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# The effect of pH, sucrose, salt and hydrocolloid gums on the gelling properties and water holding capacity of egg white gel

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## ABSTRACT

The aim of this investigation was to evaluate the effect of pH variation, sucrose, salt and hydrocolloid gums (xanthan, guar, karaya and locust bean) addition on the gelling properties and the water holding capacity (WHC) of the egg white (EW) gel. Tree levels of pH (4.5, 6.5, 8.0), sucrose/salt (1, 3, 6% w/w) and hydrocolloids (0.01%, 0.10%, 0.20% w/w) were tested in this study. Obtained results showed that mechanical properties and WHC of the EW gel were decreased significantly by pH variation. Addition of salt and sugar improved firmness and elasticity of the EW gel. However, the presence of salt decreased the WHC of the gel. Whereas, in the presence of sugar, WHC was slightly improved. The presence of hydrocolloids in EW gel is associated with the modification of the functional properties of the EW proteins. Xanthan gum decreased hardness and strength and increased WHC of the gel to 97.18% at 0.2%. Guar and karaya gums increased mechanical properties of EW gel at the lowest concentration (0.01%). Locust bean gum was shown to enhance texture profile of EW gel at all tested levels. WHC was significantly improved in the presence of food gums. Microstructure observations of EW gels were also carried out by environmental scanning electron microscopy.

## 1. Introduction

Whole egg or its constituents are key ingredients associated with unique sensory characteristics and excellent functionality for food industrial applications. Egg white proteins are known to be good foaming, emulsifying and gelling agents and they have been widely investigated for their functional properties and heat stability (Matsudomi, Takahashi, & Miyata, 2001; Rossi, Casiraghi, Primavesi, Pompei, & Hidalgo, 2010). The functionality of egg white is mainly attributed to ovalbumin (54%), which is the major egg white protein, followed by conalbumin (12%), ovomucoid (11%) and lysozyme (3.5%) (Van den Berg, Jara, & Pilosof, 2015).

In many food products formulations, pH variation, salt and/or sucrose as well as hydrocolloids gums addition, could occur together with proteins to contribute on the final structure, texture and stability (Kim, Varankovich, & Nickerson, 2016; Sadahira, Rodrigues, Akhtar, Murray, & Netto, 2016; Sadahira et al., 2015). While hydrocolloids are present as thickening and water-holding agents, salt and sucrose for sensory characteristics and pH variation for technological reasons, the overall texture and stability of food products depends not only on the properties of proteins and these compounds, but also on the nature and strength of interactions that can take place (Davis & Foegeding, 2007; Rodriguez Patino & Pilosof, 2011). Most egg products need to be heated for microbiological safety or gelling characteristics. However, such heat treatments would alter not only the physical and functional properties of proteins but also the nature of protein–protein interactions due to pH variation and ionic strength adjustment as well as protein-polysaccharide interactions upon sucrose and gums addition (Ibanoglu, 2005).

In order to improve structural and rheological properties of food products, polysaccharides are usually added. Indeed, polysaccharides enhance the long-term physicochemical stability of protein colloids (Van den Berg et al., 2015). Mixture of protein and polysaccharides can create several structures through their gelation and aggregation properties. Protein and polysaccharides molecules can associate either by covalent interactions giving rise to a strong and specific biopolymers (Benichou, Aserin, Lutz, & Garti, 2007) or by non-covalent bonds through electrostatic, hydrophobic interactions, steric exclusion and hydrogen bonding (Rodriguez Patino & Pilosof, 2011).

Egg white proteins gels in different conditions have been investigated. Recently, the impact of segregation of egg white and hydroxypropylmethylcellulose mixtures on gelation and foaming

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properties of the mixed systems was studied (Van den Berg et al., 2015). The effect of ultraviolet processing on gelling and foaming properties of egg white was investigated (Manzocco, Panozzo, & Nicoli, 2012). In our previous work, spray drying under high temperature (125 °C) and low liquid flow rate (0.2 L/h) improved gel firmness and reduced gel elasticity (Ayadi, Khemakhem, Belgith, & Attia, 2008). In a previous work, the addition of kappa, lambda, and iota carrageenan improved ovalbumin gelation (Sánchez-gimeno, Vercet, & López-buesa, 2006).

Egg white proteins coagulation and gel forming could differ versus pH, presence of polysaccharides (sucrose and gums) and salt (Ibanoglu & Ercelebi, 2007; Mine, 1996). The interaction with a polysaccharide may either give rise to stable high molecular weight complexes which increase the solubility of protein and inhibit protein-protein aggregation (Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1989) or perturb the protein structure and decrease the thermal stability of the protein (Imeson, Ledward, & Mitchell, 1977). In addition, coagulation of protein could be inhibited by pH variation which could change the electrostatic balance and leads to more stable or more instable gel structure depending on protein pI. Moreover, protein coagulation under ionic strength variation by salt addition could leads to a various gel texture. In the literature, there is relatively limited information on gelling characteristics of white egg proteins at different pH and in the presence of food additives such as sucrose, salt and hydrocolloid gums. The aim of this work is to investigate the gelling characteristics and behaviour mechanisms of egg white at different pH and in the presence of salt, sucrose and hydrocolloid gums.

## 2. Material and methods

## 2.1. Material

Fresh eggs were purchased from a local farm (hen house, Route Menzel checker km 11. Liquid egg white (EW) presented a pH of 7.0, dry matter (DM) of 12.18  $\pm$  0.06% (AFNOR, 1970, pp. 78–80), a total protein content of 90.8  $\pm$  0.03% (AOAC, 1997), total reducing sugar of 2.24  $\pm$  0.34% (Miller, 1959) and ash content of 4.95  $\pm$  0.26% (AFNOR, 1981, pp. 1–5) based on DM. Locust bean gum, guar gum and Karaya gum were purchased from Sigma-Aldrich (Germany). Xanthan gum was supplied by satiaxane, dégussat (France). Salt, sugar and chemicals used in this study were of analytical grade.

## 2.2. Sample preparation

Eggs were broken manually and the EW was separated from the yolk. A preliminary homogenization of EW was carried out using a magnetic stirrer to mix the liquid and the thick white. Three levels of pH (4.5, 6.5 and 8.0), sugar or salt (1, 3 and 6% w/w) and gums (0.01, 0.1 and 0.2% w/w) were tested in this study. pH was adjusted using citric acid powder or NaOH solution (2N). Mixtures of EW and different concentrations of sugar, salt or gums were prepared by dispersing the appropriate amount of powders in EW solution. For each operating condition, the heat induced gels were prepared in triplicate by heating 60 ml of each sample in stainless tubes (Fig. 1) in a water bath at 90 °C for 20 min, and subsequently cooled at 4 °C for 24 h.

# 2.3. Texture analysis

Cylindrical gel samples (28 mm diameter, 20 mm length) were cut in quadruplicate. A uniaxial compression test was performed until gel fracture on a texture analyzer (LLOYD instruments, England) with a 1000 N load cell, 0.005 kgf detection range, 37 mm diameter plate probe and a compression speed of 10 mm/min. Recording of force (N) and displacement (m) were converted to true axial stress  $\sigma$ ; (Equation (1)) and Hencky strain  $\varepsilon$ ; (Equation (2)).



Fig. 1. Photo of the device for the obtaining of egg white gels.

$$\sigma = \frac{F}{A} \frac{H}{Hi} [Pa] \tag{1}$$

$$\varepsilon = -\ln \frac{H}{Hi}[-] \tag{2}$$

where F = force (N), A = initial surface (m<sup>2</sup>),  $H_i =$  initial gel height (m), and H = height (m).

Fracture point values of these parameters were used for statistical analysis.

Texture profile analysis (TPA) test allowed two consecutive penetrations at a predetermined distance (50%) within the gel. Penetrations were performed with a flat-bottomed cylindrical plunger (12 mm diameter) at a speed of 40 mm/min. Texture profile analysis was carried out by measuring four parameters which are hardness (N), elasticity, cohesion strength (N) and adhesion (N) (Ayadi et al., 2008). Hardness (N) is the strength applied to deform a product, elasticity is the rate in which a deformed sample returns to its initial position, cohesion strength (N) is the capacity of a gel to maintain the structure of the network and adhesion (N) is the strength required to separate the product from the specific surface. Measurements were carried out in triplicate.

# 2.4. Water holding capacity (WHC)

Gel water-holding capacity (WHC) was analyzed by centrifugation at 10 000 g for 30 min (Ayadi et al., 2008). WHC was calculated by the relative weight according to Equation (3).

$$WHC \ (\%) = \frac{W_{after \ centrifugation}}{W_{beforecentrifugation}} \times 100$$
(3)

where W is the weight.

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#### 2.5. Microstructure observation

Small samples (1 cm long and 1 cm in diameter) were cut from the centre of the gel and used to perform microscopic observation. Egg white gel microstructure was examined by environmental scanning electron microscopy (ESEM) (Philips XL 30 ESEM, Japan). ESEM allows the observation of samples in their natural state, under controlled conditions of temperature and pressure. The environmental mode did not require any preliminary preparation; in addition, this mode removes completely the electronic loads effects on the surface and thus preserves the native structure and the water content of the sample. Microscopic observation was carried out in low vacuum mode with a pressure of 0.1 kPa. Gel sample was fixed to the support using double side adhesive tape.

## 2.6. Statistical analysis

Statistical analysis was performed using SPSS software (SPSS 24.0 for Windows, SPSS Inc., Chicago, IL, USA). Results were subject to a one-way analysis of variance (ANOVA) using the Ducan test at a

#### Table 1

Texture profile analysis of egg white gel as affected by pH. Means of the same columns followed by different lower case letters are statistically different (P < 0.05).

Egg white	Hardness (N)	Elasticity	Cohesiveness (N)	Adhesion (N)	
Control pH 4.5 6.5 8.0	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 8.69 \ \pm \ 0.13^{a} \\ 7.57 \ \pm \ 0.00^{b} \\ 8.57 \ \pm \ 0.09^{a} \\ 8.15 \ \pm \ 0.03^{c} \end{array}$	$\begin{array}{l} 0.44 \ \pm \ 0.08^{a} \\ 0.55 \ \pm \ 0.01^{b} \\ 0.40 \ \pm \ 0.02^{a} \\ 0.31 \ \pm \ 0.03^{c} \end{array}$	$\begin{array}{rrrr} 2.65 \ \pm \ 0.49^a \\ 2.37 \ \pm \ 0.36^a \\ 1.73 \ \pm \ 0.18^b \\ 1.25 \ \pm \ 0.14^c \end{array}$	

confidence level of 95% (there is a significant difference if p < 0.05).

## 3. Results and discussion

## 3.1. Effect of pH

pH is known to affect the net charges of protein molecules (Kim et al., 2016). Three different pH values (4.5, 6.5 and 8.0) were carried out to investigate the effect of protein charge on EW protein gelation. Mechanical properties of EW gels were determined by measuring four parameters (hardness, elasticity, cohesiveness and adhesion). Texture profile analysis of EW gels as affected by pH variation was given in Table 1. EW gel became less firm by varying the pH, comparing to the control (pH 7.0). The lowest hardness value was obtained at pH neighbouring the isoelectric point (Ip) of numerous EW proteins (4.5). It is well known that EW gels exhibited a low linear relationship between firmness and pH ( $R^2$  0.59) (Raikos, Campbell, & Stephen, 2007) which may explain the decrease of hardness even at basic pH (8.0). Elasticity of gels was practically none affected at pH 6.5 and 8.0. However, at pH 4.5 EW gel had a significant lower elasticity. Cohesiveness strength (N) of EW gel decreased with alkaline pH (8.0), on the other hand, this force increased with pH 4.5 (0.55 N). At this pH, EW gel maintains an intact network structure, indeed, it is able to withstand a second deformation after undergoing a first deformation. Adhesion (N) values (Table 1) where significantly decreased at pH 6.5 and 8.0 whereas this parameter was relatively unaffected at pH 4.5. Similar findings where obtained by Raikos et al. (2007) where the highest value of adhesion strength was obtained at pH 5.0 (9.46  $\pm$  0.20 mJ) comparing to pH 8.0 (6.48 ± 0.28 mJ).

Texture profile of EW gels were influenced by pH due to the variation of the net charge of proteins. Microstructure of EW gels and consequently their mechanical properties are affected by the balance between protein-protein and protein-solvent interactions (Stading, Langton, & Hermansson, 1993). Protein-protein interactions increase near the isoelectric point (Ip). Otherwise, protein-solvent interactions increase as the pH increases above the Ip.

Microscopic observation of the gel structure could explain the variation of its textural parameters. Egg white proteins form a continuous network (proteins-solvent interactions), well built, which produces a fine and uniform gel at pH 7.0 (Fig. 2A). Whereas at pH 6.5 and 4.5 (Fig. 2B and C respectively), microscopic observation showed a weakening of the protein network and a breaks of the gel obtained at pH 4.5. This coarse network with large pores can be explained by the change of proteins behavior near the isoelectric pH particularly the ovalbumin at pH 4.5 and ovotransferrin at pH 6.5. Indeed, for pH 4.5, protein aggregation represents the major phenomenon for the gel formation, whereas for the native pH (7.0), gels are composed of order linear polymers where aggregation is limited by the repulsive electrostatic forces (Bertrand & Turgeon, 2007). Previous literature showed that the EW gel network was coarse, open, and constituted of random aggregates at pH 5 (Croguennec, Nau, & Brulé, 2002), which was consistent with our results.

Water holding capacity (WHC) is an important factor in food products based on egg white gel. pH of protein dispersion influence WHC of EW gel as shown in Fig. 3A. WHC of EW gels increased gradually from 70.42% at pH 4.5-83.62% at pH 8.0. These results are similar with previous reports (Croguennec et al., 2002; Handa, Takahashi, Kuroda, & Froning, 1998). The highest value of WHC was observed for the control (90.03%). This finding can be ascribed to the small pores of the gel (Fig. 2A) and WHC was highest as free water was swiftly entrapped. The decrease of pH involved the decrease of WHC to a minimum value at pH 4.5 (Fig. 3A). This result may be attributed to the isoelectric point of ovalbumin (4.5), the most abundant protein in albumen (Mine, 1995). At this pH, the electrostatic repulsions where reduced because the net charge approaches zero and electrostatic interactions between protein and water are so reduced. As a result, rapid coagulation takes place and water is liberated. This decrease of WHC of EW gel at lowest pH values reinforces the previous drawn conclusions about the gel structure and texture. In fact, for the acid pH, EW gel became fragile and protein network was mainly based on protein-protein interactions (aggregation) which led to the liberation of water.

## 3.2. Effect of sugar and salt addition

Considering the importance of sucrose and salt in the preparation of pastry products based on egg white proteins, by their contributions to the texture, flavor and color of the final products, it is necessary to study the effect of these ingredients on the gelling properties of egg white. Texture profile analysis of egg white gels as affected by different concentrations (1%, 3% and 6%) of sugar and salt was given in Table 2. Hardness values increased in the presence of sugar and salt with one exception of the concentration 1% of salt where firmness was slightly decreased. It was also reported that NaCl increased slightly hardness of gel at pH 7.0 (native pH in our study) (Woodward & Cotterill, 1986). With respect to sucrose or salt addition, it was found that the stronger gel was obtained with 6% of sucrose. Similar results were reported by another study (Kulmyrzaev, Bryant, & McClements, 2000) and it was suggested that sucrose increased the rigidity of whey proteins gel. The presence of sugar resulted in significant increase of protein gel rigidity due to build-up of protein-protein interactions (Hao et al., 2016). Indeed, sugars protects proteins against heat denaturation by altering water structure, promoting hydrophobic interactions of proteins (Campbell, Raikos, & Euston, 2003). Mohammadi Nafchi, Tabatabaei, Pashania, Rajabi, and Karim (2013) noted that the increase of sugar concentration contributed to the increase of the protein stability of ovalbumin and ovotransferrin. Except of 6% of salt, gel elasticity increased in the presence of sugar and salt reaching a maximal value of 9.13  $\pm$  0.15 for 1% of salt (Table 2). On the other hand, gel cohesion strength decreased in the presence of different concentrations of sugar or salt. This was due to the fact that the connectivity of EW gel network was disrupted by the presence of sugar and salt, which makes the gel structure less resistant to strain. In this context, Woodward and Cotterill (1986) have demonstrated that NaCl had limited denaturation of proteins during heating which contributed to the escape of proteins in the serum. Recently, Li et al. (2018a,b) have reported that denaturation of heat-treated proteins in the presence of NaCl decreased the ordered secondary structure ( $\alpha$ -helix and  $\beta$ -sheet) by breaking hydrogen bond for EW protein gel.

According to Table 2, EW gel adhesion values were statistically comparable with that of the control with one exception (6% of sugar). It seems that sugar or salt addition did not affect if any, the adhesiveness of EW protein gels.

Gel structure is affected by a number of factors such as protein state and concentration, heating rate and temperature during gelation, pH and ionic strength (Campbell et al., 2003). Formation of intermolecular disulfide bonds during gelation, hydrophobic and electrostatic interactions contributes to the egg albumen gel network formation and texture (Hammershoj, 2001; Matsudomi, Kanda, & Moriwaki, 2003). Rupture force ( $\sigma_f$ ) and gel elasticity ( $\varepsilon_f$ ) are parameters directly related to this structure.

The effect of sugar and salt addition on mechanic properties of EW

(A)



**Fig. 2.** Microscopic images of egg white gel microstructure. (A) Control, (B) pH 6.5, (C) pH 4.5, (D) 6% of sugar; (E) 6% of salt, (F) 0.01% of xanthan, (G) 0.2% of xanthan, (H) 0.2% of guar, (I) 0.2% of karaya, (J) 0,2% of locust bean. Magnification is 800; scale bar =  $50 \,\mu$ m.

gels, evaluated by the true axial stress  $\sigma_f$  and Hencky strain  $\varepsilon_f$ , was presented in Fig. 4A and B. EW gel strength ( $\sigma_f$ ) was increased up to approximately 30 kPa in the presence of the lowest sugar concentration (1%). However, by increasing concentration, gel strength decreased until reaching the lowest value in the presence of 6% of sucrose. A similar study by Mohammadi Nafchi et al. (2013) revealed that the addition of sucrose to EW did not cause any significant difference on gel strength when heated at 120 °C for 1 h. It was also appearing that the addition of salt increased slightly the EW gel strength (Fig. 4A). Li et al. (2018a,b) reported that phosphate salts are responsible for the higher strength of the whole egg protein gels.

Hencky strain ( $\varepsilon_f$ ) is the characteristic of gel elasticity. Fig. 4B shows Hencky strain ( $\varepsilon_f$ ) of EW gel obtained by sucrose and salt addition. The addition of sucrose at a concentration higher than 1% improved the EW gel elasticity. Moreover, by adding 1% and 3% of salt to the EW, gel elasticity was increased. However, EW gel became less elastic when salt concentration reaches 6%. Hammershoj (2001) revealed that increasing ionic strength by addition of salt increased gel elasticity and did not affect gel strength, which correlate with our findings. However, Li et al. (2018a,b) reported that the addition of NaCl up to 3% decreased the fracture stress and strain of EW gels. This difference may be due to the variation of time heating which has a crucial role in the final structure



**Fig. 3.** Evolution of water holding capacity of egg white gel as a function of pH (A), Sucrose/Salt (B) and Hydrocolloid gums (C).

## of the gel (Woodward & Cotterill, 1986).

During heat treatment and prior to food additive addition, the forces within the protein aggregates are generated by intra and inter-molecular disulfide bond formation (Wongsasulak, Yoovidhya, Bhumiratana, & Hongsprabhas, 2007). In the presence of Na<sup>+</sup>, the heat-denatured protein process involves the simultaneous aggregation of soluble proteins. In fact, Na<sup>+</sup> replaces the water molecule by playing the role of binder between proteins forming the network. The aggregation rate of these proteins can be controlled by NaCl concentration (Nasabi, Labbafi, Mousavi, & Madadlou, 2017). In the case of sucrose, there are weakly interactions between sucrose molecules and proteins. Indeed, sucrose interact with the water monolayer surrounding proteins that limit electrostatic interactions. Hence, hydrophobic interactions take place, which increase the rigidity of protein gels (Hao et al., 2016; Nasabi et al., 2017).

Microscopic pictures of EW gel microstructure in presence of 6% of sugar and salt were presented in Fig. 2D and E respectively. Microscopic observation of EW gel structure as affected by 6% of sucrose showed an airy and spongy protein structure (Fig. 2D). However, development of protein aggregates in presence of Na<sup>+</sup> was clearly identified (Fig. 2E). The structure of the EW gel obtained after sugar addition could be explained by the sucrose action on the protein structure and the increasing of environment viscosity. Indeed, at low concentration, sucrose plays the role of charged molecule modifying thus the medium ionic force. This result could explain the spectacular increase of gel strength ( $\sigma_f$ ) for the fewer concentration (1%) (Fig. 4A). By increasing the concentration, sucrose induces an increase in viscosity reducing thus the free charges mobility in the environment. This result could explain the significant drop of gel strength by increasing the sucrose concentration. On the other hand, the presence of Na<sup>+</sup> in EW medium promotes protein-protein interactions in depends of protein-water interactions during gelation. Consequently, the EW gel results from an arbitrarily aggregation of proteins compared to the well-ordered structure in the absence of Na<sup>+</sup> molecules (Fig. 2A) (Nasabi et al., 2017).

EW gel is able to provide a suitable network for holding water up to 90.03% (0% NaCl). Sucrose and salt, when added, modify WHC of EW gel as shown in Fig. 3B. Sucrose enhanced WHC of the gel to roughly 94% at concentrations of 1% and 3%. The spongy and aerated gel structure obtained in presence of sucrose support the retention of water in small cavities presented in the gel network (Fig. 2D). However, the addition of salt to EW decreases significantly WHC of the gel to 65.73% in presence of 6% of salt. This finding would be explained by the phenomenon of aggregation. In fact, in absence of salt, water molecules play the role of binder between soluble proteins. The present of salt involves the substitution of water molecules by Na<sup>+</sup> and thereby the tendency of proteins to random aggregations reducing thus WHC of the gel (Campbell et al., 2003). It was also reported that the addition of NaCl to EW decreased WHC of the gel (Croguennec et al., 2002; Li et al., 2018a,b).

## 3.3. Effect of hydrocolloid gums

Texture profile analysis of EW gel in presence of xanthan, guar, karaya and locust bean gums was shown in Table 3. Results showed that gel firmness decreased by increasing xanthan concentration, however, and except of 0.20% of guar and karaya gums, gel firmness was enhanced with guar, karaya and locust bean gums addition. These findings are in agreement with those of Razi, Motamedzadegan, Shahidi and Rashidinejad (2018), who reported an improvement of the hardness of EW gel by the increase of basil seed gum concentration. Electrostatic and non-electrostatic interactions between biopolymers have a great potential on the final gel structure and firmness (Souza, Souza, Heckert, & Garcia-rojas, 2018). Low attractive forces between molecules allow to obtain weak gels. Furthermore, it is well known that carboxylated polysaccharides, such as xanthan, exhibit a limited interaction with proteins (Doublier, Garnier, Renard, & Sanchez, 2000) which could explain the decrease of gel hardness by increasing xanthan concentration. Moreover, EW gel elasticity increased in presence of these food gums. However, gel elasticity decreased with the increase of xanthan and karaya gum concentration to 0.20%. Results demonstrated that food gums addition improved some of EW gel mechanical properties but just at well defined concentrations. In fact, addition of 0.10% of xanthan gum enhances significantly gel elasticity, whereas addition of 0.20% of karaya gum decreases EW gel mechanical properties. Except of 0.20% of guar gum addition, cohesion strength values showed that xanthan, guar (at 0.01 and 0.10%), karaya and locust bean gums

#### Table 2

Texture p	rofile	analysis	of egg	white	gels as	affected	by	different	concentrations	of sugar a	nd salt.
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Egg white		Hardness (N)	Elasticity	Cohesiveness (N)	Adhesion (N)
Control		$5.98 \pm 0.02^{a}$	$8.69 \pm 0.13^{a}$	$0.44 \pm 0.08$ <sup>a</sup>	$2.65 \pm 0.49$ <sup>a</sup>
Sugar (%)	1	$6.52 \pm 0.28^{b}$	$8.73 \pm 0.09^{b}$	$0.38 \pm 0.00^{\rm b}$	$2.44 \pm 0.27^{a}$
	3	$7.42 \pm 0.35$ <sup>c</sup>	$8.75 \pm 0.48^{b}$	$0.36 \pm 0.06^{\text{b}}$	$2.57 \pm 0.68^{a}$
	6	$7.53 \pm 0.30$ <sup>c</sup>	$8.85 \pm 0.07$ <sup>c</sup>	$0.41 \pm 0.07^{a}$	$3.37 \pm 0.16^{b}$
Salt (%)	1	$5.87 \pm 0.06^{a}$	$9.13 \pm 0.15^{d}$	$0.35 \pm 0.01$ <sup>b</sup>	$2.07 \pm 0.09^{a}$
	3	$6.23 \pm 0.04^{\rm b}$	$8.78 \pm 0.28^{\rm b}$	$0.37 \pm 0.05^{\text{ b}}$	$2.33 \pm 0.34^{a}$
	6	$6.60 \pm 0.38^{b}$	$7.83 \pm 0.18^{e}$	$0.35 \pm 0.04$ <sup>b</sup>	$2.28 \pm 0.75^{a}$

Means of the same columns followed by different lower case letters are statistically different (P < 0.05).



Fig. 4. Texture evaluation of egg albumen at fracture point as function of different concentrations of sucrose/salt (A, B) and food gums (C, D): true axial stress (A, C) and Hencky strain (B, D).

addition decreased the capacity of the EW gel to maintain intact its network structure which make it less resistant to strain. Results of Table 3 showed that food gums addition decreased generally the EW gel adhesion strength. However EW gel adheres better to the specific surface in presence of the lowest concentration (0.01%) of guar, karaya and locust bean gums. According to these results, it was appeared that the addition of 0.20% of karaya gum deteriorates gel mechanical properties, however the presence of 0.01% of locust bean gum enhance EW gel mechanical properties. Likewise, a previous report has shown that xanthan has a significant effect on improving gelling properties of whey protein isolate when added at concentration as low as 0.01% (Bertrand & Turgeon, 2007).

Texture of EW gels, evaluated by the true axial stress  $\sigma_f$  and Hencky

strain  $\varepsilon_{f}$ , obtained with the addition of different concentrations of xanthan, guar, karaya and locust bean gums was shown in Fig. 4C and D. Gel strength ( $\sigma_f$ ) of EW gel was significantly decreased by the addition of xanthan gum (Fig. 4C). Indeed,  $\sigma_f$  was 2.5 times less than that of the control by adding a relatively high concentration (0.20%) of xanthan gum. Similarly, the addition of 0.20% of guar and karaya gums decreased EW gel force. However, the addition of locust bean gum didn't practically modify the strength of EW gel. Fig. 4D shows that the Hencky strain ( $\varepsilon_f$ ) was increased by 0.01% of guar gum. Karaya and locust bean gum, when added, improved the EW gel elasticity. Indeed,  $\varepsilon_f$  reaches a maximum value in presence of 0.20% of locust bean gum. However, this parameter was decreased by increasing karaya gum

#### Table 3

Texture profile analysis of egg white gels as affected by different concentrations of xanthan, guar, karaya and locust bean gums.

Egg white		Hardness (N)	Elasticity	Cohesion strength (N)	Adhesion (N)
Control Xanthan gum (%) Guar gum (%) Karaya gum (%) Locust bean gum (%)	0.01 0.10 0.20 0.01 0.20 0.01 0.10 0.20 0.01 0.10 0.20 0.2	$\begin{array}{r} 5.98 \ \pm \ 0.02 \ ^{a} \\ 5.75 \ \pm \ 0.09^{a} \\ 4.81 \ \pm \ 0.13^{b} \\ 3.41 \ \pm \ 0.20^{c} \\ 7.31 \ \pm \ 0.59^{d} \\ 6.20 \ \pm \ 0.46^{e} \\ 5.46 \ \pm \ 0.49^{f} \\ 7.16 \ \pm \ 0.10^{d} \\ 6.09 \ \pm \ 0.12^{e} \\ 5.05 \ \pm \ 0.04^{b} \\ 7.30 \ \pm \ 0.25^{d} \\ 7.13 \ \pm \ 0.29^{d} \\ 6.38 \ \pm \ 0.61^{e} \end{array}$	$\begin{array}{r} 8.69 \ \pm \ 0.13^a \\ 8.90 \ \pm \ 0.09^b \\ 9.79 \ \pm \ 0.14^c \\ 7.82 \ \pm \ 0.26^d \\ 8.66 \ \pm \ 0.42^a \\ 8.95 \ \pm \ 0.47^b \\ 8.55 \ \pm \ 0.26^a \\ 8.94 \ \pm \ 0.60^b \\ 8.81 \ \pm \ 0.33^b \\ 6.12 \ \pm \ 0.13^c \\ 9.36 \ \pm \ 0.54^c \\ 8.74 \ \pm \ 0.11^a \\ 9.78 \ \pm \ 0.37^c \end{array}$	$\begin{array}{l} 0.44 \ \pm \ 0.08^a \\ 0.33 \ \pm \ 0.01^b \\ 0.36 \ \pm \ 0.03^b \\ 0.42 \ \pm \ 0.05^b \\ 0.42 \ \pm \ 0.06^a \\ 0.42 \ \pm \ 0.00^a \\ 0.45 \ \pm \ 0.01^a \\ 0.43 \ \pm \ 0.02^a \\ 0.37 \ \pm \ 0.02^b \\ 0.25 \ \pm \ 0.04^c \\ 0.42 \ \pm \ 0.04^c \\ 0.36 \ \pm \ 0.01^b \\ 0.39 \ \pm \ 0.01^b \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

Means of the same columns followed by different lower case letters are statistically different (P < 0.05).

concentration (Fig. 4D). According to these results, it appeared that mechanical properties of EW gel depends on the nature and the concentration of the added food gum. It is well known that the protein network plays a crucial role in providing mechanical properties of the gel (Campbell et al., 2003). The balance between the attractive and repulsive forces among polymer network, polymer molecules and surrounding solvent could manipulate the strength of a protein network during gelation or the structure-forming process. Indeed, proteinpolysaccharide interactions take place at pH less than the isoelectric point of proteins (Zhang, Zhang, & Vardhanabhuti, 2014). At pH 7.0 (which is the pH of egg albumen in our study), xanthan and karaya gums exhibit a net negative charge. Hence, complexation could occur only with positive charged proteins, as lysozyme (Ip = 10.7 (Campbell et al., 2003)), or with positive patches on the proteins (Bertrand & Turgeon, 2007), and this may explain the decrease of the strength of the gel in presence of xanthan and karaya gum (Fig. 4C). Guar and locust bean gums are neutral polysaccharides widely used as agents for thickening and stabilization (Grenha & Dionísio, 2012; Zhang et al., 2014). On the other hand, the addition of food gums at different concentrations provides a mixed network with different force levels. With high hydrocolloid concentrations, protein network has the lowest mechanical properties compared to thus without additives (Table 3, Fig. 4C). The increase of the  $\varepsilon_f$  values in presence of food gums would be related to strain transmission, during mechanical test, through the polysaccharide network. Gelling is among the various functional properties of proteins and polysaccharides and the macromolecular distribution of biopolymers in mixed gels leads to either synergetic or antagonist effects on the network gel formation (Bertrand & Turgeon, 2007).

Fig. 2 shows microscopic observation of the internal EW gel microstructure in presence of hydrocolloids tested in this study. 0.01% of xanthan gum (Fig. 2F) and the highest polymer concentration, 0.2% of xanthan, guar, karaya and locust bean gums (Fig. 2G, H, I, J respectively) where chosen as examples to determine the microstructure of the gel after hydrocolloids addition. Fig. 2A shows that EW proteins form a continuous filamentous network prior to food gums addition. EW gel in presence of 0.01% of xanthan gum consisted of a linear strand-like structure where xanthan appeared to fit between protein networks. At low concentration of xanthan, positively charged protein molecules may neutralize the most of negative carboxyl groups (COOH<sup>-</sup>) of xanthan gum (Souza & Garcia-rojas, 2017), which leads to a mixed network by xanthan-proteins interactions. A similar result was reported for β-lactoglobulin/xanthan gum hydrogels, involving the formation of a three-dimension network where β-lactoglobulin bind along xanthan gum, suggesting that proteins act as crosslinking agents

(Le & Turgeon, 2013). However, no clear sign of protein network was observed on the EW gel by increasing xanthan concentration (Fig. 2G), indicating that polysaccharide network covers protein network and then proteins are enchased into this network. Similar microscopic observations where deducted from EW-guar and EW-karaya gum structures which would explain the decrease of EW gel firmness and strength in presence of 0.2% of xanthan, guar and karaya gums (Table 3, Fig. 4C). Before gelation, anionic polysaccharides (i.e. xanthan and karaya gums), in solution, can form complexes and/or coacervates with EW proteins. Soluble complexes are occurred when proteins and polysaccharides carry the same charge, while coacervation took place when biopolymers exhibit an opposite sign (Noel et al., 2007). At pH 7, ovalbumin/xanthan gum mixture showed a turbidity of 15% in presence of 0.01 mol/L NaCl indicating the formation of co-soluble polymers, however, Lysozyme/xanthan gum mixture showed a maximum value of turbidity (100%) indicating the formation of large insoluble complexes and hence coacervation (Souza & Garcia-rojas, 2017). On the other hand, phase behavior of protein/polysaccharides mixtures depends on several factors such as biopolymer mixing weight ratio, pH, total solids, the net charge of proteins and polysaccharides and ionic strength (Doublier et al., 2000; Souza et al., 2018). Indeed, it was demonstrated that a diffuse  $\beta$ -lactoglobulin–xanthan gum aggregates was initially formed and then fractal aggregates were reorganized into more compact structures. The reformation process of the complexes structure was influenced by protein-polysaccharide ratio (Laneuville, Sanchez, Turgeon, Hardy, & Paquin, 2005). Souza et al. (2018) demonstrated that Ovalbumin/Carrageenan complexes and Lysozyme/Carrageenan complexes showed different behaviors according to the influence of pH and the ratio of complexes. The reorganization process of EW proteins in presence of high concentrations of hydrocolloids may explain the obtained structure after gelation (Fig. 2G, H, I). Moreover, the protein-polysaccharide complex formation contributes to molecular changes to proteins and polysaccharide components (Turgeon, Schmitt, & Sanchez, 2007). In fact, it was shown a loss in the amount of  $\alpha$ -helix protein structure after complexation and a gain in protein secondary structure after coacervation during complex formation/coacervation processes in β-lactoglobulin/Acacia gum system (Mekhloufi, Sanchez, Renard, Guillemin, & Hardy, 2005). Molecular changes of proteins may decrease their interactions with polysaccharides. In addition, heat treatment of EW proteins in presence of hydrocolloids may contributes to thermal aggregations of proteins especially in presence of electrostatic repulsion between complexes, which prevent heat-induced protein-protein interactions (Turgeon et al., 2007).

However, in the case of locust bean gum, which is a neutral polysaccharide, it was clear that proteins clusters where associated to locust bean gum in the specific sites. The coexistence of non-electrostatic interactions contribute to a new supramolecular structure with improved interaction between biopolymers (Souza & Garcia-rojas, 2017). This finding correlate with results of EW gel mechanical properties obtained in presence of 0.2% of locust bean gum (Table 3, Fig. 4C and D). Previous report studied the interpolymer complexation of lysozyme and ovalbumin with carrageenan and suggested that EW proteins may play a crucial role as a crosslinking agents along carrageenan chains (Souza et al., 2018).

Furthermore, the decrease of gel cohesion strength (Table 3) may be due to the fact that protein network was interrupted by hydrocolloids which involves a decrease of mixed network resistance to the applied strain.

WHC of EW gels as affected by different concentrations of food gums was tested. As demonstrated in Fig. 3C, the addition of hydrocolloids generally increased WHC of the EW gel comparing to the control (90.03%). Indeed, xanthan gum enhanced significantly gel WHC that reaches a maximum value of 97.18% in presence of 0.2% of xanthan. Likewise, guar gum, when added, increased WHC up to 97.89% at 0.1%. Similar results were reported in a recent study (Horstmann, Axel, & Arendt, 2018). Moreover, Razi et al. (2018) reported an increase of WHC of the gel by adding basil seed gum to the egg albumen. However, increasing concentration of karaya and locust bean gums to a value higher than 0.01% decreased WHC of the EW gel. We can suggest that this decrease of WHC is due to the phenomenon of saturations generated by gums concentration. Indeed, there is a threshold of concentration from which gel becomes denser, less porous and consequently harder and less elastic as demonstrated microscopic experiments (Fig. 2).

## 4. Conclusion

pH variation, sugar, salt and food gums addition have an influence on the gelling properties and WHC of the EW. The change of pH of EW alter significantly the microstructure of the gel. Indeed, when the pH is near to the isoelectric point of ovalbumin (4.5), which is the most abundant protein in the albumen, EW gel becomes brittle and therefore less firm. In addition, the decrease of pH decreased significantly the WHC of EW gel. The addition of sugar or salt enhances generally the EW gelling properties. However, the presence of salt reduced significantly the ability of the gel to retain water. While in the presence of sugar, the gel WHC was slightly improved. The addition of food gums led to a spectacular modification of the EW gel microstructure. Thus, a mixed network of proteins and polysaccharides constituted the obtained gel. The gelling properties of EW depend on the polysaccharide nature and concentration. In fact, the presence of xanthan gum decreased the gel strength and firmness. However, at a concentration of 0.1%, xanthan gum enhanced the elasticity of the EW gel. In other hand, the addition of guar gum did not almost modify the EW gelling property. The presence of karaya gum at concentrations below 0.2% improved the mechanical properties of EW gel, however, the gel strength decreased by the addition of this gum. Locust bean gum addition improved the mechanical properties of EW gel. However, the gel strength decreased by the increase of its concentration to 0.2%. The EW gel WHC was significantly improved in the presence of food gums. However, an increase of the karaya or locust bean gum concentration led to a decrease of the WHC of the EW gel.

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