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Interaction of modified celluloses and pectins with gluten proteins

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ABSTRACT

Physical and chemical techniques were applied to characterize the type of interaction between hydrocolloids and the gluten network in wheat dough, with and without NaCl. Modified celluloses (microcrystalline cellulose, MCC; carboxymethylcellulose, CMC, hydroxypropylmethylcelluloses, HPMC) and pectins of low (LMP) and high (HMP) degree of methylation were utilized as hydrocolloids to interact with gluten proteins. Modified celluloses were employed at 1.5% (flour basis) and pectins at 2.0% (flour basis). By microscopy (SEM and CLSM), it could be observed that NaCl induced a more marked cross-linking and orientation of gluten network. On the other hand, the addition of hydrocolloids led to more open matrices. Molecular mobility was evaluated by ^{1}H-NMR assays and significant effects of NaCl addition and hydrocolloid type were found on relaxation times (T_2). In presence of salt, significantly higher relaxation times were observed when modified celluloses were added. Hydrocolloid addition strongly affected the secondary conformation of proteins as studied by FT-Raman. In absence of NaCl, control and MCC samples exhibited the higher α-helix conformation percentage (indicating a more ordered and compact structure), followed by dough with HMP, HPMCs, LMP and CMC. In general, doughs with modified celluloses and NaCl showed a decrease of α-helix conformation. CMC dough showed the smallest percentage of α-helix conformation, and the highest contributions of more unfolded structures. Doughs with pectins and NaCl showed similar percentages of α-helix to control one but an increase of random coil structure was observed. Electrophoresis assays confirmed that the presence of certain hydrocolloids (CMC) during gluten formation could affect protein interaction promoting subunits lability from the matrix.

1. Introduction

Hydrocolloids from different sources have been increasingly studied as breadmaking improvers to evaluate their effect on dough and bread characteristics as well as product acceptability and preservation. Among those hydrocolloids commonly used as food additives, modified celluloses and pectins have been mainly applied in beverages, desserts and dairy products. An interesting aspect of these hydrocolloids is that they are obtained by chemical modification of native polysaccharides from plants and their structure (particularly in the case of celluloses) can be tailor-made in order to obtain derivatives with different physicochemical properties. Besides, they are obtained from relatively economical, renewable sources. In spite of their extended use as food additives, their application in breadmaking is less extended respect to other hydrocolloids and bread improvers.

Several studies about the influence of hydrocolloids on wheat dough behavior and bread quality have included some modified celluloses – mainly HPMC – and pectins – particularly the high methoxylated one – (Armero & Collar, 1998; Bárcenas, De la O-Keller, & Rosell, 2009; Bárcenas & Rosell, 2005, 2007; Bollaín & Collar, 2004; Collar, Andreu, Martínez, & Armero, 1999; Correa, Pérez, & Ferrero, 2012; Guarda, Rosell, Benedito, & Gallotto, 2004; Ribotta, Ausar, Beltramo, & León, 2005; Rosell, Rojas, & Benedito de Barber, 2001). However, there is yet scarce information about their effect on wheat dough microstructure. A better knowledge about the changes induced by these hydrocolloids on gluten network could help to understand their diverse effects on dough behavior and bread quality. A comparative analysis among different modified celluloses and pectins could also contribute to associate the type of chemical structure with their efficiency as product improver.

In the case of modified celluloses, different substitutions on cellulose backbone as the presence of anionic groups in the case of carboxymethylcellulose (CMC), or a certain degree of hydrophobicity in the case of hydroxypropylmethylcelluloses (HPMC) can lead to macromolecules with very diverse properties compared to

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native cellulose. Degree of methoxylation in pectins has also influence on their physicochemical properties; high methoxyl pectins (HMP) do not exhibit gelling properties in the presence of calcium ions as low methoxyl ones (LMP) do (Glicksman, 1982).

In previous works, the effects of different types of modified celluloses and pectins on the rheological characteristics of wheat dough were analyzed and the influence of the type and level of gum could be assessed. Pectins diminished dough stability and rendered softer but more cohesive doughs. Particularly, more viscous doughs were obtained with high methoxyl pectins (Correa et al., 2012). On the other hand, the effect of modified celluloses depended not only on their intrinsic structure but also on the presence or absence of salt in dough. CMC decreased dough stability in dough without salt and HPMCs affected it in dough with salt, probably by the promotion of hydrophobic interactions. Modified celluloses softened doughs and the combination of hydrocolloids and salt led to more cohesive and less resilient systems (Correa, Añón, Pérez, & Ferrero, 2010). These results indicate that the type of modification in the case of celluloses and the degree of methoxylation in the case of pectins are influencing the interaction with dough components.

Different techniques can be applied to study the interaction of hydrocolloids with dough components, particularly with gluten proteins, at a microstructural level. The use of different microscopic techniques is a very useful approach to dough microstructure allowing a general overview of the matrix characteristics (Baier-Schenk et al., 2005; Jeckle & Becker, 2011; Li, Dobraszczyk, & Wilde, 2004; Peighambardoust, van der Goot, van Vliet, Hamer, & Boom, 2006). Techniques as NMR relaxation assays (T_2) has been applied to study water mobility in dough and bread (Engelsen, Jensen, Pedersen, Norgaard, & Munck, 2001; Esselink et al., 2003; Leung, Magnuson, & Bruinsma, 1979; Linlaud, Ferrer, Puppo, & Ferrero, 2011; Lopez-Da-Silva, Santos, Freitas, Brites, & Gil, 2007).

The characteristic viscoelastic properties of wheat dough are the result of gluten development. Under mechanical action and in the presence of water, gluten proteins undergo hydration, unfolding, orientation and there is also an interchange between sulfhydryl (S—H) and disulfide (S—S) bonds (Campos, Steffe, & Ng, 1997; Shewry, Popineau, Lafiandra, & Belton, 2001). Thus, the interactions among these proteins and hydrocolloids can have a strong influence on the secondary structure of proteins as can be inferred from FT-Raman spectra and on the lability of subunits from the gluten matrix as seen by SDS-PAGE (Linlaud et al., 2011).

The objectives of this study were (a) to apply physical and chemical techniques to characterize the interaction between modified celluloses and pectins and the gluten proteins at a microstructural level and (b) to compare the effect of these hydrocolloids and relate it to their chemical structure.

2. Materials and methods

2.1. Materials

Commercial wheat flour was used for dough preparation (Type 000, Código Alimentario Argentino, 2012). Flour composition was: protein 11.4% (Kjeldahl factor = 5.7), moisture 14.2%, lipids 1.4% and ash 0.68%. Wet and dry gluten values were 31.5 ± 1.3 (g gluten/100 g flour) and 11.0 ± 0.3 (g gluten/100 g flour), respectively. The alveographic parameters of flour were: $P = 96$ mm H_2O, $L = 93$ mm and $W = 326 \cdot 10^{-4}$ J. This type of flour is suitable for breadmaking. Two types of food grade hydrocolloids were employed: modified celluloses and pectins. The modified celluloses used were: microcrystalline cellulose (MCC) (FMC Biopolymer, Philadelphia) which was copolymerizated with 12% of carboxymethylcellulose, carboxymethylcellulose (CMC) (Latinoquímica Amtex S.A., Argentina) with a degree of substitution of 0.9% and two different types of hydroxypropylmethylcellulose (HPMC) (Dow Chemical Company, USA) with a different degree of methoxyl and hydroxypropyl substitution: HPMC F 4M with 29.3% of methoxyl groups and 6.0% of hydroxypropyl groups and HPMC F 50 with 28.6% of methoxyl groups and 5.4% of hydroxypropyl groups. Also, two types of citrus pectins (CP Kelco, USA) were used: Genu Pectin 8001 (a low methoxyl pectin — LMP) and Genu Pectin 105 (a high methoxyl pectin — HMP). Purity of both pectins was 57% (w/w) and the degree of esterification was 67% for HMP and 43% for LMP. The degree of amidation for LMP was 16% (Correa et al., 2012). Distilled water and commercial salt (NaCl) were used to prepare dough.

2.2. Methods

2.2.1. Dough preparation

Dough was formulated with flour (100 g), without and with NaCl (2 g), and water according to farinographic absorption. Modified celluloses were employed at 1.5% (flour basis) and pectins at 2.0% (flour basis); dough without hydrocolloids was used as control. Hydrocolloids levels were the maximum ones among those used in previous works (Correa et al., 2010, 2012).

Dough samples were prepared without yeast in a microfarinograph Brabender (Duisburg, Germany). For each blend the farinographic development time was used as the mixing time. Each dough formulation was prepared in duplicate.

2.2.2. Scanning electron microscopy (SEM)

Micrographs of control and samples with hydrocolloids were taken. Small portions of dough were cut, fixed in 10% glutaraldehyde and sequentially embedded in acetone solutions of increasing concentration to ensure full dehydration. Samples were dried at the critical point and coated with gold particles. A scanning electron microscope (JEOL 35 CF, Japan) was employed with magnifications of 500×, 3000×, 5000×. For comparisons among samples, 500× was selected. Nine representative fields were obtained for each formulation.

2.2.3. Confocal scanning laser microscopy (CSLM)

2.2.3.1. Sample preparation. A mixture of rhodamine B (0.001%) and fluorescein isothiocyanate (FITC) (0.01%) in distilled water was used for non-covalent labeling. The fluorescent contrast depends of dye affinity and accessibility to the different components. A small portion of dough was cut and then spread on a glass slide with a rolling pin; immediately it was imbibed with the dye solution. The sample was let to rest for an hour within a closed recipient and in darkness, and then the specimen was washed with distilled water and covered with a glass cover slip. Dough samples did not show autofluorescence.

2.2.3.2. Confocal microscopy system. A LEICA TCS SP5 (Mannheim, Germany) inverted microscope equipped with Ar and HeNe laser was used. The excitation wavelengths were 488 nm (FITC) and 568 nm (rhodamine B) and the emission wavelengths were 518 nm (FITC) and 625 nm (rhodamine B). Images were acquired using a 20× HCX PL APO CS water immersion objective and with 1024 × 1024 pixel resolution in a constant z-position. Ten photographs (5 by each replicate) with the same magnification were obtained from representative fields. Softwares Leica Application Suite Advanced Fluorescence (LAS AF), version 2.2.1. build 4842 and Image J 1.43u were employed in the image analysis. Each micrograph was RGB color split and then was corrected by shading applying FFT filtering (Walter, 2003). The corrected image was subjected to an automatic thresholding and converted in a binary image as described by Peighambardoust et al. (2006).

From binary images, protein matrix value (%) and fractal dimension (FD) were calculated. The protein matrix value was