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Microencapsulation of bioactive bilberry anthocyanins by means of whey protein gels

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Abstract

Microencapsulation is a promising possibility to stabilise anthocyanins in foods. However, the use of protein hydrogels as matrix materials for microcapsules has not yet been investigated. In this study we show that by means of the emulsion method thermally induced whey protein-based microcapsules which are applicable for the encapsulation of an anthocyanin-rich bilberry extract can be generated from whey protein solutions. The influence of manufacturing conditions, like stirrer speed and emulsifier addition, on the morphological properties of the microcapsules at pH 1.5 and pH 3 was assessed. In tensiometric measurements, the water-in-oil (w/o)-emulsifiers phosphatidylcholine-depleted lecithin (PCDL) and Span 80 significantly reduced the interfacial tension at the w/o-interface in the presence of the whey proteins in the water phase and were therefore applied for the production of microcapsules. When no emulsifier or the emulsifier Span 80 was used during the microencapsulation process, aggregated and coalesced microcapsules were obtained. This indicates a loss of the interfacial activity of Span 80 due to the conditions during microencapsulation. Only PCDL was effective in stabilising the emulsion droplets during gelation and the mean diameter of the generated microcapsules could be significantly decreased to 20-70 µm. Finally, microcapsules containing up to 10% bilberry extract with a mean diameter below 50 µm could be prepared by use of PCDL. For this, a microcapsule formed at pH 1.5 was favourable for the encapsulation of bilberry extract due to observed detrimental interactions between whey proteins and bilberry extract compounds at pH 3. The results of this study shall help to facilitate the development of innovative protein-based encapsulation systems using the emulsion method.

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Keywords: microencapsulation; thermal gelation; whey proteins; hydrogel, anthocyanins; emulsion method

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1. Introduction

Plant-derived bioactive phenolic compounds are interesting additives for functional foods. Bilberries contain high amounts of phenolic flavonoids, such as anthocyanins, which are known to display a wide range of biological activities like antioxidant, anti-inflammatory, antimicrobial or anti-carcinogenic activities, improvement of vision, induction of apoptosis and neuroprotective effects [1]. However, the application of anthocyanins as food additives is limited because after extraction these compounds are susceptible to degradation. Microencapsulation in stabilising matrices can therefore be regarded as an appropriate means for overcoming these limitations. Microencapsulated anthocyanins may be added to foodstuffs in a stabilised form whereby an adverse effect on the sensorial properties of the foods may be prevented due to the small size of the microcapsules

The commonly applied method for the microencapsulation of extracted plant phenolics, like anthocyanins, is spray drying. Mainly used matrix materials are polysaccharides like maltodextrin, inulin [2], gum Arabic, tapioca starch [3], citrus fibre [4] and other matrix materials like glucose syrup [5] and soy protein isolate [6]. By this method, the encapsulated plant phenolics are stabilised against degradation due to the impact of oxygen and light during dry storage. However, in aqueous environments, as prevalent in many foods or the digestive tract, the obtained water-soluble microparticles generally disintegrate and lose their stabilising effect for the encapsulated compounds.

Hence, alternative water-insoluble encapsulation systems appear promising which maintain their structure and core material-stabilising function after immersion in water. To date, only few studies have been carried out with the focus on the encapsulation of anthocyanins in water-insoluble matrices. This can be explained by the fact that extracted anthocyanins are mostly stable in their flavylium cation-form at very acidic conditions below pH 3 [7]. For this reason, the amount of utilisable matrix materials, and thus encapsulation methods, is limited. In fact, so far solely polysaccharide-based acidic hydrogels and lipids were used as matrix materials for the microencapsulation of anthocyanins. Ge et al. [8] microencapsulated freeze-dried anthocyanin extract by dispersing it in molten mixtures of beeswax and stearic acid with additional congealing and grinding the solidified fat into particles. Besides this study, two polysaccharide-based microencapsulation systems exist. In these studies either an acidic (exact pH not known) cold-set glucan gel [9] or a heat-set curdlan gel at pH 1.5 [10] was used for the encapsulation of the anthocyanins. In both studies, the formation of microparticles was attained by the extrusion of the specific aqueous polysaccharide solution to a stirred oil-phase that allowed curing of the droplets by avoiding aggregation. However, in general the use of polysaccharides is limited to low concentrated solutions and gels (the applied hydrocolloid concentrations were ~8.5 % for glucan and 4.3- 5.6 % for curdlan) due to their high viscosity.

Proteins can be used as a reasonable alternative matrix material because highly concentrated solutions (>10%) can be used due to their low viscosity. Consequently, upon gelation the high hydrocolloid concentration transforms to dense gel network structures, which may favour the retention of an encapsulated core material. The major fraction of the whey proteins, β-lactoglobulin, is known to be able to build water-insoluble thermal gels at anthocyanin-stabilising strongly acidic conditions ($pH \geqslant 1.5$) [11]. Furthermore, heat-induced whey protein gels are known to be suitable matrices for the encapsulation of water-soluble bioactives [12]. For this reason, whey protein-based systems seem to be promising matrices for the microencapsulation of anthocyanins. A suitable technique for the production of thermally induced proteinaceous microspheres is the emulsion method [13]. Basically, this procedure comprises the emulsification of a protein solution, which contains the core-material, in an oil phase and the heating of this emulsion to induce the gelation of the dispersed droplets. Lee & Rosenberg [14], Bhattacharjee & Das [15] and Lee et al. [16] have successfully applied the emulsion method for the generation of heat-induced water-insoluble whey protein microcapsules. However, none of these studies provides systematic information about the influence of manufacturing conditions on the properties of the generated microcapsules nor substantiates the necessity of the applied emulsifier.

The objective of this research was to investigate the applicability of the emulsion method for the generation of thermally-induced acidic whey-protein based microcapsules for the encapsulation of anthocyanin-rich bilberry extract. The influence of manufacturing conditions, emulsifier addition and bilberry extract-content on the morphological properties of the generated whey protein-based microcapsules was examined.

2. Materials and Methods

2.1. Material

Whey protein isolate (WPI, BiPro, 94 % protein) was obtained from Davisco Foods International Inc. (Le Sueur, MN, USA). Bilberry extract from *Vaccinium myrtillus* (BE, 600761 Bilberry Extract 25 %) was a gift from Kaden Biochemicals GmbH (Hamburg, Germany). Span 80 was obtained from Merck KGaA, Darmstadt, Germany. Phosphatidylcholine-depleted lecithin (DP 627, hereafter referred to as PCDL) was obtained from Cargill Texturizing Solutions Deutschland GmbH & Co KG, Hamburg, Germany. Sunflower oil was purchased from a local supermarket and was used as is without further processing.

2.2. Generation of whey protein-based microcapsules

A stock protein solution (30 % protein, w/w) was prepared by dissolving of WPI powder in deionised water. The BE was slowly added and dissolved in the stock protein solution. Consequently, diluted hydrochloric acid (3 M), HCl (Merck KGaA, Darmstadt) was added to obtain a protein content of 20 % (w/w),a final BE content of 1, 5 or 10% (w/w) and a pH of 1.5 or 3. The generated BE-WPI-solution was centrifuged at 5000 ×g for 2 min to remove insoluble fractions. The obtained supernatant was used for the production of microcapsules by means of the emulsion/heat gelation method (Fig. 1).

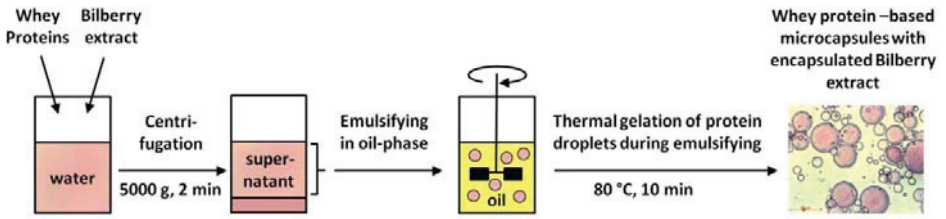

Fig. 1. Schematic depiction of the emulsion/heat gelation method for the production of whey protein- based microcapsules with encapsulated bilberry extract.

The design of the mixing system influences the emulsification process and was dimensioned according to Zlokarnik [17]. A blade impeller (six blades, diameter: 30 mm) and a beaker glass (1000 mL, diameter: 90 mm) with four standard steel baffles were used for the production of microcapsules. Stirrer speed was controlled by an IKA stirring device (RW 20 DZM, IKA-Werke GmbH & CO. KG, Staufen, Germany). Some 84 g of either WPI-solution or BE-WPI-supernatant (25 °C) was poured into 476 g of sunflower oil (w/o = 0.15) during stirring at 50 °C followed by immediate heating-up of the emulsion from 50 °C to 80°C within 6 min. The temperature was maintained at 80 °C for 10 min to facilitate gelling, then the suspension was cooled down to 20 °C. Eventually, the microcapsules suspension was centrifuged at 1000 g for 2 min and the oil-supernatant was discarded. The sedimented microcapsules were washed twice by suspending them in diluted hydrochloric acid (0.4% NaCl, pH 1.5 or pH 3,) followed by centrifugation. The obtained aqueous, oil-free suspension of microcapsules was used for further analysis.

2.3. Measurement of interfacial tension

The time-dependent interfacial tension at the w/o-interface at 25 °C was measured by an automated drop volume tensiometer (Lauda Dr. R. Wobser GmbH & Co. KG, Lauda Königshofen, Germany) in the dynamic mode. The interfacial tension was measured for two replicates. Results of all measurements are given with error bars showing the measuring inaccuracy.

2.4. Determination of microcapsule size

The volume-based median $d_{50,3}$ as well as the $d_{90,3}$ and $d_{10,3}$ (i.e. capsules representing 90% and 10% of the total sample volume have diameters $\leqslant$ the $d_{90,3}$-value or $\leqslant$ the $d_{10,3}$-value, respectively) of the resulting microcapsules was determined by laser diffraction using a Coulter LS 230 (Beckman-Coulter, Krefeld, Germany). The width of the particle size distribution was evaluated by calculating the span value: Span = $(d_{90,3}-d_{10,3})/d_{50,3}$. Microcapsule size was determined twice for replicates. Results are given as mean values with standard deviations.

2.5. Optical examination of the whey protein microcapsules

The morphology of the microcapsules was visually assessed by an optical microscope (Axioskop, Carl Zeiss AG, Jena, Germany). For this, the aqueous capsule suspension was photographed at 100-fold magnification.

2.6. Determination of protein content

The total protein content of the samples was determined by means of the Dumas method using the nitrogen gas analyser system FP-528 (LECO, Moenchengladbach, Germany). The whey protein concentration was calculated from total nitrogen content using a factor of 6.38. All measurements were carried out in duplicate.

3. Results and Discussion

3.1. Characterisation of the activity of w/o-emulsifiers in the presence of whey proteins

The production of the thermally-induced whey protein microcapsules by the emulsion method comprises the generation of a w/o-emulsion with the protein solution as dispersed phase as first step. Thereby, the droplet size of the w/o-emulsion can be influenced by increasing the energy input and by addition of an emulsifier with a low hydrophilic-lipophilic balance (HLB value) to the oil-phase. The emulsifier is furthermore intended to prevent droplet aggregation and coalescence during heating of the emulsion, which is the second step of the microencapsulation process.

Firstly, two types of food-grade oil-soluble w/o-emulsifiers, a sorbitan ester (Span 80) and a lecithin mixture (PCDL), were compared in terms of their interfacial activity in the presence of whey proteins to assess their applicability for the emulsion method (Fig. 2). Span 80 was chosen as emulsifier because sorbitan esters are typically applied for the production of heat-induced whey protein microcapsules [14, 16]. PCDL is known to synergistically interact with whey proteins at the w/o-interface [20].

Acidic WPI-solutions (pH 1.5 and pH 3) with a protein content of 20 % (w/w) were used for the experiments because it was shown in a previous study [19] that stable gels, which are applicable for the entrapment of bilberry extract, can be generated from these solutions by heating to 80 °C for 10 min.

The tensiometric measurements revealed that the sole presence of whey proteins (whey proteins in the water phase at pH 1.5 vs. pure sunflower oil) was more effective in lowering the interfacial tension at the w/o-interface than the sole presence of PCDL (PCDL in the oil phase vs. water at pH 1.5).

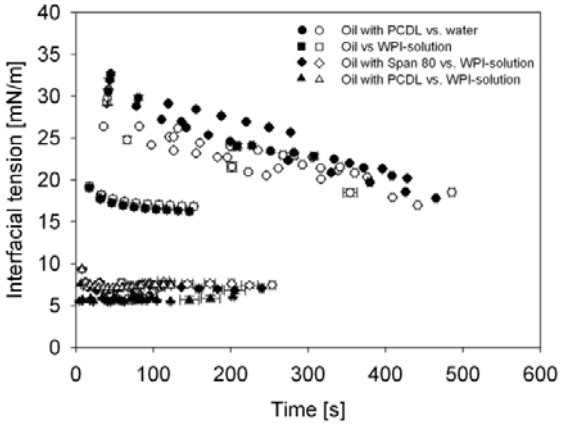

Fig. 2. Time-dependent interfacial tension at 25 °C between sunflower oil without and with 2% emulsifier (PCDL, Span 80) and water at pH 1.5 or WPI-solution (20% protein) at pH 1.5 (open symbols) and pH 3 (closed symbols).

The combination of the whey proteins in the aqueous phase with PCDL in the oil phase led to a faster and stronger reduction in the interfacial tension than when used in isolation, comparably to the activity of Span 80 in the oil phase. This is in accordance with the results of Knoth et al. [20], which showed similar results for interfacial activity by combining PCDL in the oil phase with 1.5% WPI in water at neutral pH.

Thus, the results of the present study prove that the synergistic interfacial interactions of PCDL and WPI are also effective at a high protein concentration and acidic pH, as it was the case during the microencapsulation process. Similarly, Span 80 showed a high interfacial activity which was not altered by the presence of WPI in the water phase (results not shown).

3.2. Generation and characterisation of whey protein microcapsules

A microcapsule size below 100 µm is desirable to avoid negative sensorial impacts in food [21]. To achieve this particle size by means of the emulsion method, the addition of an effective emulsifier is necessary to avoid aggregation and coalescence during gelation. The results of the tensiometric measurements indicated the applicability of the emulsifiers PCDL and SPAN 80 in the microencapsulation process. Consequently, the ability of the emulsifiers to diminish the droplet size and stabilise the emulsion during the microencapsulation process was investigated. For this, whey protein microcapsules were prepared at different stirrer speeds without added emulsifier or with the addition of 2% of emulsifiers to the oil phase (Fig. 3). By increasing the stirrer speed a reduction in the microcapsule size was expected due to the stronger comminution of droplets. The whey proteins without supplementary emulsifier were found to stabilise the droplets at 1000 and 1350 rpm, as the decrease in particle size indicates. However, by increasing the stirrer speed above 1350 rpm no further reduction in the mean particle diameter could be achieved.

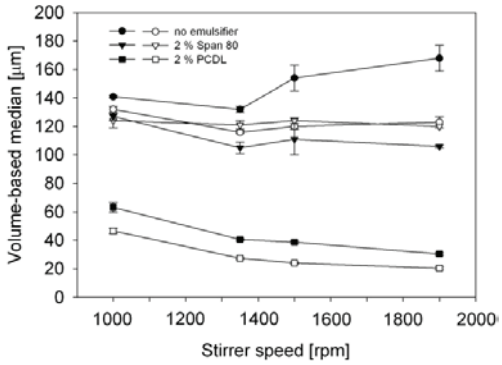

Fig. 3. Influence of stirrer speed and type of emulsifier on the mean particle size of whey protein microcapsules prepared by the emulsion/heat gelation method at pH 1.5 (open symbols) and pH 3 (filled symbols). The error bars represent the standard deviation.

Moreover, independent of the stirrer speed, Span 80 was apparently inactive at pH 1.5 and had just little effect on the size of the microcapsules at pH 3. In contrast, PCDL was effective in lowering the microcapsule size with increasing stirrer speed. The observed differences with regard to the influence of the stirrer speed on the measured particle diameters after emulsification with and without emulsifier becomes comprehensible by considering the morphology of the generated microcapsules (Fig. 4). In all cases, smaller droplets were generated at higher stirrer speed.

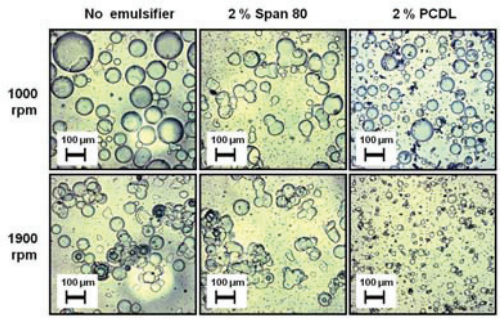

Fig. 4. Influence of stirrer speed and emulsifier addition (Span 80, PCDL) on the morphology of the whey protein microcapsules (pH 1.5) as observed by light microscopy.

However, the increased shear rate at 1900 rpm resulted in high droplet collision rates and thus induced aggregation and coalescence of the droplets [22]. In contrast to PCDL, the whey proteins alone or in combination with the emulsifier Span 80 were not able to stabilise the dispersed droplets against these phenomena especially at high stirrer speed. As a result, the mean particle size did not decrease with increasing stirrer speed because actually the diameter of the aggregated and coalesced droplets was measured. Span 80, for which an interfacial activity comparable to the PCDL-WPI-combination was measured tensiometrically at 25 °C (Fig. 2), was not active during the microencapsulation process.

In this context, the optical appearance of the generated partially coalesced microcapsules prepared with addition of Span 80 indicated an initial competitive adsorption of Span 80 and the whey proteins to the

interface [22] followed by the loss of interfacial activity due to heating above 50 °C [23]. However, Lee et al. [16] prepared heat-induced whey protein microcapsules with Span 80 as emulsifier and reported no detrimental effect of Span 80 on the morphology of the obtained microcapsules. It is likely that these contradictory results can be explained by the appearance of 1% polyglycerin polyricinolate (PGPR) in the encapsulated core material (rapeseed oil blended with egg-yolk immunoglobulins). PGPR, a potent w/o-emulsifier with restricted food-approval [24], probably migrated to the outer oil phase and superimposed the effects of Span 80. Bhattacharjee & Das [15] reported diameters of 30-50 µm for β-lactoglobulin microcapsules prepared by the emulsion method without any added emulsifier. This result, which is dissimilar to the observations of the present study, is likely attributed to the better emulsifying properties of isolated β-lactoglobulin compared to whey proteins in their native mixture [18]. Bhattacharjee & Das [15] determined the diameter of the microcapsules microscopically. The same microscopic method for size determination was used by Lee & Rosenberg [14] who applied Span 65 as emulsifier. In both studies, the size of individual microcapsules was assessed optically regardless of their possibly aggregated state. For this reason, it is difficult to draw conclusions regarding the droplet aggregation and activity of the emulsifier during these processes for comparison with the results of the present study.

Conclusively, in contrast to Span 80, PCDL can be regarded as a suitable emulsifier for the production of thermally-induced whey protein microcapsules using the emulsion method. PCDL was therefore investigated with regard to its applicability for the production of bilberry extract loaded microcapsules.

3.3. Generation of bilberry extract-loaded whey protein microcapsules

The antioxidative potential and bioactivity of bilberry anthocyanins is concentration-dependent [25]. For this reason, a high core-load of bilberry extract in the microcapsules is desirable. Microcapsules with 1, 5 and 10% w/w core material content were prepared to investigate the effect of BE addition on the microcapsule properties.

Microencapsulation of BE was carried out either without emulsifier or by addition of 2% PCDL at a stirrer speed of 1350 rpm (Fig. 5).

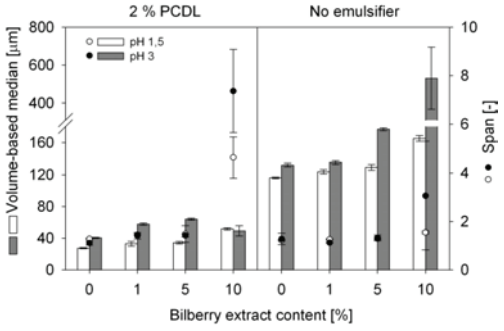

Fig. 5. Influence of bilberry extract content in the whey protein solutions at pH 1.5 and pH 3 on the mean diameter and span-value of the resulting microcapsules prepared at a constant stirrer speed of 1350 rpm with 2% (w/w) PCDL and without emulsifier. The error bars represent the standard deviation.

At this stirrer speed it was possible to produce non-aggregated and non-coalesced microcapsules without the addition of an emulsifier, as Fig. 4 shows. By assessing the morphological characteristics of the generated microcapsules, it was possible to distinguish between interactions of BE and whey proteins and possible additional interactions attributed to the presence of the PCDL emulsifier during the microencapsulation process.

Fig. 5 shows that PCDL was active in the presence of BE and that by its addition the mean diameter of the microcapsules could be significantly decreased at both pH values.

At pH 3 the particle size increased when BE was added. The span-values, as a measure of the width of the particle size distributions, however, were not dependent on BE-content, pH value and emulsifier addition at a BE-content below 10% (w/w). When 10% (w/w) BE were added the particle size distributions widened due to aggregation and precipitation phenomena. Especially at pH 3 no spherical microcapsules but irregularly shaped aggregates were obtained (Fig. 6).

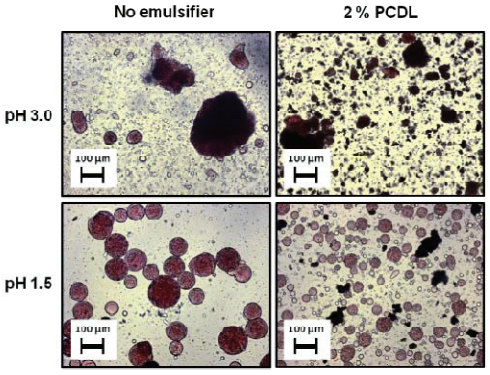

Fig. 6. Influence of bilberry extract content and addition of the emulsifier PCDL on the morphology of the whey protein microcapsules at pH 1.5 an pH 3 prepared at a constant stirrer speed of 1350 rpm as observed by light microscopy.

Generally, the detrimental effect of the high BE-content was more pronounced when PCDL was present and minimal at pH 1.5 in the emulsifier-free system.

The observed BE-induced aggregation and precipitation during the microencapsulation process is probably attributed to interactions between whey proteins and phenolic constituents of the BE. These interactions depend, amongst others, on electrostatic forces and are therefore strongly influenced by the pH value [26]. The pH dependency of the interactions would explain that at pH 1.5 the detrimental effect of BE on the morphology of the microcapsules was less pronounced. At this pH value organic acid groups of BE-constituents, like tannic acids, lose their negative charge due to protonation. Thus, their tendency to undergo electrostatic interactions with the positively charged proteins is diminished. Moreover, it is likely that by the protein-polyphenol interactions the interfacial synergism of PCDL and WPI was negatively influenced, resulting in a lower interfacial stability and thus wider particle size distributions due to aggregation. The analysis of the anthocyanin content before and after gelation in the emulsifier-free system revealed that the anthocyanin loss and thus anthocyanin-protein interactions were negligible (data not shown).

4. Conclusion

In this study, we were able to demonstrate that the emulsion method can be used to generate water-insoluble whey protein-based hydrogels for the microencapsulation of an anthocyanin-rich plant extract. Therefore, so far only water-insoluble polysaccharide-based systems were used. Owing to the applied extrusion method, the average bead diameter of the obtained microcapsules was hitherto in the range of ~0.5 mm [10] to ~2.5 mm [9]. In contrast, the emulsion method with the aid of the experimentally selected effective PCDL emulsifier allowed the production of microcapsules with mean diameters below

70 µm that appear practicable for food applications without having a negative sensory impact [21]. However, when PCDL was applied, the used sunflower oil was red-coloured after the encapsulation procedure. This indicates a transition of anthocyanins from the aqueous-phase to the oil-phase. For this reason, current investigations are focused on revealing and quantifying the observed interactions between the whey proteins, the bilberry extract and the emulsifier during the microencapsulation process. In addition, the release characteristics of the microcapsules are under investigation.

Acknowledgements

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