Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic

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A B S T R A C T
The coacervation between soybean protein isolate (SPI) and gum Arabic (GA) for sweet orange oil microencapsulation as functions of pH, ionic strength, SPI/GA ratio, core material load and micromolecules was investigated. SPI was exposed to ultrasonic to increase solubility before use and microcapsules were spray-dried after analysis. It was found that the optimum pH for SPI/GA coacervation was 4.0. High ionic strength reduced the coacervation between the two biopolymers. The highest coacervate yield was achieved in SPI/GA ratio 1:1 and the core material load for the highest microencapsulation efficiency (MEE) and microencapsulation yield (MEY) was 10%. The addition of sucrose in sucrose/SPI ratio 1:1 increased the MEY by 20%, reaching 78% compared to 65% of control. The microcapsules were spherical without holes on the surface by SEM observation and flavour components were well retained in microcapsules according to GC–MS analysis, indicating good protection for core material.

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1. Introduction

Flavours are known as the essence of foods. They play important roles in consumer satisfaction and influence the further consumption of foods. However, the stability of flavours in foods has attracted intense attentions because of its relationship with the quality and acceptability of foods. Most liquid food flavours are volatile and chemically unstable in the presence of air, light, moisture and high temperatures. Hence, it is beneficial to microencapsulate volatile ingredients prior to use in foods or beverages to limit aroma degradation or loss during processing and storage.

The methods that have been reported for flavour microencapsulation have been reviewed (Madene, Jacquot, Scher, & Desobry, 2006). Of the methods mentioned, spray drying is the most common technique to produce flavour powders from food flavour emulsion (Carolina, Carolina, Zamora, & Jorge, 2007), but coacervation is a unique and promising microencapsulation technology because of the very high payloads achievable (up to 99%) and the controlled release possibilities based on mechanical stress, temperature or sustained release (Gouin, 2004).

Complex coacervation is a phase separation process based on the simultaneous desolvation of oppositely charged polyelectrolytes induced by media modifications (Ma et al., 2009). Microcapsules produced by coacervation possess excellent controlled release characteristics and heat resistant properties (Dong et al., 2008). The complex coacervation process involves at least two differently charged polymers under set conditions. In most cases, the two biopolymers include a proteinaceous molecule and a polysaccharide molecule. The interaction between proteins and polysaccharides in complex coacervation, the phase transition, related thermodynamic studies, and various influencing factors have been reviewed by at least five articles (de Kruif, Weinbreck, & de Vries, 2004; Doublier, Garnier, Renard, & Sanchez, 2000; Turgeon, Beaullieu, Schmitt, & Sanchez, 2003; Turgeon, Schmitt, & Sanchez, 2007; Ye, 2008). The protein–polysaccharide combinations that have been reported for flavour microencapsulation by complex coacervation include xanthan gum/gelatin (Lii, Liaw, Lai, & Tom-aski, 2002), gelatin/gum Arabic (Yeo, Bellas, Firestone, Langer, & Kohane, 2005), soybean protein isolate/pectin (Mendanha et al., 2009), gum Arabic/albunin (Burgess & Singh, 1993), and many other natural components (de Kruif et al., 2004). The most classical system of complex coacervation is that gelatin is used as the positive polyelectrolyte and gum Arabic is used as the negatively polyelectrolyte. The system has been successfully used in the production of carbonless paper, scent strips, fragrance samplers and flavour ingredients. However, gelatin is quite viscous even in low concentrations and the cross-linking agent glutaraldehyde used for the system is toxic to human body. Besides, due to the emergence of new diseases such as the prion diseases, regulations concerning safety of animal-derived protein were reinforced (Chourpa, Ducel, Richard, Dubois, & Boury, 2006). These shortcomings restrict the application of this system in foods.
Soybean protein isolate (SPI) is produced from defatted soybean meal by alkaline extraction followed by acid precipitation at pH 4.5. SPI has been successfully used for casein hydrolysate microencapsulation by spray drying (Molina Ortiz et al., 2009) and was found to be more compatible with gum Arabic (GA) than other proteins (Kim & Morr, 1996). SPI is amphoteric and has an isoelectric point of pH 4.5, which is quite similar to that of gelatin. Hence, it is expected that SPI has the potential to be used as a wall material for flavour microencapsulation by complex coacervation.

Since the coacervation between SPI and GA has not been reported for flavour microencapsulation, the purpose of the current study is to study the application of SPI and gum Arabic in flavour microencapsulation by complex coacervation.

2. Materials and methods

2.1. Materials

Soybean protein isolate (SPI) of food grade was purchased from Guangzhou Qianhe Trade Co., Ltd. (Guangzhou, China). Gum Arabic (GA) of analytic grade was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sweet orange oil, a gift from Guangzhou Qianhe Trade Co., Ltd. (Guangzhou, China). Polyethylene glycol 2000 (PEG2000, with average molecular weight 2000), polyethylene glycol 4000 (PEG4000, with average molecular weight 2000), sucrose, maltodextrin and other materials were of analytical grade.

2.2. Determination of coacervate yield

The effects of pH, ionic strength, and SPI/GA ratio on SPI/GA coacervation were monitored by coacervate yield. SPI was suspended in an aqueous solution of a set ionic strength (0, 0.1, 0.5, or 1.0 mol/l NaCl) and exposed to ultrasonic for 10 min until a clear solution was obtained. Then, GA solution was added to the emulsion to make a total solid concentration of 7.5% (w/v) and final volume of 100 ml. The pH was adjusted to 2.5, 3.0, 3.5, 4.0 or 4.5 with 1 mol/l HCl solution to start the coacervation. After the solution was maintained at 50 °C and allowed to react for 30 min under magnetic stirring, the reaction mixture was centrifuged at 9000 g for 10 min and the coacervates were harvested and dried to constant weight at 160 °C. The supernatant was collected for absorbance measurement at 280 nm and the coacervate yield was calculated according to the following equation:

\[
\text{Coacervate yield} = \frac{\text{Dry weight of coacervates}}{\text{Total weight of SPI and GA in the emulsion}}
\]

2.3. Microencapsulation by complex coacervation

A total of 3.75 g SPI was suspended in 40 ml distilled water and exposed to ultrasonic for 10 min until a clear solution was obtained. After 0.75, 1.5, 2.25, 3, 3.75, 4.5, or 5.25 g sweet orange oil was added, the solution was homogenised at 6000 r/min for 5 min (T18, IKA, Germany). Then, 50 ml 7.5% (w/v) GA solution was added to the emulsion and the mixture was diluted to 100 ml with distilled water. The pH was adjusted to 4.0 with 1 mol/l HCl solution to start the coacervation. The reaction mixture was then kept at 50 °C and allowed to react for 30 min under magnetic stirring. After the reaction completed, PEG2000, PEG4000, maltodextrin or sucrose was added to the solution and mixed well. To get powdered microcapsules, the solution was fed to the spray dryer (SD-1000, EYELA, Japan). The spray-drier conditions were inlet temperature 160 °C, outlet temperature 90 °C, atomizing pressure 12 MPa, air flow 0.72 m³/min, and liquid feeder pump flow 320 ml/h. The powder was then collected and stored for analysis.

2.4. Microencapsulation efficiency and microencapsulation yield

The microencapsulation process was monitored by both microencapsulation efficiency (MEE) and microencapsulation yield (MEY). MEE was defined as the percentage of flavour load that were entrapped inside the microcapsules to the total microencapsulated flavour load and MEY was defined as the ratio of microencapsulated flavour load to the flavour load in the emulsion. The equations for the two indexes were as follows:

\[
\text{MEE} = \frac{\text{Microencapsulated flavour load} - \text{Flavour load on the surface}}{\text{Microencapsulated flavour load}}
\]

\[
\text{MEY} = \frac{\text{Microencapsulated flavour load}}{\text{Flavour load in the emulsion}}
\]

The microencapsulated flavour load was determined in the same method mentioned in a previous study (Yang, Xiao, & Ding, 2009). The flavour load on the surface was determined by n-hexane extraction. That is, 5 g of microcapsules were placed in a flask containing 10 ml n-hexane. The mixture was shaken gently and then filtered through filter paper. The microcapsules were then air dried and subject to flavour determination in the same method as microencapsulated flavour load determination.

2.5. GC–MS analysis

The original and microencapsulated sweet orange oil were analysed by using the 6980N/5975 inert GC–MS (Agilent, USA). Before analysis, 1 g original sweet orange oil or its microcapsules were suspended in 10 ml n-hexane:water (1:1) solution. After the mixture was sealed and extracted in ultrasonic for 10 min, the solution was allowed to settle and the hexane layer was collected. Then, 5 g of anhydrous sodium sulfate was added to the hexane layer and mixed well. The supernatant was used for GC–MS analysis. The GC–MS conditions were as follows:

- Column: HP-5 fused silica capillary (30 m × 0.25 mm; film thickness 0.25 μm);
- Carrier gas: helium (0.8 ml/min);
- Injection temperature 250 °C; detector temperature and type: 300 °C, total ion chromatogram (TIC); programmed column temperature: 69 °C for 1 min, 69–80 °C at 1 °C/min, 80 °C for 2 min, 80–200 °C at 10 °C/min, 200 °C for 1 min, finally 280 °C for 1 min.

2.6. Scanning electron microscope observation

Microcapsules were directly mounted on circular aluminium stubs with double-sided sticky tape, coated for 250 s with 15 nm gold, then examined and photographed in a scanning electron microscope (JSM-840 A, Jeol, Ltd., Tokyo, Japan) at an accelerating voltage of 20 kV. The sample sizes were measured using the calibrated scale bar on the micrograph.

2.7. Statistical analysis

All the experiments were performed on triplicate samples in replicates. Differences between mean values were determined using the analysis of variance (ANOVA) utilising the statistical software SAS version 8.0.
3. Results and discussion

3.1. Effect of pH on complex coacervation

Poor solubility of SPI has restricted its application. Hence, SPI was often firstly dissolved in pH 8.0 to obtain the maximum solubility (Mendanha et al., 2009; Molina Ortiz et al., 2009). To avoid the possible effect of extra ions added for subsequent pH adjustment, ultrasonic treatment was used to increase the solubility of SPI in distilled water. It was found that ultrasonic treatment resulted in a SPI concentration of up to 10% in water. Hence, SPI were exposed to ultrasonic before use.

The complex coacervation was significantly affected by the pH of the solution, since pH determines the charge density on ampholytes and may even induce structural transitions of proteins and polysaccharides (Turgeon et al., 2007). Fig. 1a shows the effect of pH on SPI/GA coacervation.

The pH of the system significantly affected the coacervation between SPI and GA, of which pH 4.0 resulted in the highest degree of coacervation and pH 2.5 resulted in the least coacervation. The charge densities of the two biopolymers seem to be stoichiometrically balanced at pH 4.0 and pH 4.0 was the electrical equivalence point (EEP) of the SPI–GA system. These results were corroborated by the absorbance values of the supernatants. When the pH value was 3.5 or lower, the coacervate yield was significantly reduced. This trend was consistent with that in GA/chitosan coacervation, possibly due to the protonation of the carboxylic groups of GA (Espinosa-Andrews, Baez-Gonzalez, Cruz-Sosa, & Vernon-Carter, 2007). When the pH was increased to 4.5, which was quite close to the pI (4.5–4.8) of SPI, a soluble protein–polysaccharide complex resulted in a SPI concentration of up to 10% in water. Hence, SPI were exposed to ultrasonic before use.

The coacervate yield was reduced significantly at pH 4.5 or lower, the coacervate yield was significantly reduced. This trend was consistent with that in GA/chitosan coacervation, possibly due to the protonation of the carboxylic groups of GA (Espinosa-Andrews, Baez-Gonzalez, Cruz-Sosa, & Vernon-Carter, 2007). When the pH was increased to 4.5, which was quite close to the pI (4.5–4.8) of SPI, a soluble protein–polysaccharide complex instead of insoluble coacervate might be formed (Turgeon et al., 2003). As a result, the coacervate yield was reduced significantly (P < 0.05).

3.2. Effect of ionic strength on complex coacervation

In addition to pH, the electrostatic force-dominated interaction between biopolymers is also dependent on ionic strength (Liu, Low, & Nickerson, 2009) and ionic strength may change the pH that initiates the biopolymer structure transition (Weinbreck, Nieuwenhuize, Robijn, & de Kruijf, 2004). It has been found that the coacervate yield of the GA–chitosan system was inversely proportional to the ionic strength (Espinosa-Andrews et al., 2007). The same trend was observed in this study, as shown in Fig. 1b. The increase of NaCl concentration significantly (P < 0.05) decreased the coacervate yield and increased the protein content in the supernatant. This was because that the presence of ionic strength screened the charges of the biopolymers, and thus influencing the formation of coacervates. When the NaCl content was increased to 1 mol/l or higher, the coacervation was nearly completely suppressed. Hence, ultrasonic treatment for higher SPI solubility was quite necessary for SPI/GA coacervation, since ultrasonic treatment could increase the SPI solubility without introducing extra ions as the pH adjustment method.

3.3. Effect of SPI/GA ratio on complex coacervation

Biopolymer mixing ratio is critical for controlling the charge balance between proteins and polysaccharides, the intensity of interactions, and the degree of self-aggregation during complexation (Ye, 2008). It should be noted that the effect of biopolymer mixing ratio on coacervation was also pH dependent (Liu et al., 2009). Since this is only a preliminary work on SPI/GA coacervation, the interaction between SPI/GA ratio and pH was not investigated here. As shown in Fig. 2, the greatest coacervate yield was obtained at the SPI/GA ratio 1:1 and 4:5; further increase or decrease of the mixing ratio significantly reduced the coacervation. When the SPI/GA ratio exceeded 1, the absorbance of the supernatant at 280 nm was even lower than that at the SPI/GA ratio 2:5 in the same total solid concentration basis. This indicated that self-aggregation of SPI might occur at high concentrations, which has been observed under high pressure (Tang & Ma, 2009).

3.4. Effect of core material load on complex coacervation

The main requirement on a good wall material is the ability to hold and seal the entire core material within its structure during processing and storage. However, high core material load often resulted in poor retention (Beristain, Garcia, & Vernon-Carter, 2001). As shown in Fig. 3, the core material load significantly affected the
MEE and MEY. The core material load of 10% and 20% resulted in the highest MEE, but led to significantly different (P < 0.05) MEY. When the core material load was increased to 50% or higher, the MEE or MEY was no longer affected. The index MEY was more susceptible to core material load variation. When the core material load was 20%, the MEE was nearly not changed, but the MEY was reduced by 20% compared with those values of the 10% core material load. In the core material load 20%, the MEY was only 68%, which is much lower than the yield 80% obtained in the spray-drying method for the same flavour (Yang et al., 2009). In the current coacervation system, SPI functioned also as the emulsifier in addition to participation in the electrostatic interaction. Due to its limited emulsifying capacity and no addition of extra emulsifier, excessive oil in the system led to incomplete emulsification, and the unemulsified oil affected the electrostatic interaction between the two biopolymers in return. As a result, the MEY was greatly reduced by the increase of core material load.

3.5. Effect of micromolecules on complex coacervation

Aldehydes and transglutaminase have been reported to enhance the stability of protein-based matrices (Prata, Zanin, & Grosso, 2008). However, few studies have been attempted on the effect of various micromolecules on the microcapsulization in the complex coacervation method. In this study, the coacervates were finally subject to spray drying to obtain microcapsules powder. Hence, sucrose and maltodextrin, two additives that are widely used in flavour microcapsulization by spray-drying or co-crystallization (Madene et al., 2006) were selected and were expected to improve the microcapsulization performance. Besides, PEG 2000 and PEG 4000, two polymers that are sometimes used as emulsion stabilizer, were also investigated. As shown in Fig. 4a, all the four micromolecules studied showed no or positive effect on the MEE, but sucrose increased the MEY in contrast to the other three micromolecules. Maltodextrin showed no effect on the MEY, but sucrose/SPI ratio 1:1 offered the effective protection of the encapsulated material. Therefore, part incomplete microcapsules were also observed amongst the populations (c and d). It could be seen that part microcapsules showed irregular morphology and some were even not entirely sealed (d), indicating that the interaction between the two biopolymers must be further enhanced.

According to the scale bar of the SEM, the particle size was not homogeneous for different microcapsules and the average particle size was 7.569 μm based on 50 particles.

3.6. SEM observation

The formation of sweet orange oil microcapsules was confirmed by SEM observation. The outer topography of the microcapsules was presented in Fig. 5. It can be observed that the microcapsules had spherical morphologies with few (a and b) or no dents (d) on the surface, but no holes were found on the surface of complete microcapsules. This was possible due to the addition of sucrose, which fills in the holes that were often found in spray-dried microcapsules (Bylaitė, Rimantas Venskutonis, & Majdelpienė, 2001). The SEM micrographs showed that the complete microcapsules guaranteed the effective protection of the encapsulated material.

However, part incomplete microcapsules were also observed in several physical changes that led to quality deterioration (Roos & Karel, 1991). Hence, the effect of sucrose on the stability and mechanical properties of the microcapsules needs further study.

3.7. Composition changes after complex coacervation

Sweet orange oil is a mixture of multiple components and each component contributes to the aroma of the oil. The loss or incomplete retention of compounds is a critical problem that restricts flavour microencapsulation. Hence, it is necessary to investigate the changes of flavour composition after microencapsulation. Table 1 listed the component profiles of both the microencapsulated and original sweet orange oil.
Fig. 5. SEM micrographs of microcapsulated sweet orange oil. (a and b): representatives of complete microcapsules; (c and d): representatives of incomplete microcapsules. The microcapsules were obtained in the sucrose/SPI ratio 1:1 and core material load 10%.

Table 1
Profile of original and microencapsulated sweet orange oil.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Relative content in original oil (%)</th>
<th>Relative content in microcapsules&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Pinene</td>
<td>1.21</td>
<td>0.84</td>
</tr>
<tr>
<td>2</td>
<td>4-Methyl-1-(1-methylethyl)-bicyclic(3.1.0)-hexane</td>
<td>0.81</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>β-Pinene</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>β-Myrcene</td>
<td>3.67</td>
<td>2.94</td>
</tr>
<tr>
<td>5</td>
<td>Octal aldehyde</td>
<td>0.79</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>3-Carene</td>
<td>0.20</td>
<td>0.23</td>
</tr>
<tr>
<td>7</td>
<td>1-Methyl-4-(1-methylethylidene)-1,3-cyclohexadiene</td>
<td>ND</td>
<td>0.07</td>
</tr>
<tr>
<td>8</td>
<td>D-Limonene</td>
<td>39.65</td>
<td>90.97</td>
</tr>
<tr>
<td>9</td>
<td>3,7-Dimethyl-1,3,7-octadecatriene</td>
<td>ND</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>3,7-Dimethyl-1,3,6-octatriene</td>
<td>0.05</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>1-Methyl-4-(1-methylethylidene)-1,4-cyclohexadiene</td>
<td>0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>12</td>
<td>1-Octyl alcohol</td>
<td>0.07</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>1-Methylene-4-(1-methylethylidene)-cyclohexene</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>14</td>
<td>2-Aminobenzoxy-3,7-dimethyl-1,6-octadien-3-ol</td>
<td>ND</td>
<td>0.78</td>
</tr>
<tr>
<td>15</td>
<td>3,7,7-Trimethyl-bicyclo[4.1.0]hept-3-ene</td>
<td>0.91</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>Butyl isocyanide</td>
<td>ND</td>
<td>0.09</td>
</tr>
<tr>
<td>17</td>
<td>Nonaldehyde</td>
<td>0.10</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>Citronellal</td>
<td>0.11</td>
<td>ND</td>
</tr>
<tr>
<td>19</td>
<td>4-Methyl-1-(1-methylethylidene)-3-cyclohexen-1-ol</td>
<td>ND</td>
<td>0.27</td>
</tr>
<tr>
<td>20</td>
<td>α,α,4-Trimethyl-3-cyclohexene-methan-1-ol</td>
<td>0.15</td>
<td>1.22</td>
</tr>
<tr>
<td>21</td>
<td>Decanal</td>
<td>0.83</td>
<td>0.68</td>
</tr>
<tr>
<td>22</td>
<td>Octyl acetate</td>
<td>0.03</td>
<td>ND</td>
</tr>
<tr>
<td>23</td>
<td>3,7-Dimethyl-2,6-octadienal</td>
<td>0.53</td>
<td>0.45</td>
</tr>
<tr>
<td>24</td>
<td>3,7-Dimethyl-2,6-octadien-1-ol</td>
<td>ND</td>
<td>0.04</td>
</tr>
<tr>
<td>25</td>
<td>Copaene</td>
<td>ND</td>
<td>0.06</td>
</tr>
<tr>
<td>26</td>
<td>α-Cadinolaldehyde</td>
<td>0.07</td>
<td>ND</td>
</tr>
<tr>
<td>27</td>
<td>Diacetoxy-1,1-dodecanediol</td>
<td>ND</td>
<td>0.13</td>
</tr>
<tr>
<td>28</td>
<td>Cyclopropane[1,2]cyclopenta[1,3]benzene</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>29</td>
<td>Tridecyl aldehyde</td>
<td>0.17</td>
<td>ND</td>
</tr>
<tr>
<td>30</td>
<td>Caryophyllene</td>
<td>0.06</td>
<td>ND</td>
</tr>
<tr>
<td>31</td>
<td>1,8-cyclic nonadiene</td>
<td>0.16</td>
<td>ND</td>
</tr>
<tr>
<td>32</td>
<td>Bisabolene</td>
<td>ND</td>
<td>0.1</td>
</tr>
<tr>
<td>33</td>
<td>Naphthalene</td>
<td>0.16</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> The microcapsules were obtained in the SPI/GA/sucrose ratio 1:1:1, pH 4.0 and core material load 10% in the absence of NaCl.

<sup>b</sup> ND = not detected.
It could be seen that the microcapsules have great flavour retention capability. D-Limonene was the major component of the original oil, with content up to 89.65%. D-Limonene was well retained in the microcapsules, with the content of up to 90.97%. The slight increase was due to the loss of other minor components during microencapsulation, which could be confirmed by the fact that 11 compounds failed to be detected in the microcapsules. It should also be noted that some “new” compounds were identified in the microcapsules, such as β-pinene. This was because that their contents in the original oil were too low to be recorded.

The great flavour retention of sweet orange oil has also been reported in another study in the spray-drying method in the presence of Tween 80 (Yang et al., 2009). It has been reported that the emulsion particle size determines the flavour retention and smaller emulsion droplet size provides more efficient flavour retention (Reineccius, 1989). However, SPI as an emulsifying agent is generally recognised to be less efficient when compared with many other food proteins or modified proteins, such as casein and whey proteins (Palazolo, Sorgentini, & Wagner, 2005; Santiago, González, Remondetto, & Bonaldo, 1998), but SPI could have an improved emulsifying activity if the proteins are partially unfolded prior to emulsification (Jiang, Chen, & Xiong, 2009). Hence, we proposed that ultrasonic treatment increased the emulsifying capacity of SPI.

The improved emulsifying capacity of ultrasonic-treated SPI was also proved by the high and less affected MEE of the coacervate. High MEE means that more oil was entrapped inside the microcapsules, which was certainly a result of strong emulsifying capacity of SPI.

### 4. Conclusion

The electrical equivalence point for SPI and GA coacervation was pH 4.0 and the optimum SPI/GA ratio was 1:1. High ionic strength was not beneficial for the coacervation, but the addition of sucrose could significantly improve the microencapsulation efficiency and microencapsulation yield. The SPI–GA coacervation showed good flavour retention and nearly no d-limonene was lost during microencapsulation. The results indicate that SPI was quite compatible with GA for complex coacervation. However, the coacervation between biopolymers involves multiple factors, such as phase separation, shearing force and temperature. Hence, the coacervation between SPI and GA deserves further study and the effect of ultrasonic exposure on the properties of SPI is another issue that should be considered.

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