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Changes in secondary structure of gluten proteins due to emulsifiers

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HIGHLIGHTS

- Changes in the secondary structure of gluten proteins were analyzed.
- Emulsifiers additions were analyzed by Raman Spectroscopy.
- Protein folding was induced by 0.25% of Sodium Stearoyl Lactylate (SSL).
- High levels of SSL and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEMs) led to more disordered protein structures.

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ABSTRACT

Changes in the secondary structure of gluten proteins due to emulsifiers were analyzed by Raman Spectroscopy. The protein folding induced by 0.25% SSL (Sodium Stearoyl Lactylate) (GS0.25, Gluten + 0.25% SSL) included an increase in α-helix conformation and a decrease in β-sheet, turns and random coil. The same behavior, although in a less degree, was observed for 0.5% gluten–DATEM (Diacetyl Tartaric Acid Esters of Monoglycerides) system. The low burial of Tryptophan residues to a more hydrophobic environment and the low percentage area of the C–H stretching band for GS0.25 (Gluten + 0.25% SSL), could be related to the increased in α-helix conformation. This behavior was also confirmed by changes in stretching vibrational modes of disulfide bridges (S–S) and the low exposure of Tyrosine residues. High levels of SSL (0.5% and 1.0%) and DATEM (1.0%) led to more disordered protein structures, with different gluten networks. SSL (1.0%) formed a more disordered and opened gluten matrix than DATEM, the last one being laminar and homogeneous.

1. Introduction

Improvements in wheat flours quality, and the requirement of maintaining good organoleptic and textural characteristics of bread, resulting in an adequate acceptance by consumers, promote the utilization of additives for improving breadmaking quality. Emulsifiers have been employed as anti-staling agents, dough modifiers, shortening sparing agents, and as improvers for the production of common and high-protein breads. Sodium Stearoyl Lactylate (SSL) and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEMs) are common emulsifiers used in breadmaking (Fig. 1). Due to their high hydrophilic/lipophilic balance these additives produce strong doughs, in which the lipophilic tail of the molecule would be bound to hydrophobic sites of gluten proteins. SSL is a surfactant known to improve volume and softness in fresh and frozen dough products [1,2]. This additive promotes emulsifying and air incorporation into dough [3]. In breadmaking, SSL improves crumb and crust texture, diminishes water loss, and decreases starch recrystallization percentage in bread [4], thus extending the shelf life of the product [5,6]. In dough pieces frozen up to 8 weeks, SSL and DATEM incorporation produced dough with higher resistance to extension and bread with significantly higher loaf volume than bread without emulsifier [7].

DATEM are anionic oil-in-water emulsifiers that are used as dough strengtheners to improve bread quality [8]. They improve mixing tolerance, gas retention, and resistance of dough to collapse. In bread, these emulsifiers increase loaf volume [9] and generate a crumb with a resilient texture, fine grain, and good slicing properties [10]. DATEM may promote the aggregation of gluten proteins in dough by binding to the protein hydrophobic surface and, through hydrogen bonds, with glutamine [11]. These interactions generate a strong protein network, which in turn will produce bread with a better texture and increased volume [12]. Hydrophilic emulsifiers may also form lamellar liquid–crystalline phases in water, which associate with gliadins. The formation of

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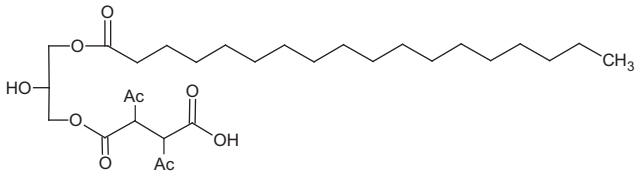
DATEM:



SSL:

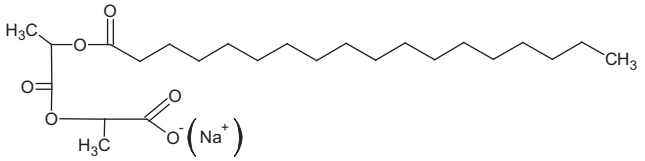


Fig. 1. Structures of the Sodium Stearoyl Lactylate (SSL) and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEMs).

such structures allows the expansion of gas cells and contributes to dough elasticity, resulting in increased bread volume [9].

Spectroscopy techniques are adequate for studying secondary and tertiary protein structure. Modifications in protein structure due to several factors (temperature, pressure, pH) as well as the interaction of certain type of molecules like emulsifiers with proteins can be analyzed [13,14]. As FT-Raman spectra of polypeptides and proteins exhibit characteristic bands, they are utilized for monitoring structural changes in food proteins. Peptide bond has nine bands, being the most important Amide I and Amide III bands. In addition, changes due to protein treatment, the conformation around the disulfide bonds, the involvement of phenol groups and Tyrosine residues in hydrogen bonding, and the degree of exposure of aromatic residues, are all reflected in Raman bands. Analysis of Amide I and Amide III bands, as well as skeletal stretching modes related to the polypeptide backbone, can be used to estimate the secondary structure of proteins [15,16] studied changes in gluten structure due to chemical amidation of proteins and found that this treatment led to transitions from ordered to random coil structures.

There is quite information about the improving effect of SSL and DATEM emulsifiers on bread quality, but few knowledge about structural changes of gluten proteins produced by these emulsifiers was reported. In a brief earlier communication [17] the analysis of the intensity of the Amide I band and some side chain bands of the gluten protein modified by the presence of the SSL emulsifier, was presented. In this paper, we present a more thorough investigation that includes conformational studies of proteins together with studies of the changes in the protein secondary structure including deconvolution and fitting process of the Amide I band to calculate the percentage contribution of the different types of conformations to the area of all the components. It complements the previously published information, including the analysis of the conformational changes produced for another common emulsifier used in breadmaking, DATEM and an exhaustive comparison of the changes the structure of the protein produced by both additives.

2. Materials and methods

2.1. Preparation of gluten samples

Wheat flour (000 type flour) for breadmaking (*Triticum aestivum* L.) was provided by an Argentinean mill (Molinos Campodónico Ltd., Argentina). Sodium Stearoyl Lactylate (SSL) and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEMs) were obtained from DANISCO (Copenhagen, Denmark) both contain calcium carbonate as anti humectants.

Gluten samples were prepared from wheat flour (control sample) or a blend composed by flour and an emulsifier: SSL, DATEM or SSL + DATEM (1:1); at levels of 0.25%, 0.5% and 1.0% (flour basis). Emulsifier levels higher than 1.0% did not allow gluten formation. Samples were codified as: G for native gluten (without emulsifier), GS for gluten–SSL, GD for gluten–DATEM and GSD for gluten–SSL + DATEM. Levels of emulsifiers were written after the codifying letters. The numbers after the letters indicate the concentrations of emulsifiers utilized for dough preparation, before obtaining gluten throughout dough washing process. Wheat dough and gluten samples were prepared in the Glutomatic equipment [18]. Samples were prepared as described previously [17]. Gluten samples, prepared in duplicate, were freeze-dried, milled, and stored at 4 °C until analysis.

2.2. Secondary structure of gluten proteins

Parameters related to secondary and tertiary structure of gluten proteins were determined by FT-Raman Spectroscopy according to [17]. 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 (1064 nm). Frequency calibration of the instrument was performed using the sulfur line at 217 cm^{-1}. Spectra were recorded at 25 °C with a laser power of 500 mW, and a spectral resolution of 6 cm^{-1}. Each spectrum was obtained after collecting and averaging 1000 scans in order to obtain spectra with high signal-to-noise ratios. FT-Raman spectra were plotted as intensity (arbitrary units) against Raman shift in wavenumber units (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 1700 and 1600 cm^{-1} was adjusted in order to calculate this band intensity. To calculate the secondary structure components, this region was truncated and fitted. The resulting fitted curve was analyzed taking into account the band assignment for the secondary structure previously reported in the literature [19–21]. The assigned structures were β-antiparallel: 1675–1695 cm^{-1}, turns: 1666–1673 cm^{-1}, α-helix: 1650–1658 cm^{-1}, random coil: 1637–1645 cm^{-1}, solvated helix: 1625–1637 cm^{-1}, β-sheet: 1613–1625 cm^{-1}. 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 Amide I area. The obtained number was taken as the proportion of the polypeptide chain in the corresponding conformation. The fitting procedure of the CH stretching band was performed by passing straight baseline through the ordinates at 3090 and 2800 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 [15–19]. In both cases the following procedure was applied: the frequencies, the number of peaks to be fitted, and the half-width of each peak to start a least square iterative curve-fitting procedures 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. 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. The intensity values obtained for the Tyrosine doublet were calculated relative to the local baseline of each peak