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Zhang, Wei, Yan, Cuie, May, Jennifer and Barrow, Colin 2009, Whey protein and gum arabic encapsulated Omega-3 lipids. The effect of material properties on coacervation, *Agro food industry hi-tech*, vol. 20, no. 4, supplement issue, pp. 20-23.

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Whey protein and gum arabic encapsulated Omega-3 lipids
The effect of material properties on coacervation

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ABSTRACT: The effect of material properties on complex coacervation of whey protein and gum Arabic from various sources was investigated. In this study, it was demonstrated that material properties of whey protein isolates and gum Arabic affect the complex coacervation process significantly. For whey protein, the coacervation capability could be correlated with their level of denaturation and calcium content. For gum Arabic, both material sources and salt content were found to be attributing factors to their coacervation capability. This study facilitated the development of Omega-3 lipids microcapsules with promising performances in certain food applications.

KEYWORDS: whey protein, gum arabic, microencapsulation, complex coacervation.

INTRODUCTION

Complex coacervation has been regarded as one of the most costly processes and been considered undesirable for food industry in general (1). However, many research groups around the world are still working to improve the cost-effectiveness of this process, including the evaluation of new materials and modified processes. The gelatin/gum Arabic system was used in the pioneering stage of complex coacervation (2-4). In recent years, a number of studies have been reported on complex coacervation systems using globular proteins and anionic polysaccharides. For example, β-lactoglobulin, bovine serum albumin, egg albumin, soy proteins, pea proteins or whey proteins have been coacervated with gum arabic (GA), carrageenan or pectin (5-8). More recently, Weinbreck et al. (9-12) and Schmitt et al. (13) studied the complex coacervation of the whey proteins/gum Arabic system in terms of how different parameters such as pH, whey protein/gum composition and ionic strength, influence the phase separation kinetics and structure. This paper describes the results as a part of the development work for Omega-3 lipids microcapsules. It focuses on the effect of material properties on complex coacervation of whey protein and gum Arabic obtained from various sources. Solid content in coacervates as well as supernatants was investigated in relation to the coacervation capability of these whey proteins and gums. Whey proteins are globular proteins that will undergo molecular reconformation and aggregation once heated above their denaturation temperature (14, 15). Therefore, the effect of heat treatment on coacervation was briefly studied. Results on properties and performance of the Omega-3 lipids microcapsules can be found elsewhere (16).

MATERIALS AND METHODS

Materials

Omega-3 rich marine lipids, in this case fish oils, were purified by Ocean Nutrition Canada Limited (ONC) and stored at -18°C. These oils contained at least 30 percent of docosahexaenoic and eicosapentaenoic omega-3 fatty acids. Four types of whey protein isolate were obtained from different manufacturers, and were coded as WPI 1 to 4 in the context. Five types of GAs were used in this study. Three types were from Senegal species, referred to as Senegal 1 to 3. Two were from Seyal species, and referred to as Seyal 1 and 2.

Metal analysis

The raw materials were subjected to metal analysis using Inductively Coupled Plasma Emission Spectrometry (ICP-ES). Representative portions of the samples (~0.5 g) are accurately weighed into PTFE microwave vessels. Concentrated high purity nitric acid (9 mL) is added. The samples are “pre-digested” in a heated Teflon coated graphite digestion block, then the vessels are sealed and are further digested using a laboratory microwave digester. The microwave digestion allows for reaction at elevated temperature and pressure which more effectively destroys organic matter and improves analytical recovery for trace elements. After cooling, the vessels are opened and the contents are quantitatively transferred into graduated polypropylene tubes. The tubes are heated in the digestion block to reduce the total solution volume to approximately 2 mL. Deionized water (~10 mL) is added and the samples are heated for an additional 30 minutes. The resulting solutions are diluted to volume (20 mL) for analysis. Each set of samples includes reagent blanks and analytical replicates which are prepared and analyzed concurrently with the samples. The results of metal analysis are shown in Table 1 with a unit of ppm.

Insoluble mass content of whey proteins

A 10 percent (w/w) whey protein solution was prepared with de-ionized water and
Separation of the solutions was carried out on a Tosohaas G3000PWx1 column (7.8 x 300mm, 6 μm) with a guard column at a flow rate of 0.6 ml/min for 30 minutes. The injection volume was 0.1 ml, giving an on-column mass of 500 μg for each injection. All solutions were injected in triplicate series with blank injections in between each set. The dn/dc constant for all solutions was set at 0.185, which is the standard for proteins. The weight-averaged Mw of whey proteins was calculated based on weight-averaging of the measured Mw of the α-lactoalbumin, β-lactoglobulin and a third fraction having higher and lower Mw in combination with their corresponding composition. The results are presented in Table 2.

Complex coacervation
A complex coacervation process was applied to produce the whey protein and gum Arabic coacervates. A mixture of WPI and GA with a total concentration of 3.3 percent was dissolved with de-ionized water in a reactor under agitation. Subsequently, coacervates were formed and harvested with centrifugation at 15,000 g for 5 minutes on a Sorvall Super T-21 centrifuge (Sorvall Products L.P., Newtown, CT, USA). 10 ml of the coacervates suspension in a 15 ml test tube was utilized for the centrifugation process. The supernatant was collected and dried in an oven to acquire the solid content. This solid content is called soluble solids. The pellets of the coacervates were collected and weighed to obtain the wet volume, and dried to obtain the solid content in these coacervates. This value was divided with its wet volume to derive the solids in coacervates.

Turbidity
Certain solution mixtures of whey protein and gum Arabic were subjected to turbidity measurements using an Analit NEP 285 turbidity probe (McVan Instruments, Melbourne, Victoria, Australia). This turbidity probe was calibrated for every measurement with a set of standard solutions provided by the manufacturer.

RESULTS AND DISCUSSION
Whey protein isolates
The results of solids in coacervates and soluble solids were obtained with four types of WPIs. Seyal 2 was used as the polyanion to form the coacervates. Figure 1 shows that solids in coacervates peaked at around pH 4.0 to 4.6, and decreased away from this pH range for all four WPIs. Furthermore, the data collected from all WPIs fell within the range of 32 to 35 percent. The results of the corresponding soluble solids are given in Figure 2. Though all WPIs showed a decline in soluble solids, WPI 1 and WPI 2 declined more steeply than WPI 3 and WPI 4. It is worth noting that a lower value of soluble solid is an indication that the total mass in the coacervates is high. This was confirmed by the results of dry mass in coacervates displayed in the lower part of Figure 2. The dry mass in coacervates followed the order of WPI 2 > WPI 1 > WPI 3 > WPI 4. A high value of dry mass in coacervates was favourable because shell material was utilized efficiently in building the microcapsules and less material was wasted in solution.

Based on the data given in Table 1 and Table 2, the material properties of these WPIs differ significantly in terms of divalent metal ions and insoluble mass content. WPI 1 and WPI 2 contain a low

**Table 1.** The results of metal ion analysis of PWIs and GAs with a unit of ppm.

<table>
<thead>
<tr>
<th>Metal</th>
<th>WPI 1</th>
<th>WPI 2</th>
<th>WPI 3</th>
<th>WPI 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Magnesium</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Potassium</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Table 2.** Weight-averaged molecular weight and insoluble mass content of WPIs.

<table>
<thead>
<tr>
<th>WPI</th>
<th>WAM/W</th>
<th>Insolubles (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46200</td>
<td>0.5 ± 0.16</td>
</tr>
<tr>
<td>2</td>
<td>46300</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>50600</td>
<td>4.31 ± 0.96</td>
</tr>
<tr>
<td>4</td>
<td>41900</td>
<td>1.23 ± 0.58</td>
</tr>
</tbody>
</table>

**Figure 1.** Effect of pH on solid content in wet coacervates for the complex coacervation of Seyal 2 with four types of WPI.

**Figure 2.** Effect of pH on soluble solids content in supernatant, and on the dry mass content in coacervates for the complex coacervation of Seyal 2 with four types of WPI. In the legend, SS refers to Soluble Solids and DM refers to Dry Mass.

Weight-averaged molecular weight of whey proteins
The molecular weight (Mw) of whey proteins was determined using size exclusion chromatography and multi angle laser light scattering (SEC-MALLS) techniques. A HP Agilent 1100 series HPLC was used for the analysis with two detectors: an Optilab DSP interferometric refractometer and a DAWN DSP laser photometer. Solutions were prepared at a concentration of 5 mg/ml in 0.1 M ammonium acetate mobile phase (pH 6.8), and were filtered to 0.1 μm.
Focus on Omega-3

level of divalent metal ions such as calcium and magnesium. It is less than 900 ppm for WPI 1 and less than 600 ppm for WPI 2. WPI III and WPI IV contain 6 to 10 fold higher levels of divalent metal ions. These divalent metal ions might function as a chelator so that the charge interactions between WPI and GA are weakened during the coacervation process. The metal ion contents appear to be process dependant, and it is possible that they play a role in the WPI denaturation process. The results of insoluble mass content provided in Table 2, show that WPI I and WPI II had less insoluble mass in comparison with that of WPI III and WPI IV, indicating that WPI I and WPI II were denatured to a lesser degree. As a consequence, these two WPIs tend to form coacervates more readily. The results of the weight-averaged Mw do not show significant difference between the WPIs. This may be explained by the fact that all samples injected to SEC-MALLS were filtered to 0.1 μm, effectively removed most, if not all, of the large denatured protein aggregates. Another possibility could be the irreversible adsorption of these protein aggregates onto the column packing material.

Gum Arabic
To examine the capability for coacervation of gum arabics obtained from different sources, turbidity measurements were performed complementary to the solid content analysis. Five types of gums were tested. WPI 1 was used as the polycation for these experiments. The turbidity results, given in Figure 3, could be divided into two groups. Senegal 1, 2 and 3 were in one group and reached their maximum turbidity around pH 4. Seyal 1 and 2 were in a separate group and maximized at around pH 5. Senegal gum has a molecular weight of about 250,000 Dalton and Seyal gum of about 750,000 Dalton, as confirmed by SEC-MALLS measurements.

The soluble solid content of various gums is given in Figure 4. WPI 2 and WPI 1 with Seyal 1 possessed soluble solids > 2 percent, while those with Senegal 1 were < 2 percent. With a heat treatment of 95°C for 5 minutes, the soluble solids decreased to below 1.2 percent for all the WPI and GA combinations mentioned above. Although the difference was small, Senegal gum still delivered a lower level of soluble solids, and produced more coacervates (data not shown). It is worth noting that besides the difference in molecular weight, Seyal gums differed from Senegal gums in terms of salt content as shown in Table 1. Senegal gums had a lower calcium content that might be one of the contributing factors to their superior coacervation capacities with WPIs. There is apparently a similarity between WPIs and GAs in this aspect. It might be reasonable to postulate that calcium can form complexes with certain functional groups of both WPIs and GAs molecules, which blocked their capability of interacting and coacervating with their counterparts.

Omega-3 lipids microcapsules
Based on this and other related studies, three types of Omega-3 lipids microcapsules were produced from complex coacervation and thermal crosslink of WPI and GA with distinguishable features (16). In certain food applications, these novel microcapsules showed promising results (17). Further studies are needed to characterize these microcapsules and to evaluate their performances in food applications.

CONCLUSION
This study demonstrated that material properties of whey protein isolates and gum arabic affect the complex coacervation process significantly. For whey proteins, the coacervation capability can be correlated with their degree of denaturation and the content of divalent metal ions. For gum arabic, both molecular weight / species and the content of divalent metal ions affect their coacervation capability. Senegal gums produced a low level of soluble solid and a high level of coacervates content in comparison with Seyal gums. Based on this study, Omega-3 lipids microcapsules have been developed with promising performances in certain food applications.

Material properties of whey protein isolates and gum arabic affect the complex coacervation process significantly
REFERENCES AND NOTES

16. W. Zhang, C. Yan et al., Dairy Science and Technology, to be published.