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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; Kobylański, 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

macroscopic phase separation at 25 °C was visually determined by recording the volume of the lower phase until complete separation had occurred. Each mixture was prepared in triplicate.

2.4. Confocal scanning laser microscopy

Microscopic images of EW (7% wt/wt)/E5LV (1% wt/wt) mixtures at pH 7 or 3 were obtained by an FV300 CLSM (Olympus Corp., Tokyo, Japan), equipped with a vertical microscope model BX61 (Olympus), used in the single photon mode with an Ar/HeNe visible light laser. The following Olympus objective lenses were used: UplanFl 10X/0.3NA/dry and UplanFl 10X/0.5NA/dry The protein was non-covalently labeled with a few drops of rhodamine B solution 0.02% (wt/v) (excitation wave length 560 nm; emission maximum 625 nm). For autofluorescence of the HPMC, wavelength 488 nm was used, which corresponds to the wave length at which cellulose particles demonstrate autofluorescence (Ray-Chaudhuri, Jayamanne, & Strong, 1998). FluoView™ image acquisition software v3.3 (Olympus) was used to acquire the images in multiple.tif format in 1024x1024 and 512x512 pixel resolutions.

2.5. Zeta-potential measurements

Zeta-potential measurements (ξ) were carried out in a Dynamic Laser Light Scattering instrument (Zetasizer Nano-Zs, Malvern Instruments, Worcestershire, United Kingdom) provided with a He–Ne laser (633 nm) and a digital correlator, Model ZEN3600. As described in Camino and Pilosof (2011), measurements were done at a fixed scattering angle of 173°. Samples of EW (7% wt/wt)/E5LV (1% wt/wt) mixtures at pH 7 or 3 were previously diluted 1:100 to a droplet concentration of 0.02% wt in double distilled water. The zeta potential was determined by measuring the direction and velocity when the droplets moved in the applied electric field at 25 °C. The zeta potential is reported as the average and standard deviation of measurements made on two samples, with ten readings made per sample.

2.6. Dynamics of gelation

Gelation dynamics measurements were performed in an MCR 300 controlled stress rheometer from Paar Physica (Graz, Austria). EW 7 or 5% wt/wt solutions, E5LV 1% wt/wt solution and their mixtures were poured onto the bottom plate of a parallel plate measuring system, gap 1 mm. The temperature of the bottom plate was controlled with a Peltier system model Viscotherm VT2, also from Paar Physica, and liquid silicone was applied to the exposed surfaces of the sample to prevent evaporation. During gelation experiments, the frequency was 1 Hz and the strain was kept constant at 0.01%, a value found to be in the linear viscoelastic region. The samples were heated from 20 to 90 °C at a rate of 10 °C/min, then kept at 90 °C during 15 min, which was enough time to allow G′ equilibration and then cooled to 20 °C at 25 °C/min. The storage modulus (G′), the loss modulus (G″) and the loss tangent (tan δ) were recorded over time. The temperature at which the storage modulus (G′) and the loss modulus (G″) crossed over was taken as the gelation temperature (T_{gel}). Experiments were performed at least in triplicate, and the average and standard deviation were reported.

2.7. Texture properties

The texture profile analysis (TPA) is an imitative test that provides standardized values of food texture by deformation of the product via a pivotal motion (resembling the human jaw). A two bite cycle is employ and the stress developed in the food sample is measured as the sample is compressed (Friedman, Whitney, & Szczesniak, 1963). The values for texture attributes were obtained by mathematical functions from the resulting force-timecurve (Rosenthal, 1999). This particular test was chosen because preliminary experiments demonstrate the existence of attributes (i.e. stickiness) that could be well described by the parameters of the TPA test (i.e. adhesiveness) (Rosenthal, 1999). Additionally, TPA has successfully been used to analyze WPC gels before (Spahn, Baeza, Santiago, & Pilosof, 2008; von Staszewski, Jagus, & Pilosof, 2011). Then, gels of single EW (7 or 5% wt/wt) and mixed with E5LV (1% wt/wt) at pH 7 or 3 were prepared for texture analysis in glass bakers (25 mm diameter × 55 mm height) containing 10 ml of sample. Then, these samples were heated for 20 min at 90 ± 0.1 °C and stored at 4 °C overnight. Before experiments, gels were brought to room temperature and were not removed from the beaker glasses on measuring. Texture profile analysis was performed at 25 °C in a Texture Analyzer model TA-XT2i from Stable Microsystems (Godalming, UK) using a cylindrical probe (13 mm diameter); gels were compressed to 30% of the initial height at a compression rate of 0.5 mm/s. For each sample the hardness and springiness were recorded. As these were defined by Bourne (1982), hardness: is the peak force of the first compression of the product; springiness: how well a product physically springs back after it has been deformed during the first compression. The assay was performed at least in triplicate, and the average and standard deviation were reported.

2.8. Foaming properties

2.8.1. Foam formation

Foams of single 7% wt/wt EW, 1% wt/wt E5LV and their mixture at pH 7 or 3 were prepared. Thus, 20 ml of each aqueous sample was transferred to a graduated 100 ml tube (35 mm diameter) and foamed at 25 °C for 3 min using a stirrer equipped with a rotating vane (25 mm diameter) at 2500 rpm (Griffin & George Ltd., Great Britain). Experiments were performed in triplicate.

2.8.2. Foam drainage and collapse

The foam height (collapse) and the volume of liquid drained to the bottom of the tubes were visually recorded over time.

Foam overrun (FO) was calculated as follows,

$$FO(\%) = \frac{V_f - V_0}{V_0} 100 \quad (1)$$

where V_f is the foam volume reached at the end of whipping and V_0 is the initial volume of the sample (Martinez et al., 2005).

Foam collapse after 1 h (FC_{1h}) was calculated as follows,

$$FC_{1h}(\%) = \frac{V_f - V_{1h}}{V_f - V_0} 100 \quad (2)$$

where V_{1h} is the foam volume after 1 h, V_f is the foam volume reached at the end of whipping and V_0 is the initial volume of the sample.

The following empirical mathematical model was applied to fit drainage over time (Elizalde, Giaccaglia, Pilosof, & Bartholomai, 1991),

$$v(t) = \frac{Vt}{B + t} \quad (3)$$

where v(t) is the drained volume at time t, V is the maximum drained volume and B refers to the time needed to drain the half of V drained maximum volume. The data was fitted by using

GraphPad Prism© 5 (GraphPad Software Inc, La Jolla, CA, USA). Estimated values for V and B were obtained. From these values the specific rate constant for drainage K_d, and the initial rate of drainage R_0 were calculated as follows,

$$K_d = \frac{1}{VB} \quad (4)$$

$$R_0 = \frac{V}{B} \quad (5)$$

All parameters were calculated for triplicates and average and standard deviation were reported.

2.9. Statistical analysis

All averages and standard deviations were calculated using Excel 2010 software (Microsoft Corp., Redmond, WA, USA). Significant differences between samples were analyzed by performing ANOVA using Statgraphics Centurion v15 software (Statpoint technologies, Warrenton, VA, USA).

3. Results and discussion

3.1. Phase behavior of EW/E5LV mixtures

In order to understand the mechanism behind changes in functionality, the phase separation behavior of EW (7% wt/wt)/E5LV (1% wt/wt) mixture was studied at pH 7 and 3; pH 7 is the EW natural pH which is above the isoelectric point of most of the proteins except lysozyme (Arzeni et al., 2012b; Erçelebi & Ibanoglu, 2009), while pH 3 is below the isoelectric point of EW proteins.

3.1.1. Macroscopic phase separation

Fig. 1 shows the kinetics of phase separation at pH 3 and 7. At pH 7 for the first 120 min no macroscopic phase separation occurred. Then the sample became turbid and the system suddenly collapsed and two phases appeared, an opaque phase on the bottom and a turbid phase above. Subsequently, the bottom phase became more concentrated and the upper phase less turbid until a certain point in which no further changes were observed.

At pH 3 macroscopic phase separation occurred gradually reaching a degree of phase separation (upper phase volume) a little lower than at pH 7 (Fig. 1). This phase separation starts earlier with a small clear upper phase and a turbid bottom phase which appeared already after about 10 min and then moved downwards very slowly.

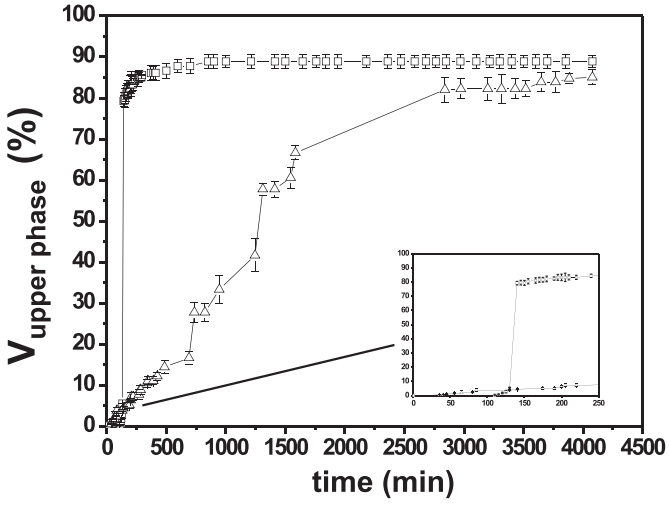

Fig. 1. Kinetics of phase separation for EW (7% wt/wt)/E5LV (1% wt/wt) mixture at either pH 7 (□) or 3 (△). Error bars indicate standard deviation.

The difference in phase separation kinetics between the samples at different pH indicates that there might be a difference in phase separation mechanism. Confocal scanning laser microscopy (CSLM) was proved to be a suitable technique to visualize the separation of aqueous biopolymer mixtures into two phases (Jara & Pilosof, 2009; van de Velde, Weinbreck, Edelman, van der Linden, & Tromp, 2003). Thus, the phase separation mechanism of EW/E5LV mixtures was further investigated at microscopic level by CSLM.

3.1.2. Microscopic phase separation

Fig. 2 shows CSLM images of the mixture EW (7% wt/wt)/E5LV (1% wt/wt) at pH 7 (Fig. 2a–b) and pH 3 (Fig. 2c–d), before complete macroscopic phase separation has taken place (Fig. 2a–c show protein-red-fluorescence while Fig. 2b–d shows the polysaccharide-green-fluorescence).

At both pHs the shape of the red protein domains is more irregular than what would be expected if there was thermodynamic incompatibility, in which more spherical particles are expected to appear (water-in-water emulsions). To investigate the location of the E5LV, images were taken at 488 nm where HPMC exhibits auto-fluorescence, (Ray-Chaudhuri et al., 1998). At pH 7 the E5LV was present almost only on the same spots where it had also found the protein. This could indicate a complex formation instead of thermodynamic incompatibility since otherwise E5LV would be only present in the matrix. At pH 3 the intensity of E5LV auto-fluorescence is also higher on the same spots as where fluorescence of the protein was seen. However, the green color is more visible in the matrix in between the protein domains. This probably means that less E5LV participates in complex formation. According to the CLSM images the protein/polysaccharide complex would form at both pHs, but at pH 7 they are formed in a higher amount and flocculate to form increasing bigger particles that after a certain time separate (Fig. 2a–b). CLSM images over time (data not shown) have shown an increase of size at pH 7. At pH 3, the complexed particles are smaller than at pH 7 (Fig. 2c–d) and the rate of phase separation is much slower (Fig. 1), probably because the particles do not flocculate as much with time. Therefore, the macroscopic phase separation would be caused by sedimentation of the complexes (de Kruif, Weinbreck, & de Vries, 2004), which occurs at a higher rate (pH 7 compared to pH 3) with bigger complexes (Figs. 1 and 2). Moreover, this flocculation may be enhanced by a low surface charge of particles. Thus, the complexes net superficial charge was determined by measurements of zeta-potential of mixtures at both pHs, being -17.6 ± 0.5 mV and $+17.4 \pm 1.8$ mV for pH 7 and 3, respectively. From the zeta potential of the mixtures at both pHs it cannot be concluded that flocculation should be more pronounced at pH 7, as the absolute value was similar at both pHs. Depletion of complexes due to small amounts of non complexed polysaccharide may be considered as a possible cause of the flocculation observed at pH 7.

Although HPMC is considered to be a non-ionic polysaccharide it was found in previous research that E5LV exhibits a small negative charge (-1.71 ± 0.05) mV at pH 3 and a smaller positive charge at pH 6 ($+0.78 \pm 0.1$) mV (Camino et al., 2012). At low temperatures, the water molecules are situated around the methyl groups in cage-like structures. It could be possible that the ions in solutions were interacting with the cage-like structures and, therefore, giving charge to HPMC molecules (Camino, Sánchez, Rodríguez Patino, & Pilosof, 2011). Moreover, EW presents zeta-potential values of (-15.9 ± 1.1) mV and ($+22.7 \pm 0.5$) mV, at pH 7 or 3, respectively (Arzeni, Pérez, & Pilosof, 2012a). Therefore, opposite charges

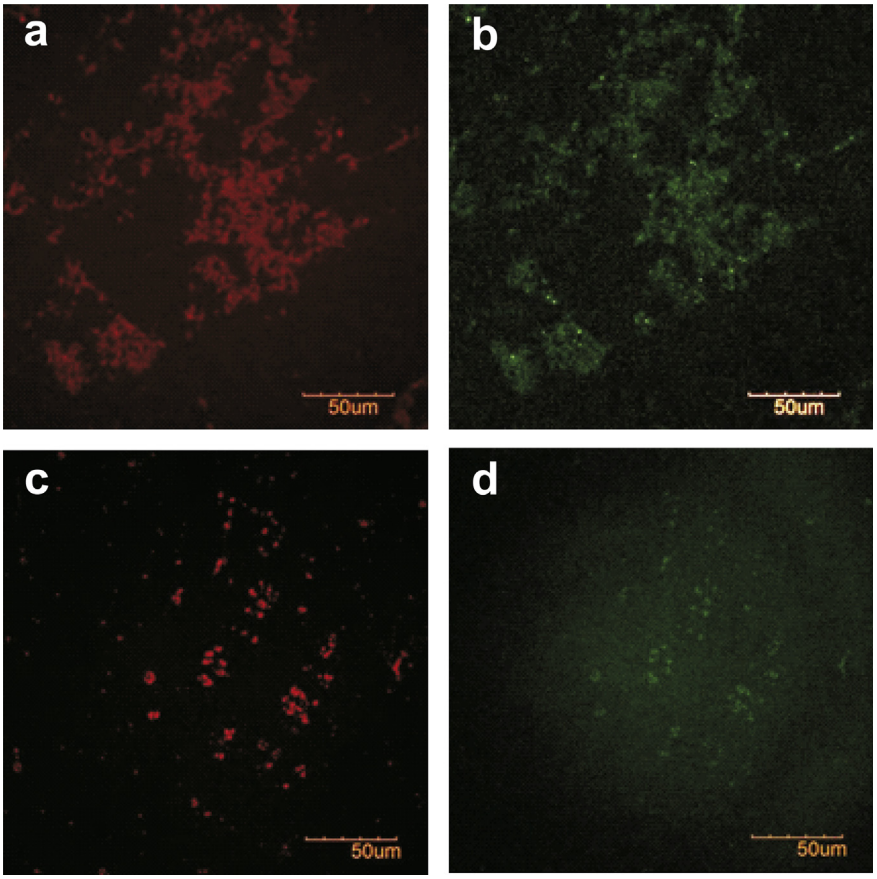

Fig. 2. CSLM pictures of EW 7% (w/w)/E5LV 1 (wt/wt) at pH 7 (a,b) or 3 (c,d); red color indicates protein fluorescence (a,c); green color indicates polysaccharide fluorescence (b,d). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

between EW and E5LV can support the possibility of weak electrostatic complexation at both pHs. Furthermore, complexes also may be formed by hydrophobic interactions between methyl groups on HPMC and hydrophobic domains of EW proteins that could be pH-modulated. Hydrophobic interactions of HPMCs are strongly reduced at pH 3 as reported by Camino et al. (2011) when studying the HPMC self-association in solution and at oil-water interfaces. Briefly, these authors proposed that the methyl groups interact with ions present in the solution, avoiding HPMC self-association driven by hydrophobic interactions. Also, Tritt-Goc and Pilewski (2002) found that at low pH predominate a higher number of hydrogen bonds between the HPMC and the protons of the solvent which further indicates that there is a decrease of hydrophobicity. Thus, this HPMC pH-modulated hydrophobicity could explain the lower extent of HPMC interaction with EW protein observed by CSLM at pH 3 (Fig. 2). In addition, a difference in conformation and self-association of the proteins at pH 7 or 3 might cause a difference in accessibility (i.e. hydrophobicity) of the protein to the polysaccharide and therefore affect the possibility of complex formation by hydrophobic interactions (Li & Huang, 2013; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003).

3.2. Gelation behavior of EW/E5LV mixtures

3.2.1. Gelation kinetic

3.2.1.1. Gelation temperatures. For the rheological experiments, mixtures were prepared with 1% wt/wt of E5LV and two different EW concentrations, 5% and 7% wt/wt, at pH 7 or 3 Also, the experiments were performed on single compounds. The gelation temperatures (T_{gel}) of the different mixtures and single compounds at both pHs are shown in Table 1.

At pH 7, EW T_{gel} slightly increased with increasing of protein concentration from 62.0 °C at 5% to 65.4 °C at 7% wt/wt, but it was not a significant difference. In literature it was found that EW T_{gel} is around 64 °C, though it is influenced by heating rate, pH and protein concentration (Arzeni et al., 2012b; Leksrisompong & Foegeding, 2011). For E5LV T_{gel} value was found to be around 52 °C which corresponds to the pre-gel-regime according to literature (Pérez et al., 2006; Silva et al., 2008). The T_{gel} values for both mixtures were similar to the gelation temperature of the component with the lowest T_{gel} value, E5LV.

Table 1
Gelation parameters obtained by dynamic rheometry for EW, E5LV and their mixtures at pH 7 or 3.

	Sample	T_{gel} (°C)	G'_{eq} (Pa)
pH 7	E5LV 1%	52.0 ± 2.0	33.1 ± 1.2
	EW 5%	62.0 ± 2.0	42.4 ± 1.3
	EW 7%	65.0 ± 4.0	436.0 ± 40.5
	EW 5%/E5LV 1%	49.0 ± 2.5	92.6 ± 2.3
	EW 7%/E5LV 1%	48.0 ± 2.0	614.0 ± 27.5
pH 3	E5LV 1%	48.0 ± 2.0	53.5 ± 5.0
	EW 5%	66.0 ± 2.0	338.0 ± 37.7
	EW 7%	63.30 ± 0.01	1677.0 ± 83.5
	EW 5%/E5LV 1%	47.5 ± 2.0	1747.0 ± 81.4
	EW 7%/E5LV 1%	45.2 ± 0.6	4710.0 ± 264.0

Average ± SD, n = 3.
%: % wt/wt.
T_{gel} is the gelation temperature.
G′eq is the plateau value of G′ at 90 °C.

At pH 3, EW T_{gel} decreased with increasing protein concentration from 66.0 °C at 5% wt/wt to 63.3 °C at 7% wt/wt. E5LV T_{gel} value at pH 3 (48 °C) was lower that at pH 7 (52 °C), but there was no significant difference between both pHs (Table 1). For mixtures, just like at pH 7, the gelation point is dominated by the component with the lowest gelation point, E5LV.

3.2.1.2. Evolution of storage modulus upon heating. Fig. 3a shows the storage modulus (G′) time development for EW (5% wt/wt)/E5LV (1% wt/wt) mixture at pH 7, compared to the single components. Upon time, G′ tends to an equilibrium value G'_{eq} which was reached around 5–7.5 min according to each sample. The EW concentration is around the minimum concentration needed for EW to gel; however it did form a non-reversible gel which was confirmed as no cross-over of loss modulus (G″) and storage modulus (G′) occurs on cooling (data not shown). As previously reported the G′ of single E5LV first increases upon heating because of gelation and then decreases again on cooling and the storage modulus and loss modulus (G″) cross again (Jara et al., 2010; Pérez et al., 2006; Yuguchi et al., 1995) due to its thermoreversibility. For the mixture, G′ development was initially dominated by the E5LV; then G′ was dominated by the protein and continued to increase gradually reaching a higher final value compared to single EW.

The G′ time evolution for EW (5% wt/wt)/E5LV (1% wt/wt) mixture compared to the single components at pH 3 can be seen in Fig. 3b. In this case, G'_{eq} was reached around 5–10 min depending on each sample. Again, single E5LV gels on heating and reverse upon cooling as at pH 7. EW protein forms non-reversible gels as there was not cross-over between G′ and G″ upon cooling (data not shown); however G′ increased more steeply at pH 3 than 7 (Fig. 3a–b). The mixture at pH 3 also follows first the rheological behavior of the HPMC up to almost 5 min and there was more influenced by the EW (Fig. 3b) but G′ was much higher. This indicates that there is a synergistic effect which is evidenced by the increase of G'_{eq} value five times for mixture over added single components (Table 1). Moreover, the G′ of mixtures continued to increase on cooling down due to strengthening of hydrogen bonds (Jara et al., 2010; Yang, Irudayaraj, Otgonchimeg, & Walsh, 2004) indicating that all water was used' by the protein and not available for rehydration of the HPMC.

The storage modulus (G′) development over time for EW (7% wt/wt)/E5LV (1% wt/wt) mixture at pH 7, compared to the single components, are showed in Fig. 4a. Here, G'_{eq} equilibrium values were reached around 5–10 min depending on each sample. Focusing on single EW which is now at a higher concentration, it can be seen that the G′ increases in two subsequent steps. This was reported before by Pérez & Pilosof (2003). The gel point corresponds to the gelation temperature of conalbumin and the second

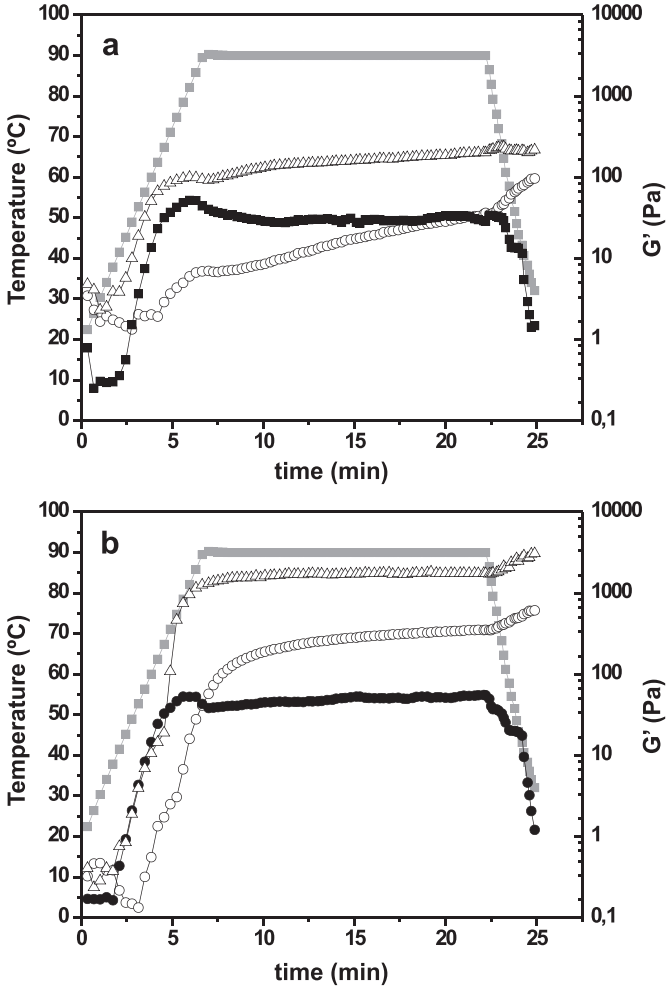

Fig. 3. G′ time evolution of EW (5% wt/wt)/E5LV (1% wt/wt) mixture at pH 7 (a) or 3 (b) compared to single components. EW (○), E5LV (■), mixture (△), Temperature (■).

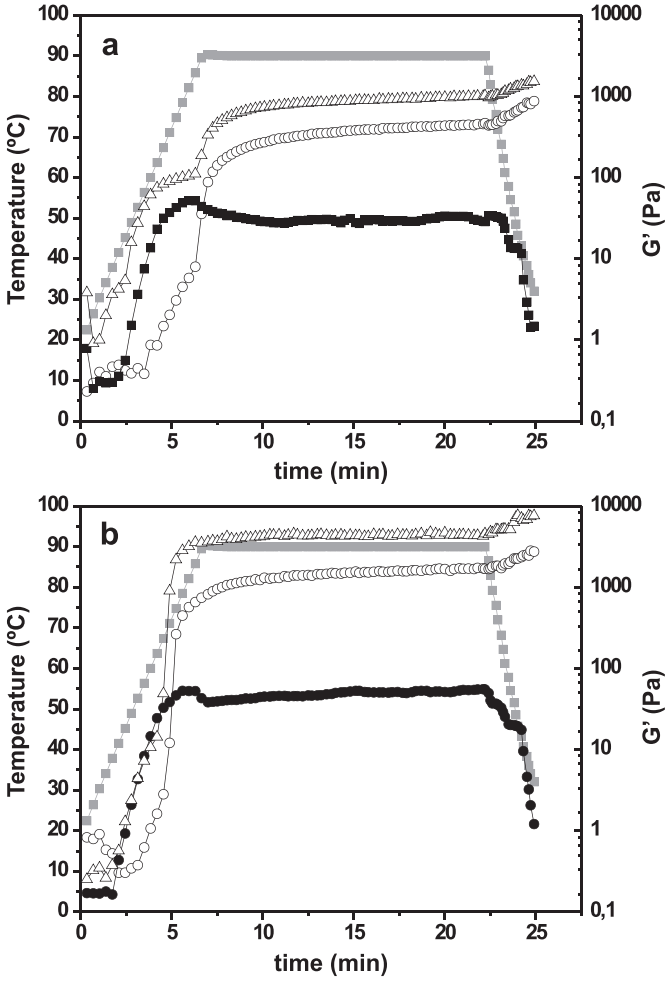

Fig. 4. G′ time evolution of EW (7% wt/wt)/E5LV (1% wt/wt) mixture at pH 7 (a) or 3 (b) compared to single components. EW (○), E5LV (■), mixture (△), Temperature (■).

increase in G′, which is more pronounced, corresponds to the gelation of ovalbumin (Arzeni et al., 2012b; Pérez et al., 2003). The mixture behaves in the same way as with 5% wt/wt of EPW; it initially follows the behavior of the E5LV. At the point where a second increase in G′ was found for single EW (around 7 min), the mixture followed the behavior of EW. This indicates a separate gelation of the different components in the mixture; first gelation of the HPMC takes place and then the ovalbumin starts to dominate the mixture. On the other hand, conalbumin, has not an important influence on the mixture rheological behavior, which can be explained by the fact that ovalbumin is more abundant and has the ability to form disulfide bonds which are more important for the gel structure (Arzeni et al., 2012b; Pérez et al., 2003). The G'_{eq} of the mixture seems to be slightly higher than added single components values, but there was not a significant synergistic effect (Table 1).

Fig. 4b shows the storage modulus (G′) time development for EW (7% wt/wt)/E5LV (1% wt/wt) mixture at pH 3, compared to the single components. It can be seen that the G′ for EW increases continuously reaching G'_{eq} value of 1677 Pa. The mixture behaved similarly to pH 7; it followed a similar G′ behavior to that EW. However, G'_{eq} reached a value of three times the added single components values, which evidenced a synergistic effect on the mixed gel (Table 1).

Finally, the behavior of the mixture compared to the single EW was very different with 5% or 7% wt/wt of protein (Figs. 3a and 4a). As EW hardly forms a gel at 5%, the addition of HPMC in this case is more significant, strongly promoting gelation.

3.2.2. Texture properties of mixed gels

Table 2 shows the results of textural assays of mixed gels, for hardness and springiness, which are parameters for gel strength and elasticity, respectively. It can be seen that the presence of 1% wt/wt of E5LV at pH 7 did not modify the hardness of EW gel, but at pH 3 the addition of HPMC improved gels hardness, being it upturn higher as protein concentration increased. Regarding to the springiness at pH 7 there was not any significant difference by adding E5LV. However, at pH 3 the mixed gels compared to the single EW gel had a significantly higher springiness at both protein concentrations, being higher in the mixture with 7% wt/wt EW.

The results for the texture properties were similar to the results for gelation dynamics. At pH 3 higher synergistic effects were found regarding storage modulus (G_{eq}; Table 1), hardness and springiness (Table 2), than at pH 7. This reveals that small complexed particles with low sedimentation rate, pH 3 (Fig. 2 c–d), are favorable for obtaining stronger gels, because this allows the gelation of protein before any macroscopic phase separation. Moreover as these protein-HPMC particles are more uniformly distributed when gelation takes place they could benefit the formation of a more homogenous network including the continuous phase. Compared to the single protein, the network could in this way be reinforced by the HPMC complexes.

Table 2
Textural parameters for EW/E5LV mixed gels at pH 7 or 3.

	Sample	Hardness (N)	Springiness (a.u.)
pH 7	EW 5%	15.0 ± 2.4^{a}	0.90 ± 0.02^{a}
	EW 7%	131.5 ± 9.0^{b}	0.93 ± 0.03^{a}
	EW 5%/E5LV 1%	19.3 ± 2.0^{a}	0.91 ± 0.02^{a}
	EW 7%/E5LV 1%	135.2 ± 15.6^{b}	0.94 ± 0.01^{a}
pH 3	EW 5%	45.0 ± 4.0^{a}	0.93 ± 0.01^{a}
	EW 7%	133.4 ± 6.0^{b}	0.92 ± 0.01^{a}
	EW 5%/E5LV 1%	63.0 ± 2.1^{c}	0.955 ± 0.004^{b}
	EW 7%/E5LV 1%	194.0 ± 8.4^{d}	0.98 ± 0.02^{b}

Average ± SD, n = 3.
%: % wt/wt.
Different letters on superscript indicate significant differences ($P < 0.05$) between samples (within pH group).

3.3. Foaming behavior of EW/E5LV mixtures

3.3.1. Foam drainage

In Fig. 5a the drainage of foams of single EW 7% wt/wt, E5LV 1% wt/wt and their mixture at pH 7 can be seen. Drainage is the increase in volume of the liquid phase that appears at the bottom of the foam. It was expected that EW formed a stable foam at pH 7 (Arzeni et al., 2012b) and the drainage is indeed very slow. E5LV is also surface active and can form a stable foam (Camino, Pérez, Sanchez, Rodriguez Patino, & Pilosof, 2009), though it is less stable than the EW foam since drainage occurred faster. The drainage of the foam that was made from the mixture appears in between of drainage curves for the single components but closer to drainage of E5LV. Thus, addition of E5LV to EW at pH 7 impairs the drainage of foams and has therefore a negative effect. Fig. 5b shows the drainage of the foams at pH 3; again for single components and their mixture. Although the stability regarding drainage of the E5LV foam was not significantly affected by pH, the EW foam was less

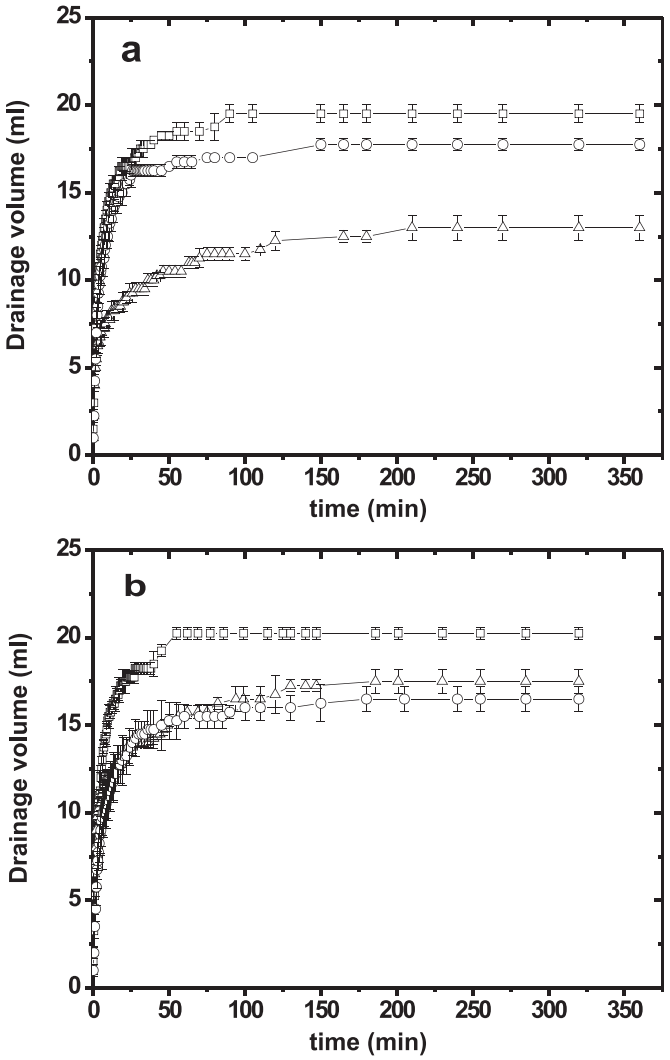

Fig. 5. Drainage of foams of EW 7% wt/wt, E5LV 1% wt/wt and their mixture at pH 7 (a) or 3 (b). EW (△), E5LV (□), mixture (○). Error bars indicate standard deviation.

stable at pH 3 than 7. Contrarily to what happened at pH 7, at pH 3 the drainage of the mixture was very similar to the drainage of EW foam, but lower. Then, in this mixture there is a slight improvement in foam stability. In order to calculate the specific rate constant for drainage (K_d) and the initial rate of drainage (R_0), the data was fitted with a two-parameter equation (3) developed by Elizalde et al. (1991), following by the equations (4) and (5), for K_d and R_0 calculation, respectively (see Section 2.8.2). Results are shown in Table 3. The K_d and R_0 for drainage of the foams at pH 7 are very similar to each other. The only significant difference was found between R_0 of EW and E5LV; as expected R_0 of mixture was in between of single components. At pH 3 the mixture seemed to have a lower K_d and R_0 than both single EW and E5LV. However, this difference was not significant. Thus, the drainage of mixed foam was not improved by E5LV adding at both pHs.

Drainage is dependent on bulk viscosity and surface films properties. Generally, this process is retarded by increasing bulk viscosity, effect imparted by the polysaccharides. HPMC due to its high surface activity (Pérez et al., 2009) may compete with proteins for the A/W interface. Thus, at pH 7, where interactions between EW and HPMC are weak, HPMC could hide the adsorption of EW at the A/W interface, resulting in a mixed foam with a drainage behavior similar to HPMC foam. Previous works on the behavior of HPMC in admixture with whey proteins (Pérez et al., 2009) or soy proteins (Martinez et al., 2007) at the A/W interface, have shown that HPMC could dominate the mixed interface due to its unusual surface activity (Rodríguez Patino & Pilosof, 2011). On the other hand, at pH 3, condition for stronger associative interactions between EW and HPMC, could enhance mixed film properties (Rodríguez Patino & Pilosof, 2011) contributing to lower drainage. Finally, depending on pH different drainage rates of mixed systems can be obtained, determined probably by a particular balance between interfacial behavior of mixed film and viscosity effect imparted by the polysaccharide.

3.3.2. Foam collapse

For the collapse of the foams at pH 7 similar results were found as for drainage (Fig. 6a). The single EW foam was very stable while the E5LV foam collapsed quickly. Again, the collapse of the mixed foam was in between the collapse of single components foam, but closer to EW foam. Thus, the effect of E5LV addition to 7% wt/wt EW was slightly negative.

In Fig. 6b the collapse of foams at pH 3 can be seen. As was seen with the drainage stability, the stability against collapse is similar at both pHs for E5LV but is lower at pH 3 than 7 for EW foam. The mixture was very stable against foam collapse at pH 3, compared to

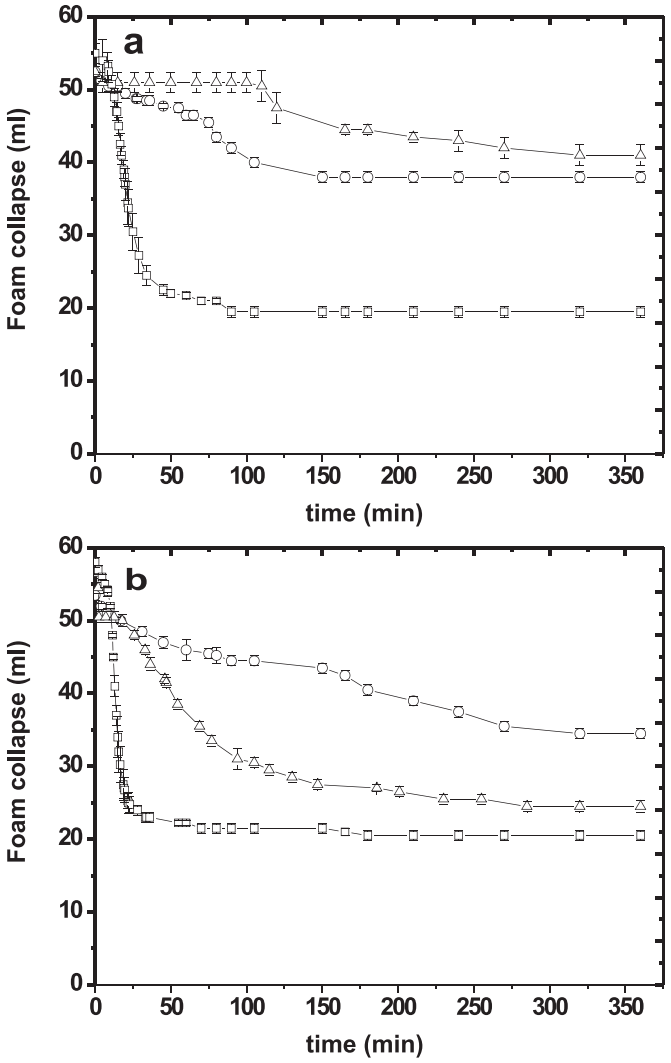

Fig. 6. Collapse of foams of EW 7% wt/wt, E5LV 1% wt/wt and their mixture at pH 7 (a) or 3 (b). EW (△), E5LV (□), mixture (○). Error bars indicate standard deviation.

both single components. This indicates a synergistic effect since collapse of the mixture occurs much slower.

The foam overrun (FO) and foam collapse after 1 h (FC_{1h}) were calculated by equations (1) and (2), respectively (see Section 2.8.2), and the results are summarized in Table 3.At pH 7, EW 7% (wt/wt) and E5LV 1% (wt/wt) had similar FO. However, the FO value of the mixture was significantly lower indicating a negative effect on foam overrun by addition of HPMC at pH 7. Nevertheless, the negative effect was not found in foam collapse after 1 h (FC_{1h}) since there was no significant difference between single EW and the mixture while E5LV performed significantly worse. At pH 3 the FO and FC_{1h} values of mixture were both significantly lower than for both single components. A negative effect is therefore found in foam overrun, while the effect on collapse was strongly positive.

In conclusion, at pH 7 no beneficial effect was found by E5LV addition to EW, at none of the foam properties. However, it can therefore be said that there was a synergistic effect at pH 3 on foam collapse while foam overrun was slightly negative and drainage did not show differences compared to single EW. Thus, the synergistic effect is thought to be caused by the formation of small complexes at pH 3 which once adsorbed at the air–water interface would form better films according to the above results on gel formation, thus offering an improvement against collapse of surface films

Table 3
Foam parameters for 7% wt/wt EW, 1% wt/wt E5LV and their mixture at pH 7 or 3.

	Sample	K_d (ml min)$^{-1}$	R_0 (ml/min)	FO (%)	FC_{1h} (%)
pH 7	E5LV 1%	0.017 ± 0.001^a	6.2 ± 0.2^b	202.5 ± 3.5^b	96.0 ± 1,0^b
	EW 7%	0.022 ± 0.005^a	3.1 ± 1.1^a	205.0 ± 7.1^b	27.0 ± 2,4^a
	EW 7%/E5LV 1%	0.016 ± 0.004^a	5.2 ± 1.2^{a,b}	180.0 ± 2.4^a	26.4 ± 2.0^a
pH 3	E5LV 1%	0.016 ± 0.003^a	6.4 ± 1.3^a	205.0 ± 1.3^b	99.0 ± 2.0^c
	EW 7%	0.03 ± 0.01^a	6.6 ± 1.4^a	190.0 ± 1.2^b	56.0 ± 5.0^b
	EW 7%/E5LV 1%	0.014 ± 0.003^a	3.8 ± 1.0^a	185.0 ± 0.5^a	27,0 ± 7.4^a

Average ± SD, n = 3.
%: % wt/wt.
Kd is the rate constant for drainage.
R0 is the initial rate of drainage.
FO is the Foam Overrun.
FC1h is the Foam Collapse after 1 h (%).
Different letters on superscript indicate significant differences between samples (within pH group).

and foams. The main reasons for using protein-polysaccharide complexes as emulsion or foam stabilizers are their high surface activity, their ability to increase the viscosity of the dispersion medium and their ability to form gel-like charged and thick adsorbed layers (Rodriguez Patino & Pilosof, 2011). Miquelim, Lannes, and Mezzenga (2010) demonstrated that using pH conditions and protein/polysaccharide pairs capable to undergo ionic coacervation, is a robust protocol to stabilize air–water interfaces and the corresponding foams. Moreover, the molecular properties of egg white ovalbumin in a complex with pectin in the bulk solution and at air/water interface were studied by Kudryashova, Visser, Van Hoek, and De Jongh (2007). They found that complexation of ovalbumin with pectin in the bulk phase resulted in the formation of a compact structure with a different spatial arrangement depending on the protein/pectin ratio. Interaction with pectin in the bulk solution resulted in a significantly slower adsorption of the protein to the air/water interface.

Since the synergistic effect was found at pH 3 but not at pH 7, this limits the type of products in which HPMC could be used to improve the functionality of EW. It would therefore be more suited for the development of new products, such as foams or gels of low pH as those.

4. Conclusions

From CLSM determinations it was found that EW formed complexes with HPMC, since fluorescence of EW and HPMC at pH 7 or 3 was found on the same locations. At both pHs HPMC existed in the continuous phase as well. The complexes were strongly flocculated at pH 7 leading to a more rapid phase separation than at pH 3. It was concluded that the macroscopic phase separation did not occur through thermodynamic incompatibility but rather through complex formation and sedimentation of these complexes. Even if weak electrostatic interactions between EW and HPMC could take place, it seems more probable the existence of hydrophobic interactions which are pH-modulated.

Upon gelation of mixtures with EW in concentrations of 5 or 7% (wr/wt) and ELV5 1% (wt/wt) independently of pH a synergism between EW and E5LV was observed, which was higher at pH 3. Additionaly, there was not any improvement on gel strength and elasticity of the gels by addition of HPMC at pH 7 but a synergistic was found at pH 3.

At pH 7 EW formed very stable foam and no beneficial effect was found by HPMC addition. At pH 3 the EW foam was less stable and HPMC addition strongly improved foam stability.

Overall, it can be concluded that it is possible to improve the functionality of EW regarding gelation and foaming by using it in admixture with HPMC. This does however depend on conditions such as pH. In this research a net improvement was only found at pH 3, below the iso-electric point of the proteins. For the implementation of these results in actual products, due to the low pH value at which the synergism was found, HPMC and EW mixtures might be suitable to be used as functional ingredients in for instance fruit foams or gels or even marshmallows.

Acknowledgments

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