Vitro* Release Systems.** *In vitro* release kinetics of anthocyanins from the capsules and degradation kinetics of nonencapsulated anthocyanins were monitored during incubations in vessels that were continuously agitated, at 10 rpm about their horizontal axes, in an end-over-end shaker equipped with a temperature control system. Capsules and release media were warmed prior to the start of the experiment to 37 ± 1 °C, and they were maintained at this temperature throughout each incubation. Samples of both nonencapsulated BE and BE-loaded capsules were added to the incubation media so that the initial total anthocyanin concentration was 0.7%. This concentration was set as 100%, and concentrations in all samples were related to this value. Following standard pharmacopoeia methods (Ph. Eur. 6.8, USP 32), the compounds were incubated for 120 min in total in SGF. After a change to a medium with higher pH, the pharmacopoeias recommend an incubation time of at least 45 min to assess the release of tested compounds. Here, nonencapsulated BE and capsule systems were incubated for 150 min in the FeSSIF medium because anthocyanins continued to be released from the investigated capsule systems after 45 min incubation in it.

At preset time points (0, 10, 20, 30, 45, 60, and 120 min for incubations in SGF; 0, 10, 20, 30, 60, 120, and 150 min for those in FeSSIF), the pH was adjusted to its initial value of 6.8 for the FeSSIF mixtures (the pH of the SGF did not change, so no such adjustment was required) and 100 μL samples were taken.[50] Each sample was diluted with 900 μL of a mixture of formic acid/methanol/bidistilled water (10/50/40, v/v/v) to stop the enzyme reaction and stabilize the anthocyanins. Subsequently, the samples were centrifuged using an Eppendorf mini spin instrument (Eppendorf, Germany) at 11300*g* for 5 min to separate the dispersed components. The resulting supernatants were transferred to HPLC glass vials and stored at −20 °C in the dark until analysis (see below). All experiments were performed in triplicate (n = 3).

HPLC-UV/Vis Analysis. The anthocyanins were chromatographically characterized and quantified using an HPLC-UV/Vis system consisting of a PU-2080 intelligent HPLC pump, a LG-2080-02 ternary gradient unit, a UV-2075 plus intelligent UV/Vis detector (detection wavelength, 520 nm), a DG-2080-53 3-line-degasser, an AS-2055 plus intelligent sampler (all from Jasco, Gross-Umstadt, Germany), a Luna 3 μm C18 (2) 100 Å, 250 mm × 4.6 mm column (Phenomenex, Aschaffenburg, Germany), and a security guard column. Following injection of 20 μL samples, 15 anthocyanins and the internal standards were separated and quantified using this system and a mobile phase consisting of acetonitrile/water/HCOOH (87/3/10 v/v/v; solvent A) and acetonitrile/water/formic acid (50/40/10 v/v/v; solvent B) at a flow rate of 0.5 mL/min and an elution gradient consisting of 2−14% B (0−20 min), 14% B held (20−40 min), 14−15% B (40−50 min), rising to 19% B (50−55 min), then to 20% B (55−65 min), and finally washing and reequilibration with 2% B (65−75 min).

Anthocyanin Identification and Quantification. The anthocyanins in the samples were identified by comparing their retention times with those of authentic standards.[41] In addition, they were quantified (as cy-3-glc equivalents) by comparing the ratios of their peak areas to those of an internal standard (0.08 mg/mL delphinidin-2,5-*O*-diglucopyranoside) to calibration ratios obtained from analyses of solutions of pure cy-3-glc in solvent A, at concentrations ranging from 0.262 to 64 μg/mL, with the same internal standard. Limits of quantification (LOQ) and limits of detection (LOD) were defined as concentrations yielding signal-to-noise (S/N) ratios of 10:1 at 0.3 mg/L and 3:1 at 0.1 mg/L, respectively.[51]

RESULTS AND DISCUSSION

To compare the stability of nonencapsulated and encapsulated bilberry extract (BE), and assess the release of anthocyanins in conditions imitating those of the upper gastrointestinal tract, the preparations were incubated in simulated gastric fluid (SGF) and fed state simulated intestinal fluid (FeSSIF) in vessels agitated in an end-over-end shaker. This type of shaker has several advantages compared to the paddle or rotating basket apparatuses recommended in the pharmacopoeias; notably, it generates weaker, but constant, shear stress. In contrast, when using paddles, the shear stress strongly varies with location in the agitated system, and the paddles may kick the delivery system and cause a burst release. Use of rotating baskets can also be disadvantageous because they cause high abrasion due to mechanical interaction between the baskets and release systems, leading to higher variability in the release results. Further benefits of using an end-over-end shaker to monitor release kinetics are that smaller volumes of release media and, hence, smaller samples are required, and due to the mild agitation, the results are highly reproducible. The other conditions, such as temperature and permanent contact between the incubation media and samples, are identical to those applied in the methods recommended in the cited pharmacopoeias. Samples were drawn at preset time points, and the anthocyanins were stabilized by adding 10% (v/v) formic acid and analyzed by HPLC-UV/Vis to characterize and quantify the 15 anthocyanins present in the BE.[10,41] To minimize analytical errors, in further analyses, the total anthocyanin contents of the final capsules were determined and set to 100%. The total anthocyanin content of each sample was related to this value and then calculated and plotted as cy-3-glc equivalents.

To determine the stability of the BE anthocyanins under stomach-simulating conditions, samples were incubated in SGF. During these incubations, no pH changes of the medium were observed. The changes in anthocyanin concentrations observed during incubation with nonencapsulated extract are shown in Figure 3 (light gray curve). In the stomach mimetic medium,

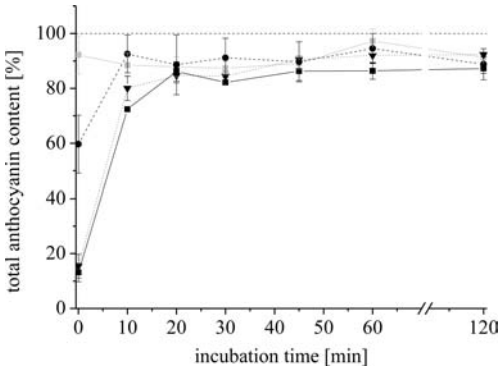

Figure 3. Total anthocyanin concentration [%] (0.7% BE is equivalent to 100% anthocyanins) during incubation with preparation of simulated gastric fluid (SGF; without enzyme) measured by HPLC-UV/vis at 520 nm. Control: nonencapsulated BE (■)(light gray curve). Encapsulated BE: system 1, PA (■); system 2, WPI (●); system 3, SL (▼). Values are means of three determinations ± SD.

the anthocyanins were stable, and their concentrations remained roughly constant for 120 min, in accordance with previous reports that anthocyanins are stable at low pH. In addition, the stability of anthocyanins in three BE encapsulation systems, and their release kinetics from the systems, were investigated and compared to the stability of those in the nonencapsulated extract (see Figure 3). Incubations with systems PA and SL resulted in very similar release profiles, with the anthocyanin concentration rising to a maximum, indicative of compete release, within the first 20 min, and then remaining stable for the following 100 min. The WPI capsule system also yielded similar results, but the release was much

faster and completed within the first 10 min. The higher observed release rate from the WPI system can be mainly attributed to the smaller mean capsule diameter of the capsules (180 μm) compared to those of the SL- (250−500 μm) and PA- (1800 ± 200 μm) systems. The protein based WPI-capsules swell, pH-dependently, on hydration, but this does not cause release because the hydrogels are used in prehydrated form; thus, the swelling-induced alteration of the hydrogel mesh size, which would induce release if the gel was used in dried form, is negligible.

Since the total anthocyanin concentration did not decrease during the incubation time, the results are consistent with previous indications that anthocyanins are not degraded under SGF conditions.[52,53] None of the capsule systems tested here were able to prevent early release of anthocyanins from BE into the stomach-mimicking medium, even the SL-system, although shellac-coated microcapsules reportedly have a strong protective effect.[33] This could be due to pore-formation in the shellac coating resulting from the incubation media dissolving the HPMC of the film, with consequent loss of the coating layer's gastric-resistant properties and liberation of the incorporated anthocyanins. Another factor that may promote release is the nonuniform surface area of the particles (see Figures 2 and 4)

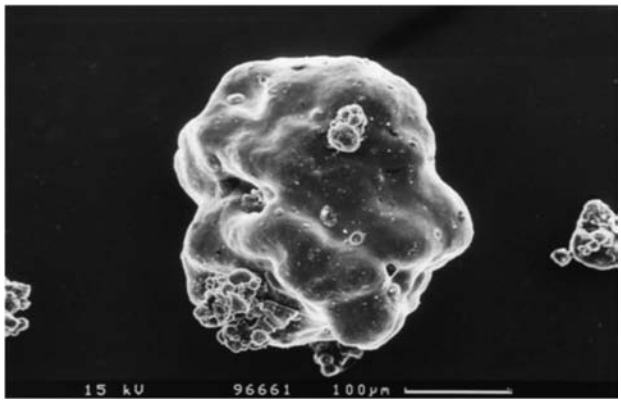

Figure 4. Scanning electron micrograph of a shellac-coated pectin amide/maltodextrin capsule (system 3, SL).

and the associated lack of continuity of the coating layer.[54] If the capsules had been coated with a uniform shellac film with no pore former, anthocyanin release into the stomach-mimicking medium may have been inhibited more robustly.[33] Since anthocyanins incorporated in the investigated capsule systems were released within the first 10−20 min, the analysis was not repeated with the addition of pepsin, which would otherwise have been especially pertinent for the WPI-system. In addition, the capsules were not transferred into the intestine-simulating medium following their incubation in the SGF medium, due to the complete release of the anthocyanins into that medium.

The next section of the GIT, after the stomach, is the small intestine, which was simulated by fed state simulating media (FeSSIF). A major difference between the stomach and the small intestine is that the pH of the latter is substantially higher (6.8 versus approximately 1−2). Thus, during the FeSSIF incubations, the pH of the mixtures was monitored. Only minor shifts to lower pH-levels were detected, and the initial value of pH 6.8 was restored on each sampling occasion using sodium hydroxide (1 N). The time concentration profile in FeSSIF of nonencapsulated BE total anthocyanins is shown in Figure 5 (light gray curve). Initially the detected concentration was 60%

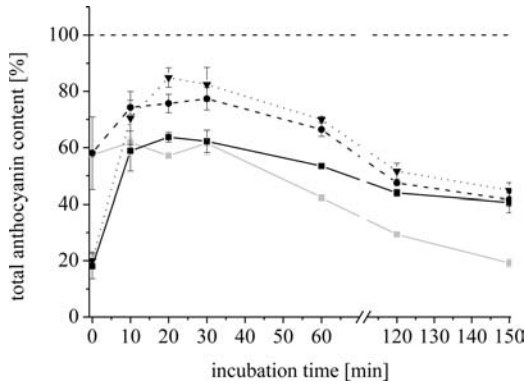

Figure 5. Total anthocyanin concentration [%] (0.7% BE is equivalent 100% anthocyanins) during incubation with fed state simulated intestinal fluid (FeSSIF) measured by HPLC-UV/vis at 520 nm. Control: nonencapsulated BE (■)(light gray curve); encapsulated BE: system 1, PA (■); system 2, WPI (●); system 3, SL (▼). Values are means of three determinations ± SD.

of the total theoretical anthocyanin concentration, it remained roughly constant until 30 min and decreased to 19% by the end of the incubation. Thus, as expected and previously reported,[11,52,55] the total anthocyanin concentration decreased over time due to degradation at neutral pH conditions. Anthocyanins are known to differ in their stability in the small intestinal milieu.[41] However, these differences should not have affected the results, since the total anthocyanin content of each sample was analyzed and related to the final total anthocyanin concentration of the associated capsule systems or nonencapsulated BE. For further studies of the three capsule systems, they were also incubated with FeSSIF (see Figure 5). In all three cases, release of BE anthocyanins was detected during the first 20 to 30 min of incubation, followed by a time-dependent reduction in the total anthocyanin concentration. However, in contrast to their incubation in SGF, the maximum total concentration of anthocyanins released from the three systems differed; reaching 64% after 20 min, 77% after 30 min, and 85% after 20 min for the PA, WPI and SL systems, respectively, and then declining to similar levels (approximately 42%) by the end of the incubation. Nevertheless, the total anthocyanin concentration remained higher during the incubation for the capsulated BE than for the nonencapsulated BE.

Taken together, our data indicate that encapsulating anthocyanins from BE protects them from early degradation in the small intestine. During incubation, the three investigated capsule systems exhibited varying release characteristics, which can be attributed to the differences in capsule types and sizes (see Figure 2 and Table 1). In both the PA (hydrogel-shell with liquid core) and WPI (hydrogel matrix) systems, surface binding of anthocyanins is induced by the production process.[56] Thus, anthocyanins at the surfaces of both systems are released earlier than those in their inner core and matrix, respectively. In contrast, the additional coating layer of the SL-capsules was added after matrix formation. Thus, all of the anthocyanins were located in the inner matrix and were not in contact with the outer surface. However, the shellac coating layer of the SL capsules did not provide a complete diffusion barrier and was thus unable to prevent anthocyanin release into the stomach-

mimetic medium. As shown in Figures 3 and 5, 15−20% of the total anthocyanins were rapidly released from the SL and PA capsules, in both test media, and 60% from WPI capsules. This considerable difference can be explained by the smaller mean capsule size of the WPI-system (180 μm). The capsule surface area, which was accessible to the release media, was larger for WPI than for the other systems during incubations of comparable sample volumes, resulting in a higher initial release. Generally, the detected amount of released anthocyanins in FeSSIF was less than 100% of the encapsulated amount, and some pH-dependent degradation is assumed to have occurred.[52] This assumption is corroborated by the continuous reduction in concentration of nonencapsulated BE anthocyanins (see Figure 5, light gray curve) observed during the last parts of their incubation in FeSSIF. In addition, anthocyanins have a known capacity to bind to proteins, which may have affected their release from the WPI-system.[57,58] Nevertheless, at the end of incubation, the anthocyanin concentrations of all capsule systems (SL, PA, WPI) exceeded the nonencapsulated concentrations by about 20%, strongly indicating that encapsulation can protect anthocyanins in the small intestine, provided that release in the stomach can be prevented.

The objective of the presented study was to assess the ability of the tested encapsulation systems to inhibit the degradation of BE anthocyanins in simulated gastric and small intestinal fluids. Under simulated gastric conditions, the nonencapsulated and released anthocyanins were stable. However, in the simulated small intestinal fluid, their concentration decreased rapidly due to the shift in pH, mimicking pH changes within the GIT. Further, none of the tested capsule systems appears to be able to prevent early release within the stomach, although they all provided the anthocyanins some protection from early degradation in the small intestine-mimicking fluid (final anthocyanin concentrations being 23 ± 2% higher in the incubations of the capsulated anthocyanins). These results can be attributed to the retarded release and consequently enhanced stability of the incorporated anthocyanins. In summary, encapsulation can potentially stabilize these and other bioactive compounds in the gastrointestinal tract. However, since the investigated capsule systems do not appear to be able to fully prevent early release of anthocyanins from encapsulated BE into the stomach, they require further optimization in terms of matrix density and other relevant parameters, e.g. the addition of a dense shellac coating layer with no pore-forming substance, or a lipid coating with a high melting point. Nevertheless, the results clearly show the potential utility of encapsulating bioactive ingredients in food technology.

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ABBREVIATIONS

BE, bilberry extract; Cy-3-gal, cyanidin 3-*O*-galactopyranoside; Cy-3-glc, cyanidin 3-*O*-glucopyranoside; Cy-3-ara, cyanidin 3-*O*-arabinofuranoside; DE, dextrose equivalent; Del-3-gal, delphinidin 3-*O*-galactopyranoside; Del-3-glc, delphinidin 3-*O*-glucopyranoside; Del-3-ara, delphinidin 3-*O*-arabinofuranoside; FeSSIF, fed state simulated intestinal fluid; GIT, gastrointestinal tract; IS, internal standard; LOD, limit of detection; LOQ, limit of quantification; Mal-3-glc, malvidin 3-*O*-glucopyranoside; Mal-3-ara, malvidin 3-*O*-arabinofuranoside; Peo-3-gal, peonidin 3-*O*-galactopyranoside; Peo-3-glc, peonidin 3-*O*-glucopyranoside; Peo-3-ara, peonidin 3-*O*-arabinofuranoside; Pet-3-gal, petunidin 3-*O*-galactopyranoside; Pet-3-glc, petunidin 3-*O*-glucopyranoside; Pet-3-ara, petunidin 3-*O*-arabinofuranoside; Ph. Eur, European Pharmacopoeia; SGF, simulated gastric fluid; USP, United States Pharmacopoeia; WPI, whey protein isolate

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