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Performance of egg white and hydroxypropylmethylcellulose mixtures on gelation and foaming

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ABSTRACT

The aims of this research were: i) to investigate phase separation behavior of egg white (EW) and hydroxypropylmethylcellulose (HPMC) mixtures at pH 7 (EW natural pH) or 3 (below EW proteins isoelectric point); ii) to study the impact of this segregation on gelation and foaming properties of the mixed systems as compared to single EW. A sudden phase separation took place at pH 7, while at pH 3 occurred gradually and slowly. In confocal microscopy, fluorescence of EW and HPMC was found on the same locations, indicating complex formation. At pH 7 complexation was more pronounced and the complexes flocculated to form bigger particles bringing on the sudden macroscopic phase separation. At pH 3 the complexes were smaller and did not flocculate with time. The mixtures gelation temperature (T_{gel}) was similar to HPMC T_{gel}; however, the storage modulus (G') initially similar to that of HPMC was then dominated by the protein. A synergism between EW and HPMC regarding G' was found at both pHs, being this effect higher at pH 3. For textural properties, an improvement on hardness and springiness was found at pH 3. Regarding foaming properties, there was a synergistic effect on foam collapse at pH 3, while foam overrun slightly decreased and drainage did not show differences as compared to single EW. Thus, although depending on pH conditions, it is possible to improve gelation and foaming of EW by adding HPMC. Improvement was mostly found below the iso-electric point of EW.

1. Introduction

Egg white (EW) has multiple functional properties as food ingredient, foaming, emulsification, gelation upon heating and binding adhesion. Therefore EW is used as functional ingredient in many food products like meringues, mousses or bakery products (Mine, 1995). The functionality of EW depends on hydrophobicity and electrostatic interactions. However, it is not completely understood since EW comprises about 40 different proteins, and interactions between these proteins contribute to its functionality (Arzeni, Pérez, & Pilosof, 2012b; Mine, 1995; Yang, Berry, & Foegeding, 2009). The most important constituent proteins for the functionality are ovalbumin, conalbumin, ovomucoid and lysozyme (Arzeni, Pérez, & Pilosof, 2012b). Ovalbumin represents 54% of EW; contains one disulfide bond, has a molecular weight of 45 kDa and an isoelectric point (pI) of 4.5 (Arzeni et al., 2012b; Mine, 1995; Weijers, Sagis, Veerman, Sperber, & Van Der Linden, 2002). Conalbumin constitutes 12% of the EW and contains 15 disulfide bridges. It has a molecular weight of 77.8 kDa, a pI of 6.1 and is easily heat-denaturable. Ovomucoid constitutes 11% of EW, has a molecular weight of 28 kDa, a pI of 4.1 and a denaturation temperature of 77 °C (Arzeni et al., 2012b; Mine, 1995).

In order to improve long-term physicochemical stability of protein colloids, polysaccharides are often added. However in these mixtures, different types of protein/polysaccharide interactions can take place (Rodriguez Patino & Pilosof, 2011). These interactions can result in synergistic effects and thus help in the improvement of food products and in reducing their cost-price (Baeza, Carp, Bartholomai, & Pilosof, 2002). In proteins/polysaccharides aquous mixtures the following phenomena can occur: thermodynamic incompatibility, complexation, cosolubility and segregation (Martinez, Baeza, Millán, & Pilosof, 2005). Cosolubility occurs at

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low concentrations. At higher concentrations, different types of complexation can take place, by non-covalent interactions such as electrostatic or hydrophobic, hydrogen bonding and steric exclusion. (Dickinson, 2008; Rodriguez Patino & Pilosof, 2011). Electrostatic interactions typically take place when charged polysaccharides such as alginate, pectate or carrageenan are involved. Another interesting phenomenon regarding protein-polysaccharide interactions is phase separation, which can occur through insoluble complex formation or thermodynamic incompatibility (Rodriguez Patino & Pilosof, 2011). Insoluble complex formation or complex coacervation can occur because of the existence of electrostatic complexes in solution which spontaneously phase separate into a solvent-rich and a solvent-depleted phase. On the other hand, thermodynamic incompatibility involves repulsive interactions between chemically different polymers, arising from the tendency of polymers to prefer neighbors of similar structure. This is more likely to occur with non-ionic polysaccharides since the entropy that is involved is lower (Norton & Frith, 2001). The net repulsion between the two polymers leads to their mutual exclusion from the local vicinity of the other (the excluded volume effect), which increases their thermodynamic activity. It can cause either macroscopic or microscopic phase separation, depending mostly on the concentration of each polymer. In addition to the concentration of the polymers, the degree of incompatibility depends on pH and/or ionic strength. At pH above the isoelectric point of the protein, incompatibility will be higher than below the isoelectric point (Baeza et al., 2002).

A very interesting polysaccharide regarding its functional properties is the non-ionic cellulose derivative hydroxypropylmethylcellulose (HPMC) (Coffey, Bell, & Henderson, 1995). The functionality regarding structure is based on four important properties: surface activity, the ability to form thermo-reversible gels, efficient thickening and film forming ability. For example, it is used for controlled drug-release matrixes in the pharmaceutical industry and for the improvement of baked products in the food industry (Pérez, Wargon, & Pilosof, 2006). The surface activity of HPMC arises from the fact that hydroxypropyl groups are hydrophilic, while the methyl groups are hydrophobic and constitute hydrophobic zones along the cellulose backbone (Pérez, Carrera-Sanchez, Rodriguez-Patino, & Pilosof, 2007; Pérez et al., 2006). Generally, polysaccharides are used in admixture to proteins mainly to enhance stability of dispersed systems. Most high molecular weight polysaccharides, are hydrophilic in nature and do not adsorb to the air–water interface. However, they can strongly enhance the stability of protein foams by acting as thickening or gelling agents at the interface (Dickinson & McClements, 1995). Nevertheless, as mentioned before, due to its surfactant character, HPMC can be adsorbing in a competition with different proteins (Martínez, Farías, & Pilosof, 2011; Pérez, Sánchez, Pilosof, & Rodríguez Patino, 2009). Thus, it could influence on admixtures foam behavior by complexation, or indirectly by a depletion mechanism in the vicinity of the interface (Martínez, Ganesan, Pilosof, & Harte, 2011).

Another important functional property of HPMC is that it exhibits thermo-reversible gelation; it gels upon heating, and returns again to liquid state upon cooling (Yuguchi, Urakawa, Kitamura, Ohno, & Kajiwara, 1995). HPMC has been studied in combination with various proteins. It has been found that HPMC can improve the stability of soy protein based food products (Martinez, Carrera Sánchez, Pizones Ruíz-Henestrosa, Rodríguez Patino, & Pilosof, 2007). A synergistic effect with whey protein at neutral pH in both surface activity and gelation characteristics has been found as well, which occurred due to thermodynamic incompatibility (Jara, Perez, & Pilosof, 2010; Pérez et al., 2006, 2007). Camino, Sanchez, Rodríguez Patino, and Pilosof (2012) compared the behavior of mixtures of HPMC with β-lactoglobulin at pH above and below the isoelectric point of the protein. They observed the existence of thermodynamic incompatibility above the isoelectric point, and complex formation below the isoelectric point, due to the small charge of the HPMC. The complex formation caused an antagonistic effect of HPMC at pH 3. In a study where different proteins were added to a gluten-free bread formulation containing HPMC, it was found that EW could improve loaf volume and thermal performance, in certain concentrations (Crockett, Ie, & Vodovotz, 2011; Kobylañski, Pérez, & Pilosof, 2004).

However, the combination of HPMC with EW in other systems has not been studied yet. Thus the aims of this research were: i) to investigate the phase separation behavior of mixtures of EW with a commercial HPMC at pH 7 (EW natural pH) or 3 (below of EW pI); ii) to study the impact of this segregation on the gelation and foaming properties of the mixed systems compared to the performance of single EW.

2. Materials and methods

2.1. Materials

Egg white (EW) powder gently provided by Ovoprot (Buenos Aires, Argentina) was used as starting material. The protein content (total basis) of the powder was 88.93 ± 1.18 (N × 6.25) (AOAC., 1995). Commercial hydroxypropylmethylcellulose (HPMC), Methocell™ E5LV (Dow Chemical Company®) was gently donated by Colorcon-Argentina. This is a food quality HPMC and was used without further purification. All other chemical reagents used were of analytical grade.

2.2. Preparation of stocks solutions and mixtures

2.2.1. Stock solutions

EW stock solution was prepared by gently dissolving 20% (wt/wt) protein powder in double distilled water while stirring. To prevent microbial growth, 0.02% (w/w) sodium azide was added. After all powder was dissolved, the solutions were stirred for 30 min. Subsequently, they were centrifuged for 1 h at 12,857 × g and 20 °C (Model 5810 R, Eppendorf AG, Hamburg, Germany) in order to remove non-dissolved solids. The supernatant was stored at 4 °C until use.

E5LV stock solutions 4% (wt/wt) were prepared by dispersing the appropriate mass powder in double distilled water previously heated to 85 °C, with agitation to allow complete dissolution of the powder. Then, the solutions were cooled at ambient temperature and stored at 4 °C over night to allow the polysaccharide to reach its maximum hydration.

Stock solutions were heated up to room temperature prior to the experiments.

2.2.2. Preparation of mixtures

Mixtures with different concentrations of EW (5–7% wt/wt) and 1% wt/wt E5LV were prepared using the stock solutions and double distilled water. For the mixtures at pH 7, the pH was left unadjusted. To obtain mixtures with pH 3, EW and E5LV stock solutions were mixed with part of the water, the pH was adjusted with HCl (1N), and then water was added to complete to the desired volume. The pH of mixtures was measured whit a pH meter model 3 STAR (ORION RESEARCH, Beverly, CA, USA).

2.3. Phase separation studies

EW (7% wt/wt)/E5LV (1% wt/wt) mixtures at pH 7 or 3 were transferred to graduated 10 ml tubes and the evolution over time of