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Microencapsulation of Peppermint Oil by Complex Coacervation and Subsequent Spray Drying Using Bovine Serum Albumin/Gum Acacia and an Oxidized Starch Crosslinker

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Abstract: Most liquid food flavours such as essential oils are volatile and prone to degradation in the presence of oxygen, light, moisture and high temperatures. Microencapsulation of volatile ingredients prior to use in food or beverages is a commonly used process to limit loss and degradation of flavours and aromas during processing and storage. Here, peppermint essential oil was microencapsulated via complex coacervation using a combination of bovine serum albumin and gum Acacia as wall materials. The resulting core-shell microcapsules were chemically crosslinked with a modified food-grade starch, and subsequently spray dried, resulting in dry microcapsules which could be easily redispersed in aqueous solutions. Microcapsule formation and stability, as well as microencapsulation yield of peppermint oil, were investigated as a function of polymer concentration, core material load/wall thickness and crosslinker concentration. The crosslinked peppermint oil microcapsules were spherical and mononuclear both before and after spray drying and redispersion, whereas control coacervate samples without crosslinker did not withstand the spray drying process. Microencapsulation yield as analysed by GC-MS showed no loss of peppermint oil during or after complex coacervation, and 54% loss after spray drying for the best combination of Polymer:Oil ratio and crosslinker concentration used here, indicating good overall protection of the core material.

Keywords: complex coacervation; bovine serum albumin; gum Acacia; peppermint oil; microencapsulation; spray drying; food grade crosslinker

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1. Introduction

Essential oils (EOs) are frequently used to provide flavour and aroma to a wide range of food and cosmetics and are increasingly used as antimicrobial agents and feed additives [1–5]. EOs are highly volatile and mostly insoluble in water, and prone to degradation by exposure to oxygen, light, water and heat. Microencapsulation, wherein one material or mixture of materials (i.e., core or active ingredient) is coated with or entrapped within another wall material or shell, is an established technique to protect active ingredients such as EOs against the external environment. A wide range of microencapsulation methods have been used for encapsulation of flavours and aromas—see, for example, the review by Dordevic [6] and references therein. Peppermint oil is one of the most commonly used essential oils, with applications in food, perfumery, pharmaceutical and flavouring products [7]. Microencapsulation of peppermint oil has been performed using, for example, complex coacervation and spray drying [8–10], electrosprayed alginate capsules [7,11], entrapment in cellulose nanocrystals [12], and free radical interfacial polymerization [13]. Additionally, microfluidics [14] and especially supercritical fluid (SCF) techniques [15,16] are emerging as alternative methods for micro- and nanoencapsulation of volatile ingredients.

While spray drying is by far the most common microencapsulation method for protecting volatile ingredients such as essential oils from degradation, complex coacervation is an attractive and scalable alternative method offering higher payloads (up to 99%), better hydrothermal resistance and controlled release properties [17,18]. Complex coacervation is a liquid-liquid phase separation phenomenon that occurs upon mixing oppositely charged weak polyelectrolytes, typically a protein and a polysaccharide (see, e.g., the excellent review by de Kruif et al. [19] for a thorough description). Microcapsules produced by complex coacervation form core-shell structures, either mononuclear, polynuclear or aggregated "grape" coacervates depending on parameters such as the Reynolds number during capsule formation and hardening, and the cooling/hardening rate [20]. As the core is protected from water in the external phase, complex coacervation is well suited for encapsulation of hydrophobic substances such as essential oils. The protein-polysaccharide combination of gelatin and gum Acacia remains the most widely studied set of wall materials for microencapsulation of essential oils via complex coacervation [1,17,18]. However, any oppositely charged polyelectrolyte pair can in principle be used, provided the charge density on the polymeric species allows for liquid-liquid phase separation—see, for example, de Kruif [19] and references therein for a non-exhaustive overview of combinations of wall materials reported in the literature. For microencapsulation of peppermint oil, reported wall materials for complex coacervation typically use gelatin as the protein, combined with either gum Acacia [8], pectin [10] or mixtures containing chitosan [9].

One inherent limitation of complex coacervation is that the process is limited to a relatively narrow solvent-rich corner of the ternary phase diagram. For example, for the system gelatin-gum Acacia, the concentration of both polymers has to be below 8% (*w*:*w*) in order for coacervation to occur [20]. Consequently, there is often a need for post-processing in the form of, for example, freeze drying or spray drying in order to reach sufficiently high concentrations of active ingredients as well as to provide further protection from hydrothermal degradation. However, this also entails additional thermal gradients and shear forces, necessitating crosslinking of the capsules. In food and nutraceutical applications, there is a severely limited choice of crosslinking agents regarded as safe, with the enzyme transglutaminase being the most widely used [6]. Thus, there is a need for new crosslinking agents which can be applied to complex coacervate capsules intended for food applications and adjacent areas.

In this study, we have encapsulated peppermint oil via complex coacervation using a novel combination of bovine serum albumin (BSA) and gum Acacia (GA). The coacervate capsules were chemically crosslinked using a novel method based on oxidized starch, and the capsules were subsequently spray dried in order to investigate stability and payload retention under various conditions.

2. Materials and Methods

2.1. Materials

Bovine serum albumin (BSA), gum Acacia (GA, acacia tree), ascorbic acid, sodium hydroxide (NaOH), Zulkowsky potato starch, sodium metaperiodate, and Peppermint oil food grade were purchased from Sigma Aldrich Chemicals (MilliporeSigma, Merck KGa, Munich, Germany) and used as received.

2.2. Synthesis of Oxidized Starch Crosslinker

The oxidized starch was prepared from Zulkowsky starch using sodium metaperiodate as oxidizing agent according to established protocols [21]. The periodate anions oxidize specifically carbons 2 and 3 of the repeating unit of starch, generating two aldehyde groups with the cleavage of the bond between the carbons 2 and 3. The reaction was carried out at room temperature, in the dark under an inert atmosphere. The crude product was purified by dialysis against DI water. Briefly, Zulkowsky starch (10 g) was dissolved in water (150 mL). Sodium metaperiodate (4 g) was dissolved in water (150 mL) and added to the starch solution. The mixture was stirred in the dark, at room temperature under

an inert atmosphere for 26 h. The crude product was purified by dialysis in DI water and freeze dried to isolate the solid oxidized starch. The final product was characterized by ^{1}H NMR.

2.3. Microencapsulation of Essential oils by Complex Coacervation

Essential oil-loaded complex coacervate microcapsules were prepared using a modification of the procedure reported by Lemetter et al. [20].

2.3.1. Polymer Dissolution

4–8 wt% solutions of BSA and GA in DI water were prepared separately (BSA:GA 1:1 *w*:*w*) and allowed to hydrate under gentle stirring at 40 °C for 1 h before the two polymer solutions were mixed and the pH was adjusted to >5 to avoid premature formation of coacervate nodules.

2.3.2. Emulsification

Peppermint essential oil was added to the polymer solution, and the reaction mixture was emulsified using an Ultraturrax (T25 digital, IKA, Staufen, Germany) at 5000 RPM for 2 min and transferred to a reactor under stirring (250 RPM) for 30 min at 40 °C. The amount of essential oil and thus the wall to oil (Polymer:Oil) ratio was varied from 1:2 to 2:1 with respect to the total polymer concentration.

2.3.3. Coacervation

In order to initiate coacervation, the pH was lowered slightly below the isoelectric point of BSA (pI = 4.6–4.8) to pH 4.2, with continued stirring at 40 °C. The reaction mixture was gradually cooled to 5 °C at a rate of ~0.5 °C/minute.

2.3.4. Crosslinking

Chemical crosslinking was done via reaction with an oxidized food starch (see Section 2.2 above) wherein the aldehyde groups were reacted with residual amino groups on the protein. While maintaining the temperature (5 °C) and stirring rate from the coacervation step, the pH was adjusted to 9 using a 5 wt% aqueous NaOH solution, and an aqueous solution of oxidized food starch was added at 2.5–10% with respect to protein. The adducts formed by reaction between aldehyde groups and protein residual amino groups was stabilized by addition of ascorbic acid.

2.4. Spray Drying

Spray drying of the essential oil complex coacervates was undertaken with a Büchi B290 Minispray dryer fitted with a 1.4 mm tip and a 2-fluid nozzle (Büchi, Flawil, Switzerland). The inlet air temperature was varied from 135 °C to 145 °C and the aspirator rate was maintained at 100%. The rotameter height was kept at 40 mm, which corresponds to the spray gas flow rate at 473 L/h. After conditioning, the samples were delivered to the nozzle using an integrated peristaltic pump at a feed flow from 7–11 mL/min (28–34% of the maximum pump rate). The resultant spray-dried samples were collected and stored at 5 °C for further analysis.

2.5. Extraction of Peppermint Oil

Three parallel samples of coacervate (3 × 3 mL) were treated with dichloromethane (9 mL) mixed (15 min) on a Vortexer (VX-2500, VWR, Radnor, PA, USA), then acetonitrile (6 mL) was added, and the slurry was vortexed for additional 15 min. The samples were then centrifuged for 5 min at 1000 rpm. The extracts were analysed by GC-MS. The spray dried particles (0.05 g) were rehydrated with water (1 mL) and then extracted in the same way as the coacervates.

2.6. Characterization

^{1}H NMR of the oxidized starch (see Section 2.1) was undertaken at 600 MHz in D_2O, using a Bruker 600 MHz Avance III HD (Bruker, Billerica, MA, USA) equipped with a 5-mm cryogenic CP-TCI z-gradient probe. Data were reprocessed using ACDLabs spectrus software (version 2019.0.1, ACD/Labs, Toronto, ON, Canada, 2019) and the spectra referenced to the residual solvent peak (4.79 ppm for D_2O). 1H NMR (600 MHz, D_2O, ppm): 3.50–4.40 (m, 5 H), 4.50–4.88 (m, 0.5 H), 4.90–5.50 (m, 1.5 H), 8.48 (s, 0.03 H), 9.31 (s, 0.03 H), 9.74 (m, 0.03 H).

Optical microscopy images were collected using an Olympus BX43 equipped with an Olympus XM-10 digital camera (Olympus Corporation, Tokyo, Japan). Capsule size measurements were performed using a Beckman Coulter LS230 Laser Diffraction Particle Size Analyzer (Beckman Coulter Inc., Brea, CA, USA) with water as the mobile phase.

GC MS analysis of peppermint: The samples were analysed on a Gas Chromatography—Mass Spectrometry (GC-MS) system from Agilent Technologies (Santa Clara, CA, USA). The GC-MS system consisted of an Agilent 7890A gas chromatograph and an Agilent 5975C mass spectrometer. The GC was equipped with a split/splitless inlet. The inlet was run in split mode with a split ratio of 25:1, temperature was set at 250 °C. The column used in this analysis was an Agilent J&W DB-WAX GC Column, 30 m, 0.25 mm, 0.25 µm. The column was run with constant pressure mode at 16.1 psi. The oven was programmed with a temperature gradient: 40 °C for 4 min, then 5 °C/min to 50 °C for 1 min, then 18 °C/min to 200 °C for 0 min, then 25 °C/min to 245 °C for 1 min. The total runtime was 18.33 min. The mass spectrometer was operated in scan mode, with a scan range from 35–550 mass to charge ratio (m/z). Data analysis was performed with Agilent MassHunter Quantitative Analysis (Version B.09.00, Agilent Technologies, Santa Clara, CA, USA, 2017).

The microencapsulation efficiency (*MEY*) was calculated as the percentage of peppermint oil entrapped in capsules relative to the total amount added according to Equation (1).

$$MEY\ (\%) = \left(\frac{Microencapsulated\ peppermint\ oil}{Peppermint\ oil\ initially\ added} \right) \times 100 \quad (1)$$

3. Results and Discussion

3.1. Oxidation of Starch Crosslinker

The oxidation resulted in a complex mixture of modified oxidized starches, as evidenced by the scrambling of the CH/CH_2 protons (3.5–4.4 ppm) in ^{1}H NMR spectra, and the formation of the key C2 and C3 aldehyde groups could be observed at 9.75 and 9.3 ppm respectively.

3.2. The Role of pH during Complex Coacervation

One of the most critical parameters in microencapsulation of oils by complex coacervation is obtaining the proper pH, where oppositely charged wall materials form approximately neutral coacervate nodules which self-assemble on the oil-water droplet interface. When using a combination of a weakly anionic polyelectrolyte such as GA and a protein, this is typically achieved by lowering the pH slightly below the isoelectric point of the protein [19], as previously reported, for example, gelatin type A and B [17,20,22], whey protein isolate [23,24] and soybean protein isolate [25]. Here, we adapted this approach to a combination of bovine serum albumin (BSA) and gum Acacia (GA), using a ratio of BSA:GA = 1:1 (*w*:*w*) as wall materials, using different polymer concentrations, Polymer:Oil ratios and crosslinker concentrations. A summary of the reaction conditions and obtained results is shown in Table 1. Figure 1 shows typical optical microscopy images of four of the critical steps in microencapsulation of peppermint essential oil using BSA:GA (1:1 *w*:*w*); during emulsification (Figure 1A, pH > 5), coacervation (Figure 1B, pH = 4.2), during crosslinking (Figure 1C, pH = 9) and for fully formed crosslinked capsules (Figure 1D, pH = 7). Lowering the pH below the isoelectric point of BSA (pI_{BSA} = 4.6–4.8) results in the formation of loosely associated flocs (Figure 1B), which are easily redispersed into single

capsules upon increasing pH above pI_{BSA} during crosslinking (Figure 1C). The complex coacervation process described herein results in well-dispersed spherical mononuclear microcapsules, with similar sizes as the parent emulsions.

Table 1. Overview of system parameters, capsule size and microencapsulation yields (*w*:*w*).

			Mean Number Average Diameter			Microencapsulation Yield	
Polymer:Oil (*w*:*w*)	**[Crosslinker] (wt%)**	**[Polymer] (wt%)**	**Coacervates (μm)**	**After Spray Drying (μm)**	**SD Yield (%)**	**CC (%)**	**SD (%)**
1:2	0	3	7 ± 3	-	-	-	-
1:2	5	3	7 ± 3	6 ± 3	26	100	17 ± 1
1:1	5	3	7 ± 3	7 ± 3	21	100	19 ± 1
1:1	5	6	7 ± 4	6 ± 4	32	100	27 ± 3
2:1	2.5	6	7 ± 4	6 ± 3	29	100	54 ± 2
2:1	5	6	5 ± 3	6 ± 3	31	100	47 ± 1
2:1	10	6	22 ± 4	27 ± 4	35	100	31 ± 1

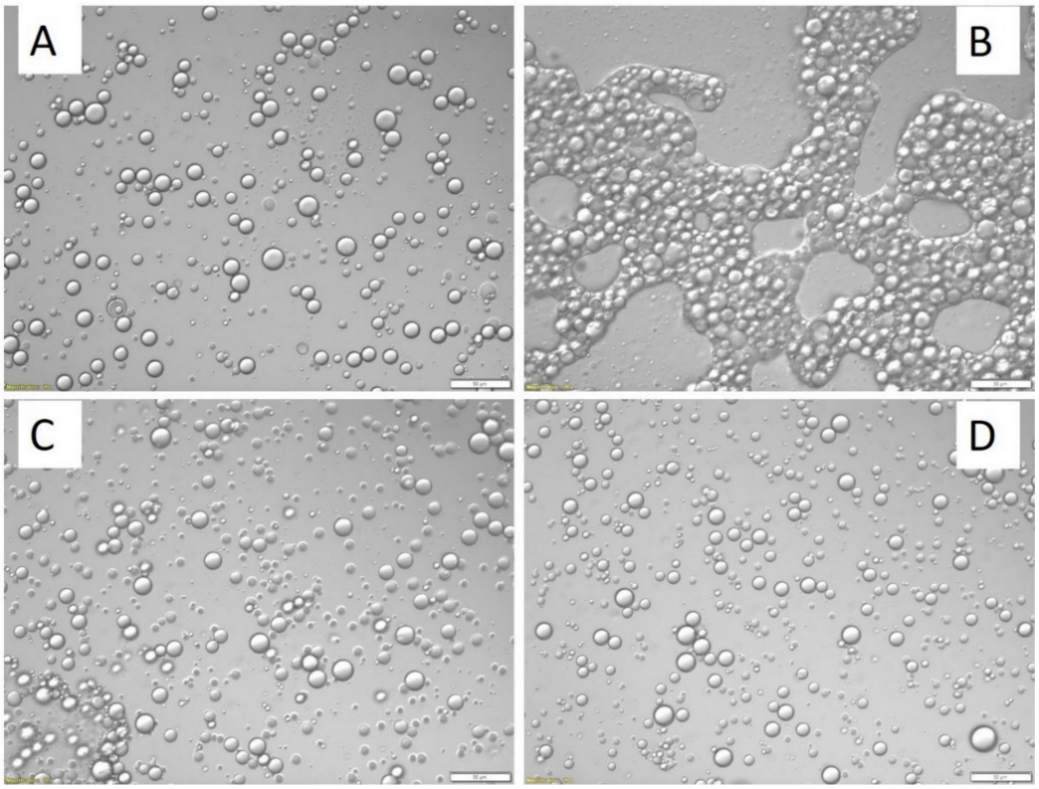

Figure 1. Optical microscopy images of process steps during complex coacervation of peppermint essential oil: oil-in-water emulsion at pH > 5 (**A**), coacervation at pH = 4.2 (**B**), crosslinking at pH 9 (**C**) and crosslinked capsules (**D**). The scale bar corresponds to 50 μm.

3.3. Effect of Spray Drying on Complex Coacervate Microcapsules

In order to evaluate the effect of spray drying on the stability, size distribution and potential loss of encapsulated peppermint oil, the complex coacervate microcapsules were spray dried and subsequently resuspended in water. Samples without the crosslinker were destabilized during spray drying, and so were not included in further analyses. Figure 2 shows typical optical microscopy images before (Figure 2A) and after (Figure 2B) spray drying and resuspension, together with the corresponding differential number and volume size distributions (Figure 2C,D respectively). From optical microscopy and the differential number size distributions (Figure 2A–C), there is an apparent loss of the largest capsules during spray drying, resulting in a narrowing of the microcapsule size. While

the optical microscopy images and the differential number distributions largely are in agreement regarding capsule size, differential volume size distributions (Figure 3D) are significantly shifted towards larger diameters. Thus, the number size distributions likely reflect single or primary capsules, whereas the differential volume size distribution reflects partial flocculation as the microcapsules flow in DI water. However, the effect of spray drying can also be seen from a narrowing of the differential volume size profile as well as a loss of an intermediate floc population after spray drying.

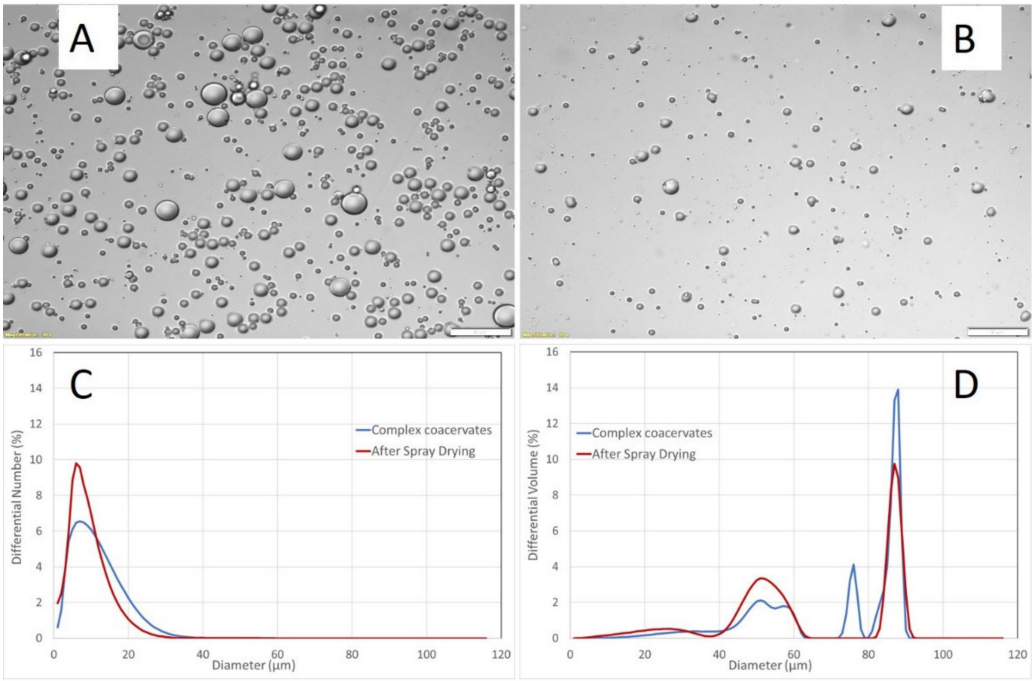

Figure 2. Optical microscopy images of peppermint oil complex coacervate microcapsules before (**A**) and after (**B**) spray drying, as well as their respective size distribution according to differential number (**C**) and differential volume (**D**). The scale bar corresponds to 50 µm. Samples shown here: Polymer:Oil = 2:1 and crosslinker concentration = 5% (*w*:*w*) with respect to BSA.

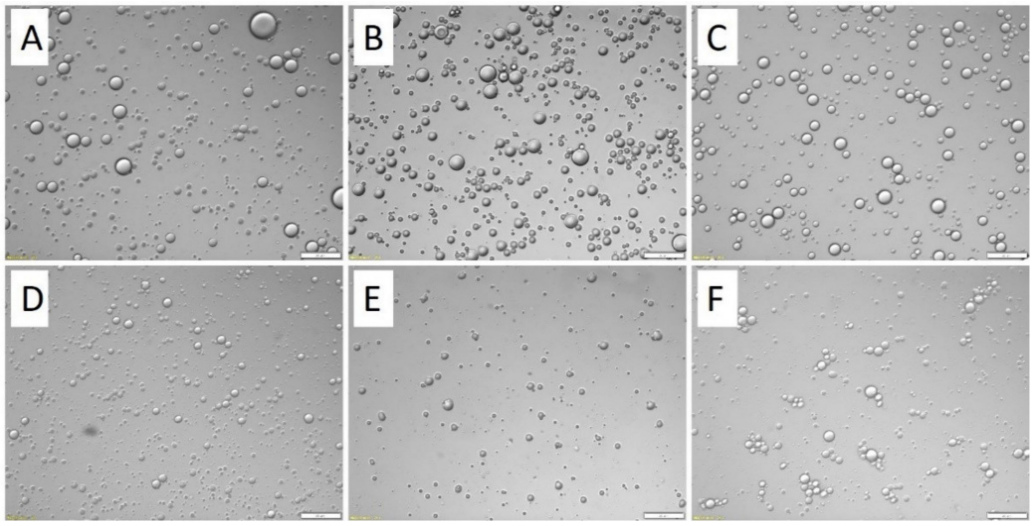

Figure 3. Optical microscopy images of peppermint oil complex coacervate microcapsules with different Polymer:Oil ratios before (upper row) and after (bottom row) spray drying. Polymer:Oil = 1:2 (**A**,**D**), Polymer:Oil = 1.1 (**B**,**E**), Polymer:Oil = 2:1 (**C**,**F**). Crosslinker concentration = 5% (*w*:*w*) with respect to BSA for all capsules. The scale bar corresponds to 50 µm.

3.4. Effect of Polymer:Oil Ratio

As complex coacervation results in the formation of core-shell microcapsules, one of the advantages of this process compared to conventional spray drying is a high loading (see, for example, the review by de Kruif [19]). Here, we have investigated the degree of loading as determined by the Polymer:Oil ratio used in complex coacervation, and to what extent this affects size distribution and retention of peppermint oil following spray drying. Polymer:Oil ratios were varied from 1:2 to 2:1. Optical microscopy images and size distributions for microcapsules with different Polymer:Oil ratios before and after spray drying are shown in Figures 3 and 4 below, respectively.

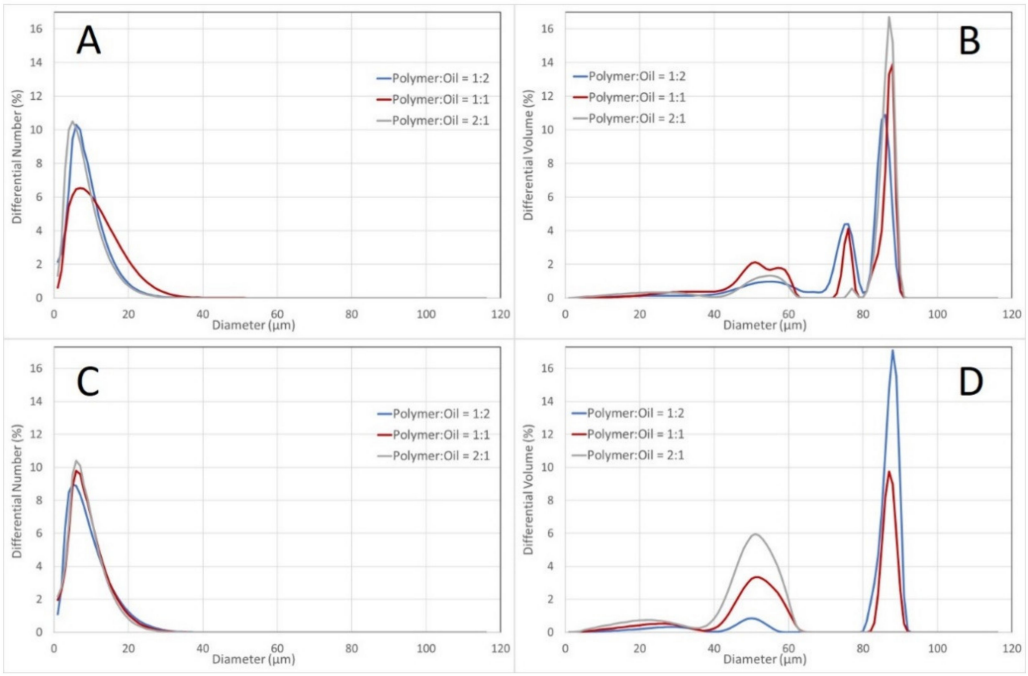

Figure 4. Size distribution of peppermint oil complex coacervate microcapsules with different Polymer:Oil ratios before (**A**,**B**) and after (**C**,**D**) spray drying as measured using laser diffraction. Crosslinker concentration = 5% (*w*:*w*) with respect to BSA for all capsules.

From the optical microscopy images in Figure 3, the tendency towards apparent loss of the largest capsules during spray drying is less for the highest Polymer:Oil ratio, with a similar narrowing of the differential number size distribution as discussed above (Figure 4). The differential volume size distributions show that the fraction of the largest floc population decreases with increasing Polymer:Oil ratio, with the population of flocs > 80 μm completely absent for Polymer:Oil = 2:1. This observed difference in dispersion flow behaviour might reflect changes in the viscoelastic properties of the polymer shell, with a thicker shell resulting in more rigid capsules with lower sticking probability.

The microencapsulation yield as a function of Polymer:Oil ratios after spray drying is shown in Figure 5. The coacervates prepared with ascorbic acid were stable during spray drying. None of the systems studied here showed any measurable loss of peppermint oil following the complex coacervation process (Table 1). In a 2004 study, Weinbreck et al. [26] encapsulated sunflower, lemon and orange oil by complex coacervation using whey protein isolate and gum Acacia as wall materials, reporting less than 20% loss of oil following the emulsification and coacervation steps. In order for better comparison of parameters such as wall thickness and crosslinker concentration, the spray drying parameters were kept constant for all the samples studied here. Also, unlike for conventional spray drying where microcapsules are formed during drying of the sprayed droplets, retention and encapsulation yield of oil in microcapsules formed by complex coacervation are expected to be less susceptible to spray drying parameters. From Table 1 and Figure 5 below, the mi-

croencapsulation yield after spray drying generally increases with Polymer:Oil ratio, which is in agreement with other published work on microencapsulated essential oils [23,25]. Note that for the data presented in Figure 5, crosslinker concentration was kept constant at 5 wt% with respect to BSA. At lower Polymer:Oil ratios—especially for Polymer:Oil = 1:2, the high amount of peppermint oil relative to polymer suggests the formation of very thin microcapsule walls, which do not impart sufficient stability to prevent loss of oil during spray drying. Still, it should be noted that a Polymer:Oil ratio of 1:2 is much higher than what is generally found in the literature for spray drying of complex coacervate microcapsules, where Polymer:Oil ratios of up to 10:1 are reported [25]. Interestingly, it also appears that total polymer concentration affects microencapsulation yield, as shown for Polymer:Oil = 1:1 in Table 1 and Figure 5. Specifically, increasing the polymer concentration also increases the microencapsulation yield from 19% to 27% for 3 and 6 wt% polymer, respectively. This might be attributed to increased partitioning at the oil-water interface of coacervate nodules at higher polymer concentrations. Based on the above, a Polymer:Oil ratio of 2:1 and a total polymer concentration of 6 wt% was used for further analysis on the effect of crosslinker concentration.

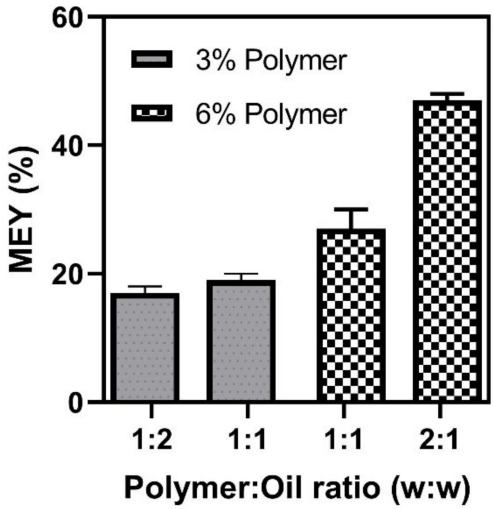

Figure 5. Microencapsulation yield of peppermint essential oil after spray drying for different Polymer:Oil ratios. Note that crosslinker concentration was kept constant at 5 wt% with respect to BSA.

3.5. Effect of Crosslinker Concentration

In order for complex coacervate microcapsules to withstand the thermal and mechanical stress of spray drying, the capsule walls are commonly hardened using crosslinkers like transglutaminase [8,27,28], glutaraldehyde [14,27,29], tannic acid [10] or tripolyphosphate [29], or via simple addition of, for example, Maltodextrin [23], corn syrup [5] or even poly(ethylene glycol) [25] or non-ionic surfactants such as lutensol [9] prior to spray drying. Here, we report the effect of a crosslinker based on an oxidatively modified Zulkowsky type food starch. The Zulkowsky starch was oxidized according to a standard procedure using sodium metaperiodate [21]. This method results in the ring-opening of the glucose units within the starch between the C2 and C3 carbons, with concurrent formation of a dialdehyde, making it an ideal candidate for the crosslinking of protein domains. In order to assess the effect on complex coacervate capsule size, stability and microencapsulation yield, three different crosslinker concentrations were used; 2.5, 5 and 10 wt% with respect to BSA. As mentioned above, control samples without crosslinkers did not withstand spray drying, and so were excluded from further analysis. Optical microscopy images of complex coacervate microcapsules before (upper row) and after (bottom row) spray drying are shown in Figure 6, with corresponding size distributions as measured by laser diffraction shown in Figure 7. As discussed above, the general trend is that spray dry-

ing results in a narrowing of the microcapsule size distribution. Interestingly, while the size distributions for 2.5 and 5 wt% crosslinkers are almost identical, the microcapsules obtained with a 10 wt% crosslinker are significantly larger. Moreover, for the 10 wt% crosslinker, spray-dried microcapsules were found to aggregate significantly within 24 h after redispersion in DI water, whereas no such dispersion instability was observed for the lower crosslinker concentrations. This indicates that above a threshold concentration, the crosslinker increases microcapsule stickiness. Since the crosslinker only acts on the protein component of the wall materials (BSA), one explanation could be that increasing crosslinker concentration could induce surface defects or even porosity, by selective contraction of the polypeptide domains.

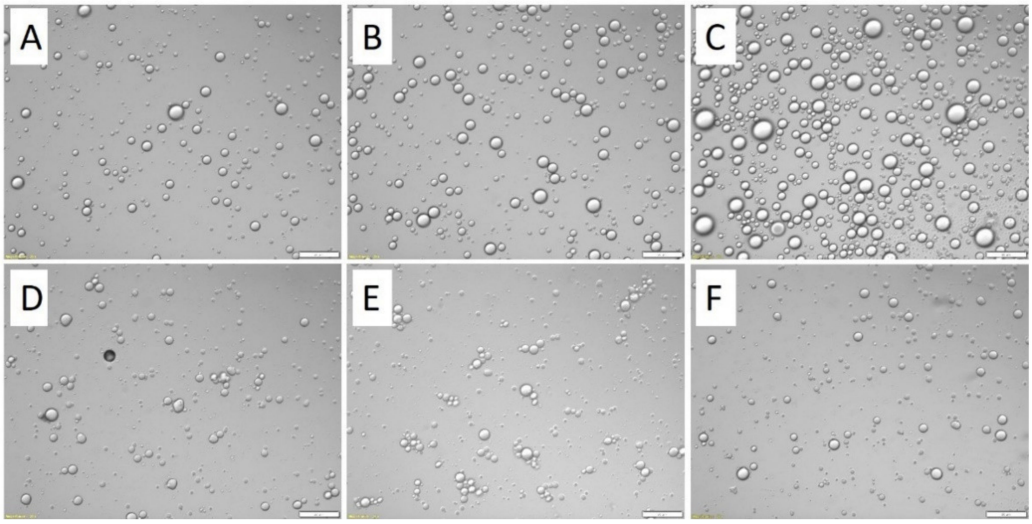

Figure 6. Optical microscopy images of peppermint oil complex coacervate microcapsules with different concentrations of crosslinker before (upper row) and after (bottom row) spray drying. The polymer concentration and Polymer:Oil ratio was held constant at 6% and 2:1, respectively. (**A**,**D**) = 2.5% crosslinker, (**B**,**E**) = 5% crosslinker, (**C**,**F**) = 10% crosslinker. The scale bar corresponds to 50 μm.

The effect of crosslinker concentration on microencapsulation yield is shown in Table 1 and Figure 8. Spray drying is often challenging for coacervates due to their typically soft-shell nature and the high shear forces experienced in the peristaltic feed pumps and the narrow nozzle, to minimize such problems we used a wider-than-standard tip for all the samples contained in our study (1.4 mm in place of 0.7 mm). To minimize the evaporation of the peppermint oil, inlet and outlet temperatures were kept as low as possible whilst still obtaining dry particles, as such, inlet temperature was held between 135–145 °C, resulting in outlet temperatures of 54–60 °C. The lowest crosslinker concentration used here—2.5 wt% with respect to BSA—resulted in the highest microencapsulation yield observed after spray drying, at 54%. Rojas-Moreno et al. [23] have reported complex coacervation and subsequent spray drying of orange essential oil using a wall combination of whey protein isolate (WPI) and GA at WPI:GA = 1:2 and a Polymer:Oil ratio of 2:1, using Maltodextrin DE 10 (Maltodextrin:WPI = 2:1 *w*:*w*) as a hardener, giving a microencapsulation efficiency of 53%. Using soybean protein isolate (SPI) and GA as wall materials (SPI:GA = 1:1), a Protein:Oil ratio of 2:1 and Maltodextrin and PEG as additives, Xiao et al. [25] reported microencapsulation yields of 30% of the complex coacervate microcapsules after spray drying with comparable inlet temperature (160 °C vs. 135–145 °C used here). Thus, the results reported here compare favourably to the literature.

Upon increasing crosslinker concentration, the microencapsulation yield decreases. While only a slight decrease is observed between 2.5 and 5 wt% (54% and 47% microencapsulation yield, respectively), increasing the crosslinker concentration to 10% results in a markedly decreased yield (31%). Taken together with the observed difference in size

distribution, this supports the hypothesized induced defects or porosity above a threshold concentration of crosslinker, which leads to loss of encapsulated material and increased capsule stickiness from exposure to hydrophobic domains.

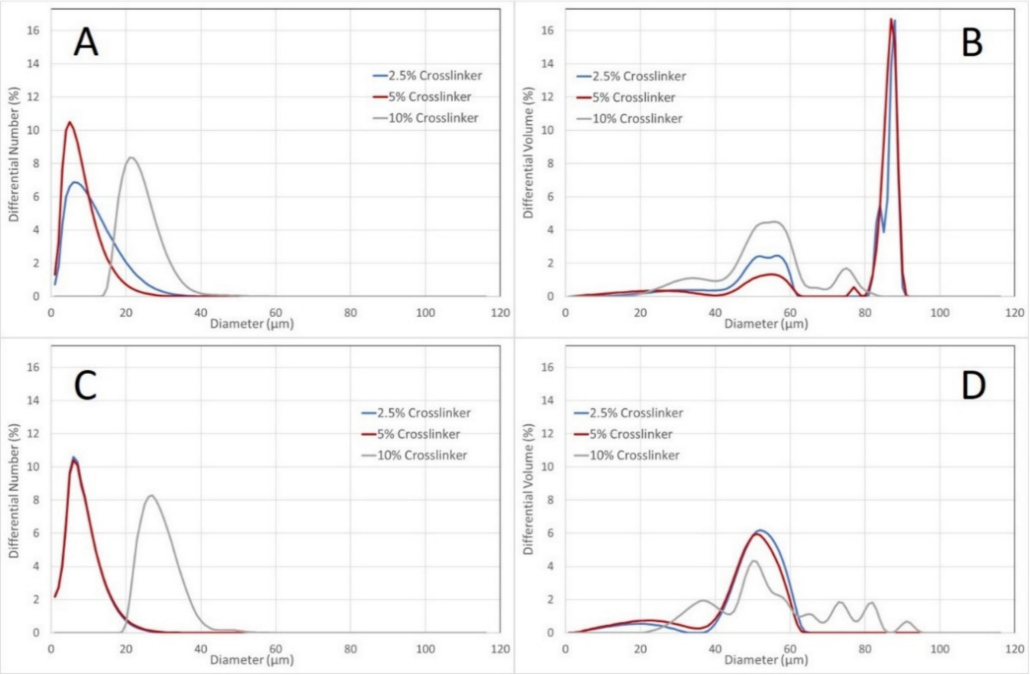

Figure 7. Size distribution of peppermint oil complex coacervate microcapsules with different crosslinker concentrations before (**A**,**B**) and after (**C**,**D**) spray drying as measured using laser diffraction.

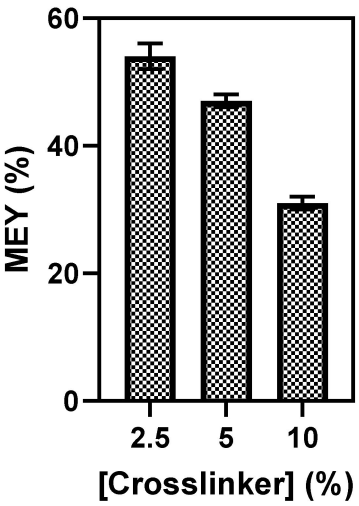

Figure 8. Microencapsulation yield of peppermint essential oil after spray drying for different crosslinker concentrations. Here, Polymer:Oil ratio was kept constant at 2:1 and total polymer concentration was 6 wt%.

4. Conclusions

Peppermint essential oil was microencapsulated via complex coacervation, using bovine serum albumin and gum Acacia as wall materials, and an oxidatively modified food-grade starch as crosslinker. Samples without crosslinkers were included as controls. After complex coacervation, all the reactant ratios used here resulted in stable spherical mononuclear core-shell capsules, with no measurable loss of peppermint oil compared to

the parent emulsion. Samples without crosslinker did not withstand spray drying, thus demonstrating the need for reinforcing the complex coacervate walls with a crosslinker or with a common additive such as a sugar. Crosslinked microcapsules retained their size and morphology following spray drying and redispersion in water, albeit with some loss of peppermint oil. Polymer:Oil ratios were varied from 1:2 to 2:1, with thicker capsule walls providing higher microencapsulation yield and thus better protection of the peppermint oil after spray drying. Upon increasing the crosslinker concentration, the microencapsulation yield decreased for spray-dried samples. This can likely be attributed to the formation of domains with weakened interphases, as the crosslinker only acts on the protein component of the microcapsule walls. The results reported here show good protection of the volatile payload with high core material loads using novel wall material and crosslinker combinations.

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References

1. Carvalho, I.T.; Estevinho, B.N.; Santos, L. Application of microencapsulated essential oils in cosmetic and personal healthcare products—A review. *Int. J. Cosmet. Sci.* **2016**, *38*, 109–119. [CrossRef] [PubMed]
2. Chawda, P.J.; Shi, J.; Xue, S.; Quek, S.Y. Co-encapsulation of bioactives for food applications. *Food Qual. Saf.* **2017**, *1*, 302–309. [CrossRef]
3. Matthews, R.F.; Braddock, R.J. Recovery and Applications of Essential Oils from Oranges. *Food Technol.* **1987**, *41*, 57–61.
4. Stevanovic, Z.D.; Bosnjak-Neumuller, J.; Pajic-Lijakovic, I.; Raj, J.; Vasiljevic, M. Essential Oils as Feed Additives: Future Perspectives. *Molecules* **2018**, *23*, 1717. [CrossRef] [PubMed]
5. Wang, S.J.; Shi, Y.; Han, L.P. Development and evaluation of microencapsulated peony seed oil prepared by spray drying: Oxidative stability and its release behavior during in-vitro digestion. *J. Food Eng.* **2018**, *231*, 1–9. [CrossRef]
6. Dordevic, V.; Balanc, B.; Belscak-Cvitanovic, A.; Levic, S.; Trifkovic, K.; Kalusevic, A.; Kostic, I.; Komes, D.; Bugarski, B.; Nedovic, V. Trends in Encapsulation Technologies for Delivery of Food Bioactive Compounds. *Food Eng. Rev.* **2015**, *7*, 452–490. [CrossRef]
7. Koo, S.Y.; Cha, K.H.; Song, D.G.; Chung, D.; Pan, C.H. Microencapsulation of peppermint oil in an alginate-pectin matrix using a coaxial electrospray system. *Int. J. Food Sci. Technol.* **2014**, *49*, 733–739. [CrossRef]
8. Dong, Z.J.; Ma, Y.; Hayat, K.; Jia, C.S.; Xia, S.Q.; Zhang, X.M. Morphology and release profile of microcapsules encapsulating peppermint oil by complex coacervation. *J. Food Eng.* **2011**, *104*, 455–460. [CrossRef]
9. Irma, K.K.; Qurrota, A.; Hanina, M.; Marsasi, M.R.A. Encapsulation of Peppermint Oil with Carboxymethyl kappa Carrageenan-Gelatine-Chitosan. In *International Conference on Condensed Matters and Advanced Materials*; Mufti, N., Ed.; IOP: Bristol, UK, 2019; Volume 515.
10. Muhoza, B.; Xia, S.Q.; Zhang, X.M. Gelatin and high methyl pectin coacervates crosslinked with tannic acid: The characterization, rheological properties, and application for peppermint oil microencapsulation. *Food Hydrocoll.* **2019**, *97*, 105174. [CrossRef]
11. Yilmaztekin, M.; Levic, S.; Kalusevic, A.; Cam, M.; Bugarski, B.; Rakic, V.; Pavlovic, V.; Nedovic, V. Characterisation of peppermint (*Mentha piperita* L.) essential oil encapsulates. *J. Microencapsul.* **2019**, *36*, 109–119. [CrossRef]
12. Kasiri, N.; Fathi, M. Entrapment of peppermint oil using cellulose nanocrystals. *Cellulose* **2018**, *25*, 319–329. [CrossRef]

13. Jiao, X.; Zhao, D.; Zhang, Y.; Wu, Q.; Qiu, G.; Lu, X.H.; Shi, X.D. Synthesis and studies of poly(ethylene glycol dimethacrylate) microcapsule. *Colloid Polym. Sci.* **2016**, *294*, 639–646. [CrossRef]
14. Du, Y.; Mo, L.; Wang, X.; Wang, H.; Ge, X.H.; Qiu, T. Preparation of mint oil microcapsules by microfluidics with high efficiency and controllability in release properties. *Microfluid. Nanofluid.* **2020**, *24*, 42. [CrossRef]
15. Prosapio, V.; De Marco, I.; Reverchon, E. Supercritical antisolvent coprecipitation mechanisms. *J. Supercrit. Fluids* **2018**, *138*, 247–258. [CrossRef]
16. Soh, S.H.; Lee, L.Y. Microencapsulation and Nanoencapsulation Using Supercritical Fluid (SCF) Techniques. *Pharmaceutics* **2019**, *11*, 21. [CrossRef] [PubMed]
17. Leclercq, S.; Harlander, K.R.; Reineccius, G.A. Formation and characterization of microcapsules by complex coacervation with liquid or solid aroma cores. *Flavour Fragr. J.* **2009**, *24*, 17–24. [CrossRef]
18. Xiao, Z.B.; Liu, W.L.; Zhu, G.Y.; Zhou, R.J.; Niu, Y.W. Production and characterization of multinuclear microcapsules encapsulating lavender oil by complex coacervation. *Flavour Fragr. J.* **2014**, *29*, 166–172. [CrossRef]
19. de Kruif, C.G.; Weinbreck, F.; de Vries, R. Complex coacervation of proteins and anionic polysaccharides. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 340–349. [CrossRef]
20. Lemetter, C.Y.G.; Meeuse, F.M.; Zuidam, N.J. Control of the Morphology and the Size of Complex Coacervate Microcapsules During Scale-up. *Aiche J.* **2009**, *55*, 1487–1496. [CrossRef]
21. Rinaudo, M. Periodate Oxidation of Methylcellulose: Characterization and Properties of Oxidized Derivatives. *Polymers* **2010**, *2*, 505–521. [CrossRef]
22. Burgess, D.J. Practical Analysis of Complex Coacervate Systems. *J. Colloid Interface Sci.* **1990**, *140*, 227–238. [CrossRef]
23. Rojas-Moreno, S.; Cardenas-Bailon, F.; Osorio-Revilla, G.; Gallardo-Velazquez, T.; Proal-Najera, J. Effects of complex coacervation-spray drying and conventional spray drying on the quality of microencapsulated orange essential oil. *J. Food Meas. Charact.* **2018**, *12*, 650–660. [CrossRef]
24. Weinbreck, F.; de Vries, R.; Schrooyen, P.; de Kruif, C.G. Complex coacervation of whey proteins and gum arabic. *Biomacromolecules* **2003**, *4*, 293–303. [CrossRef]
25. Xiao, J.X.; Yu, H.Y.; Yang, J.A. Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. *Food Chem.* **2011**, *125*, 1267–1272.
26. Weinbreck, F.; Minor, M.; De Kruif, C.G. Microencapsulation of oils using whey protein/gum arabic coacervates. *J. Microencapsul.* **2004**, *21*, 667–679. [CrossRef]
27. Dong, Z.H.; Xia, S.Q.; Hua, S.; Hayat, K.; Zhang, X.M.; Xu, S.Y. Optimization of cross-linking parameters during production of transglutaminase-hardened spherical multinuclear microcapsules by complex coacervation. *Colloids Surf. B Biointerfaces* **2008**, *63*, 41–47. [CrossRef]
28. Gharibzahedi, S.M.T.; George, S.; Greiner, R.; Estevinho, B.N.; Fernandez, M.J.F.; McClements, D.J.; Roohinejad, S. New Trends in the Microencapsulation of Functional Fatty Acid-Rich Oils Using Transglutaminase Catalyzed Crosslinking. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 274–289. [CrossRef]
29. Prata, A.S.; Grosso, C.R.F. Production of microparticles with gelatin and chitosan. *Carbohydr. Polym.* **2015**, *116*, 292–299. [CrossRef]