Interactions of Different Carrageenan Isoforms and Flour Components in Breadmaking

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The aim of this study was to compare the effects of carrageenans with different sulfate contents on bread volume and dough rheological properties. Results showed that only lambda carrageenan, the most sulfated isoform, produced a significant increase in bread volume. In contrast, the different carrageenans induced a negative effect on the cookie factor. Alveographic and farinographic analyses indicated that dough rheological properties were differentially modified depending on whether lambda carrageenan was added to flour and then hydrated or vice versa. Analysis of the interaction between lambda carrageenan and flour components by infrared spectroscopy and SDS−PAGE indicated that a pool of low molecular weight hydrophobic gluten proteins interact with carrageenan. This interaction drastically changes their physicochemical properties since carrageenan−gluten protein complexes show a hydrophilic behavior. In addition, the results indicate that carrageenan sulfate groups and probably the amino groups of glutamines present in the primary structure of gluten proteins are involved in the interaction.

Keywords: *Flour additives; iota; kappa and lambda carrageenans; baking; hydrocolloids*

INTRODUCTION

Baking products are made with flour, usually obtained from wheat, which is mixed with water and other ingredients and then fermented with yeast or other leavening agents, followed by heating. Proteins and carbohydrates are the major components of wheat and wheat flour. The properties of these components have been extensively studied (Preston, 1998). The intimate dough characteristics are dependent on the interactions that take place among the different components of the mix, the mechanical action of mixing, and the subsequent thermal effects of cooking (He and Hoseney, 1991). It appears that the driving and restrictive forces that modulate dough rheological properties and bakery quality are strongly influenced by the interaction between gluten proteins and other dough components.

Different macromolecular compounds that include nonwheat flours (Lorenz and Coulter, 1991; Mustafa, et al., 1995; El-adawy, 1995) and hydrocolloids (Anderson and Andon, 1988; Belitz and Grosch, 1997) have been used as a supplement to wheat flour to modify bakery foods to achieve certain goals, such as nutritional properties. Hydrocolloids are used in two different ways, as simple dietary additives for the management of diabetic and metabolic disorders (Ellis et al., 1988; Gatti et al., 1984) or as an agent of water retention (Anderson and Andon, 1988).

One of the most widely employed hydrocolloids as a food additive is carrageenan (Pilnik and Romboust, 1985; Trius and Sebranek, 1996; Ozawa et al., 1984; Hansen, 1968; Schmidt and Smith, 1992). Carrageenans are a group of related red algae linear sulfated biopolymers (MW 200−800), with a structure of the alternating repeat type. The main gel formers iota (ι) and kappa (κ) carrageenans share a similar repeat structure: galactose and 3,6-anhydrogalactose, with the galactose residue sulfated at position 4. In addition, in ι carrageenan, the anhydrogalactose is sulfated in position 2. The relevant conformational data is that these carrageenans tend to be ordered as a double helix (Morris et al., 1989; Trius and Sebranek, 1996; Belitz and Grosch, 1997).

On the other hand, lambda (λ) carrageenan is quite different from the others in having virtually no anhydrooxygen bridge residues and also a higher sulfate content, because one galactose is sulfated at position 2 and the other is sulfated at positions 2 and 6. It is remarkable that the conformation of λ carrageenans is of the kind of single coils, and they seem to be unable to form gels under any experimental condition (Morris et al., 1980; Trius and Sebranek, 1996; Belitz and Grosch,1997).

At present, the interaction of different carrageenan isoforms with flour components has not been studied in detail. The aim of this study was to compare the effects on breadmaking of adding different carrageenan isoforms to dough and to determine some of the potential interactions between the biopolymer and the flour components.

MATERIALS AND METHODS

Reagents. Carlos Boero Romano SAIC supplied wheat flour 000, without additives. Flour characteristics were 13% protein content, 13.7% moisture, 0.75% ash, 30% wet gluten, and 336 s Falling Number. Pressed fresh yeast was from Calsa SA. Kappa (type III), iota (type V), and lambda (type IV) carrageenans were from Sigma Chemical Co.

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Breadmaking. Bread samples were baked by a direct process with the following recipe: flour 100%, water 63%, yeast 3%, salt 1.8%, ascorbic acid 0.015%, and sodium propionate 0.2%. Ingredients were mixed in a Argental L-20 mixer (Argentina) for 10 min and allowed to relax for 15 min at 30 °C and 80% relative humidity. The bulk dough was degassed in a Mi-Pan vf roller containing two rolls of 50 × 12.7 cm (Argentine), divided by hand into pieces of 70 ± 5 g each, and then proofed for 90 min at 30 °C and 100% relative humidity. Baking was done in an electric oven at 180 °C for 17 min. Bread samples were cooled at room temperature for 75 min.

The optimum proof time for each sample was determined using dough pieces (50 g) that were loaded in calibrated fermentation flasks. The increase in volume was measured every 15 min until the optimum level was reached. Different carrageenans at 0.5% (flour basis) were added alternatively to flour or to water.

Bread Loaf Volume. Bread volumes were measured by millet seed displacement, and their specific volumes were calculated as volume/weight (cm^3/g).

Preparation of Cookies. Cookies were prepared according to León et al. (1996). Ingredients used were flour (45 g); caster sugar (27 g); vegetable fat (20.2 g); powdered milk (2.25 g); $NaHCO_3$ (0.5 g); NaCl (0.42 g); and water (8.5 mL). Different carrageenans were added at 0.5% (flour basis) within the flour. Then, cookies were baked at 200 °C for 10 min. The cookie factor was defined as the ratio of width/height of four cookies.

Farinograms and Alveograms. To evaluate dough rheological properties, we obtained farinograms and alveograms of control and carrageenan supplemented flours, using a Brabender Farinograph and Chopin Alveograph (AACC, 1995).

Studies of the Interaction between Lambda Carrageenan with Flour Components. To study the interaction between carrageenan with flour components, samples were processed as follows: (1) Commercial dry gluten (100 mg) and different amounts of λ carrageenan (5−100 mg) were mixed before the addition of 5 mL of distilled water (this procedure is now defined as condition A). (2) Gluten (100 mg) was suspended in 5 mL of water containing different amounts (5−100 mg) of λ carrageenan (this procedure is now defined as condition B). (3) Wheat starch (100 mg) and λ carrageenan (5−120 mg) were mixed before the addition of 5 mL of distilled water. In all cases, the resulting mix was shaken during 5 min and sonicated for 5 min in a Branson sonifier in point 5 and then centrifuged for 10 min at 1000*g*. The different supernatants were taken and analyzed by infrared spectroscopy and SDS−PAGE.

Infrared Spectroscopic Analyses. Infrared measurements were performed in a FTIR system (Shimadzu 6501) furnished with horizontal attenuated total reflection (hATR) and diffuse reflectance infrared fourier transform (DRIFT) accessories. Measuring the amide I peak absorbance at ca. 1650 cm^{-1} (Torrii and Tasumi, 1996), we carried out quantification studies of protein samples processed as described above. Aqueous samples were dried on a stainless steel sampling support (homemade) attached to a conventional DRIFT accessory. Additionally, some spectra were taken by placing the aqueous phase over an ATR cuvette fitted with a ZnSe crystal.

SDS−Polyacrylamide Gel Electrophoresis. Protein samples were run on 12% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) (Laemmli, 1970). The electrophoresis was conducted for 1 h at a constant voltage of 200 V. A Mini Protean II Slab Cell (Bio-Rad Laboratories, Richmond, CA) was used. Proteins were stained with Coomassie Brilliant Blue.

Statistical Analysis. All data were expressed as the mean ± standard error (SE). Significant differences were evaluated by using Tukey's test. A value of $p < 0.05$ was considered significant.

RESULTS

Effect of Dough Supplementation with Different Carrageenan Isoforms on Bread Volume. Bread made according to the procedure described in Materials and Methods has a specific volume value of 3.70 cm^3/g, which was taken as 100% (Table 1).

Table 1. Effect of Dough Supplementation with Carrageenan Isoforms on Bread Volume and Cookie Factor[a]

sample	specific bread volume (cm^3/g)	cookie factor	width four cookies (cm)
control	3.70 ± 0.06 (100 ± 0%)[a]	7.34 ± 0.04[a]	23.5 ± 0.9[a]
κ in flour	3.75 ± 0.05 (101 ± 3%)[a]	5.54 ± 0.03[b]	21.6 ± 0.2[b]
ι in flour	3.80 ± 0.03 (102 ± 2%)[a]	6.44 ± 0.08[c]	23.1 ± 0.4[a]
λ in flour	4.75 ± 0.04 (128 ± 2%)[b]	6.29 ± 0.08[c]	23.3 ± 0.3[a]
κ in water	3.75 ± 0.04 (101 ± 2%)[a]	-	-
ι in water	3.90 ± 0.04 (105 ± 2%)[c]	-	-
λ in water	5.10 ± 0.07 (138 ± 4%)[d]	-	-

[a] Values are the average of three independent determinations. Values followed by the same letter in the same column are not significantly different ($p < 0.05$). Carrageenan in flour means condition A. Carrageenan in water means condition B.

Table 2. Effects of Carrageenan Supplementation on Dough Rheological Properties[a]

	alveograph				farinograph
sample	*P*	*L*	*W*	*P/L*	WA
control	120 ± 7	85 ± 2	380 ± 15	1.41 ± 0.03	63.2 ± 0.1
0.5 κ in flour	138 ± 4	70 ± 2	378 ± 8	1.97 ± 0.02	64.6 ± 0.2
0.5 ι in flour	156 ± 5	62 ± 3	385 ± 11	2.52 ± 0.06	65.0 ± 0.2
0.5 λ in flour	148 ± 3	90 ± 2	437 ± 7	1.64 ± 0.02	68.9 ± 0.3
0.5 κ in water	118 ± 8	35 ± 2	184 ± 13	3.37 ± 0.12	-
0.5 ι in water	138 ± 5	28 ± 2	169 ± 17	4.93 ± 0.20	-
0.5 λ in water	160 ± 6	43 ± 1	239 ± 14	3.72 ± 0.10	-

[a] WA = water absorption. Values represent the average of two separate determinations. Carrageenan in flour means condition A. Carrageenan in water means condition B.

The three carrageenan isoforms were supplemented in dough at 0.5% (flour basis) in two different experimental conditions: (1) carrageenan was mixed with flour and then hydrated (condition A) or (2) carrageenan was previously hydrated and then added to the flour (condition B). In both cases, the hydration process was done at room temperature. We observed that in both conditions, only λ carrageenan significantly increased bread specific volume by 28% and 38%, respectively (Table 1). Kappa and iota carrageenans did not show any significant effect.

The effects of different carrageenan isoforms on the cookie factor (ratio width/height) are also shown in Table 1. In contrast to bread, a negative effect was observed when different carrageenan isoforms at 0.5% (flour basis) were added to dough. The λ and ι carrageenan effects on the cookie factor are due to an increase in height value, whereas kappa carrageenan produces both an increase in height and a decrease in width.

Effect of Carrageenan Supplementation on Dough Rheological Properties. Results from alveographic and farinographic analyses, after flour supplementation with different carrageenan isoforms, are shown in Table 2. The supplementation of carrageenan was done in a form similar to that described above**.** When flour and carrageenan isoforms were mixed and then hydrated (condition A), a 15% increase in *W* value was detected only for lambda carrageenan.

In contrast, when carrageenans were solubilized in water before adding them to the flour (condition B), a reduction of *W* values was observed, which was higher for the κ and ι isoforms than for the λ isoform. An analyses of *P* values in condition A and condition B showed that in most cases these volumes were slightly

higher than control but very similar for each carrageenan in both conditions. With respect to *L* values, it can be seen that no significant changes were observed when different carrageenan isoforms were added to flour in condition A; however, a significant reduction of about 50% in the *L* value was detected when flour was mixed with water containing different carrageenan isoforms in condition B. The *P*/*L* ratio clearly shows that higher values obtained in condition B are exclusively due to the contribution of *L* values at the same conditions. These results indicate a loss of elasticity because of an increase in the tenacity/extensibility ratio that renders a more rigid dough when carrageenans are previously hydrated before adding to dough.

Farinographic analyses demonstrate that the addition of different carrageenans to dough produces an increase in water absorption with respect to control; however, as can be seen in Table 2, λ carrageenan shows the highest capacity of water absorption (WA).

Taking into account these results, they indicate that the physical state of λ carrageenan added to flour is a factor that modulates the different rheological parameters as well as the amount of free water that can be used to hydrate it, without affecting the final bread volume (Table 1).

Studies by Infrared Spectroscopy and SDS−PAGE of the Interaction between Lambda Carrageenan with Flour Components. We analyzed whether interactions between λ carrageenan and some flour components, in addition to hydration capacity, are also involved in the improving effect of the biopolymer. To do this, quantitative analyses by infrared spectroscopy of λ carrageenan interaction with gluten were performed. Samples of supernatants from the gluten−carrageenan interactions done in condition A and condition B (see Material and Methods) were loaded on a solid support and dried as films. The results clearly show maximum amide I peak absorbance values in both conditions (Figure 1); this maximum corresponds to 5%. It should be noted that at higher carrageenan concentrations, the amide I absorbance decreases; however, it remains substantially higher than in the absence of λ carrageenan. This results suggest that when hydrophobic gluten proteins and λ carrageenan are allowed to interact, a hydrophilic complex of carrageenan−gluten is formed since it remains soluble in water. To support this observation and also with the aim to analyze the possible carrageenan−starch interactions, we carried out other studies using the ATR (Figure 2) to detect changes in the spectra of the sulfate group region of the carrageenan peak at 1220 cm^{-1} (Roeges, 1995). Figure 2 shows that the profiles of the sulfate group of λ carrageenan alone and of the carrageenan mixed with starch did not show any significant difference, demonstrating that no interaction via sulfate group occurs. However when λ carrageenan was mixed with gluten, a clear shift in the sulfate peak was detected, indicating that this group may be involved in the biopolymer−protein interaction.

To know which of the gluten proteins are involved in the gluten−λ carrageenan interaction demonstrated by the above infrared studies, we analyzed by SDS PAGE the profile of proteins present in the supernatant fraction of samples prepared in condition A. Figure 3 shows that λ carrageenans interact selectively with a group of low molecular weight hydrophobic gluten proteins.

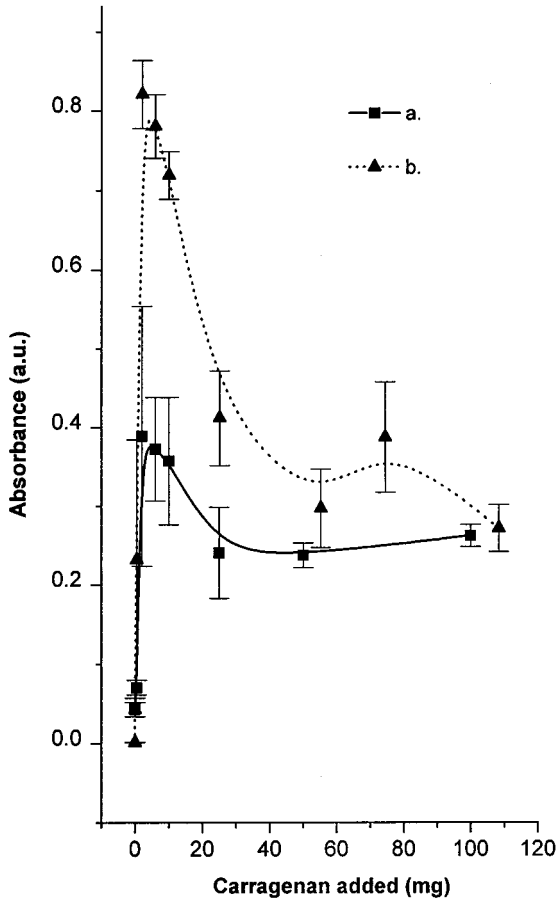

Figure 1. Interaction between λ carrageenan and gluten proteins. Analyses by infrared spectroscopy of amide I peak. (a) Samples containing 100 mg of gluten protein were mixed with different amounts of λ carrageenan, and then 5 mL of water were added (condition A). (b) Samples containing 100 mg of gluten protein were mixed with 5 mL of water containing different amounts of λ carrageenan (condition B). Absorbance for amide I peak was measured at ca. 1650 cm^{-1}; a.u., arbitrary units.

DISCUSSION

The main contribution of this study was to show that a particular carrageenan isoform, λ carrageenan, has the best improving effect on dough and bread properties. When λ carrageenan was added to the dough formulation, there was a significant increase in bread volume. In addition, rheological data from alveographic and farinographic determinations showed that dough containing the λ carrageenan isoform has a higher capacity to increase dough strength and water absorption. These results could be explained by the ability of this polysaccharide to take in more water at room temperature, since ι and κ carrageenans are fully hydrated at temperatures higher than 50 °C, and on the other hand, by a potential carrageenan−gluten protein interaction. Physicochemical and biochemical approaches were used to study this interaction. The analyses by FTIR of amide I peak (Figure 1) show the changes in the physicochemical behavior of hydrophobic gluten proteins that appear as a hydrophilic component due to the carrageenan−gluten interaction.

In addition, the infrared spectroscopy using ATR (Figure 2) shows the changes in the spectra of the sulfate group region of carrageenan when it was incubated with gluten proteins but not with starch. These results strongly suggest that carrageenan−gluten in-

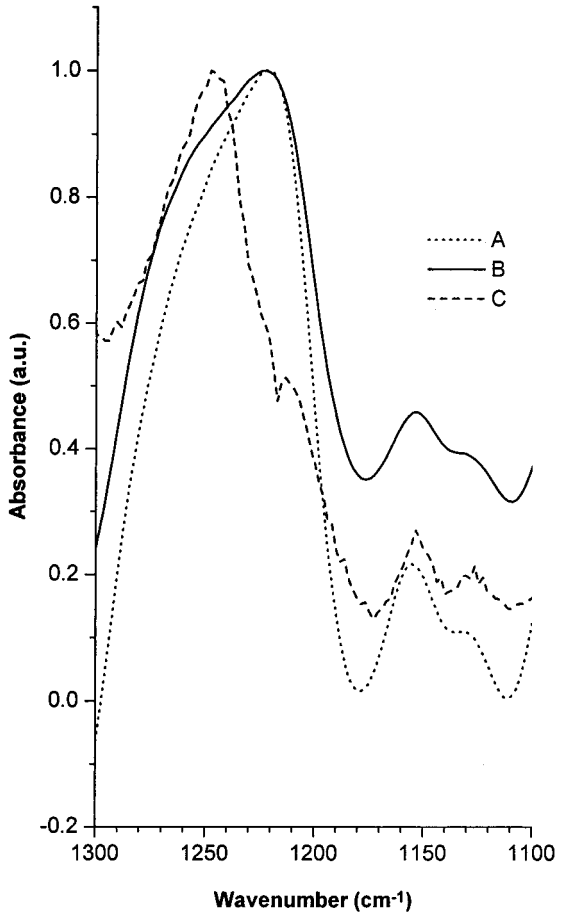

Figure 2. Analyses of the interaction between λ carrageenan with starch and gluten protein using infrared spectroscopy for sulfate spectra. Samples containing 5 mg of λ carrageenan were mixed with 100 mg of starch or gluten, and then 5 mL of distilled water was added to the mix. Then the samples were processed as described in Materials and Methods. The sulfate group region was analyzed at ca. 1220 cm^{-1}; a.u., arbitrary units; (A) λ carrageenan−water interaction (control sample); (B) λ carrageenan−starch interaction; (C) λ carrageenan−gluten protein interaction.

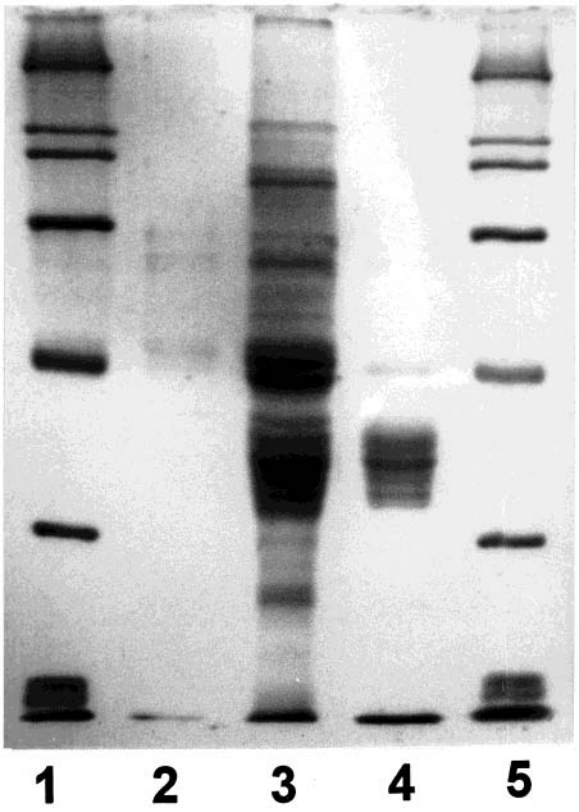

Figure 3. Analysis of the interaction between λ carrageenan and gluten proteins by SDS−PAGE. Samples of gluten were incubated in the absence or in the presence of λ carrageenan as described in condition A. Mixes were processed as described in Materials and Methods. Similar volumes of supernatant samples were loaded onto the gel. Lanes 1 and 5, molecular weight standards (from the top, molecular weights are 200, 116, 97, 66, 45, 31, 21, and 14); lane 2, supernatant of gluten incubated with water; lane 3, total gluten proteins; and lane 4, supernatant of gluten incubated with λ carrageenan.

teraction occurs via sulfate group, probably with the ϵ amino group of glutamine, which is abundant in the primary structure of gluten proteins.

On the other hand, using SDS−PAGE (Figure 3), we demonstrated that the hydrophilic carrageenan−gluten proteins complex appears to be selective for a population of low molecular weight hydrophobic gluten proteins. However, we could not rule out the possibility that carrageenan also interacts with other gluten proteins and remains as an insoluble complex.

In contrast to an improving effect on bread, the addition of different carrageenan isoforms to cookie dough produces a negative effect on its quality (Table 1). This negative effect could be explained by presence of other factors that modulate carrageenan properties such as the amount of water (8.5% in cookie dough in comparison to 63% in bread) and the high amount of caster sugar (27%). In these conditions, it is possible that λ carrageenan produces an increase in dough strength that induces more height to cookies and consequently a decrease in the cookie factor.

In conclusion, from this study two main views should be considered to explain the improving effect of λ carrageenan on bread volume. One of them is the higher capacity of this biopolymer as compared to ι and κ carrageenans to be more hydrated at room temperature when mixed with dough ingredients. The other is the role of the λ carrageenan structure, which has the highest sulfate and the lowest anhydrogalactose content per molecule, showing a spatial conformation that reduces the tendency for self−self interactions, making possible the interaction with gluten proteins that could regulate dough rheological properties.

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