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Short communication

Structural changes in gluten protein structure after addition of emulsifier. A Raman spectroscopy study

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ABSTRACT

Food protein product, gluten protein, was chemically modified by varying levels of sodium stearoyl lactylate (SSL); and the extent of modifications (secondary and tertiary structures) of this protein was analyzed by using Raman spectroscopy. Analysis of the Amide I band showed an increase in its intensity mainly after the addition of the 0.25% of SSL to wheat flour to produced modified gluten protein, pointing the formation of a more ordered structure. Side chain vibrations also confirmed the observed changes.

1. Introduction

Changes in quality of wheat flours and the requirement of maintaining good organoleptic and textural characteristics of bread, and adequate acceptance by consumers promote utilization of additives for improving breadmaking quality. Some authors have demostrated that emulsifiers exercised desirable effects in breadmaking process [1,2]. These additives increase force and extensibility of gluten and therefore enhace air retention in dough during fermentation [3]. In breadmaking, they improve crumb and crust texture and diminish water loss. Emulsifiers also decrease cristalization percentage of starch [4] and consequently extend shelf-life and textural characteristics of products [5]. Gluten is composed of two major proteins, gliadins and glutenins, which form a continuous viscoelastic network within the dough [6]. Gluten has a structure in which the high molecular weight glutenin subunits (HMW-GT) form an 'elastic backbone' consisting largely of head-to-tail polymers with inter-chain disulphide bonds. This backbone forms a basis for low molecular weight subunit 'branches' (linked by disulphide bonds). Gliadins may also interact with the glutenin polymers by non-covalent forces, although these interactions are traditionally considered to contribute to gluten viscosity rather than elasticity [6].

Sodium stearoyl lactylate (SSL) is a common emulsifier used in breadmaking. Due to their high hydrophilic–lypophilic balance, this additive develops strong dough where the lypofilic tail of the molecule (Fig. 1) would be bound to hydrophobic sites of gluten proteins. There is quite a lot of information about the effect of the emulsifier on improving bread quality, but no reports were found about changes in structure of gluten proteins produced by SSL. For this reason the objective of this research was to investigate the effectiveness of using Raman spectroscopy to study the extent of gluten protein modification by the addition of sodium stearoyl lactylate (Fig. 1) in order to improve its functional properties.

2. Material and methods

2.1. Materials

Sodium stearoyl lactylate was obtained from DANISCO (Copenhagen, Denmark) and wheat flour (000 flour) for breadmaking (*Triticum aestivum* L.) was provided by an Argentinean mill (Molinos Campodónico Ltd., Argentina).

2.2. Sample preparation for FT-Raman spectroscopy

Gluten samples were prepared with wheat flour (control sample) or a mix composed by flour and emulsifier (SSL) at levels of 0.25, 0.5 and 1.0 %w/w (g SSL/100 g wheat flour). Wheat dough was obtained incorporating 4.9 mL of water to 10 g of dry mix and mixing during 1 min in a Glutomatic equipment. Dough formed was then washed with distilled water to obtain gluten. After dough washing, starch and soluble proteins were eliminated together with the water. Following this process an elastic protein matrix (gluten)

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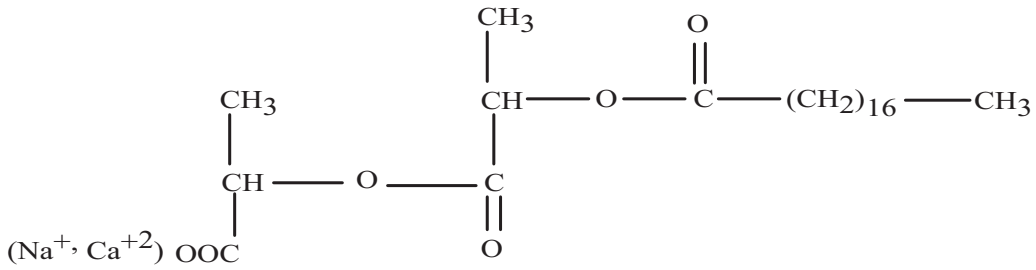

Fig. 1. Structure of the Sodium stearoyl lactylate (SSL) emulsifier.

was obtained mainly formed by gliadins and glutenins with 62% of moisture content. No gluten was formed at levels of emulsifier higher than 1%. Gluten samples were freeze dried, milled by hand with a pestle in a mortar and stored at 4 °C.

2.3. FT-Raman spectroscopy

Raman spectra of dried gluten samples were collected on a Bruker IFS 113 FT-IR spectrophotometer provided with the NIR Raman attachment equipped with an Nd:YAG laser at 1064 nm laser. Frequency calibration of the instrument was undertaken using the sulfur line at 217 cm^{-1}. Spectra were recorded at room temperature with a laser power of 500 mW, and spectral resolution of 6 cm^{-1}. Each spectrum was obtained after collecting and averaging 1000 scans in order to obtain high signal-to-noise ratio spectra. FT-Raman spectra were plotted as intensity (arbitrary units) against Raman shift in wavenumber (cm^{-1}). All spectra were vector normalized to the intensity of the phenylalanine band at 1004 cm^{-1}. In Amide I region, a straight baseline passing through the ordinates at 1715 and 1625 cm^{-1} was adjusted in order to calculate this band intensity. The intensity values obtained for the tyrosine doublet were calculated relative to the local baseline of each peak (830 and 850 cm^{-1}). Band assignment of the major vibrational motions of the side chains or the peptide backbone was based on comparison to Raman data reported in the literature [7]. Fitting of the CH stretching band was performed based in the following procedure: the frequencies, the number of peaks to be fitted, and the half-width of each peak to start a leastsquare iterative curve-fitting procedure were those obtained from the second derivative of the original spectra. The areas of the bands were calculated by integration of the corresponding fitted band. A straight baseline passing through the ordinates at 3090 and 2800 cm^{-1} was adjusted as an additional parameter to obtain a best fit. The curve-fitting procedure was performed by stepwise iterative adjustment towards a minimum root mean-square error of the different parameters determining the shape and position of the Raman bands. It was carried out by assuming an initial mixed Lorentzian–Gaussian line-shape function, with full width band at half-height (FWHH) of 10–13 cm^{-1} and a maximum resolution factor. Band intensities, base line corrections, normalization, derivation, curve fitting and area calculation were carried out by means of Grams/32 (Galactic Industries Corporation, USA) software, OPUS 3.1 and Perkin-Elmer software. The resulting fitted curve was analyzed taking into account the band assignment for the secondary structure previously reported in the literature [8]. In order to calculate the percentage contribution of the different types of conformations to the area of all the components, bands assigned to a given conformation were summed and divided by the total area. All analyses were performed in three independent experiments, and the results were reported as averages of these replicates. Before spectra were analyzed the raw data were subjected to a quality test using Opus software. Spectral quality was ensured by taking into account the signal-to-noise ratios (r_{s1} and r_{s2}). The signals were taken as peak heights of the band S_1 and S_2 at the 3000–2800 cm^{-1} and 1700–1600 cm^{-1} regions, respectively; and the noise was determined between 2500 and 2300 cm^{-1} as peak-to-peak. Signal-to-noise ratios were calculated by the quotient between the corresponding signals and noise values.

3. Results and discussion

3.1. Amide I band

Raman bands corresponding to Amide I and III can be used to characterize protein backbone conformation. Usually, proteins having (-helix contents show an Amide I band centered around 1650–1660 cm^{-1} and a weak Amide III band located in a broad region from 1260 to 1300 cm^{-1} [7]. Fig. 2(A) shows Amide I band obtained for gluten. This result is in concordance with previously reported data [9] indicating that secondary structure of gluten is dominated by α-helix conformation. Attachment of additives to food proteins affects electrostatic and hydrophobic interactions, producing conformational changes. In this case, modifications are showed in Fig. 2(B–D). The calculated band intensities of the Amide I band were 0.196 ± 3.10^{-3} for gluten (control sample) and 0.322 ± 4.10^{-3}, 0.273 ± 3.10^{-3} and 0.221 ± 3.10^{-3} for gluten pre-

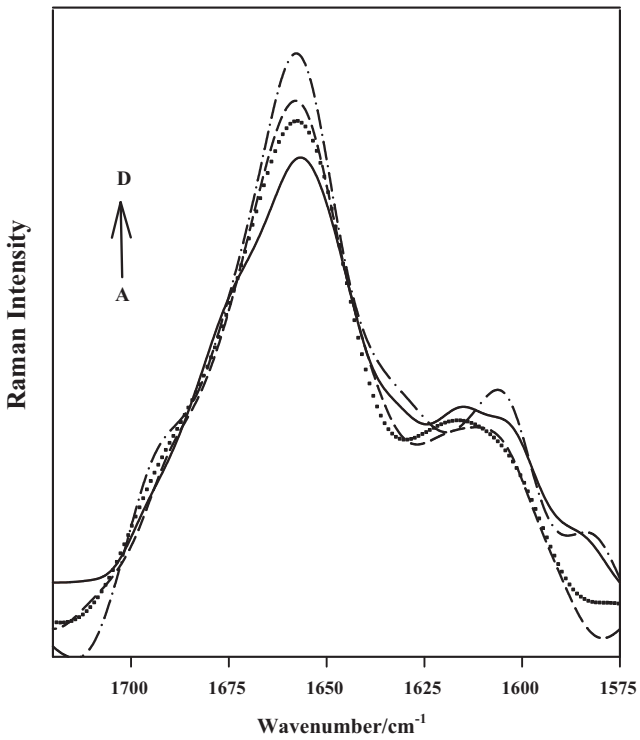

Fig. 2. (A) Amide I band of gluten, (B) Amide I band of 1% SSL-gluten system, (C) Amide I band of 0.5% SSL-gluten system and (D) Amide I band of 0.25% SSL-gluten system.

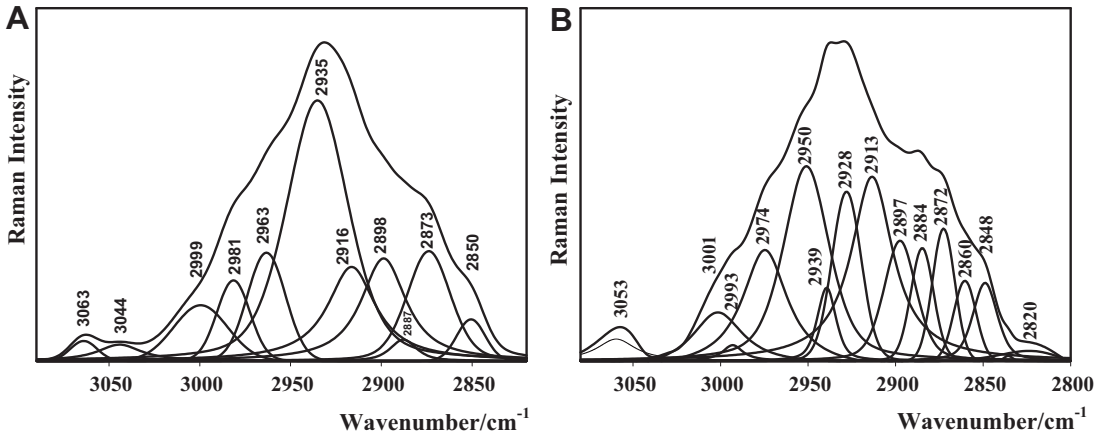

Fig. 3. CH stretching region (A) gluten, (B) 0.25% SSL-gluten system.

pared with SSL at different concentrations 0.25%, 0.5%, and 1%, respectively. An increase of the intensity of Amide I band of SSL-gluten was observed, presenting a maximum at 0.25% level. A higher intensity of the band, assigned to (-helix conformation, can be related to a high proportion of this conformation in the secondary structure of the protein [10].

Mingduo et al. observed that gluten protein-emulsifier interactions lead to a more cohesive dough structure with a high gluten protein cross-link degree. This type of network formed is in agreement with the more ordered structure observed for 0.25% SSL-gluten sample [11].

In Fig. 2 it is also possible to observe that the modifications in the intensity of the Amide I bands (assigned at 1657 cm^{-1} to α-helix conformation) were accompanied by a decrease in intensity of the band assigned to β-sheet component (1613–1625 cm^{-1}) in the 1.0% and 0.5% SSL-gluten samples with practically the disappearance of this band in 0.25% SSL-gluten sample. This behavior was in accordance with the proposed conformational change [10].

3.2. Side chain vibrations analysis

3.2.1. Tyrosine doublet

After SSL treatment, significant changes were observed in gluten samples. The detected changes indicated that the microenvironments of tyrosyl groups were greatly altered by the interaction of the additive with the gluten protein. The ratio of the tyrosyl doublet around 850 and 830 cm^{-1} ($I_{850/830}$) is known as a good indicator of the hydrogen bonding of the phenolic hydroxyl group. A decrease of $I_{850/830}$ ratio was reported to reflect an increase in buriedness, suggesting possible involvement of tyrosyl residues in intermolecular or intramolecular interactions. When tyrosine residues are exposed, the 850 cm^{-1} band becomes more intense than the 830 cm^{-1} band [12]. In comparison with control gluten (1.42 ± 0.04), only in the mixture containing 1% of the SSL the $I_{850/830}$ ratio (1.56 ± 0.05) was increased. For the others samples with 0.5% and 0.25% SSL contents the $I_{850/830}$ ratio calculated were 1.25 ± 0.03 and 1.12 ± 0.03, respectively, resulting lower than the control sample. The last behavior is in concordance with the burial of tyrosine groups and with folding of gluten protein. The unusual high intensity ratio value for the 1% level suggests that the tyrosine residue, in an extremely strong hydrogen bond, acts as a positive charge acceptor. In this case, this behavior possible could be related to an excess of metal ions (Na^+ and Ca^{+2}) at this concentration of SSL (1%) [13].

3.2.2. CH stretching region

In order to investigate the CH stretching region, the fitting of this band corresponding to native gluten and 0.25% SSL-gluten, was performed; considering that one of the most significant changes was observed at the lowest level of SSL. Fig. 3 shows the CH stretching region of native gluten (A) and 0.25% SSL-gluten (B). The considerable changes are associated primarily to the bands related to the contribution of Tyrosine (Tyr) moiety among others aminoacids, mainly those containing OH groups [8]. The relevant band of native gluten, located at 2935 cm^{-1} with a 37% of contribution to the total area was split into two components located at 2939 and 2950 cm^{-1}

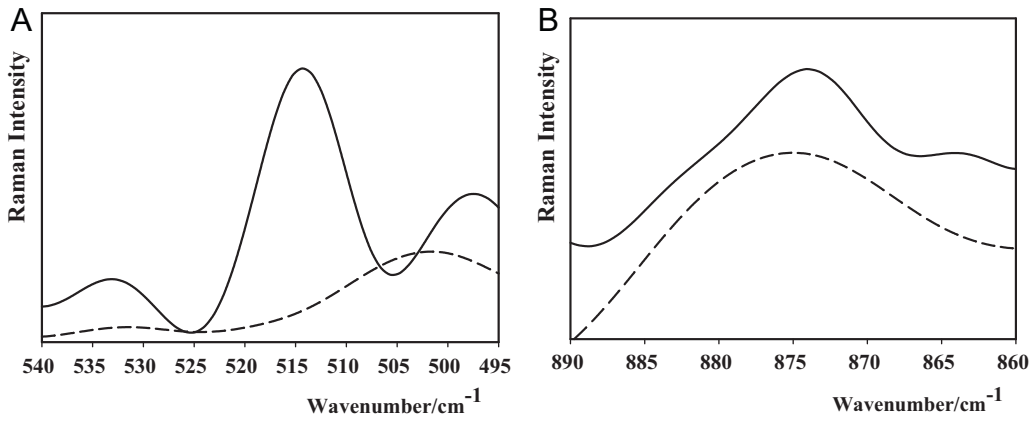

Fig. 4. (A) Disulfide stretching vibrational bands, control gluten (dotted line), 0.25% SSL-gluten (solid line). (B) Tryptophan ca. 875 cm^{-1} band: control gluten (dotted line), 0.25% SSL-gluten (solid line) (17 number of smoothing points-OPUS Program).

in the presence of SSL and a significant lowering in the percentage of the total area contribution of each component leading to 2.7% and 17.5%, respectively, was also detected. The decrease of the area percentage would mainly be related with the buriedness of Tyr and the shifting of the bands with the inter or intra-molecular H bond.

3.2.3. Disulfide region

Raman spectroscopy provides a direct analysis of the disulfide region, showing S–S stretching vibrational bands in the region 500–550 cm^{-1} As it can be seen from Fig. 4(A) in control gluten (dotted line) those bands associated to these vibrational modes are located at 534 cm^{-1} and 503 cm^{-1}. These bands are related to trans–gauche–trans (t–g–t) rotamer and gauche–gauche–gauche (g–g–g) form, respectively [14,15].

We can observe that g–g–g gives the most intense band. With the addition of the perturbing additive (0.25% SSL), three bands appeared at 533, 514 and 497 cm^{-1}. The first one is related to the t–g–t configuration and the others are probably associated to two types of S–S configuration, intrachain and interchain disulfide bounds, respectively. Similar behavior was observed in insulin protein [7]. These results are in agreement with protein folding phenomenon suggested for the gluten protein-additive interaction.

3.2.4. Tryptophan modes

A sharp line at 1361 cm^{-1} has been suggested as an indicator of buried tryptophan residues. Thus, the presence or absence of a line in this region of the spectrum of proteins containing tryptophan, suggests buried or exposed tryptophan groups [16]. However, other tryptophan bands (880 cm^{-1}, 760 cm^{-1}) have been proposed to be used as a monitor for the strength of H-bonding and the hydrophobicity of the environment of the indole ring. The addition of the emulsifier caused an increase of intensity of these bands. In the Fig. 4(B) it is possible to observe the increase in intensity of the band center at 875 cm^{-1}. These results suggest that in the presence of SSL, tryptophan residue turns from a middle exposed hydrophobic micro environment to a buried one, and it contributes to the formation of the new more ordered structure.

4. Conclusions

The present data show that the native conformation of the gluten protein is affected after the treatment with SSL emulsifier. The modified protein showed a significant change with a substantial increase in the intensity of the Amide I band mainly for the 0.25% SSL addition pointing to a more ordered structure. In addition, conformational variations of disulfide bounds were detected together with the variations of the intensity ratio of the tyrosine doublet bands, tryptophan and CH stretching band, being in concordance with the conformational change tendency to a more ordered structure. This behavior was also observed with studies performed to gluten proteins: changes in free sulfhydryls and aggregation of proteins due to emulsifier presence (study in progress).

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