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Complex coacervation between flaxseed protein isolate and flaxseed gum

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Abstract

Flaxseed protein isolate (FPI) and flaxseed gum (FG) were extracted and the electrostatic complexation between these two biopolymers was studied as a function of pH and FPI-to-FG ratio using turbidimetric and electrophoretic mobility (zeta potential) tests. The zeta potential values of FPI, FG and their mixtures at the FPI-to-FG ratios of 1:1, 3:1, 5:1, 10:1, 15:1 were measured over a pH range 8.0-1.5. Alteration of the secondary structure of FPI as a function of pH was studied using circular dichroism. The proportion of α-helical structure decreased, whereas the both β-sheet structure and random coil structure increased with the lowering of pH from 8.0 to 3.0. The acidic pH affected the secondary structure of FPI and the unfolding of helix conformation facilitated the complexation of FPI with FG. The optimum FPI-to-FG ratio for complex coacervation was found to be 3:1. The critical pH values associated with the formation of soluble (pHc) and insoluble ($pH_{\phi 1}$) complexes at the optimum FPI-to-FG ratio were found to be 6.0 and 4.5, respectively. The optimum pH (pH_{opt}) for the optimum complex coacervation was 3.1. The instability and dissolution of FPI-FG complex coacervates started ($pH\phi_2$) at pH 2.1. These findings contribute to the development of FPI-FG complex coacervates as delivery vehicles for unstable albeit valuable nutrients such as omega-3 fatty acids.

Introduction

The process of complex coacervation or associative phase separation in protein polysaccharide mixtures occurs due to electrostatic attraction of oppositely charged molecules, eventually leading to a solvent-rich and a biopolymer-rich phase (Schmitt & Turgeon, 2011; Tolstoguzov, 1991). Other factors influencing the complex coacervation are charge density, relative ratio and total concentration of biopolymers, pH, and temperature of the solvent (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Protein-polysaccharide mixtures form electrostatic complexes in a narrow pH range. Proteins are positively charged below their isoelectric point (pI) and can undergo complexation with negatively charged polysaccharides, resulting into soluble complex coacervates at pH_c, where pH_c is defined as the pH at which noncovalent interaction between protein and polysaccharide initiates (Aryee & Nickerson, 2012). Further reduction in mixture pH results into the formation of insoluble complexes at $pH_{\phi 1}$, where $pH_{\phi 1}$ is defined as the pH at which interaction between protein and polysaccharide is strong enough to cause macroscopic phase separation (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). The yield of complex coacervates is highest at pH_{opt}, where the net charge on the system is zero. The dissolution of complex coacervates back to solution state due to the protonation of polysaccharide occurs at $pH\phi_2$, where $pH\phi_2$ is defined as the pH beyond which interaction between protein and polysaccharide starts decreasing. (Elmer, Karaca, Low, & Nickerson, 2011). Determination of these important pH values (pH_c, $pH_{\phi 1}$, pH_{opt} and $pH_{\phi 2}$) for any protein-polysaccharide combinations provide better understanding of complexation behaviour as a function of pH. Kruif, Weinbreck, and Vries (2004) suggested that pH induced changes in the conformation of protein also influence the complexation of polymers with proteins.

A number of studies have reported that plant proteins are capable of forming complex coacervates in the presence of polysaccharides. Pea protein is the most widely studied protein for complex coacervation (Ducel, Richard, Saulnier, Popineau, & Boury, 2004; Elmer et al., 2011; Klemmer, Waldner, Stone, Low, & Nickerson, 2012; Liu, Low, & Nickerson, 2009; Liu, Elmer, Low, & Nickerson, 2010). Other plant proteins considered appropriate for coacervation include soy protein (Jaramillo, Robert, & Coupland, 2011), canola protein (Klassen, Elmer, & Nickerson, 2011) and corn protein (Quispe-Condori, Saldana, & Temelli, 2011).

There are plant proteins that are theoretically known to possess favourable characteristics for coacervation but are unexplored practically. Dickinson, (2003) reported that charge density and droplet size are two important characteristics required for the stabilization of an emulsion. Wang, Li, Wang, Adhikari, and Shi, (2010) observed that flaxseed protein concentrate when compared to soy protein concentrate, possessed higher surface charge and smaller emulsion droplet size. Recently, Kuhn, Silva, Netto, and Cunha, (2014) found that flaxseed protein isolate (FPI) based emulsion are more stable than mixed FPI-whey protein isolate stabilised emulsions. In addition, the amino acid profile of flaxseed protein is nutritionally desirable and it is considered nutritionally similar to other oil seed proteins such as soybean (Oomah, 2001). However, the complexation behaviour of flaxseed protein with its own polysaccharide or with other polysaccharides has not been studied.

Flaxseed gum (FG) is another plant polymer identified as a good emulsifier (Cui, Ikeda, & Eskin, 2007). FG is a heteropolysaccharide composed of xylose, arabinose, glucose, galactose, galacturonic acid, rhamnose and fucose (Cui, Mazza, Oomah, & Biliaderis, 1994). Functional properties of flaxseed gum are comparable to those of gum Arabic and hence it can be used to replace gum Arabic in emulsions (Mazza & Biliaderis, 1989). Moreover, consumption of flaxseed gum as dietary fibre is reported to reduce the blood glucose level thereby reducing the risk of coronary artery disease (Oomah & Mazza, 2000).

The important nutritional characteristics of flaxseed protein and gum mean that they can be economical source of functional foods (Oomah, 2001). A thorough study on the complexation behaviour of these two biopolymers would help produce novel FPI-FG complex coacervates which can be preferentially used to microencapsulate active bio-ingredients such as omega-3 oils. This study determines the optimum pH range, FPI-to-FG ratio and total biopolymer concentration required for the formation of soluble and insoluble complexes between FPI and FG. In order to gain greater insight into the formation of these complex coacervates, the underlying structural change of flaxseed protein as a function of pH was also investigated. Except for this work, the complexation behaviour of flaxseed protein and flaxseed gum has not, so far, been reported.

2. Materials and Methods

2.1 Materials

Golden flaxseeds (*Linum usitatissimum*) were received from Stoney Creek Oil Product Pty. Ltd (Talbot, VIC, Australia). FG and flaxseed protein isolate (FPI) were

extracted in the laboratory at Federation University, Australia. All other chemicals used in this study were purchased from Sigma-Aldrich Australia (Sydney, New South Wales, Australia) and were analytical grade.

2.2.1 Extraction of flaxseed gum

FG was extracted from whole raw flaxseed using the method of Cui et al. (1994) with slight modification (Fig. 1). Briefly, the flaxseed was soaked in Milli-Q water at a flaxseed-to-water ratio of 1:18 at 50 °C with continuous and gentle stirring for 2h for each of two consecutive cycles of extraction. The soaked seeds were filtered and the water containing the dissolved gum was treated with three volumes of 95% ethanol to precipitate the gum. The precipitated gum was collected by centrifuge at 4,000g for 10 min. The precipitated gum was vacuum dried at 50 °C and stored at 4 °C.

2.2.2 Extraction of flaxseed protein isolate

Flaxseed protein was extracted from whole raw flaxseed following Oomah, Mazza and Cui (1994)'s method with minor modifications. Firstly, the flaxseeds were demucilaged as described in section 2.2.1. The demucilaged seeds were dried in a hot air oven at 50 °C for 24h and pulverized using a coffee grinder (EM0415, Sunbeam Corporation Ltd. NSW, Australia). The crushed meal was defatted for 3 hours using hexane at a flaxseed-to-hexane ratio of 1:6. The hull was separated from the kernel by screening the tailings using a 0.15mm sieve to further reduce the interference of the mucilage during protein extraction. This fat extracted powder was subsequently soaked in 0.1M tris buffer (pH 8.6 with 0.1M NaCl) for 6h at a powder-to-buffer ratio of 1:16. The large residues were then separated from the protein extract using double layered cheesecloth. This filtered sample was centrifuged at 9,000g for 20 min using an ultracentrifuge (Sorvall Instruments, Wilmington, DE). The supernatant was collected and the pH was adjusted to 4.2 using 0.1 M HCl to precipitate the flaxseed protein. Once the pH was adjusted the sample was stored at 4 °C for 16h in order to provide sufficient time for protein to precipitate completely. The precipitated protein was recovered by centrifuging at 12,000g for 20min. The recovered solid mass was redispersed in Milli-Q water and was neutralized using 0.1 M NaOH. Finally, the FPI was obtained by freeze drying the sample at -45 °C compressor temperature and 0.5mm vacuum pressure using a freeze drier (DYNAVAC, Dynavac Engineering, Australia). The freeze-dried FPI was ground, vacuum sealed and stored at 4 °C.

2.3 Chemical analysis of FPI and FG

Chemical analyses on all materials were performed according to AOAC Methods 925.10 (moisture), 923.03 (ash), 920.87 (crude protein) and 920.85 (lipid) (AOAC, 2003). Carbohydrate content was calculated on percent differential from 100%.

2.4 Identifying pHc, $pH_{\phi 1}$, pH_{opt} and $pH_{\phi 2}$ by turbidimetric analysis

FPI (1% w/w; pH 8.4) and FG stock solutions (0.3%, w/w; pH 7.0) were prepared by dispersing FPI and FG powders in tris buffer(0.1M pH 8.4) and Milli-Q water, respectively, followed by stirring at 500 rpm for 16 h at room temperature (21–22 °C), and 1 h at 40 °C to dissolve the protein. Tris buffer at pH 8.4 was used to prepare FPI stock solution to get better solubility. The FG concentration was fixed based on the preliminary experiments as higher concentrations (< 0.3%) showed high turbidity and viscosity. Moreover, past studies have indicated that FG dissolves completely at concentration within 0.2%-0.3% (w/w) (BeMiller, Whistler, Barkalow, & Chen, 1993). Therefore stock of 0.3% FG was made and used further in mixtures of FPI and FG at different ratios. Turbidity measurements were performed on individual FPI and FG systems and mixed systems at FPI-to-FG mass ratios of 1:1, 1.5:1, 3:1, 5:1, 10:1 and 15:1. The total solid concentration (FPI+FG) was maintained at 0.5% (w/w). The pH range in these tests was 8.00–1.50. In order to measure the turbidity in terms of light absorption, a UV spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 700 nm was used. The plastic cuvettes provided a path length of 1 cm. The mixture was acidified by adding 0.3M HCl dropwise to bring the pH to 6.0. Further decrease in pH to 2.0 was achieved by adding 0.1M HCl dropwise. To reach pH 1.5, 0.3M HCl was used. A different concentration of HCl was used to go to pH 1.5 so as to minimize the effect of dilution. Structure-forming transitions (pHc, $pH_{\phi 1}$ and $pH_{\phi 2}$) were determined graphically from the curve according to Weinbreck, Nieuwenhuijse, Robijn, & Kruif (2004), whereas pH_{opt} corresponds to the pH value at which the highest optical density at 700 nm was observed. All these measurements were carried out in triplicate.

2.5Measurement of zeta potential

A Zetasizer (ZS-90, Malvern instruments Ltd, UK) was used to measure the zeta potential values of individual (FPI, FG) solutions and mixed FPI-FG formulations as a function of pH within the range 7.0-2.0. The apparatus measures the direction and the velocity of the charged particles by applying an electric field and calculates their zeta

potential using Smoluchowski model (Kirby & Hasselbrink, 2004). In these tests, the individual FPI (0.5%) and FG (0.2%) solutions were diluted by a factor of 100 using MilliQ water. Mixed FPI-FG formulations at different FPI-to-FG ratios (1:1, 3:1, 5:1, 10:1, 15:1) were prepared maintaining total solid (FPI+FG) concentration of 0.5% (w/w) then diluted by a factor of 100 using MilliQ water. Triplicate measurements were made for each sample and average values were reported.

2.6 Circular Dichroism

Circular dichroism (CD) spectral tests of FPI were carried out under nitrogen atmosphere at room temperature using a Jasco J-815 CD spectrophotometer (Jasco Corporation, Japan) using a quartz cell of 0.1 cm path length (Starna Pty. Ltd., Atascadero, CA, USA). Protein solution was prepared at a concentration of 2 mg/ml in deionized water (pH=8.4) and then injected in the required amounts into previously prepared citric acid buffer solutions. The concentration and pH of the final test samples were maintained at 1 mg/ml and 3.0-8.0, respectively. Absorption spectra of the above samples were recorded between 190 and 250 nm using the following instrumental parameters: bandwidth = 1.0 nm, time constant =1.0s and scanning rate=20 nm/min. Each acquired spectrum represented an average of three consecutive scans. The composition (%) of α-helix, β-sheet, β-turn and unordered structures in the test specimen were calculated as suggested by Raussens, Ruysschaert, and Goormaghtigh (2003).

2.7 Statistical analysis

Results were expressed as the mean value ± standard deviation of three replicate experiments. The analysis of variance (ANOVA) was conducted using Minitab statistical software package (Minitab Inc., ver. 17, 2014). The significant difference between two mean values were calculated using the Tukey method at 95% confidence level ($P<0.05$).

3. Results and discussion

3.1 Chemical analysis of FPI and FG

Chemical analyses of the extracted FG showed: 4.42±0.47% moisture, 9.35±0.84% protein (%N×6.25), 1.75±.022% lipid, 3.17±0.43% ash and 81.31% carbohydrate.

Analysis of extracted FPI showed: 90.60±1.31% protein, 4.2±0.3% moisture, 2.20±0.24% ash, and 1.06±0.18% lipid and 1.94±0.37% carbohydrate. All of the above results are on weight (w/w) basis.

3.2 Effect of pH on the secondary structure of flaxseed protein

Fundamental understanding of pH induced conformational changes in protein structure provides insight on complexing behaviour of the protein. Mapping of the protein conformation changes aids in identifying the optimum conditions its functionality is preseserved before and after its conjugation with other polymers (Shang, Wang, Jiang, & Dong, 2007).

As shown in Table 1, the secondary structure of flaxseed protein is clearly affected by the variation of pH within the tested range. The lowering of the pH 8.0-3.0 promoted the formation of mostly β-sheets and random coils to an extent at the expense of α-helix. Both α-helix and β-sheet are regular form of secondary structure, held together with amide back-bone of hydrogen bonds. However, in α-helix they are formed within a single strand with an average length of 1.5A whereas in a β-sheet, they are formed between different strands with an average length of 3.5A (Walsh, 2012). Hence providing β-sheet structure, some extra flexibility and stability than α-helix. In the current study as the solvent conditions of FPI were changed from low alkaline (pH 8.0) to low acid (pH 6.0) a significant proportion of helices (50% of the original content) were dissociated. In contrast, the proportion of the β-sheets increased by 50 % from the original content observed at pH 8.0. With further lowering of the pH, the same trend continued for α-helical and β-sheet forms.. With respect to the random coil content, there is a gradual but statistically insignificant ($P>0.05$) increase from pH 8.0-3.5. However, on lowering the pH from 3.5 to 3.0, the random coil content increased significantly ($P<0.05$), indicating considerable disorder in the structure of FPI in association with the helical unwinding. The amount of β-turns is essentially unchanged at 12.1-12.5% ($P>0.05$) across the pH range.

Similar changes in the secondary structure of the wheat protein α-gliadin due to change in pH were reported by Chourpa, Ducel, Richard, Dubois, and Boury (2006). However, the authors attributed this change (more of alpha helices and random coil above pH 3.0 and more of β-sheets below pH 3) to the complexation of gum Arabic with α-gliadin. In the present study we confirmed that the changes in the conformation of FPI are a result of

pH change and not complexation. The observed unfolding of flaxseed protein may facilitate its complexation with other polymers.

3.3 Complex coacervation of FPI and FG

3.3.1 Effect of pH

Changes in turbidity (measured as optical density (O.D.)) during an acid titration were measured for individual FPI (0.5%) and FG (0.3%) solutions (Fig. 2a), as well as for mixtures of FPI and FG (Fig. 2b) at FPI-to-FG ratios of 1:1, 1.5:1, 3:1, 5:1, 10:1, 15:1. The total solid content (FPI+FG) in these tests was maintained at 0.5% (w/w).

For FPI alone, O.D. started increasing near pH 6.0 (Fig. 2a) and the highest O.D. was observed at pH 3.4, after which it decreased with further lowering of pH down to 1.5. The increase in O.D. can be attributed to the decrease in solubility of FPI in that pH range. In separate experiments it was observed that the pI of FPI was 4.2; thus, the highest value of O.D. was observed below the pI of FPI. This may be due to the fact that some low molecular weight fractions of FPI have their pI at 3.5 (Oomah et al., 1994). Moreover, the presence of carbohydrates (approx. 2%) in FPI as mentioned in section 3.1 also might have contributed to this observation. These carbohydrates are basically the acidic fraction of flaxseed gum, composed of rhamnose and glacturonic acid (Cui, Kenaschuk, & Mazza, 1996).

In the case of FG, the O.D. started increasing near pH 6.0 (Fig. 2a); however, the increase was slow and the highest O.D. value (0.267) was quite low when compared to that for FPI (0.024-0.529). The initial increase of O.D. in FG takes the form of a plateau between pHs ~3.75 and 3.00 (corresponding to a maximum O.D. value of 0.267), before starting to decrease in magnitude from pH 2.9, with a minimum of 0.177 at pH 1.5. Unlike other polysaccharides such as gum Arabic (Aryee & Nickerson, 2012), FG showed an increase in O.D. with decrease in pH most probably due to the presence of protein (10%).

In the case of mixtures of FPI and FG, the trend of variation of O.D. with pH was similar to that of FPI, except for the 1:1 mixture, in which the O.D. value of the mixture was lower than that of the pure protein. However, the highest O.D. values of the mixtures were significantly higher than that of FPI and FG alone (Fig. 2b). This can be attributed to the formation of complexes between these two polymers. Interestingly, the process of complexation started at the relatively high pH value 6.0, albeit the process was very slow. Though this pH is higher than the pI of FPI (4.2) and FPI is still negatively charged at this

pH, the reason for this interaction can be attributed to electrostatic attraction between anionic FG and cationic patches of FPI. Similar findings have been observed in whey protein-gum Arabic systems (Weinbreck, Vries, Schrooyen, & Kruif, 2003). The FPI-FG mixtures became slightly turbid due to this interaction; however, as no phase separation occurred the complex coacervates formed in this condition were believed to be soluble. Further acidification of this mixture, adjusted the pH towards the pI of FPI (4.2) and hence resulted in FPI having a net positive charge. The electrostatic attraction between the positively charged FPI and negatively charged FG resulted in the formation of insoluble complexes (at pH =4.5) and the O.D. of the mixture continuing to increase. The highest O.D. (0.913) was observed in the case sample with FPI-to-FG ratio of 15:1 at pH 3.3. When the pH was lowered further, slow dissociation of complex coacervates began to occur due to the protonation of reactive sites in the anionic polysaccharide.

3.3.2 Effect of FPI-to-FG ratio

Protein to polysaccharide ratio is another important factor affecting the process of complex coacervation. Different mixing ratios influence the charge balance between protein and polysaccharide, ultimately affecting the intensity of interaction and complexation (Liu et al., 2009).

The formation of complex coacervate at different FPI-to-FG ratios was investigated as a function of pH by varying the ratio from 1:1 to 15:1. The data presented in Fig. 2b illustrates that the O.D. of the mixtures increased with the increase in the proportion of FPI. The variation of O.D. of the mixtures as a function of FPI-to-FG ratio in terms of the highest O.D. values is presented in Fig. 3a.

The peak O.D. values for FPI-to-FG ratios of 3:1, 5:1, 10:1 and 15:1 were 0.831, 0.869, 0.875 and 0.913, respectively are not significantly different ($P>0.05$). This observation indicates that the formation of complex coacervates does not increase above the FPI-to-FG ratio of 3:1 as all of the negative sites available with the FG have complexed with the positive sites of FP and the remaining positive sites of FP are in excess in the mixture. Hence, optimum complexation between FPI and FG was achieved at the FPI-to-FG ratio of 3:1. Similar observations were made by Elmer et al. (2011) using a base titration, for cationic polysaccharide chitosan and pea protein isolate.

3.3.3 Identification of pHc, $pH_{\phi 1}$, pH_{opt} and $pH_{\phi 2}$

As described in Section 1, identification of the phase boundaries pHc, $pH_{\phi 1}$, pH_{opt} and $pH_{\phi 2}$ is important for determining the critical pH values associated with structure forming

events observed in the complex coacervation process. In the present study we selected the optimum FPI-to-FG ratio (3:1) to identify the critical pH values for complexation of FPI and FG. The process of complexation follows two structure-forming events associated with the formation of soluble and insoluble electrostatic complexes (Aryee & Nickerson, 2012). As shown in Fig. 3b, the soluble FPI-FG complex coacervates were formed at pH_c = 6.0, as indicated by the slight increase in the O.D. due to increase in acidity. As the pH is lowered further, formation of insoluble complex coacervates started at pH 4.5. Both FPI and FG are negatively charged at this $pH_{\phi 1}$ = 4.5. However, as explained in Section 3.3.1, these interactions were assumed to be between negative sites of FG and the positive patches present in the molecular structure of FPI. As the pH approaches the pI of FPI (4.2), the process of complexation between oppositely charged polymers accelerated and the O.D. reaches its highest value at pH_{opt} (3.1). When the pH of the mixture was reduced further, it resulted in a decline in O.D. indicating the dissolution of complexes due to the protonation of negatively charged sites of FG. The dissolution of electrostatic complexes started near pH 2.1 as indicated by a steep decrease in the O.D. and continued until pH 1.5 ($pH\phi 2$) to give an O.D. value of 0.289. This O.D. at pH 1.5 is still higher than the starting O.D., because at pH 1.5 solubility of FPI and FG is not 100% as indicated by their individual turbidity plots in Fig. 2a in the previous section

Phase diagram (Fig. 3c) of FPI-FG system is used to demonstrate the effect of pH and FPI-to-FG ratios on the complex coacervation. Among these phase boundaries (pH_c, $pH_{\phi 1}$, pH_{opt}, and $pH_{\phi 2}$) $pH_c$and $pH_{\phi 2}$ were found to be independent of FPI-to-FG mixing ratios ($P>0.05$). In contrast, $pH_{\phi 1}$ and pH_{opt} were influenced by the change in the mixing ratios. Specifically, $pH_{\phi 1}$ values shifted to lower pHs as mixing ratios (FPI-to-FG) increased from 1:1 to 3:1; however, $pH_{\phi 1}$ became independent when mixing ratio increased further ($p>0.05$). The pH_{opt} values shifted to higher pHs for the mixing ratios ranging from 1:1 to10:1 and was stable when the rations were increased further. Similar trends for pH_c and $pH_{\phi 2}$ were reported by Mattison, Brittain, and Dubin (1995) in BSA-polydimethyldiallylammonium chloride system and by Aryee and Nickerson (2012) in lentil protein-gum Arabic system, respectively.

In other studies some differing trends have also been reported regarding the dependence of phase boundaries with the biopolymer ratio. For example, Weinbreck et al. (2003) observed that in the case of whey protein isolate-gum Arabic system, out of all the phase boundaries only pH_c was independent of biopolymer ratio. In contrast, Liu et al. (2009) found that pH_c was dependent on the biopolymer ratio in pea protein isolate-gum Arabic

mixtures. The differences in the above findings can be attributed to the different characteristics of the protein and polysaccharides mixed systems (Weinbreck et al., 2003).

3.4 Effect of pH on the charge density

The charge density of individual FPI, FG solutions and their admixtures at FPI-to-FG ratios of 1:1, 3:1, 5:1, 10:1, 15:1 were determined as a function of pH in order to confirm the pH_{opt} for each individual ratio of FPI and FG mixed systems.

Net surface charge density of FPI and FG as a function of pH (8.0-2.0) is shown in Fig. 4a. Electrostatic complexes are formed under solvent conditions when the participating polymers have opposite charges. As shown in Fig. 4a, the net charge of FPI and FG solutions is zero at pH 4.20 and 2.25, respectively. This means effective formation of complex coacervates between FPI and FG can occur between pH values of 4.20 and 2.25.

The points of electrical equivalence (a pH value at which the net charge of the oppositely charged moieties is zero) of different FPI-to-FG ratios are shown in Fig. 4b. As shown, the net neutrality (zeta potential = 0 mV) of FPI-FG mixed systems at FPI-to-FG ratios 1:1 to 15:1 occurred within the pH range of 2.9 and 3.4. It can be observed from Fig. 4b that when the proportion of FPI in FPI-to-FG ratio increased, the point of neutrality or the point of electrical equivalence shifted to higher pH values. These data sets provide important insights to determine the optimal protein-to-polysaccharide ratio in order to optimize the complex coacervation process. Fig. 4b also shows that the pH of neutrality for all the FPI-to-FG ratios except 1:1 was same as pH_{opt} determined through optical density tests, showing that both these methods are capable of determining the optimum pH and optimum FPI-to-FG ratio for maximum yield of complex coacervates.

4. Conclusions

This study demonstrated that flaxseed protein isolate (FPI) and flaxseed gum (FG) can be successfully complexed at optimized conditions. The variation of pH affected the secondary structure configuration of FPI and the unfolding of helix conformation facilitated the complexation of FPI with FG at lower pH values. The optimum FPI-to-FG ratio and pH value for complexation between FPI and FG were 3:1 and 3.1, respectively and the resultant complex coacervates were found to be stable at low pH values up to 2.1.

These FPI-FG complex coacervates can be preferentially used as novel, economic and nutritionally valuable delivery vehicles for active and unstable food ingredients. Further research is needed to study the practical applications of this delivery matrix and the ability to

microencapsulate and protect sensitive ingredients such as omega-3 oils, vitamins or probiotics.

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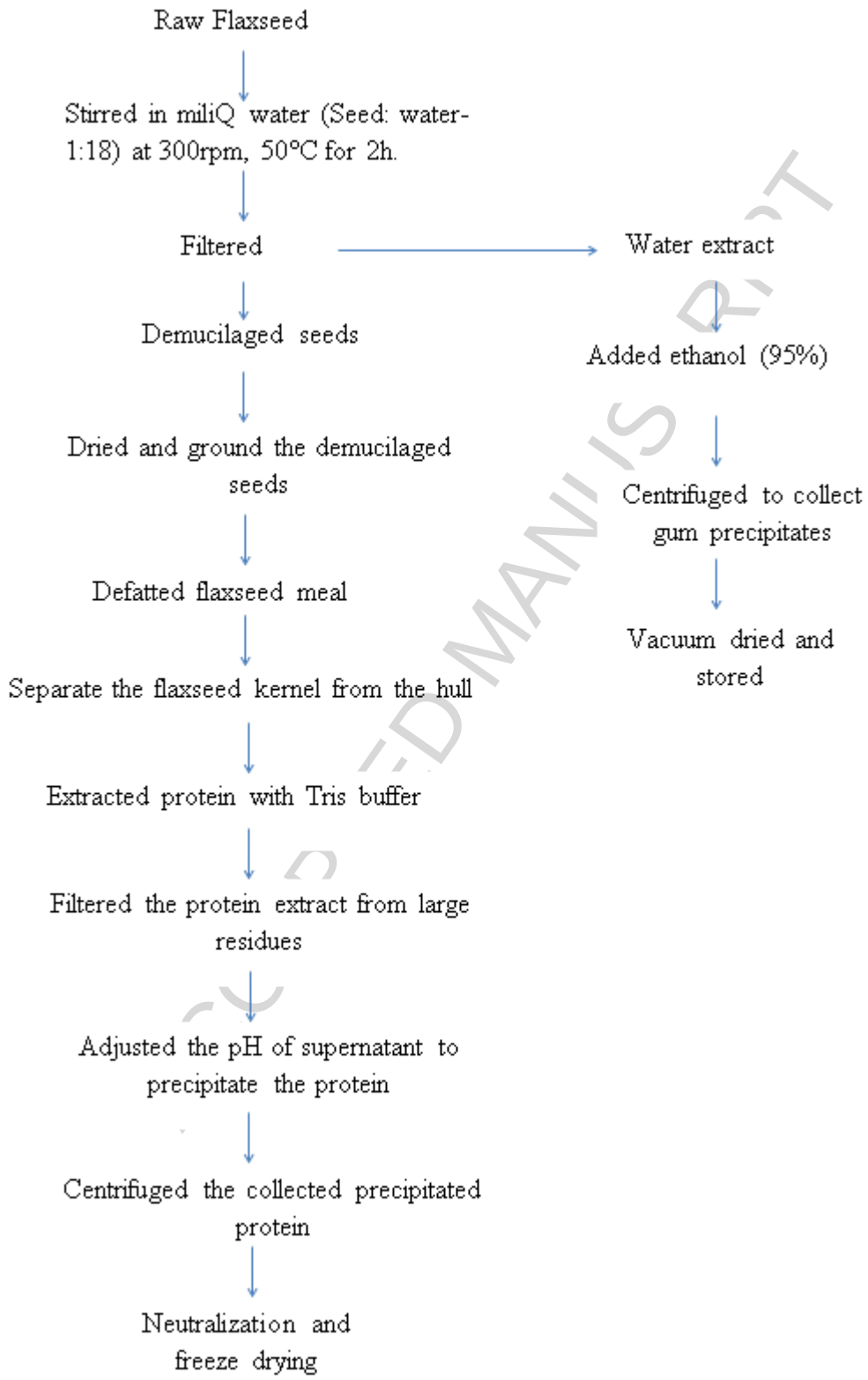


Figure 1

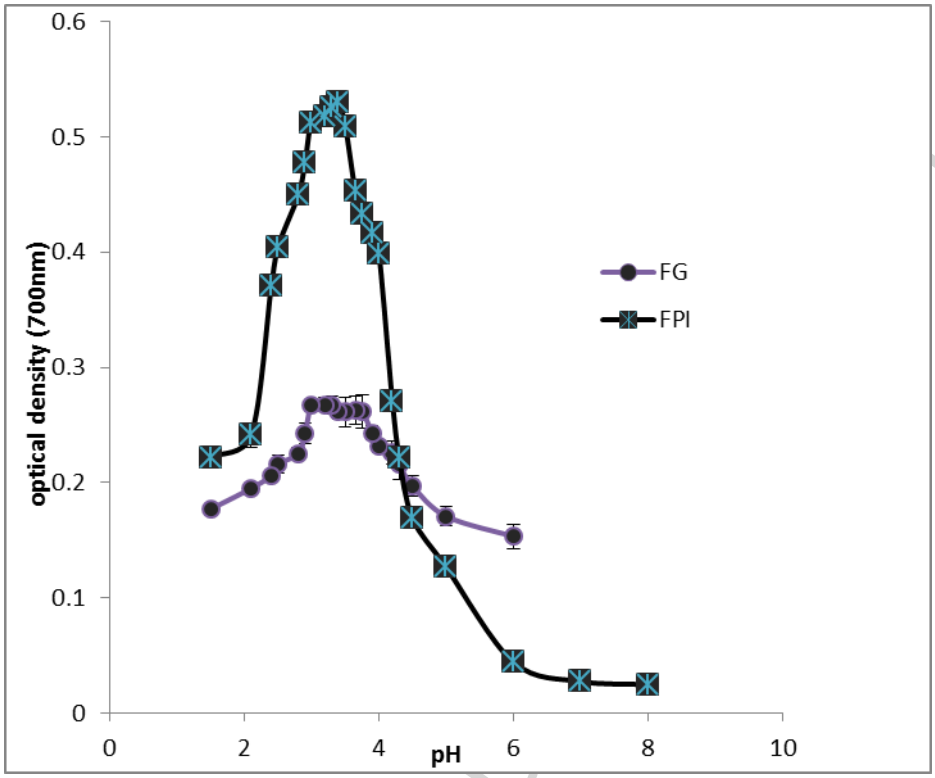
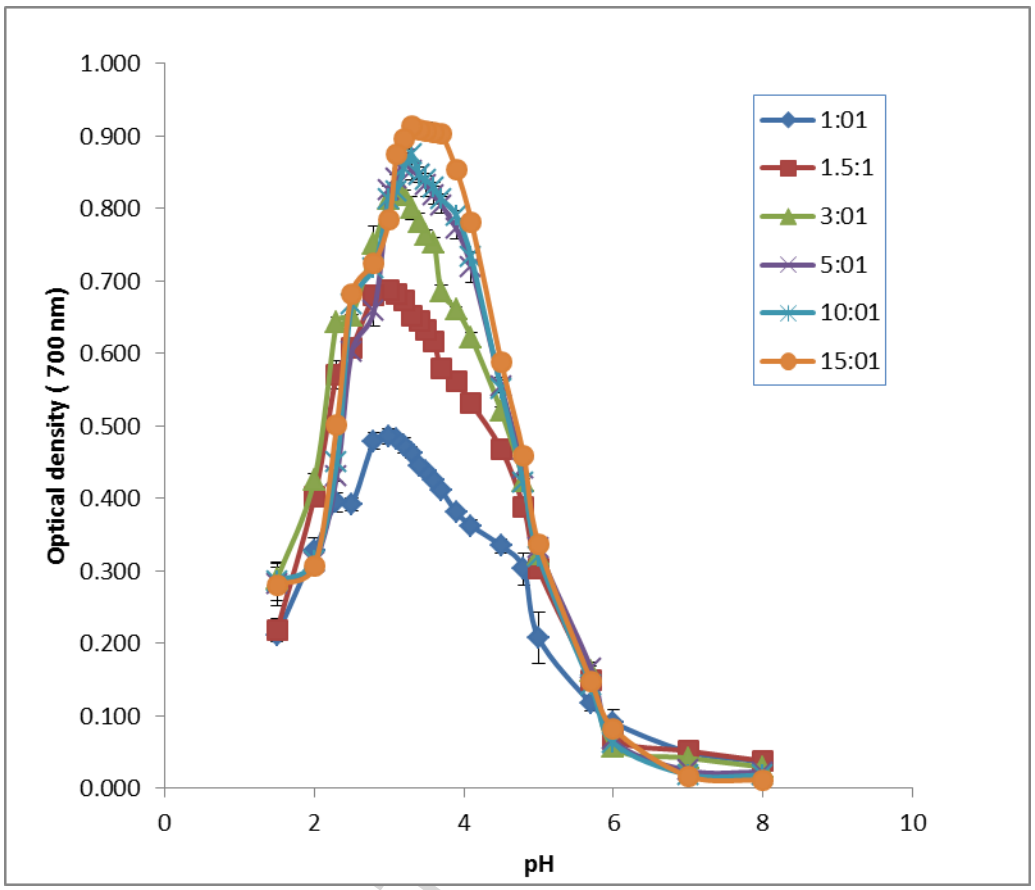


Figure 2a



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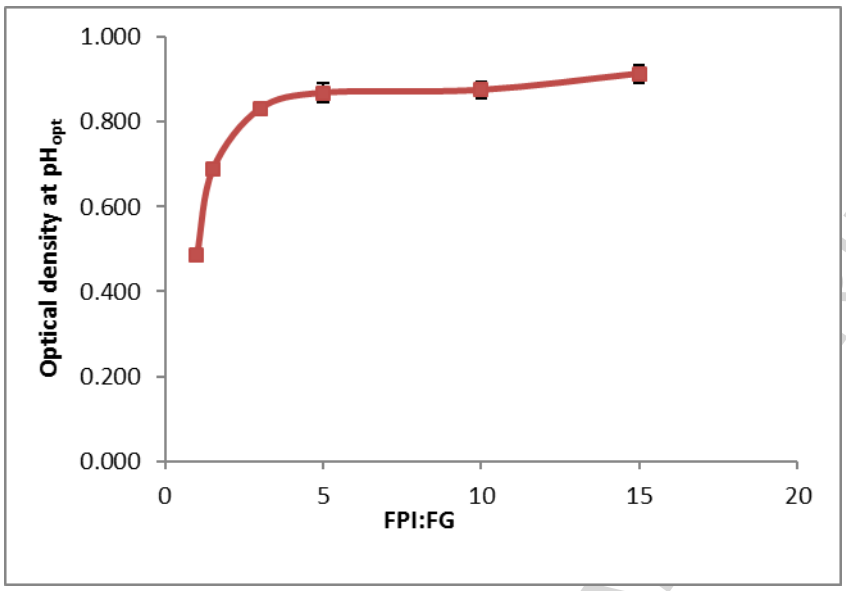

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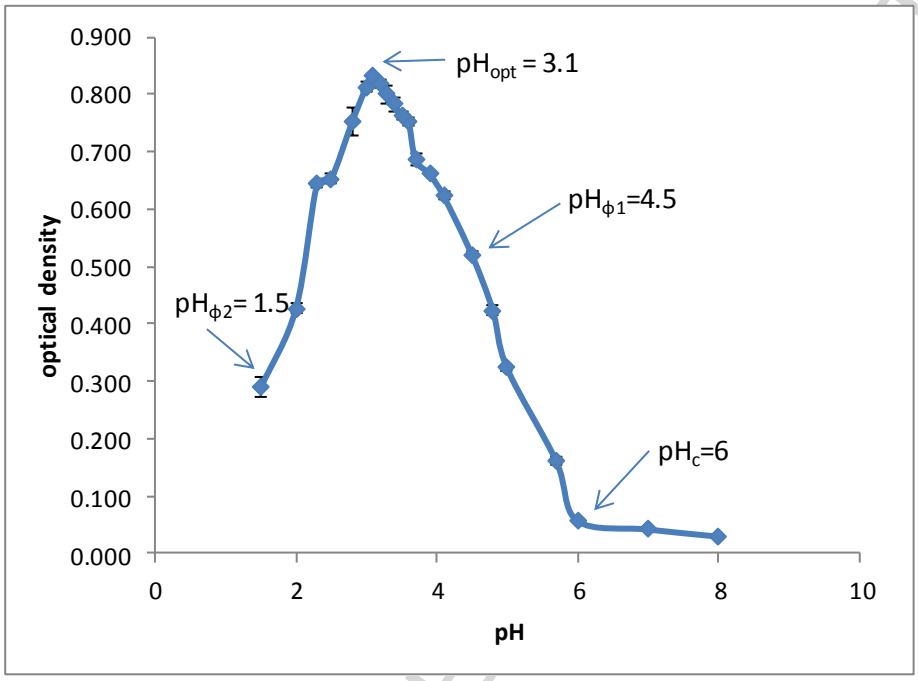

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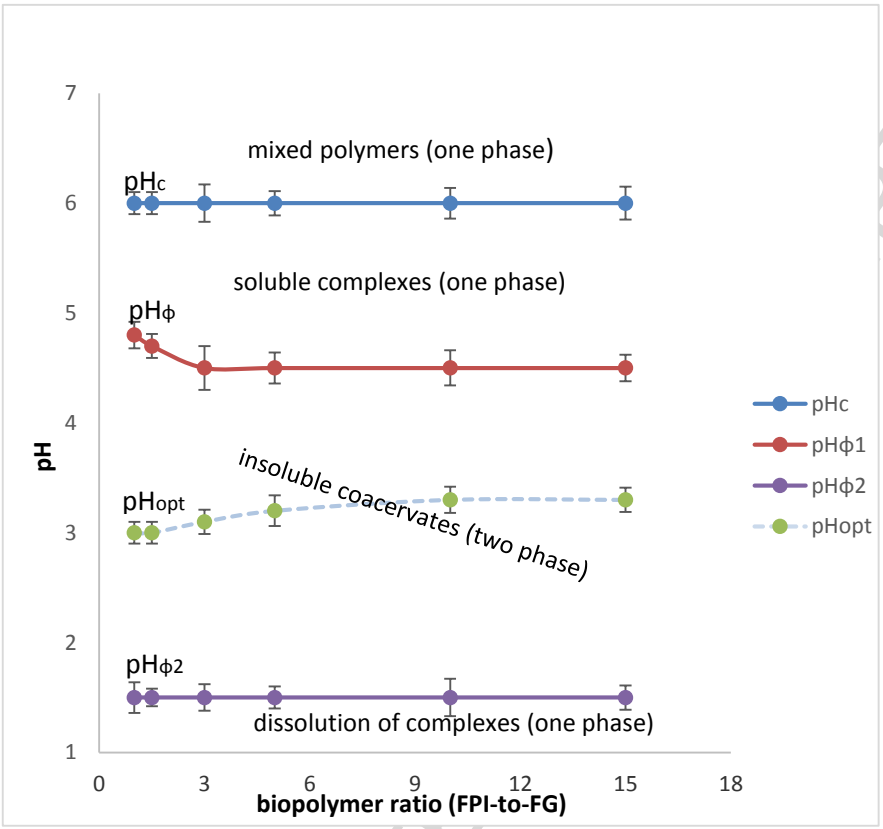

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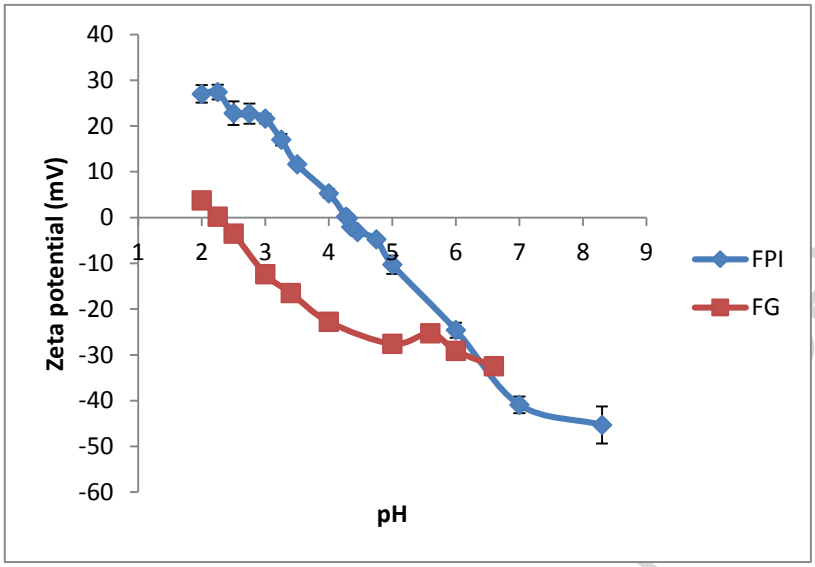

Figure 4a

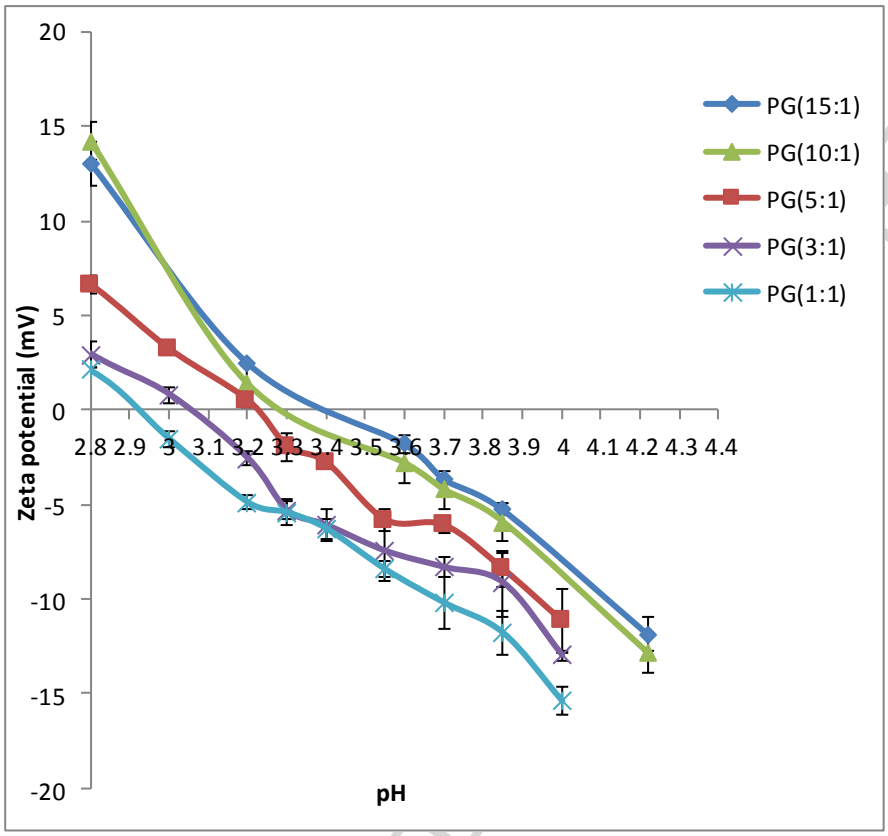

Figure 4b

Table 1. The composition (%) of secondary structural features of FPI in a pH range of 8.0-3.0

Structure (%)	pH						
	8	7	6	5	4.2	3.5	3
α-Helix	23.6[a]	19.0[b]	15.0[c]	13.5[c]	13.0[c]	12.0[c]	7.5[d]
β-sheet	13.9[c]	29.4[b]	30.2[b]	37.9[a]	32.9[b]	35.9[a]	31.6[b]
Turn	12.1[a]	12.3[a]	12.3[a]	12.2[a]	12.2[a]	12.1[a]	12.5[a]
Random coil	31.8[b]	34.7[b]	35.4[b]	34.0[b]	35.5[b]	34.9[b]	39.9[a]

Values in a row that do not share a letter are significantly different (at 95% confidence level)

Highlights

1. Flaxseed protein and gum were extracted from raw flaxseed.
2. Interactions of protein and polysaccharide were studied as function of pH and different mixing ratios.
3. Different phase boundaries related to structure forming events were determined.
4. Changes in the secondary structure of protein with lowering of pH were found facilitating the interactions of protein and polysaccharide.
5. Optimum pH and protein –to– polysaccharide ratio for complexation was found to be 3.1 and 3:1.