Interactions of hydrocolloids and sonicated-gluten proteins

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Abstract

The aims of this article were to explore the nature of the interactions between certain commercial hydrocolloids and sonicated-gluten proteins and to evaluate their relation to bread quality. Dough rheology and bread quality were affected in different ways by the addition of hydrocolloids. Pectin and λ-carrageenan strengthened wheat dough and sodium alginate augmented the extensibility of dough. In addition, sodium alginate and pectin improve loaf volume and all the hydrocolloids tested decreased the initial bread crumb firmness and chewiness. This work demonstrated that carrageenan isoforms and pectin (sulphated and carboxylated hydrocolloids, respectively) can form hydrophilic complexes with gluten proteins and the capacity of complexation appears to be related to the density of the anionic group in the hydrocolloid.
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Keywords: Baking; Hydrocolloid; Rheology; Protein; SDS-PAGE

1. Introduction

The effects of hydrocolloids on the functional properties of wheat bread have been investigated; in such products gums improve dough stability, bread performance and bread shelf life (Christenson, 1976; Christianson, Gardner, Warner, Boundy, & Inglett, 1974; Collar, Andreu, Martínez, & Armero, 1999; Davidou, Le Meste, Debever, & Bekaert, 1996; Guarda, Rossel, Benedito de Barber, & Galotto, 2004; Mettler & Seibel, 1993; Rosell, Rojas, & Benedito de Barber, 2001; Sidhu, Singh, & Bawa, 2000). The effects of hydrocolloids on the functional properties of dough and bread quality depend on the nature, origin and particle size of the hydrocolloid, and the dosages of the hydrocolloid incorporated into dough formulations. Protein and polysaccharide functions are greatly affected by their interactions with each other and with other components of food systems (Dickinson, 1998; Preston, 1998). The nature of protein–polysaccharide interactions can vary widely due to wide variations in biopolymer structure and solvent conditions. Depending on these conditions, macromolecular interactions may be specific or non specific, weak or strong, repulsive or attractive (Tolstoguzov, 2003).

Only a few papers are available on interactions between hydrocolloid and gluten protein. Huebner and Wall (1979) found associative interactions among microbial polysaccharides, carrageenan and alginate with purified gluten protein. Howell, Bristow, Copeland, and Friedli (1998) showed an increase in viscosity of mixtures of deamidated gluten and sodium alginate. They mention two possible explanations: phase separation and electrostatic interactions between carboxyl groups and amide groups. In addition, in a previous study, León et al. (2000) found that a fraction of hydrophobic gluten protein interacted with λ-carrageenan, and they suggested that sulphate groups of hydrocolloids and the amino groups of glutamines present in the gluten protein are involved in the interaction.

Because of the different baking qualities of wheat flours, the new technologies and the different properties of baked goods elaborated all over the world, the use of additives to control their rheology and texture have become a common practice in the baking industry. One group of additives used successfully in food industry to modify the rheological and textural properties of emulsions, suspensions and foams are hydrocolloids. In the baking industries, hydrocolloids are of increasing importance but there is a lack of information on the interactions between different hydrocolloids and gluten

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proteins. More studies are necessary to explain the effects of hydrocolloids on bread quality.

The aims of this article were to explore the nature of the interactions between hydrocolloids and sonicated-gluten proteins and to evaluate their possible relation to bread quality.

2. Materials and methods

2.1. Materials

Commercial bread flour was obtained from the local market (Carlos Boero Romano SAIC, Argentine). Flour characteristics were protein 10.9%, ash 0.65% and moisture content 14%. Vital gluten was purchased from Avebe Argentina S.A. Hydrocolloids were obtained from of different source. Low molecular weight sodium alginate (AL), κ-carrageenan (TYPE III) (κ-C), ι-carrageenan (type V) (ι-C) and λ-carrageenan (type IV) (λ-C) were purchased from Sigma Chemical Co. Carob gum (CG) and guar gum (GG) were purchased from Lucid Group Ltd (India), high methoxyl-pectin (PE) was obtained from Danisco (Brazil) and xanthan gum (XG) was purchased from Jungbunzlauer (Switzerland).

2.2. Micro-extension procedure

Measurements were performed with a TA.XT2i texture analyser (Stable Micro Systems, Surrey, UK) using the SMS/KIEFFER RIG for dough extensibility measurements. Doughs were prepared using a standard dough formulation (100% flour, 1.8% sodium chloride and 60% of water). Hydrocolloids were incorporated at a 0.5% (flour base) level. Ingredients were mixed in a Philips HR 1495 mixer (Philips, Argentina) for 2 min and rested for 15 min in a cabinet at 30 °C and 70% rh. Salt was previously dissolved in water and the remaining ingredients were added as solids. Rounded dough (20 g) was pressed by the strip form and allowed to relax for 40 min. Ten strip by batch were placed on the platform, trimmed and extended until their elasticity was exceeded and the dough broke. The dough strips were extended to 3.3 mm/s (Suchy, Lukow, & Ingelin, 2000). Resistance to extension (R_m, maximum resistance), extensibility (E, maximum extensibility) and area under the curve (A) were automatically calculated from the curves by the software supplied with the texturometer.

2.3. Baking procedure

The recipe and breadmaking process followed here are those currently employed in our country in the preparation of bread. The dough formulation used in this study comprised: 100% wheat flour (protein 13.2%, water 11.8%, ash 0.7%), 3% compressed yeast (CALSA, Buenos Aires, Argentina), 1.8% sodium chloride, 0.2% sodium

propionate, 0.015% ascorbic acid and 63% of water. Hydrocolloids were incorporated at 0.5% (flour base) level. Water addition was based on a faringraph test using the 500 BU line. Ingredients were mixed in an Argental L-20 mixer (Argental, Santa Fe, Argentina). Yeast and salt were separately dissolved in water and the remaining ingredients were added as solids. The resulting dough was allowed to rest for 15 min in a cabinet at 30 °C and 70% rh, and, then the bulk dough was sheeted in a Mi-Pan vf roller (Mi-Pan, Cordoba, Argentina) having two rolls of 50×12.7 cm^2. The dough was then divided into 80 g pieces, hand-molded, proofed at 30 °C (96% rh) up to its maximum volume increment (Armero & Collar, 1998) and baked at 200 °C for 18 min.

Bread loaf specific volume was determined by rapeseed displacement and weighed 24 h after baking.

2.4. Bread crumb texture

Texture profile analysis (TPA) parameters were determined by using a TA-XT2i texturometer (Stable Microsystems, Surrey, UK) equipped with 5 kg load cell. A cylinder probe with 3.6 cm of diameter was attached to moving crosshead.

The bread loaves were wrapped up into polyethylene bags and stored at 20 ± 1 °C and 75 ± 5% rh. At timed intervals (0, 3 and 7 days), 3 bread loaves were cut into 2 slices (2.5 cm thick) and the ends were discarded. Each slice was subjected to a double cycle of compression, under the following conditions: crosshead speed, 100 mm/min and maximum deformation, 40%. The texture profile parameters were determined using the Texture Expert 1.22 (Stable Microsystems, Surrey, UK). The bread crumb firmness (force required to compress a substance between incisor teeth) and chewiness (the quantity to simulate the energy required to disintegrate a solid food to a state ready for swallowing) were calculated from a force-distance graph (Carr & Tadini, 2003; SMS, 2001).

Six slices were analysed per point, and average values were reported.

2.5. Gluten−hydrocolloid interactions

To study the interaction between hydrocolloids and gluten proteins, commercial vital gluten (100 mg) and hydrocolloids (5 mg) were mixed before the addition of 2.5 ml of distilled water (pH 6.5). The resulting mix was vortexed for 5 min, sonicated for 5 min in a Branson sonifier at point 5, and centrifuged for 10 min at 1000g. Besides, hydrocolloid−gluten mixtures were centrifuged at 1000g, 6000g and 12,000g, when the causes of protein solubilization were studied.

The supernatants were tested for viscosity and content of soluble proteins ($N \times 5.7$). The proteins were characterized by SDS-PAGE.

2.5.1. Viscosity

Viscosity of the prepared hydrocolloid, of the gluten and of the gluten–hydrocolloid suspensions (supernatant) was measured using a Brookfield viscometer with temperature control (Stoughton, MA, USA). The pure hydrocolloid solutions and pure gluten protein solutions were made following the same procedure described for gluten–hydrocolloid suspensions. All measurements were carried out at 38/s shear rate, at 30 °C, and 1 ml sample volume.

2.5.2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

One volume of supernatants were mixed with two volumes of sample buffer (0.063 M Tris–HCl pH 6.8, 1.5% (w/v) SDS, 3% mercaptoethanol, 10% (v/v) glycerol and 0.01% (w/v) blue bromophenol) and the suspensions were heated in a boiling water bath for 3 min and allowed to cool. A constant volume of each sample was loaded into the gel. Molecular weight standards were obtained from Bio-Rad (SDS-PAGE MW standards, Broad range, Bio-Rad Laboratories, Hercules, USA). SDS-PAGEs were performed according to Laemmli (1970) using gels of $T = 12\%$ and $C = 2.7\%$. The gels were 0.75 mm thick and consisted of a 2 cm stacking gel and 8 cm running gel. Twenty microlitre of each sample was loaded onto each slot. The electrophoresis was conducted at a constant voltage of 150 V until the front reached the end of the gel (approximately in 90 min). A mini Proten II Slab Cell (Bio-Rad Laboratories, Richmond, CA) was used. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol:water:acetic acid (4:5:1 v/v) and distained in the same solvent.

2.6. Statistical analysis

All analyses were done in duplicate unless otherwise indicated. The data obtained were statistically treated by variance analysis while the means were compared by the LSD Fisher test at a significance level of 0.05, in both cases using the INFOSTAT statistical software (Facultad de Ciencias Agropecuarias, UNC, Argentina).

3. Results and discussion

3.1. Effects of hydrocolloids on dough extension and bread quality

The effect of hydrocolloid addition on the micro-extensigraph measurements after 45 min resting time is shown in Table 1. The maximum resistance (R_m) and the area under the curve (A) were taken as measure of dough strength, with larger values indicating greater strength. R_m to extension augmented significantly ($P < 0.05$) with the addition of λ-C and PE while AL decreased this parameter.

Table 1
Breadmaking quality of dough elaborated with hydrocolloids

Sample	R_m (g)	E (mm)	A (g s)	SLV (cm³/100 g)
Control	49.1 bc	33.1 bc	366.9 a	330 bc
κ-C	47.1 bc	35.5 cd	384.3 ab	338 c
ι-C	50.2 bc	37.1 de	447.3 c	327 b
λ-C	79.2 e	26.5 a	426.8 c	337 c
AL	36.1 a	49.6 f	437.1 c	376 e
CG	46.3 b	39.7 e	436.9 c	336 c
GG	47.3 bc	38.2 de	420.4 bc	360 d
PE	60.7 d	31.7 b	425.4 bc	355 d
XG	50.4 c	31.1 b	352.2 a	304 a

Values followed by the same letter in the same column are not significantly different ($p < 0.05$). κ-C, κ-carrageenan; ι-C, ι-carrageenan; λ-C, λ-carrageenan; AL, low molecular weight sodium alginate; CG, Carob gum; GG, Guar gum; PE, high methoxyl-pectin, XG, xanthan gum; R_m, maximum resistance; E, maximum extensibility; A, area under the curve; SLV, specific loaf volume.

AL, CG, GG and ι-C addition yielded an increase ($p < 0.05$) of dough extensibility (E) while λ-C decreased it. PE, κ-C and XG did not affect dough extensibility.

A was augmented by the presence of ι-C, λ-C, AL, CG, GG and PE. Conversely, XG and κ-C did not affect either R_m or A.

Loaf volume increased significantly ($p < 0.05$) by the presence of AL, GG and PE and the highest increment was produced by AL. XG caused a drop of loaf volume in agreement with the results of Christenson (1976), while the rest of hydrocolloids did not affect it (Table 1).

Previous studies have showed the effect of these hydrocolloids on bread quality. Guarda et al. (2004) observed that specific loaf volume was improved by the addition of κ-C and XG, but AL adversely affected it. These discrepancies could be the consequence of wheat flour quality used, the amount of water used in this bread recipe because these authors worked with constant dough consistency whereas we worked at a constant water percentage; the different chemical structure and origin of the hydrocolloids tested and the different bread making process utilized.

The effect of hydrocolloid addition on bread crumb firmness are shown in Fig. 1. The addition of hydrocolloids decreased significantly ($p < 0.05$) the initial crumb firmness and AL promoted the largest effect. The effects of alginate on crumb firmness are related with the loaf volume increment (Pearson coefficient, $r = -0.58$). The differences in initial crumb firmness among breads with hydrocolloid were slight. Carrageenan isoforms and PE yielded similar results while CG, GG and XG displayed the highest values of initial crumb firmness from bread with hydrocolloids. In addition, firmness was lower after 3 days of storage in the presence of hydrocolloids; the exception was the sample with XG, which showed similar firmness to that of the control (without hydrocolloid) according to Christianson et al. (1974). On the basis of the bread formulation employed in this study, the acceptability of the final product is lost within 3 days. However, after 7 days of storage, only
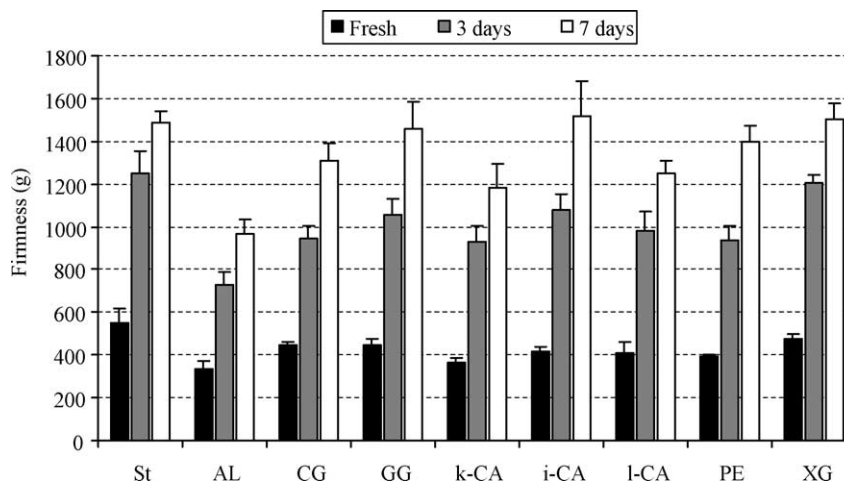
Fig. 1. Effect of hydrocolloid addition on bread crumb firmness during bread staling. κ-C, κ-carrageenan; ι-C, ι-carrageenan; λ-C, λ-carrageenan; AL, low molecular weight sodium alginate; CG, Carob gum; GG, Guar gum; PE, high methoxyl-pectin, XG, xanthan gum. Error bars show standard deviation.

AL, κ-C and λ-C yielded significant lower firmness than that of the standard bread. With regard to rate of bread firming, only the addition of AL decreased it in comparison with control sample.

The influence of hydrocolloids on firming might result from changes occurring in the amorphous part of the starch - perhaps by inhibiting gluten–starch interactions or the development of macromolecular entanglement- or from the capacity of the gums to retain water even after baking (Davidou et al., 1996; Heflich, 1996).

The addition of hydrocolloids decreased the initial chewiness of the bread crumb (Fig. 2), and AL produced the highest effect again; indicating that bread with hydrocolloids require lesser energy to disintegrate that control bread. The differences in initial chewiness among breads with hydrocolloids were slight and had similar behavior to the crumb firmness. During the first 3 days of storage, hydrocolloids affected the chewiness in different way, while some of them (AL, GG, CG, PE, κ-C and λ-C) continued to have low values of chewiness, others (ι-C and XA) seemed

to lose the improving effect on chewiness. After 7 days of storage, only AL yielded significant by lower chewiness than the control bread.

3.2. Effects of hydrocolloids on gluten protein solubility and viscosity

In order to investigate the interaction between gluten protein and hydrocolloids, we studied the viscosity, the protein content and the protein pattern of the supernatant from mixtures of vital gluten and different hydrocolloids. The viscosity of hydrocolloid solutions and of hydrocolloid–gluten supernatants was analysed at one shear rate to know if the presence of protein changed the hydrocolloid viscosity and to obtain additional information of hydrocolloid–protein interaction. The effect of hydrocolloid addition on the viscosity measurements is summarized in Table 2. Water viscosity was increased by the hydrocolloid addition. XG and λ-C showed the highest viscosity, while CG, AL and PE had the lowest values. λ-C showed higher
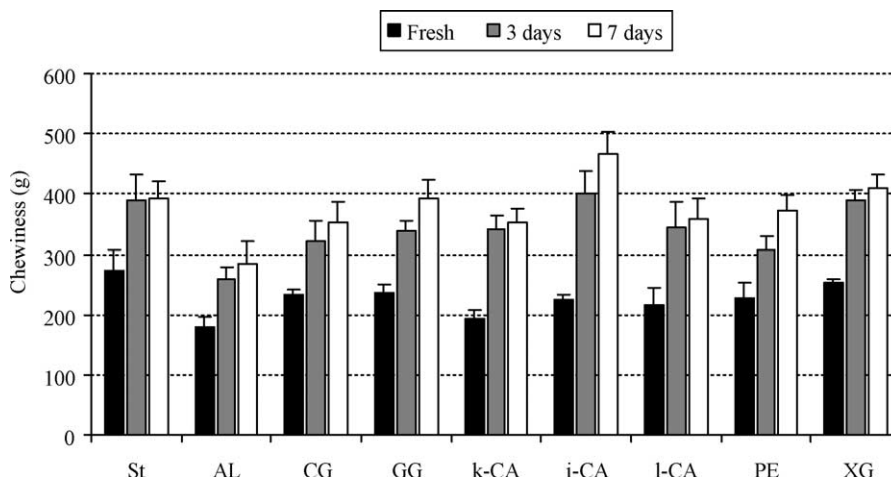

Fig. 2. Effect of hydrocolloid addition on bread crumb chewiness during bread staling. κ-C, κ-carrageenan; ι-C, ι-carrageenan; λ-C, λ-carrageenan; AL, low molecular weight sodium alginate; CG, Carob gum; GG, Guar gum; PE, high methoxyl-pectin, XG, xanthan gum. Error bars show standard deviation.

Table 2
Effects of gluten supplementation with hydrocolloids on the amount of protein extracted and on the viscosity

Sample	Viscosity (mPa s)		Protein[a] (mg/ml)
	HS[b]	HS + gluten[c]	
Control	–	1.22 a	1.61 m
κ-C	3.17 cde	4.90 fg	4.59 o
ι-C	4.74 fg	15.20 k	2.46 n
λ-C	8.95 i	13.33 j	6.42 q
AL	3.62 def	4.13 efg	2.39 n
CG	2.57 bcd	1.86 ab	1.67 m
GG	4.99 g	6.37 h	1.62 m
PE	3.65 def	2.14 abc	5.42 p
XG	12.15 j	27.10 l	2.40 n

Values followed by the same letter in the same column and in the same row are not significantly different ($p < 0.05$). κ-C, κ-carrageenan; ι-C, ι-carrageenan; λ-C, λ-carrageenan; AL, low molecular weight sodium alginate; CG, Carob gum; GG, Guar gum; PE, high methoxyl-pectin; XG, xanthan gum.

[a] Protein content of solutions.
[b] Hydrocolloid solution.
[c] Hydrocolloid–gluten solutions.

viscosity than both κ-C and ι-C, which is in agreement with the higher solubility of λ-C in cold water.

The rheology of the hydrocolloid solutions depended on the molecular mass, the shape and rigidity of the macromolecule. Two main types of behaviour can be identified: (i) very branched or globular macromolecules which occupy a very small volume and can be assimilated into spheres, in this respect, as there is little to hinder the mobility of the solution, viscosity is low and the behaviour of the solution is close to Newtonian behaviour; and (ii) unfolded macromolecules which can occupy a very large volume, so they can limit the mobility of the solutions, and consequently, these solutions have high viscosity and the behaviour is of a pseudoplastic type (Linden & Lorient, 1999). It is important to mention that in our work the hydration of the hydrocolloids could not be total since the solutions were prepared by mixing and they were not heated.

Water-soluble gluten proteins had the lowest viscosity and the addition of different hydrocolloids increased it, except CG and PE, which did not show significant ($p < 0.05$) changes.

The viscosity of hydrocolloid–gluten supernatants was different for each gum. XG-, GG-, and carrageenan isoforms–gluten solutions exhibited a significant ($P > 0.05$) increment of the viscosity in comparison with solutions of pure hydrocolloids, while CG- and PE–gluten supernatants showed a decrease in the viscosity. The changes in the viscosity could be attributed to different concentration of hydrocolloid and protein in the supernatant phase after mixing and centrifugation of the blends, and modifications on the hydrocolloid and protein molecular structure due to attractive or repulsive interactions between biopolymers. The Pearson correlation between the increase

in the protein concentration as that of the starting sample (protein content of gluten solution), and the changes in the viscosity as that of the initial viscosity (pure hydrocolloid solution) was determined. The correlation coefficient ($r = -0.18$) indicated that the increase in the protein concentration did not explain the viscosity differences. Besides, none of the hydrocolloid–gluten solutions had higher amount of hydrocolloid than pure hydrocolloid solutions. Consequently, the viscosity increase could be related to the interactions.

Previous observations on κ-C-protein systems showed synergistic effects between the two polymers on apparent viscosity, gelation temperature and storage module of the gels at pH above the isoelectric point of protein (Baeza, Carp, Pérez, & Pilosof, 2002). These authors and Tolstoguzov (1995) suggest that these changes in the behaviour could be due to: (i) excluded volume effects that increased the effective concentration of both the hydrocolloid and the protein in separate microphases, and (ii) electrostatic interaction between both biopolymers in solution.

León et al. (2000) showed that a pool of low molecular weight hydrophobic gluten proteins interacted with carrageenan, and this interaction changed the protein physicochemical properties since carrageenan–gluten protein complexes showed a hydrophilic behaviour.

In order to determine if soluble complexes were formed, samples of supernatant from biopolymer mixtures were analysed by the Kjeldahl method and characterized by SDS-PAGE.

The amount of soluble-gluten protein increased by the addition of the hydrocolloid with anionic functional groups ($-COO^-$ to PE, AL and XG; $-OSO_3^-$ to carrageenan isoforms) but neutral gums (CG and GG) did not improve gluten protein solubility (Table 2). PE and λ-C showed the highest effect on protein solubilization. The increase in the amount of soluble gluten protein could indicate (i) associative interactions between these macromolecules, or (ii) a great amount of gluten protein in suspension due to an increase in a system's viscosity and/or due to suspension stabilization by hydrocolloid addition.

In order to determine the causes of soluble-protein increment hydrocolloid–gluten mixtures were centrifuged at different speeds ($1000g$, $6000g$ and $12,000g$). For this experiment, λ-C and AL were chosen because both hydrocolloids augmented the amount of protein in the supernatant, but they modified in a different way the viscosity of the gluten supernatant. The amount of soluble-protein did not show significant differences when the centrifugation speed changed (data not shown), i.e. the increment in soluble-proteins was not due to an increase in suspension stabilization. Therefore, the results indicate the formation of hydrocolloid–gluten protein soluble complexes, which have a hydrophilic behaviour.

The protein profiles of the supernatant fraction are shown in Fig. 3. The stain intensity showed the same trends as the protein content of the supernatant; all the hydrocolloids with
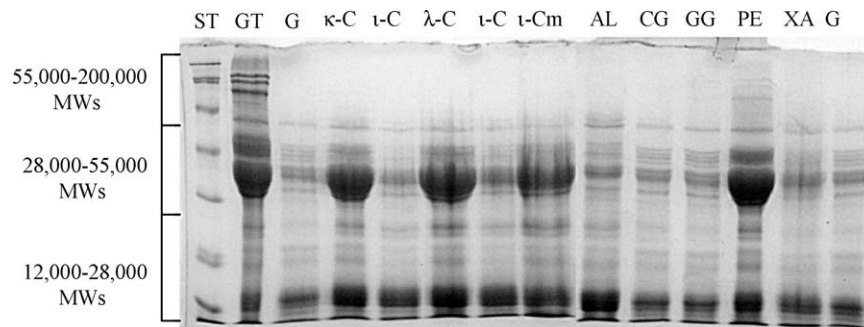
Fig. 3. Electrophoretic patterns of water-soluble proteins extracted of gluten–hydrocolloid mixtures. ST, molecular weight standard, GT, total gluten extraction (SDS and $2 +$ -mercaptoethanol), G, supernatant of gluten. Supernatant from gluten–hydrocolloids (κ-C, κ-carrageenan; ι-C, ι-carrageenan; λ-C, λ-carrageenan; AL, low molecular weight sodium alginate; CG, Carob gum; GG, Guar gum; PE, high methoxyl-pectin, XG, xanthan gum) mixtures. ι-Cm, gluten-ι-CA cloudy suspension.

anionic functional groups augmented the stain intensity by comparison with gluten extraction while GG and CG had similar intensity. Carrageenan isoforms and PE had different protein patterns, which showed a selective interaction with a group of medium molecular weight gluten proteins (30,000–42,000) in agreement with a previous work (León et al., 2000). Besides, PE seemed to interact, though in a minor proportion, with high (78,000–105,000) and low (16,000–26,000) molecular weight gluten proteins.

ι-C-gluten formed a cloudy suspension by mixing, and after the mix was centrifuged it formed a gel-like cloudy precipitate on insoluble gluten protein phase. This fact could explain the great differences in the amount of soluble protein in comparison with λ-C and κ-C. When the proteins of clear supernatant and gel-like precipitate formed in ι-C-gluten mix were separated by SDS-PAGE, similar patterns were found in both phases, but a greater proportion of protein–polymer complexes were present in the cloudy precipitate (Fig. 3, line ι-Cm).

AL and XG did not show a selective interaction with gluten proteins though they incremented the amount of water-soluble proteins. GG and CG did not change the profiles of soluble gluten proteins.

To investigate whether the selective interaction of carrageenan and PE with medium molecular weight gluten proteins depend on polysaccharide–protein ratios, proteins solubilized from mixtures with different hydrocolloid (λ-C and Pe)/gluten ratios were characterized by SDS-PAGE (Fig. 4). We could observe an increment in the stain intensity of a gluten protein fraction (30,000–42,000 MWs) while the intensity of the rest of solubilized protein did not change when the hydrocolloid/gluten ratio was increased. The interaction appeared to depend on a relative percentage of the polysaccharide; a greater proportion of λ-C and PE provoked an increment in these soluble proteins.

The significant ($p < 0.05$) increment in the amount of soluble-gluten proteins and the viscosity showed that the relative intensities of protein/κ-C and λ-C interactions (ι-C was not included in the comparison because of the phase separation mentioned previously) depended on the relative density of sulphate groups on these anionic polysaccharides (order of charge densities is λ-C > ι-C > κ-C). The same trends were found between bovine serum albumin and carrageenan isoforms by Galazka, Smith, Ledward, and Dickinson (1999). These results support the hypothesis that anionic hydrocolloids can form an electrostatic complex with some fractions of gluten proteins. Grinberd and Tolstoguzov (1997) described the formation of soluble protein-sulphated polysaccharide complexes. They attributed it to the formation of ionic pars between ionised sulphated groups of the hydrocolloids and ε-amino groups of protein. Our results indicated that, free carboxyl groups could interact with gluten proteins in the same way as the sulphated group.

The great capacity of the λ-C and PE to form complexes through ionic interaction with gluten proteins is a factor that may explain the increment detected in R_m. Hydrogen bonding may also play an important role in the polysaccharide–gluten interactions; the hydrocolloids tested offer extensive hydroxyl sites to form noncovalent links to the numerous amide groups on gluten proteins. Hydrogen bonding may account for the action of the neutral hydrocolloids on dough rheology and bread performance. In addition, it is possible that hydrocolloids interact with
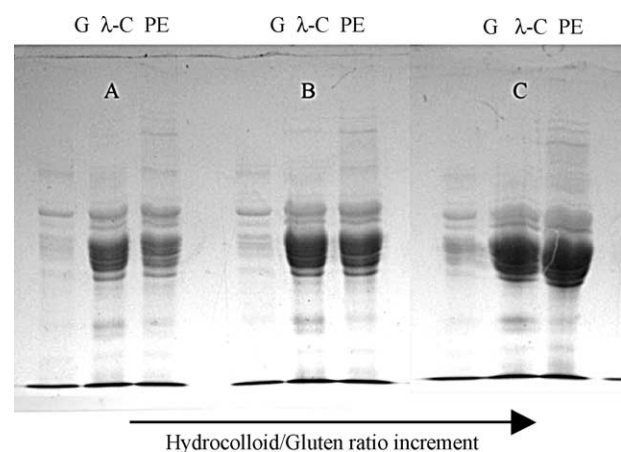

Fig. 4. Electrophoretic patterns of water-soluble proteins extracted from λ-carrageenan (λ-C) and high methoxyl-pectin (PE)–gluten mixtures. Hydrocolloid/gluten ratios: 1/30 (A), 1/20 (B) and 1/10 (C).

other gluten proteins and the resulting complexes are not water-soluble: however, new studies are needed to verify these observations.

4. Conclusions

Dough rheology and bread quality were affected in different ways by the addition of hydrocolloids tested. Pectin and λ-carrageenan strengthened wheat dough and sodium alginate augmented the extensibility of dough. In addition sodium alginate and pectin improved loaf volume and all the hydrocolloids tested decreased the initial bread crumb firmness and chewiness.

It has been clearly demonstrated that carrageenan isoforms and pectin (sulphated and carboxylated hydrocolloids) can form hydrophilic complexes with gluten proteins and the capacity of complexation appears to be related to the density of the anionic group in the polysaccharide.

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