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Low methoxyl pectin/sodium caseinate interactions and composite film formation at neutral pH

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ABSTRACT

The behavior of mixed negatively charged sodium caseinate (CAS) and low methoxyl pectin (LMP) at pH 7.0 was presented by phase diagram. The existence of interactions between CAS and LMP as a function of increasing protein concentration at neutral pH was studied by turbidity and zeta potential measurements. The observed significant increase of turbidity value for higher CAS/LMP ratios as well as the obtained zeta potential profile confirmed the formation of complexes at pH 7.0 at which the two macromolecules are negatively charged. Then, composite films were elaborated from CAS and LMP mixtures by increasing protein to polysaccharide ratio at neutral pH. The results revealed that the incorporation of protein to pectin-based films significantly increased the stiffness of films (Young's modulus) and decreased their flexibility ($p < 0.05$). Water content of composite films decreased significantly as the concentration of CAS increased ($p < 0.05$) and reached its lowest value for CAS/LMP ratios of 2 and 5 ($7.50 \pm 0.10\%$ and $7.63 \pm 0.19\%$) compared to LMP film ($19.82 \pm 0.19\%$). The prepared films, having tunable properties by varying the ratio of CAS/LMP, seem to be adequate for the packaging of moist foods.

1. Introduction

Stability as well as structural and textural properties of protein/polysaccharide mixed films significantly depend on the type of interactions between the used biopolymers. The presence of various intermolecular forces between the different segments and side chains of the two macromolecules determine the overall interactions between the two biopolymers (Tolstoguzov, 1997). Establishing of attractive or repulsive protein/polysaccharide interactions can be attributed to the distribution of the various types of functional groups, such as charged and hydrophobic groups, and processing conditions, such as pH. It is important to distinguish the difference between the associative complex coacervation and the segregative phase separation. The first phenomenon occurs between biopolymers carrying opposite charges: for example, protein/anionic polysaccharide complexes (at a pH lower than the protein isoelectric point (*pI*)) precipitated in the lower phase whereas the upper phase is consisted of solvent (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). However, thermodynamically unstable protein/polysaccharide solutions with macroscopic phase separation (upper phase rich in polysaccharide and lower phase rich in protein) are formed at pH values higher than the protein *pI* at which both polymers carry the same net charge (Grinberg & Tolstoguzov, 1997).

Edible packaging films are mainly made of proteins and polysaccharides (Wang, Liu, Holmes, Kerry, & Kerry, 2007). The high nutritional value, the good sensory properties as well as the capability of protecting food products from their surrounding environment can be considered as the main reasons for the application of casein-based films in the food industry. Despite their high content of non-polar amino acids (35–40% of total amino acid residues), caseins are soluble in water. In order to improve the properties of native caseins, caseinates are produced from coagulated casein micelles, which are washed with water and dissolved in an alkali solution to increase the pH up to 7.0, and subsequently spray-dried. In the case of using sodium hydroxide (NaOH) for increasing the pH, sodium caseinate will be obtained (Fabra, Talens, & Chiralt, 2010). Na-caseinate is made up of four types of phosphoproteins, α_{s1}-, α_{s2}-, β-, and κ-caseins (Aoki, Uehara, Yonemasu, & El-Din, 1996).

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Caseinate-based edible films can be formed through hydrogen bonds, electrostatic interactions and hydrophobic forces due to the random coil structure of this protein (Chen, 2002; Schou et al., 2005). However, the low mechanical properties along with the limited moisture barrier ability of casein and caseinate based films are known as the main disadvantages of these type of films when compared with the films prepared from the commonly used synthetic plastics (Pereda, Amica, Racz, & Marcovich, 2011).

On the other hand, polysaccharide based films have low gas permeability and low moisture barrier properties which can be attributed to their hydrophilic nature as well as the structure of polymeric chains of polysaccharides (Coma, 2013). Pectin (E440) is known as a heterogeneous grouping of acidic structural polysaccharides, which are commonly extracted from citrus peel and apple pomace. The main structure of this anionic polysaccharide is made up of β-1,4-linked D-galacturonic acid residues with either fully or partially methyl esterified of the uronic acid carboxyls (HMP, high methoxyl pectin and LMP, low methoxyl pectin, respectively) (Skurtys et al., 2010). Pectin is a food-grade polymer that can produce environment friendly edible films due to its biocompatibility, edibility, and various physicochemical properties such as gelation and selective gas permeability (Otoni et al., 2014). It is worth mentioning that films made out of pectin have some disadvantages including low water barrier at high humidity and being less flexible than synthetic packaging films (Gohil, 2011).

Composite films can be produced by mixing several components with different ratios that influence barrier and mechanical properties of the films (Tharanathan, 2003). It is thought that elaboration of pectin/protein composite films can lead to the enhancement of physical, biological and chemical properties of films compared with protein-free pectin films. In addition, the structural properties of composite films revealed that proteins were embedded in the pectin matrices indicating the formation of compatible composites (Liu et al., 2006). These composite films showed higher tensile strength and elastic modulus values with higher moisture barrier properties than pectin and pectin/starch films (Liu et al., 2006). In another study, it was reported that the incorporation of fish skin gelatin or soybean flour protein into pectin films resulted in improvement of mechanical properties including strength and stiffness, also reduction of water vapor transmission rate in comparison to pectin control films (Liu, Liu, Fishman, & Hicks, 2007). Recently, we have investigated the coacervation between sodium caseinate and low methoxyl pectin at pH 3 as a function of protein/polysaccharide ratio (Eghbal et al., 2016). The properties of films based on these complex coacervates were then studied and it was shown that coacervation resulted in decreasing water content and water sorption of films as the protein concentration increased. Moreover, the mechanical properties of films were highly influenced by the formation of electrostatic complexes (Eghbal et al., 2016).

In the current study, phase behavior of sodium caseinate and LM pectin mixtures at room temperature and neutral pH was exhibited by phase diagram where both biopolymers have net negative charges. Moreover, the turbidity and ζ-potential values of CAS/LMP mixtures with different protein/polysaccharide ratios prepared at pH 7.0 were measured. Finally, the effect of increasing Na-caseinate/LM pectin ratio on the various physical, mechanical and structural properties of elaborated composite films at neutral pH was studied.

2. Materials and methods

2.1. Materials

Low methoxyl pectin (LMP) (Unipectine™ OF 305 C SB, degree of esterification from 22% to 28% and degree of acetylation from 20% to 23%) was purchased from Cargill (Baupte, France). Sodium caseinate (CAS) powder was from Fisher Scientific (United Kingdom). CAS total protein content determined by the Kjeldahl method was 93.20% (nitrogen conversion factor N = 6.38). Analytical grade imidazole ($C_3H_4N_2$), acetic acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), Nile blue and Bradford reagent were purchased from Sigma-Aldrich Chimie (St Quentin Fallavier, France). Distilled water was used for the preparation of all solutions.

2.2. Sodium caseinate and LM pectin solutions preparation

Stock solutions of LMP (2 g L^{-1}) and CAS (10 g L^{-1}) were prepared by dispersing powders in an imidazole-acetate buffer (5 mmol L^{-1}) at pH 7.0 and stirred for at least 3 h with a magnetic stirrer until the proper hydration was reached. The pH was adjusted by adding HCl (1 mol L^{-1}) or NaOH (1 mol L^{-1}). Stock solutions were then centrifuged (10000 × g; 30 min) (Sigma 3K18, Bioblock Scientific, Illkirch, France) to remove insoluble residues.

2.3. Sodium caseinate/LM pectin phase diagram

First, different CAS and LMP mixtures at pH 7.0 were visually observed to see if macroscopic phase separation occurred. Various solutions with different concentrations of these two biopolymers, based on weight percentage (w/w), were prepared by mixing different amounts of stock solutions and buffer into 15 mL tubes. The pH was then checked again. After vortexing for obtaining homogeneous mixtures, tubes were centrifuged at 5000 × g, 15 min, and 20 °C with the aim of having clear phase separations through enhancing macroscopic phase separation. Complete macroscopic separation was achieved when the volume of the upper phase was no longer decreasing. Protein concentration in each phase was measured by Bradford method with 1:1 ratio of solution:-Bradford reagent and the absorbance was measured at 595 nm. The amount of polysaccharide was determined based on the following formula:

$$\%\ \text{polysaccharide} = \%\ \text{dry matter} - \%\ \text{protein} \quad (1)$$

Dry matter was measured by oven-drying at 103 °C until constant weight. For this calculation, it was assumed that biopolymers were distributed evenly in the upper and lower phases. The biopolymer concentrations in each separated phase were shown on the diagram for the delimitation of the co-solubility and the incompatibility zones. The bimodal delimitation line was drawn according to the method described by Tolstoguzov (2003). The binodal line was obtained by placing the points representing the composition of the two phases in each mixture. Therefore, these points were modeled by an order 3 polynomial equation (R^2: 0.96).

2.4. Preparation of CAS/LMP mixtures and turbidity measurement

Biopolymer mixtures containing sodium caseinate (0.00, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, and 5.00 g L^{-1}) and LM pectin (1 g L^{-1}) were prepared by mixing different ratios of the stock solutions with imidazole-acetate buffer at pH 7.0. The resulting suspensions were mixed for 1 min using a vortex mixer and their pH was adjusted again. Turbidity measurements were performed as a function of the CAS/LMP ratio with a UV/Vis spectrophotometer (Jenway 3705, Villepinte, France). The absorbance was measured at 600 nm at room temperature (25 °C) against LMP solution in imidazole-acetate buffer (5 mmol L^{-1}; pH 7.0) (Bayarri, Oulahal, Degraeve, &