The synthesizing of defatted chia- chitosan beads for drug delivery

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Abstract: Chia (Salvia hispanica L.), Lamiaceae (Mint) family's long-year herbaceous, tropical and subtropical climates an angiosperm plant. The Chia seeds are a good source of fat, they contain protein, dietary fiber, minerals and polyphenolic compounds. After removing the oil, defatted chia seeds are mainly covered with a layer of muslin which consists of carbohydrates and protein. At optimal conditions, these seeds can be produced mucilages. These kind of mucilages are hydrophilic carbohydrate polymers of high molecular weights and consisted of many monosaccharide units joined by glucocidic bonds. Hydrophilic polymers are the most suitable materials for drug delivery systems. Chia seed mucilage has good water adsorption capacity, gel formation, emulsion stabilizing properties. Because of these structural and chemical properties, chia can be used in many areas such as; bakery compounds, nutritional supplement, thickener in food industry and coating materials as edible films. Also, natural mucilages like chia seed mucilage are biocompatible, cheap, easily available, low cost and lack of toxicity. In this study, we aimed to prepare the drug delivery material with defatted chia seed mucilage chitosan beads. The chia seed was defatted by Soxhlet extraction. To get best defatted the chia seed mucilage was incubated at different buffer (acetate, phosphate) and water at different ratios. After the defatted chia mucilage was ready, the different seed: buffer/ water ratio and coating materials as edible films. Also, natural mucilages like chia seed mucilage are biocompatible, cheap, easily available, low cost and lack of toxicity. In this study, we aimed to prepare the drug delivery material with defatted chia seed mucilage chitosan beads. The chia seed was defatted by Soxhlet extraction. To get best defatted the chia seed mucilage was incubated at different buffer (acetate, phosphate) and water at different ratios. After the defatted chia mucilage was ready, the different seed: buffer/ water ratio, pH and temperature were studied to get best form of beads for drug delivery. The water adsorption capacity and a model study for drug delivery with medicine was tested.

Keywords: Chia seed, drug delivery, natural mucilage, biopolymer

1. Introduction

Chia (Salvia hispanica L.), Lamiaceae (mint) family’s long-year herbaceous, tropical and subtropical climates from the grain which is an angiosperm plant. Chia cultivation dates back to the pre-Columbus period. Today, annual production around the world is about 30,000 tons. Salvia hispanica produces white or purple flowers that grow significantly for seeds. The plant grows in numerous small brown, black and beige (without pigmentation) seeds, which are oval shaped. The seed consists 30 g fat/100 g seed weight and contains mostly unsaturated fatty acids. The composition of fat to tal fat in chia seeds is α-linolenic acid (64%), linoleic (21%), oleic acid, stearic acid and palmitic acids. Chia seeds are also a good source of protein (19-27 g/ 100 g). Protein content (19-23%) in chia seeds is higher than most commonly used grains. Basic amino acids, such as leucine, isoleucine and valine, constitute 42.2% of total amino acids in chia seeds. Another benefit of the chia seed is its high fiber content, which is 6% soluble. (Capitani et al., 2012; Porras-Loaiiza et al., 2013).

The main physiological effect of chia fiber is to swell ability due to the presence of carbohydrates with hydrophilic bonds. This chemical structure is important for getting gel formation and therefore leads to an increase in the volume of stool which induces more frequent peristaltic movements in the intestine. The carbohydrate composition of Chia seed gum was mainly of xylose, glucose and methyl glucuronic acid. Chia seeds polysaccharide (CSP), which is named as chia seed mucilage (CSM) as seen was the water soluble anionic hetero polysaccharide extracted from the seed layer (Dick et al., 2015; Xing et al., 2017).

Chia is used in many areas today. Chia fiber flour is used as a component in the bakery products and beverage industries because of its nutritious and functional properties. Chia seeds can be used in the production of bar, breakfast cereals and cookies in many countries as well as in nutritional supplements. There are many health-promoting properties of chia seed was known such as lowering blood sugar levels, controlling blood pressure, developing cardiovascular and colonial health, regulating intestinal emptying, and reducing gastro-esophageal reflux and heartburn. Chia seeds have potential as a functional ingredient to be used as a thickener in food and have been used to prepare blisters, coatings and edible films taken from the seed. In general, because of their water retention capabilities, it is widely used in different applications in the foods. The chia seed can also be used such as glue remover, gel builder and chelator. (Coorey et
al., 2014; Felisberto et al., 2015; Salgado-Cruz et al., 2013; Oliveira-Alves SC et al., 2017).

Chia seeds are good source of natural mucilage. The natural mucilage has excellent water retention properties, gel forming, cutting thinner and emulsion stabilizing properties, and they can be used as a functional additive for drinks. As a result of water retention capacity and viscosity, the chia mucilage can be used a foam stabilizer, suspension material, emulsifier in foods. The mucilage taken from chia seeds is a good source of polysaccharide based biopolymers and can produce edible films and coatings. The chemical composition, molecular structure and thermal stability or jellification ability of chia are important factors that determine the suitability of pits usage in the food and drug industry.

Given that the different conditions were occurred, polysaccharide mucilage are usually negatively charged in the wide pH range, while proteins are positively charged under their isoelectric point. The chia seed protein is positively charged under pH3. The Chia seed mucilage (CSM) is an anionic polysaccharide and negatively charged between pH 2.0-12.0. Under appropriate conditions, these counter-charged polymers in aqueous solutions interact with each other and form complex coacervated. (Timilsena et al., 2016a; Timilsena et al., 2016c; Sandavol-Oliveros et al., 2013).

The aim of the present study was to synthesize defatted chia seed chitosan beads and to identify its potential for the development of drug delivery material. By Soxhlet extraction, defatted chia seed was taken and after completely dried chia seeds was incubated in different buffer (phosphate, acetate) and water at different ratio, pH and temperature for obtaining the mucilage’s of the bead which was used. The water adsorption capacity and a model study for drug delivery with medicine was tested.

2.Material and Method

2.1. Extraction of chia seed mucilage

Obtaining the chia mucilage two assay studied; defatting and mucilage extraction. The appreciate amount of chia seed (dry) were defatted to get chia seeds without oil content. Defatted chia seeds were taken after solvent extraction which were extracted with acetone in a Soxhlet by thermal cycles at 50°C for 5 hours. The excessed solvent was removed at 65°C for 3 days in incubator. The oil content was gravimetrically determined by this equation (dry weight/wet weight) (Timilsena et al., 2016d)

Mucilage extraction studied with defatted dry seeds. The mucilage extraction was performed with different seed: distilled water; buffer; temperature; ionic strength conditions (w/v). The selected temperatures were room temperature and 60°C and all the experiments were studied at these temperatures. The defatted chia seeds were (0.5 g) placed in tubes and distilled water was added in 1:20; 1:30; 1:50 proportions. The buffers were acetate (1M pH 4.5) and Pi (1 M pH 7) were chosen and studies as in described for distilled water (same proportions). To analyze the effect of ionic strength for mucilage extraction different sets for chosen. The distilled water, Pi, acetate buffer containing 0.5 % NaCl and water, Pi and acetate KCl % 0.5 were studied. The same procedure was studied for all of these solutions (Campos et al., 2016; Munoz et al., 2012)

The distilled water, salt-free buffers and containing salt buffers (all proportions) were incubated at room temperature and at 60°C for 30 minutes. After incubation, the samples were centrifuged at 6000 rpm for 30 minutes. The aqueous phase was separated and mucilage was used as fresh.

2.2. Preparation of chia mucilage- chitosan beads (CM-CB)

Stock solution of chitosan was chosen 1 % (prepared with acetic acid). The various weight ratios (1:1; 1:1.5; 1:1.8) of the chia mucilage (all of the prepared which ones are; in distilled water, salt-free and salt-containing buffers) and chitosan solutions were mixed and stirred 30 min. The mixture was dropwise into the NaOH (5 M) and stirred till inclusion body (beads) observed at room temperature. The beads were observed nearly 10 min. after dropping to analyze if they dissolved or not.

2.3 The water adsorption capacity of chia mucilage-chitosan beads

The swelling behavior of CM-CB were determined under different conditions. The samples of 40 mg CM-CB were hydrated in 1 M of Pi (pH 7) and acetate buffer (pH 4.5) for 24 h. The experimental runs were performed in triplicate. After 24 h, each solution was centrifuged at 2000 rpm for 5 min and taken the weight of sample CM-CB. The average of water adsorption capacity was calculated by the percentage of dividing the difference of sample weight of CM-CB to original CM-CB weight to original CM-CB weight. The result was expressed as g water adsorbed per g bead (Timilsena et al., 2016b)

3.RESULTS

3.1. Defatted Chia Seed Beads

The reason why chia is used in our study is because of its chemical content. Chia seeds contain (15-29%) protein, (30-33%) fat, (26-41%) carbohydrates (40-42%), high dietary fiber. In the external part of the seed shell, the mucilage polysaccharides form 5% to 6% of the seed's dry weight. Early studies have shown that chia seeds are a good source of protein, fat and polysaccharides, and this feature provides them with excellent thermal stability and water retention capacity (Goh et al., 2016). Recently, the benefits of chia seed for human health and nutrition have been defined and bioactive components have been found to promote harm to health, improve biological markers associated with dyslipidemia, inflammation, cardiovascular disease, glucose homeostasis and insulin resistance, and have no adverse effect. Chia seeds have no known allergic and negative effect on the use of chia increases the area. Recent awareness of the health benefits of chia seed beads have led to an increase at academic research as a food additives and drug delivery. In this study, we prepared defatted chia seed
beads and optimized all conditions whether it can be a drug delivery material.

3.2. Optimization studies of CM-CB

The most important point was observing transparency chia seed beads. While defatting process, the outer shell of chia was removed by Soxhlet extraction (Timilsena et al., 2016d). The remain polysaccharide and protein skeleton has an ability of being gelling at different pHs and some solvents. In this study, we prepared buffers and salty buffers and brought together with defatted chia seeds and waited at room temperature and 60°C. According to media conditions gel was determined before pre-centrifuge and also after centrifuge. The observed gel was visible and no color (transparency). As known, the chia beads were in color from dark coffee to beige and soaked in water, a clear mucilaginous gel is exuded. In our study, we optimized conditions of getting more chia seed gel. All studies were carried out with defatted chia beads. After all, mucilage’s were taken at room temperature and 60°C were mixed with chitosan as a crosslinker and dropwise in 5 M NaOH for getting defatted chia seed beads for drug delivery. The mucilage which were prepared at different solutions (water, buffer, buffers with salt) were also tested without chitosan for checking the bead observation was occurred or not. All of mucilage’s, which were prepared at different ratio of seed: water/ buffers (phosphate and acetate)/ buffers with KCl/ NaCl, were observed but cannot be stable beads without chitosan after dropwise in 5 M NaOH. Dark color mucilage was observed in acetate buffer. Because of this reason, the mucilage which were prepared in acetate buffer, did not use at further studies.

According table 1, at 60°C the getting chia beads were increased. The reason of this can be the protein structure of the chia beads. Because, the stability at high temperatures can be shown that hydrophobic bonds between the amino acids can be stabilized the polymer network at a high temperature (60 °C). According to Grancieri et al, the thermal stability of chia proteins was improved (108°C) when it interacted with chia seed mucilage by complex coacervation. We also found similar result. (Grancieri et al., 2019; Timilsena et al., 2016b)

Figure 1. Chia seed and defatted chia seed: chitosan bead

Table 1. The optimization conditions of defatted chia mucilage- chitosan beads

<table>
<thead>
<tr>
<th>Temperature</th>
<th>CM: chitosan (w/v)</th>
<th>P&lt;sub&gt;i&lt;/sub&gt;</th>
<th>P&lt;sub&gt;i&lt;/sub&gt; (NaCl)</th>
<th>P&lt;sub&gt;i&lt;/sub&gt; (KCl)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>1:20</td>
<td>1:30</td>
<td>1:50</td>
<td>1:20</td>
</tr>
<tr>
<td>Room temp</td>
<td>1:1.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1:1.8</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1:1.5</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:1.8</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>60°C</td>
<td>1:1.5</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1:1.8</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

- : no bead formation
+ : observation bead formation but not very stable
++ : obtained very stable beads
Table 2. Water adsorption capacity of CM: CS

<table>
<thead>
<tr>
<th></th>
<th>(Seed: P;: Chitosan) WAC (%)</th>
<th>(Seed: P: (NaCl/ KCl): Chitosan) WAC (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>Room temperature</strong></td>
<td></td>
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<tr>
<td>(1:30):1.8</td>
<td>2.75</td>
<td>(1:30(NaCl)):1.8</td>
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<td>(1:50):1.8</td>
<td>3.50</td>
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<td>(1:30(KCl)):1</td>
<td>0.96</td>
<td>(1:30(KCl)):1</td>
</tr>
<tr>
<td>(1:30(KCl)):1.5</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td><strong>60°C</strong></td>
<td></td>
<td></td>
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<tr>
<td>(1:20):1.5</td>
<td>3.15</td>
<td></td>
</tr>
<tr>
<td>(1:20):1.8</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>(1:30):1.5</td>
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<td></td>
</tr>
<tr>
<td>(1:30):1.8</td>
<td>1.35</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. The optimal defatted chia seed: chitosan beads

3.3 Water adsorption capacity (WAC)

The water adsorption capacity and fragmentation rate of the beads were determined by hydration in the buffers (Pi, acetate, buffers with NaCl/ KCl) and water (Table 2).

As known, chia seeds were hydrated very fast. But CM-CB was hydrated for a long time period. The polysaccharide skeleton of defatted chia seeds was incorporated with chitosan. By the effect of amino groups on the surface CM-CB were become more resistant to pH. The fragmentation or decrease on water adsorption capacity maybe the poor viscosity of CM-CB. The water adsorption capacity of the CM-CB was depending on the concentration and type of salt. The monovalent salts decrease in water holding capacity. Because salt concentration changed the osmotic pressure between the forming bead and watery phase. The presence of salts was the reason of loss of the hydrophilic and hydrophobic balance of beads polymer network. As known; the chia mucilage can absorb little or no water in the presence of ions. (Munoz et al., 2012) We also found same result. The beads were swollen easily when their polymer networks disrupted. The high-water capacity was desirable. This can be more useful while using drug delivery of these CM-CB. They can be delivered the molecule easily to the media.

In this study, we choose a model drug to test defatted chia seed chitosan can be have a potential as drug delivery material. An herbal food supplement (proanthocyanin tablet) was crushed and dissolved in water (1mg/ ml). The beads which were prepared at 1 (1:30 P; KCl (mucilage)): 1.5 chitosan was used and incubated in Pi buffer (1M pH 7). As a result of time-dependent controlled delivery test; in 10 minutes 42 % and in 12 hours 100 % of drug was delivered to media.

4. Conclusion

The complex coacervation between defatted chia seed mucilage and chitosan was optimized in terms of pH, temperature, mucilage: chitosan ratio and salts. It was found that the complexation between defatted CM and chitosan was pH and the best CM: CS ratio was 1:30 at P; KCl (mucilage): 1.5 chitosan was used and incubated in Pi buffer (1M pH 7). The structural stability of CM:CS beads was much better compared to that of chia seed mucilage in NaOH after the complex coacervation. The water adsorption capacity of beads was shown that CM-CS system has irregular shape and porous microgel particles. The natural mucilages like chia seed mucilage are biocompatible, cheap, easily available, low cost and lack of toxicity. These results indicated that CM-CS beads can be used for encapsulation and delivery of molecules.

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References


