

**EFFECT OF FORMULATION AND PROCESS ON
PSYSSICOCHEMICAL PROPERTIES OF
CEREAL BASED FOODS**

Ph.D. dissertation

by

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**Dedicata a voi mamma e papà,
certezze della mia vita.**

**Non sono i frutti della ricerca scientifica che
elevano un uomo ed arricchiscono
la sua natura, ma la necessità di capire
e il lavoro intellettuale**

Albert Einstein

Summary

Food products are complex materials whose quality and stability are strongly dependent upon the way their constituent structural elements interact and assemble at multiple time-space domains. The ability to predict and control quality and stability of food requires a thorough understanding of the properties and the dynamics at multiple space-time levels characterizing food materials. The formulation of a product defines the type and the amount of structural elements available to build the food materials while the processing conditions determine the way the constituent building blocks interact and assemble.

It is well recognized that water plays an important role not only in food processing operations but also in defining quality and stability of food. A thorough understanding of water status and water dynamics is one fundamental element to understand quality and stability of food items.

The effect of formulation and processing in tortillas and fresh pasta have been studied in respect to products' quality and stability with a multianalytical approach to describe multiple attributes at different time-space levels. Macroscopic product quality and stability indicators were found to be related to different measurable parameters underlying that the "relevant scale" that determines different properties must be identified.

The description of the water status with different parameters such as water activity, moisture content, "frozen water" content (macromolecular level) and ^1H NMR mobility (molecular level) was found to be a very valuable tool for a better understanding of properties and stability in relation to formulation and processing.

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Introduction

A food is an assemble of a variety of “building blocks” that are held together by multiple type of interaction forces resulting in a large variety of microstructures that will ultimately define product quality and stability. All definitions of food microstructure emphasize three key aspects: (i) the presence of identifiable discrete elements or domains, (ii) some kind of organization among these elements in space (architecture), and (iii) the presence of interactions (Aguilera and Lillford, 2008).

The type if building blocks and forces involved depend on the structural level of interest, e.g. nano-scale (0.1-100 nm), micro-scale (0.1-100 μm), or macro-scale (0.1-100 mm). Some of the most common building blocks found in foods are listed below:

- Nano-scale: atoms, ions, molecules (e.g., proteins, polysaccharides, lipids, water), micelles, microemulsions, molecular assemblies.
- Micro-scale: lipid droplets, fat crystals, air bubbles, starch granules, cells.
- Macro-scale: bulk phases (e.g., oil, water, air).

A variety of forces act between these building blocks, which also depends on the scale (McClements, 2005):

- Nano-scale: covalent interaction, physical interaction (i.e., intermolecular van der Waals, electrostatic...).
- Micro-scale: physical interactions (i.e., colloidal van der Waals, electrostatic, hydrogen bonding and hydrophobic forces), gravity, electrical forces, mechanical forces.
- Macro-scale: gravity, electrical forces, mechanical forces.

Identification and characterization of the most important elements and forces present in a food is necessary to understand its physicochemical, sensory and nutritional properties. The interpretation of the results and the

understanding is extremely difficult because foods are both compositionally and structurally complex systems and are also subjected to very different environmental conditions.

The relationship between structure and final properties of food is a key factor in material science, product engineering and plant design that has to be taken in consideration to accelerate the prototyping stages, decrease times of production, reduce costs in an effort to deliver a product with desired properties, functionality and stability.

However, scientific literature in which there is an evident relation between microstructure and physical, sensorial and nutritional properties is not easy to find. When attempting to relate microstructure and food properties, the main issue is the choice of the scale at which elements interact to produce a given behaviour or effect. As regards complex and multicomponent systems such as food, this is a major mission because interactions may occur at different lengths scales from molecules to the macroscale, and may extend as well many decades on the time scale (Aguilera and Lillford, 2008). The trend of this type of research should to apply non-destructive and non-invasive techniques, and coupling imaging with physical and chemical probing to examine structures formation/collapse.

The formulation of a product defines the type and the amount of structural elements available to build food materials while processing conditions determines the way the constituent building blocks interact and assemble.

The high abundance as well as its contribution to functional, technological, and nutritional properties in foods made water molecule one of the most critical component of foods. Water is a small and dynamic molecule that has a heterogeneous spatial distribution within foods, and that exhibits significant variations in properties and reactivity depending on location. Water generally influences texture, flavour release and safety in food. In food processing, water plays a key role in determining quality and stability

of the final product. Most of processing operations are influenced by water compartmentalization and microscopic redistribution, which, in turn, affects macroscopic properties and food functionality (Vittadini and Vodovotz, 2007). Water plays a role in the definition of all levels of food structure: at the molecular level it can interact with other molecules (through hydrogen bonds, hydrophobic interactions, ...) and affect their conformation, mobility, plasticity and functionality. At an ultrastuctural level water can modulate the association/breakdown of macromolecules as well as the formation of natural assemblies. At a microstructural level, where colloidal phenomena predominate, the role of water is critical in the formation of droplets (e.g. emulsion), crystals (e.g. ice formation), air cells (e.g. foams), etc. Finally, all these structural interactions manifest themselves at a macrostuctural level (Vittadini and Vodovotz, 2007).

Water has to be taken in consideration as a key factor in food stability. In particular, controlling water availability for microbial growth has been one of the oldest food preservation technologies. Physical parameters have been used to express water availability or the relative degree of binding to food components. Water activity is the only water availability measure parameter actually used in food industry to predict the shelf-life and controlling the quality of foods. Water activity is expressed as the ratio of vapour pressure referred to pure water, depending on the degree of water binding to the solid interface. Unfortunately, foods are complex systems and in many cases are multiphase systems where different domains coexist, possibly, with different water activity. Franks (1991) emphasized the fact that availability may be related to the dynamic properties of water such as diffusional (translational) mobility. Slade and Levine (1988) proposed the glass transition (T_g) as the parameter to measure the diffusional dynamics of water and therefore its availability. Another approach to study the dynamic properties of water is to consider the short-range motions (or

"molecular relaxation") and not only the long-range relaxation (or "structural relaxation"), that can be done with molecular spectroscopy such as Nuclear Magnetic Resonance (NMR), Electron Spin resonance (ESR) and dielectric relaxation. NMR offers a non-invasive determination of the dynamic properties of water in complex systems.

Large interest has recently risen in the development of "functional" foods, products that affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way relevant to improved state of health and well-being, reduction of risk of diseases, or both (Riccardi et al., 2005). Foods rich in antioxidants and low glycemic index (GI) effect can reduce in combination the risk of increased post-prandial oxidative stress (constituent of the onset of several chronic diseases). (Monnier et al., 2006; Jenkins et al., 2006). Fiber-rich diets are associated with lower serum cholesterol concentrations, lower risk of coronary heart disease and certain forms of cancer, reduced blood pressure, enhanced weight and glycemic control, and improved gastrointestinal function (Marlett et al., 2002; Jones, 2008). Addition/substitution of functional ingredients to/in food formulations are expected to affect physicochemical properties and water status of the product at different levels and, consequently, its stability. Water redistribution is one of the most important phenomena that occurring during storage and contribute to staling process. To better understand the effect of formulation on physicochemical properties and water redistribution during storage is fundamental a multianalytical approach to identify multiple attributes at different time-space levels.

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Objective

The objective of this work was to study the effect of formulation and processing on the physicochemical properties and water status in nutritionally enhanced tortillas and fresh pasta to investigate the role of the different structure and mobility levels in establishing properties and stability of the products.

SECTION A

EFFECT OF PROCESSING
IN FRESH PASTA

EFFECT OF SHAPING

Effects of different shaping modes on physicochemical properties and water status of fresh pasta

Eleonora Carini, Elena Vittadini, Elena Curti and Franco Antoniazzi

1. Abstract

Fresh pasta is a very common food in Italy and it is mainly produced with a lamination process. Very little information is available in the literature about the physicochemical properties of fresh pasta as a function of the processing conditions. The object of this work was to evaluate the effect of different shaping process (extrusion, lamination and lamination with the application of vacuum) on physicochemical properties of fresh pasta.

Different shaping modes significantly ($p < 0.05$) affected macroscopic physicochemical properties (i.e. colour, cooking loss and texture) of fresh pasta whereas water status (moisture content, water activity, frozen water content and ^1H NMR mobility) was only slightly affected by the processing conditions.

The application of vacuum during lamination improved the “fresh pasta quality” indicators perceived by the consumers as it was characterized by a yellow colour and a tenacious and extensible texture.

2. Introduction

According to the Italian legislation “Pasta” is defined as the product obtained by extrusion or lamination and successive drying (to 12.5% maximum water content) of a dough made of durum wheat semolina and water (DPR, 2001). The Italian legislation allows the use of soft wheat flour in the “fresh pasta” recipe and requires storage of the product at temperatures < 4 °C. If “fresh pasta” is packed before sale, it should fulfil additional requirements: it must be subject to a pasteurization treatment, stored at temperatures $< 4 \pm 2$ °C, and have moisture content > 24 % and water activity in the 0.92 – 0.97 range (DPR, 2001).

The pasta-making process consists of few steps starting from mixing and kneading of semolina and water to supply the mechanical energy necessary to form a viscoelastic dough. The viscoelastic dough is then formed into the desired shape with either an extrusion or a lamination step. The product, characterized by its own shape, may then be stabilized with a pasteurization process and sold as “Fresh Pasta” or it can be dried to obtain “Pasta”.

The pasta manufacturing process can be considered a “mature technology” given not only its world-wide-spread diffusion, but also the very limited innovation applied to this process in the last fifty years. The literature on the effect of pasta processing on product quality is quite scarce and focused mainly on the role of raw materials (D’Egidio et al., 1990; Del Nobile et al., 2005, Vignaux et al., 2005), drying (Sannino et al., 2005, Berteli and Marsaioli 2005) and extrusion conditions (Pagani et al., 1989; Sarghini et al., 2005; Zardetto et al., 2005).

Pasta shaping by means of lamination is currently the preferred forming technology employed by the Italian fresh pasta manufacturers. To the authors’ best knowledge no reports comparing properties of laminated and extruded “fresh pasta” are present in the scientific literature and only a

couple compare the effect of shaping on either “pasta” (dry spaghetti, Pagani et al., 1989) of “fresh egg pasta” (Zardetto and Dalla Rosa, 2006). Pagani et al. (1989) reported that dried (45 °C, 16 hours) spaghetti (moisture content 12,5 %) formed by pressure extrusion (60 atm, 37 °C) exhibited a more extensive protein network breakage, a less porous and more compact structure which may be responsible for its poor cooking quality if compared with dried spaghetti formed by sheeting-rolls. Zardetto and Dalla Rosa (2006) recently reported that pasteurized fresh egg pasta (eggs: 19 % w/w) produced by lamination or extrusion differed in colour (extruded product was more yellow) and gelatinization level (more extensive in the extruded product) but adsorbed water similarly upon cooking. A different matrix-water association and starch-gluten interaction was suggested by the authors.

An innovative step has recently been proposed for the pasta manufacturing process that consist in the application of vacuum during lamination (EPA, 2006). At the authors’ best knowledge, no scientific data about the effect of lamination under vacuum on pasta (either “fresh” or “dry”) properties is available in the scientific literature.

The objective of this work was, therefore, the study the effects of different shaping modes (extrusion, lamination and lamination with the application to the vacuum) on selected physicochemical properties of fresh pasta.

3. Materials and Methods

Fresh pasta production

Fresh pasta was produced using durum wheat semolina (Molino Grassi di Fraore PR, Italy; moisture content = 14.5 % dry weight and 12.75 % protein)

and water at a 100:30 ratio. Semolina and water were mixed with a traditional dough mixer (Storci Spa, Collecchio, Italy) for 10 minutes.

Fresh pasta dough was then subjected to a different shaping processes to obtain a fresh pasta sheet 2.6 ± 0.1 mm thick. A schematic illustration of the different forming processes is shown in Figure 1.

- Extrusion using a V70-N (Storci Spa, Collecchio, Italy, 50 atm, 30 rpm). The dough was lad by a screw (798 mm long with a diameter to 72 mm) towards the extrusion head for an average time to 30 - 40 s subjecting the product to 50 - 55 atm of pressure and temperatures of 38 - 40 °C. This sample was named "**Ext**".
- Lamination using a STF540 TV dough sheeter (Storci Spa, Collecchio Italy). The dough was passed among grooved rollers almost instantaneously with very low stress applied to the product that had a temperature of ~ 29 °C at the end of the process. This sample was named "**Lam**".
- Lamination using a STF540 TV dough sheeter (Storci Spa, Collecchio Italy) with simultaneous application of vacuum (to 60 cm Hg to the laminating chamber). This sample was named "**Lam-v**".

Three fresh pasta productions were carried out for each shaping mode on different days.

Fresh pasta samples were kept in sealed plastic bags at 25°C and analyzed 2 hours after production.

Fresh pasta characterization

Fresh pasta macroscopic properties

Colour

Colour determination was carried out on the surface of fresh pasta samples using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka Japan). The spectral curves were determined over the 400-700 nm range using illuminant D₆₅ and for a 2 degree position of the standard observer. L* (lightness), a* (redness), b* (yellowness) values were measured (CIE, 1978). Ten punctual colour determinations on two samples of each past type were taken for each fresh pasta production.

Texture

Texture of fresh pasta samples was analysed using a TA.XT2 Texture Analyzer (Stable Micro Systems, Goldalming, U.K.) with a two-dimensional extensibility test (Bejosano et al., 2005). The test was carried out using a TA-108 Texture fixture that was attached to the texture analyzer platform and an acrylic probe (2.54 cm diameter at edges) attached to the analyzer arm. The test was conducted in compression mode at a constant speed of 3 mm/s. Force at rupture (maximum force [N] required to shear the sample) and extensibility (deformation at breakage [mm]) were obtained. Textural properties of ten samples of each fresh pasta type were analysed for each fresh pasta production.

Cooking loss

Cooking loss (the amount of solid substance lost to cooking water) was determined according to the method AACC (1999). The analysis was

performed in triplicate for each fresh pasta type (differently shaped) for each fresh pasta production.

Water properties

Water activity

Water activity (a_w) of fresh pasta samples was measured at 25 °C using a Decagon Aqualab meter TE8255 (Pullman, WA). Fresh pasta was broken into small pieces immediately before water activity measurement. Water activity of two samples of each fresh pasta type was analyzed for each fresh pasta production.

Moisture content

Moisture content (MC, g water / g product) of fresh pasta was determined from weight loss by drying in a forced-air oven at 105 °C. Moisture content of each fresh pasta type was analyzed in duplicate for each fresh pasta production.

“Frozen” water content

“Frozen water” (FW) content was obtained using a differential scanning calorimeter (DSC Q 100 TA Instruments, New Castle, DE, USA), calibrated with indium and n-dodecane. Fresh pasta samples (8 -10 mg) were placed into hermetically sealed stainless steel pans (Perkin Elmer, Somerset, NJ, USA), equilibrated at -50 °C and heated to 120 °C with a heating rate of 5 °C/min. Thermograms were analyzed with a Universal Analysis Software, Version 3.9A (TA Instruments, New Castle, DE) and enthalpy (ΔH , J/g), onset (T_{on}), and offset (T_{off}) temperatures of the transitions were obtained. Percent “Frozen water” (at the given conditions; FW) was calculated from the endothermic peak at about 0 °C (ice melting) using the following

equation (Vittadini et al, 2004; Vittadini and Vodovotz, 2003, Vittadini et al., 2002, Baik and Chinachoti, 2001; Baik and Chinachoti, 2000):

$$FW = \text{Enthalpy}_{\text{Ice Fusion}} \times \left(\frac{1}{\text{latent heat ice fusion}} \right) \times \left(\frac{1}{MC} \right) \times 100$$

FW = Frozen Water [%, g frozen water / g water]

Enthalpy_{Ice Fusion} [J / g product]

Latent heat of ice fusion = 334 J / g ice

FW content of each fresh pasta type was analyzed in duplicate for each fresh pasta production.

¹H NMR Mobility

¹H NMR Mobility was measured by low resolution (20 MHz) ¹H NMR spectrometer (the miniSpec, Bruker Biospin, Milano, Italy) to study a wide range of proton molecular mobility by measuring the free induction decay (FID, mobility of the most rigid components), transverse (T₂) and longitudinal (T₁) relaxation times (more mobile ¹H fractions). Approximately 3 g of sample were placed into a 10 mm NMR tube that was then sealed with parafilm to prevent moisture loss during the NMR experiment. All measurements were made at 25.0 ± 0.1 °C. FIDs were acquired using a single 90° pulse, followed by dwell time of 7 μs and a recycle delay of 0.4 s. T₂ (transverse relaxation time) was obtained with a Carr Purcell Meiboom Gill (CPMG) pulse sequence with a recycle delay of 0.4 s (≥ 5 T₁) and interpulse spacing to 0.04 ms (Carr and Purcell 1954, Meiboom and Gill 1958). T₁ (longitudinal lattice relaxation times) were determined by the inversion recovery pulse sequence with an inter pulse spacing ranging from 1 ms to 600 ms depending on the sample relaxation time and a recycle delay of 0.4 s (≥ 5 T₁). (Derome, 1987). The number of data points acquired was 300 for the FIDs, 20 for the Inversion Recovery and 1500 for the CPMG sequence. T₂ and T₁ relaxation time distributions

curves were analyzed as quasi-continuous distributions of relaxation times using a UPEN software (Borgia et al., 1998, Borgia et al., 2000).

Duplicated analyses on two fresh pasta sample for each different shape were carried out for a total of 12 NMR determinations for each experiment for each fresh pasta sample.

Statistical Analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 13.0, SPSS Inc., Chicago, IL, USA). SPSS was used to verify significant differences of evaluated parameters among fresh pasta samples produced with different shaping modes at the same storage time by one-way-analysis of variance (ANOVA) followed by least significant difference test (LSD) at $p \leq 0.05$.

4. Results and Discussion

The three shaping modes used in this study for fresh pasta production were characterized by intrinsically different processing conditions (processing times and kneading action) resulting in different levels of stress (pressure and temperature) applied to the forming pasta. It could, therefore, be assumed that fresh pasta obtained with a lamination process was generally less stressed than the extruded sample because it was not only exposed to lower temperatures and pressures, but also to a shorter processing time. Fresh pasta samples were characterized for multiple physicochemical properties to verify the effect of the different shaping processes on product quality.

Macroscopic Properties

Fresh pasta colour is the first important quality characteristic that greatly influences consumer acceptance and it is highly related to semolina properties (genetic and agronomic origin, carotenoid content, degree of milling; Borrelli et al., 1999; Dexter and Matsuo, 1978) and the conditions of the pastification process (De Stefanis and Sgrulletta, 1990; Acquistucci and Pasqui, 1992). The colour of the fresh pasta samples considered in this study are reported in Table 1 and the observable differences are ascribable only to the shaping processing step since both the ingredients used and the other processing variables (mixing and storage) were the same for all samples. The *Ext* sample was found to have, all colour coordinates ($L^* = 70.3$, $a^* = 1.2$, $b^* = 18.5$) significantly lower of the *Lam* sample (Table 1). Application of vacuum during product lamination further increased the differences among products (Table 1). In particular, the *Lam-v* sample was characterized by a more yellow colour while the *Ext* product was the least yellow. A more yellow colour in fresh pasta is generally considered an important quality attribute for the product. All colour parameters were significantly affected by the different shaping process and may be ascribable to an altered oxidation pattern of the coloured molecules (e.g. semolina's carotenoids) due to different temperature and oxygen content in the three processing modes considered and/or to a different microstructure of the fresh pasta matrix resulting, for example, from the elimination of air from micropores (or, even, their collapse) in the *Lam-v* product.

The amount of residue in the cooking water is widely used as an indicator of fresh pasta quality. Low amounts of residue indicate high fresh pasta cooking quality. Significant differences were found among the samples as result of the fresh pasta shaping process (Figure 2). The larger cooking loss was observed in the *Ext* sample (2.25 ± 0.09 g solids released / 100 g

product) while the smallest in the *Lam* product (1.62 ± 0.06 g solids released / 100 g product); *Lam-v* fell in the middle (2.04 ± 0.14 and 2.4 ± 0.1 g solids released / 100 g product, respectively). The higher stress applied on the *Ext* sample during processing may have either caused some damage to the starch phase (i.e. breakage of starch granules that would easy starch loss in the cooking water) or altered some domains of the continuous phase favouring solid loss during cooking. The milder conditions characteristic of the lamination process may have lead to the formation of a more stable and less damaged gluten-starch matrix that resulted in a lower release of solids during cooking. Previously, Pagani et al (1989) reported higher protein network breakage (observed by Scanning and Transmission Electron Microscopy) in extruded spaghetti as compared to roll-sheeted or hand made dry spaghetti.

Textural attributes (force at rupture and extensibility) of fresh pasta samples were summarized and reported in Figure 3 and found to be significantly affected by the different shaping processes considered. *Ext* and *Lam-v* samples resulted significantly higher (but comparable themselves) in force at rupture than *Lam* sample. Extensibility was also significantly affected by the shaping mode with the *Ext* product being the most extensible followed by *Lam-v* and finally *Lam*. It is well know that overall texture of fresh pasta is strongly affected by the gluten network developed during processing. It may be hypothesized that the higher “stress” intrinsic to the extrusion process may have favoured the formation of a more tenacious and extensible continuous phase (i.e. gluten network) by forcing interactions among biopolymers (within themselves and/or with water) as compared to the milder conditions found in the lamination process. The application of vacuum during lamination may have favoured the formation of a stronger and more extensible matrix than in the traditional lamination process. It may be hypothesized a reduction of the free volume among

molecules (through air removal and a possible micro-structural “collapse”) resulting them coming to closer vicinity and favouring their interaction. Similar results were also reported by Pagani et al (1989) who found higher breaking strength in extruded dry spaghetti as compared to cooked roll-sheeted or hand made dry spaghetti.

Water Properties

Water is known to play a key role in quality and stability of food products as it can interact with other molecules through hydrogen bonds, hydrophobic interactions and it can affect their conformation, mobility, plasticity and functionality. Different processing conditions can strongly influence these molecular interactions and, consequently, alter the water-solid interactions and, ultimately, the quality and stability of the final products. Water properties of the fresh pasta considered in this study were characterized with a multi-analytical approach (in terms of moisture content, water activity, frozen water content and ^1H NMR mobility), in order to investigate the status of water at different length-time scales.

Ext (29.8 ± 1.2 water/g fresh pasta) and *Lam-v* (29.4 ± 0.4 g water/g fresh pasta) samples were found to have a slightly higher moisture content (i.e. water extractable at 105°C to constant weight) if compared to *Lam* sample (27.8 ± 1.2 g water/g fresh pasta) even if the same semolina:water ratio was used in all formulations (Table 2). Water activity (Table 2) ranged from 0.983 to 0.990 in the three samples considered in this study, with little, but significant differences due to the shaping process. *Ext* and *Lam-v* were characterized by a lower water activity (0.983 ± 0.003 and 0.985 ± 0.005 , respectively) while *Lam* by the higher water activity (0.990 ± 0.002). The water activity values of the samples are higher than the legal limit ($0.97 a_w$, DPR, 2001) but it must be taken into consideration that the product object

of this study did not undergo pasteurization and light drying that is used in industrial fresh pasta production that can induce a reduction of fresh pasta's water activity (Zardetto et al., 2005).

Thermal properties (FW, T_{on} and T_{off}) of the ice melting peak measured from thermograms obtained by DSC were reported in Figure 4. The ice melting endothermic peak had a lineshape and onset temperature (T_{on}) comparable among all fresh pasta samples. On the contrary, the shaping process affected T_{off} : in the *Ext* sample T_{off} was found significantly higher than in the laminated products (Figure 4) possibly suggesting that a fraction of the total water was better phase separated (i.e. "pure ice") and less interacting with the solids. The frozen water content (under the selected experimental conditions, Table 2), ranged from 35 to 39 % (g frozen water / 100 g water) with no significant differences among samples shaped in different modes.

Molecular mobility was studied by low resolution ^1H NMR and multiple experimental techniques were used in an attempt to cover a large range of molecular relaxation events. It must be emphasized that the ^1H NMR analysis is not specific for water as the signal detected may arise from any proton present in the sample relaxing in the time frame characteristic of the experiment (Halle and Wennerstroem, 1981; Schmidt and Lai, 1991; Colquhoun and Goodfellow, 1994; Ruan and Chen, 2001).

Characteristics ^1H FID decays for the three fresh pasta samples are shown in Figure 5A and they indicate the presence of a fast relaxing solid-like ^1H population in all fresh pasta samples. In the *Lam-v* sample a slightly slower decay was found as compared to *Ext* and *Lam* samples (Figure 5A), but these slight differences were not significant since the ^1H T_2^* relaxation times (calculated with a quasi-continuous distributions of relaxation times, Borgia et al., 1998; Borgia et al., 2000) were comparable (^1H $T_2^* = 9\text{-}10 \mu\text{s}$, insert in Figure 5A). Doona and Baik (2007) reported a proton population

relaxing at $\sim 10 \mu\text{s}$ in starch, gluten, starch/gluten mixtures (76:12, dry basis) and dough samples (41.1% moisture content) and similar results were reported in a biscuits dough (19.4% moisture content, 25 °C) by Assifaoui et al. (2006). The authors suggested that this population could be associated to solids components such as starch, protein and water molecules tightly associated with those of solids. Different shaping modes of fresh pasta samples considered in this study did not affect the less mobile proton fraction suggesting a similar molecular mobility of protons of the starch, proteins and water tightly associated in all samples.

^1H T_2 and ^1H T_1 relaxation decays were analyzed as quasi-continuous distributions of relaxation times (Borgia et al., 1998; Borgia et al., 2000) and the results were summarized in Figure 5B and 5C, respectively. ^1H T_2 distribution spectra were analyzed for $T_2 \geq 0.089 \text{ ms}$ (2 interpulse spacing + instrument dead time) in order to consider only real data points and no extrapolated values. T_2 distribution curves were extremely reproducible for the three fresh pasta analyzed. All fresh pasta samples were characterized by the presence of a major ^1H T_2 population ($\sim 80 \%$ to the total detectable protons) in the $\sim 2 - 20 \text{ ms}$ range and of a second faster relaxing population (peak at $\sim 0.15 \text{ ms}$). The mobility of all protons was comparable among the three types of fresh pasta considered in the T_2 time-frame window. To the authors best knowledge, the only study of ^1H NMR mobility of pasta products was reported by Zardetto et al. (2005), who observed, in extruded and laminated fresh egg pasta a monoexponential T_2 decay with relaxation times of 4 - 6 ms with no significant difference between samples. Doona and Baik (2007) reported in uncooked wheat dough (33.1% moisture content) the presence of two ^1H T_2 populations: the faster relaxing population (0.1 ms) was not affected by increasing water and was attributed to the water molecules closely associated with solids while the slower relaxing population was found to be moisture dependent. In particular, this

^1H T_2 population broadened and shifted from 3 to 10 ms as moisture content increased from 33.1 to 47.2 % (wb) and was related to a variation of the chemical and physical states of water molecules in the dough. Similarly, in flour-water mixtures (22 – 40% water [wb], manually blended) Assifaoui and co-workers (Assifaoui et al., 2006), indicated the presence of a ^1H T_2 population that shifted to longer relaxation times with increasing moisture content and related this increased mobility to an increase in free volume (higher mobility of starch) and to the proton population (gluten, starch and sucrose) likely associated with water (Assifaoui et al., 2006).

The ^1H T_2 results were also reflected by analysis of the ^1H T_1 distribution (Figure 5C) that indicated the presence of only one ^1H T_1 population in all samples. This suggested that the protons were in a “fast-exchange” regime in the T_1 experimental time window. The ^1H T_1 population observed in fresh pasta samples was characterized by different relaxation time ranges of the protons: ^1H T_1 ranged between 50 – 100 ms (peak at 66 ms), 40 – 140 ms (peak at 54 ms), and 25 – 180 ms (peak at 60 ms) in *Ext*, *Lam*, and *Lam-v*, respectively, suggesting a more homogeneous molecular/microstructure in the *Ext* product.

5. Conclusions

Different shaping modes (extrusion, lamination and lamination with the application to the vacuum) were found to significantly affect macroscopic physicochemical properties (i.e. colour, cooking loss and texture) of fresh pasta whereas water status (moisture content, water activity, frozen water content and ^1H NMR mobility) was only slightly affected by the processing conditions.

The “higher stress” characteristic of the extrusion process may have favoured the formation of a more plastic continuous phase (by forcing

interactions among biopolymers and water) resulting in a more extensible and firmer material that may be partially damaged in some domains favouring solid loss during cooking.

The milder conditions found in the lamination process resulted in a softer and less extensible material that better retained solids during cooking. The application of vacuum during lamination improved the “fresh pasta quality” indicators perceived by the consumers as it was characterized by a more yellow colour and had extensibility and firmness similar to the extruded fresh pasta. The application of vacuum during lamination induced air removal from the forming fresh pasta, reduced the free volume among molecules forcing them to closer interactions (e.g. solid-solid and water-solid).

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7. List of Tables

Table 1: Brightness (L*), redness (a*) and yellowness (b*) of fresh pasta samples produced with different shaping modes.

Table 2: Water activity and moisture content of fresh pasta samples produced with different shaping modes.

Table 1: Lightness (L^*), redness (a^*) and yellowness (b^*) for fresh pasta samples produced with different shaping modes.

	L^*		a^*		b^*	
	average	st. dev.	average	st. dev.	average	st. dev.
<i>Ext</i>	c 70.3	1.1	c 1.2	0.1	c 18.5	0.7
<i>Lam</i>	b 72.1	3.5	b 1.7	0.2	b 22.3	1.1
<i>Lam-v</i>	a 87.6	2.2	a 1.9	0.1	a 23.7	1.5

Different superscript small letters preceding numbers indicate significant differences of the column parameter among fresh pasta samples produced with different shaping modes ($p \leq 0.05$).

Table 2: Moisture content and water activity of fresh pasta samples formed with different process.

	moisture content g H ₂ O / g sample	water activity
<i>Ext</i>	29.8 ± 1.2	0.983 ± 0.003
<i>Lam</i>	27.8 ± 1.2	0.990 ± 0.002
<i>Lam-v</i>	29.4 ± 0.4	0.985 ± 0.005

Different superscript small letters preceding numbers indicate significant differences of the column parameter among fresh pasta samples produced with different shaping modes ($p \leq 0.05$).

8. List of Figures

Figure 1: Schematic illustration of the different shaping processes used for fresh pasta production.

Figure 2: Cooking loss of extruded, laminated and laminated under vacuum fresh pasta.

Different letters above histogram bars indicate significant differences among fresh pasta produced with different shaping modes ($p \leq 0.05$).

Figure 3: Force at rupture (A) and extensibility (B) of extruded, laminated and laminated under vacuum fresh pasta.

Different letters above histogram bars indicate significant differences among fresh pasta produced with different shaping modes ($p \leq 0.05$).

Figure 5: ^1H NMR characterization of fresh pasta samples produced with different shaping modes:

A) ^1H FID decays and corresponding ^1H T_2^* relaxation times;

B) ^1H T_2 distribution curves;

C) ^1H T_1 distribution curves.

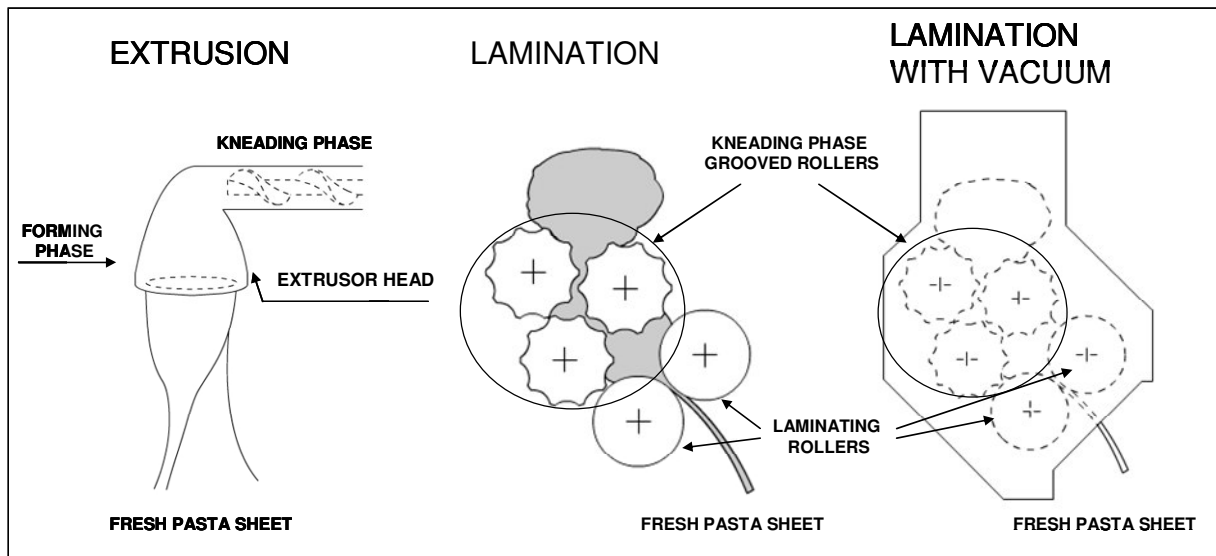


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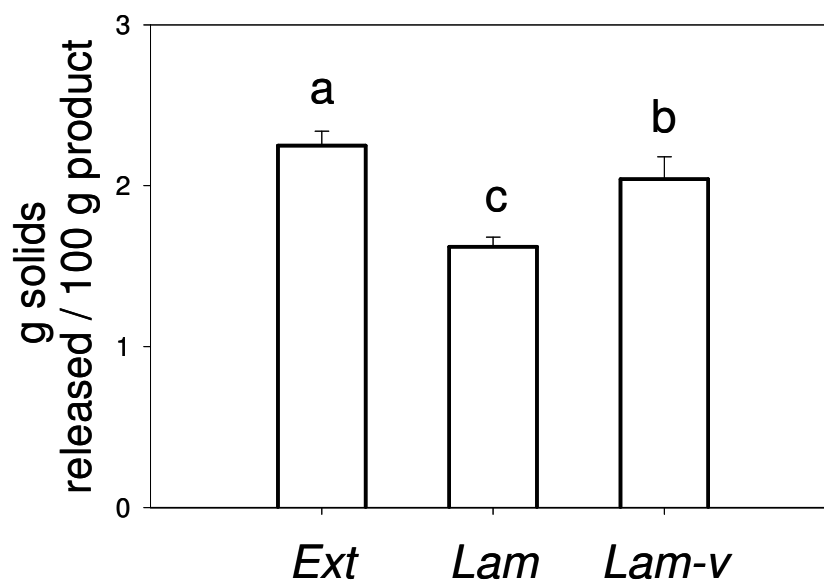


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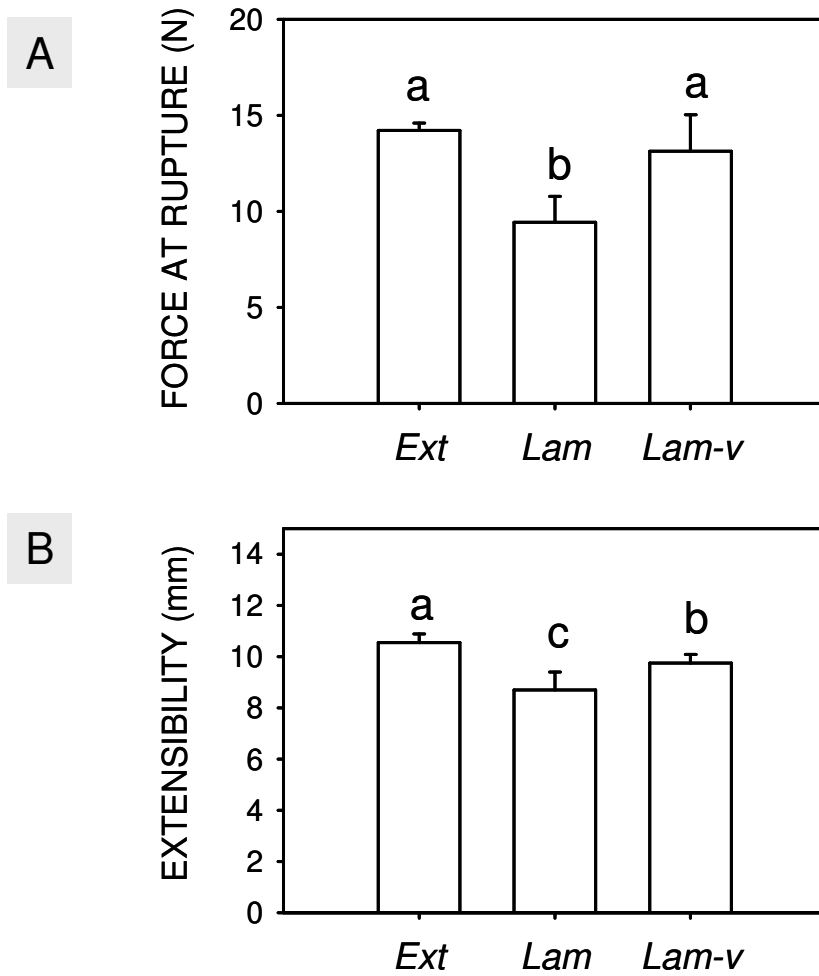


Figure 3: Force at rupture (A) and extensibility (B) of extruded, laminated and laminated under vacuum fresh pasta.

Different letters above histogram bars indicate significant differences among fresh pasta produced with different shaping modes ($p \leq 0.05$).

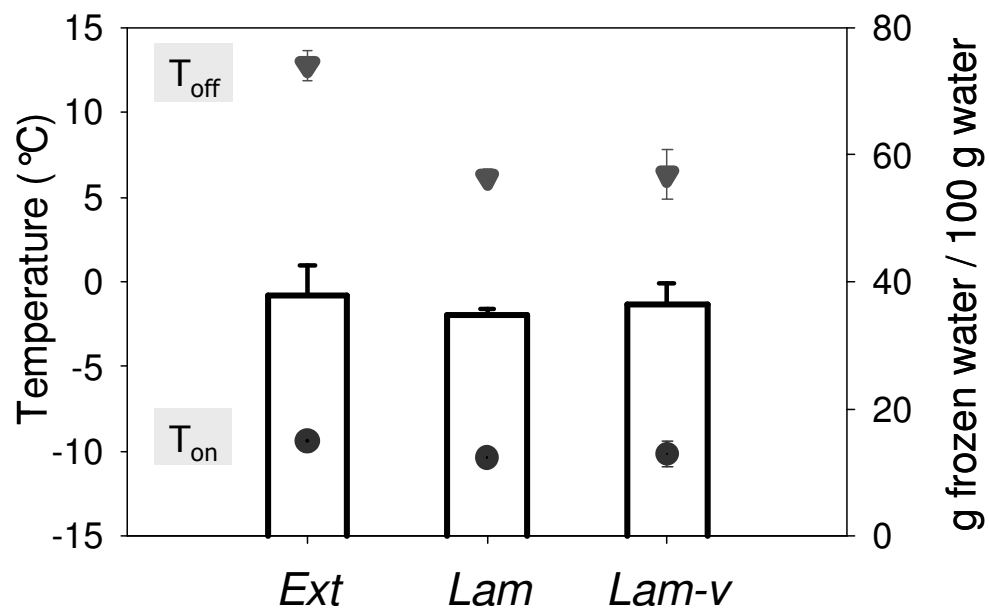


Figure 4: FW (bars), T_{on} (circle) and T_{off} (triangle) of ice melting peak for fresh pasta samples produced with different shaping modes.

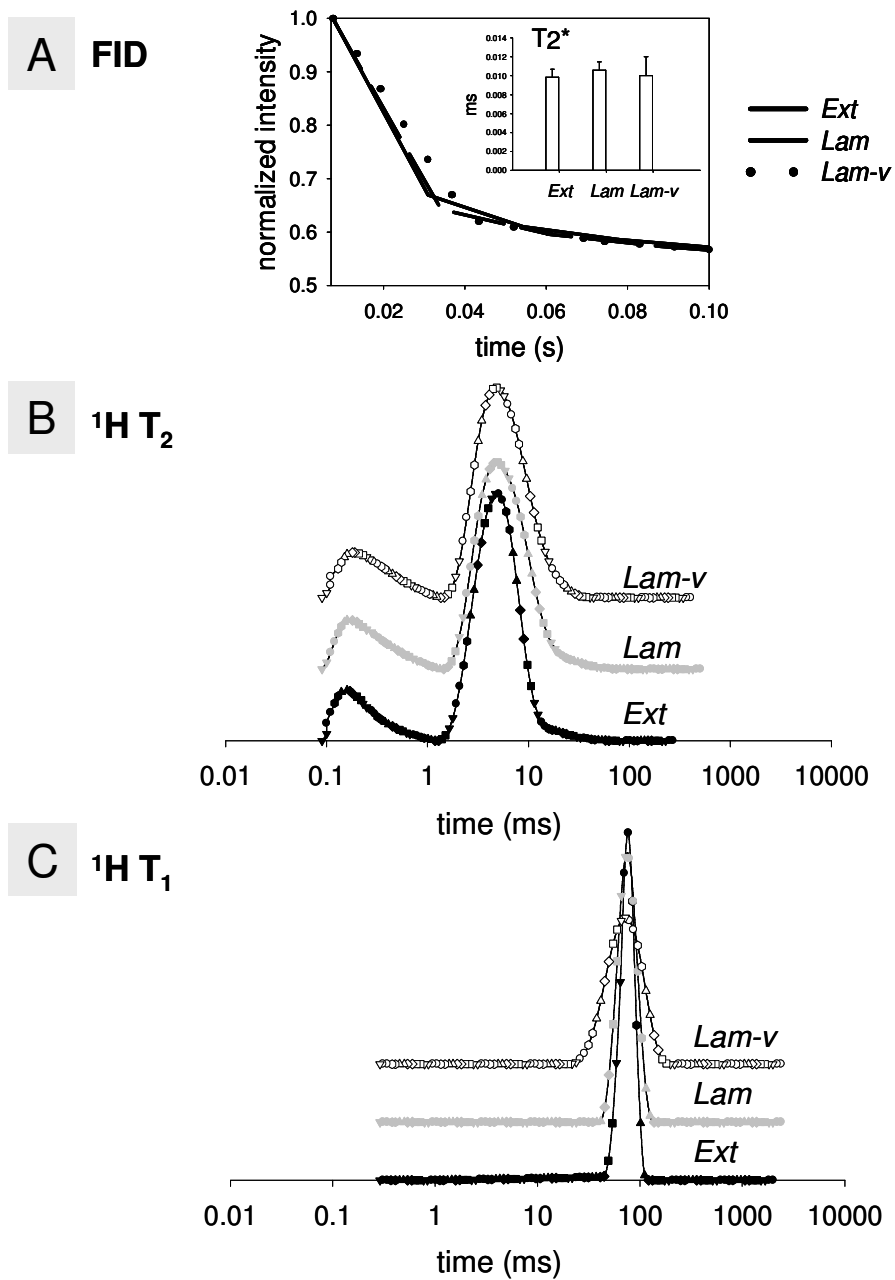


Figure 5: ^1H NMR characterization of fresh pasta samples produced with different shaping modes:

- A) ^1H FID decays and corresponding ^1H T_2^* relaxation times;
- B) ^1H T_2 distribution curves;
- C) ^1H T_1 distribution curve

EFFECT OF MIXING

Physicochemical properties of extruded and laminated fresh pasta produced with innovative mixers

Eleonora Carini, Elena Vittadini, Franco Antoniazzi, Elena Curti

1. Abstract

A recently designed innovative mixer (Premix®) that induces a quick and uniform hydration of the solids and allows for the formation of a dough in 2-5 s was used in extruded and laminated fresh pasta production. Premix® was also modified by the insertion of a “low pressure extruder” connecting the mixer to the extruder (Bakmix®). The effect of the innovative mixers on physicochemical properties of extruded and laminated fresh pasta sheets was evaluated.

Mixing of the ingredients with the innovative mixers (Premix® and Bakmix®) affected the physicochemical properties considered more markedly in the extruded than laminated products.

Experimental evidences suggest that the innovative mixers may have not allowed the formation of a well developed gluten matrix able to prevent solids loss during cooking and to result in a plastic and extensible texture. The harsher processing conditions of the extrusion step seem to enhance the effect of the mixing process.

The water status of fresh pasta was not affected by the mixing process in the laminated products. On the contrary, a stronger water-solids interaction measured (both in the macromolecular [FW] and the molecular [lower ¹H mobility of FID, larger % pop A, bimodal T₁ dispersion] scales) was induced by the innovative mixers in the extruded products.

2. Introduction

According to the Italian legislation “Pasta” is defined as the product obtained by extrusion or lamination and successive drying (to 12.5 % maximum water content) of a dough made exclusively of durum wheat semolina and water (DPR n. 187, February 9th 2001, Art. 6). “Fresh pasta” is not subjected to drying, allows the use of soft wheat flour in the formulation and must be stored, if sold unpackaged, at temperatures < 4 °C. If “fresh pasta” is packed before sale it should be subject to a pasteurization treatment and have moisture content > 24% and water activity in the 0.92 – 0.97 range.

The pasta-making process consists of few steps starting from mixing and kneading of semolina and water to supply the mechanical energy necessary to form a viscoelastic dough. The viscoelastic dough is then formed into the desired shape with either an extrusion (more commonly) or a lamination step. The product obtained, characterized by its own shape, can be either sold as it, stabilized with a pasteurization process and sold as “Fresh Pasta” or dried to a moisture content < 14.5% to obtain “Pasta”.

The pasta manufacturing process can be considered a “mature technology” given its world-wide-spread diffusion, and the very limited innovation applied to this process in the last fifty years. The literature on pasta extrusion is quite scarce and focused mainly on the effect of raw materials (D’Egidio et al., 1990; Del Nobile et al., 2005, Vignaux et al., 2005), drying (Sannino et al., 2005, Berteli et al., 2005) and extrusion conditions (Pagani et al., 1989; Sarghini et al., 2005; Zardetto et al., 2005) on pasta processing and quality.

Mixing is the initial step in a pasta making process and the manner in which mixing is carried out (type of mixer, speed, time and pressure of mixing) determines the state of dispersion of ingredients, their interactions,

and, in turn, the efficiency of processing and quality of the final product. Water is known to play a key role in quality and stability of final products as it can interact with other molecules and affect their conformation, mobility, plasticity and functionality. Characterization of the properties of water in food products, as well as the effect of processing (e.g. mixing) and storage is a very important tool to understand the fundamental causes that define food quality and acceptability.

Mixing of semolina and water during pasta production is generally carried out in a mixer consisting in a horizontal chamber inside which rotates a mixing shaft with suitably shaped blades. Formation of the viscoelastic dough and optimal hydration of semolina is achieved in about 10- 15 minutes.

An innovative mixer (Premix®) has recently been designed (Patent DE102005025016) and it has been proposed for pasta manufacturing applications. The Premix® mixer (Figure 1A) provides the simultaneous introduction of semolina (stored in [2] and volumetrically dosed [3]) and water (dosed with a pump and delivered through [4]) in a chamber [5] containing a stirring mechanism. Semolina and water are subjected to a centrifugal force that causes their dispersion in air as dust and aerosol, respectively. The dispersed materials come in contact in a chamber [5] inducing an uniform hydration of the surface of each individual semolina grain and leading to the formation of a homogenous semolina-water mixture that is immediately extracted from the chamber through [6] in the form of an incoherent matter. The total mixing time of semolina and water in chamber [5] is about 2-5 seconds, significantly reduced from the 10-15 minutes of a traditional mixing operation. The incoherent matter obtained from the Premix® is generally allowed to rest for a few minutes (to match the timing of the traditional mixer) and then formed into the desired shape using an extruder [9]. A variation to the described design was developed by

linking the Premix® mixing chamber to a twin-screw “low pressure extruder” ([7], operating at 5 atm and 100 rpm, Figure 1B) that is then connected to an extruder [9] to form the pasta into the desired shape. The “low pressure extruder” step not only eliminates the resting time by directly linking the mixer to the extruder [9], but also favours the transformation of the incoherent wet semolina mass in a “pasta dough” with little stress applied to the product as it may be observed extracting the product from a large dye [8]. This modification of the Premix® design was named “Bakmix®”.

This innovative mixing processes are significantly different from the traditional mixing not only for the lack of extensive kneading action but also for a more even exposure of semolina particles to water and for the significantly shorter processing time. It is hypothesized that this innovative mixing process may affect water-solid interactions, the state of water in the dough and, consequently, influence pasta properties and quality.

The Bakmix® was previously used to produce white bread and the product was compared with a standard (produced with a traditional mixer) bread; the bread obtained with the innovative mixer was found softer than standard bread at longer storage times (≥ 5 days; Curti et al., in press) and this evidence was tentatively attributed to a better plasticization of the solids due to stronger water-solids interactions in Bakmix® (Curti et al., in press). A different water-solids interaction is, therefore, anticipated also in the fresh pasta produced to the innovative mixers, and its effect on product quality should be verified.

Pasta quality attributes include an amber-yellow colour without white (originating from uniform hydration) or brown spots and low cooking loss that are indicative of uniform mixing of the ingredients and proper gluten network development.

The objective of this work was, therefore, the study of the effect of the innovative mixers (Premix® and Bakmix®) in fresh pasta production and to evaluate product quality in terms at macroscopic, macromolecular and molecular levels.

3. Materials and methods

Pasta production

Pasta was produced using durum wheat semolina (Molino Grassi di Fraore PR, Italy; moisture content = 14.5 % dry weight, 12.75 % protein) and water at a 100:30 ratio.

Semolina and water were subject to mixing and the pasta dough was then shaped into 2.6 ± 0.1 mm thick pasta sheets either by extrusion (V70-N, Storci Spa, Collecchio, Italy; 50 atm, 30 rpm) or lamination (STF540 TV dough sheeter, Storci Spa, Collecchio Italy).

Mixing of the ingredients was carried out in three different modes:

- A- Traditional dough mixer (V50, Storci Spa, Collecchio, Italy) for 10 minutes. This sample was named "**Std**".
- B- Premix® (Storci Spa, Collecchio, Italy) for 2 seconds and rest for 10 minutes before extrusion. This sample was named "**Pre**".
- C- Bakmix® (Storci Spa, Collecchio, Italy) for a total time to 20 seconds (2 seconds in the Premix® and 18 seconds in the "low pressure extruder"). This sample was named "**Bak**".

Three pasta productions were carried out for each mixing mode on different days. Pasta samples were placed in sealed plastic bags immediately after production, kept at room temperature and analyzed after two hours.

Pasta characterization

Macroscopic properties

Water activity

Water activity (a_w) of pasta samples was measured at 25 °C using a Decagon Aqualab meter TE8255 (Pullman, WA). Pasta was broken into small pieces immediately before water activity measurement. Water activity of two samples of each pasta type was analyzed in duplicate for each pasta production.

Moisture content

Moisture content (MC, g water / 100 g product) of pasta was determined from weight loss by drying in a forced-air oven at 105 °C. Moisture content of two samples of each pasta type was analyzed in duplicate for each pasta production.

Colour

Color determination was carried out on the surface of pasta samples using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka Japan). The spectral curves were determined over the 400-700 nm range using illuminant D65 and for a 2 degree position of the standard observer. L^* (lightness), a^* (redness), b^* (yellowness) values were measured (CIE, 1978) and the color difference (ΔE) from the *Std* sample (of equal resting time) was calculated using the following equation:

$$\Delta E = \sqrt{(L^*_{\text{sample}} - L^*_{\text{control}})^2 + (a^*_{\text{sample}} - a^*_{\text{control}})^2 + (b^*_{\text{sample}} - b^*_{\text{control}})^2}$$

Ten punctual color determinations on two samples of each pasta type were taken for each pasta production.

Texture

Texture of pasta samples was analysed using a TA.XT2 Texture Analyzer (Stable Micro Systems, Goldalming, U.K.) with two-dimensional extensibility test (Bejosano et al., 2005) using a TA-108 Texture fixture that was attached to the texture analyzer platform and an acrylic spherical probe (2.54 cm diameter at edges) attached to the analyzer arm. The test was conducted in compression mode at a constant speed of 3 mm/s. Force at rupture (maximum force [N] required to shear the sample) and extensibility (deformation at breakage [mm]) were taken. Textural properties of six samples of each pasta type (different mixing) were analyzed for each pasta production.

Cooking loss

Cooking loss (the amount of solid substance lost to cooking water) was determined according to the AACC Method 66-50 (1999). The analysis was performed in triplicate for each pasta type for each pasta production.

Macromolecular properties: Thermal analysis

Thermal properties were studied using a differential scanning calorimeter (DSC Q 100 TA Instruments, New Castle, DE, USA), calibrated with indium and n-dodecane. Pasta samples (8 -10 mg) were placed into hermetically sealed stainless steel pans (Perkin Elmer, Somerset, NJ, USA), equilibrated at -50 °C and heated to 120 °C at a 5 °C/min heating rate. Thermograms were analyzed with a Universal Analysis Software, Version 3.9 A (TA Instruments, New Castle, DE). The thermal event observed (endothermic

peak at about 0 °C) was characterized for enthalpy (ΔH , J/g), onset (T_{on}), and offset (T_{off}) temperatures of the transitions.

“Frozen” water (FW) content was calculated using the following equation:

$$FW = \text{Enthalpy Ice Fusion} \times \left(\frac{1}{\text{latent heat ice fusion}} \right) \times \left(\frac{1}{MC} \right) \times 100$$

FW = Frozen Water [%, g frozen water / g water]

Enthalpy Ice Fusion [J / g product]

Latent heat of ice fusion = 334 J / g ice

Three DSC scans of two samples of each pasta type for each pasta production were analyzed.

Molecular properties: ^1H NMR mobility

^1H NMR mobility was measured by low resolution (20 MHz) ^1H NMR spectrometer (the miniSpec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1 °C to study ^1H molecular mobility. Approximately 3 g of sample were placed into a 10 mm NMR tube that was then sealed with parafilm to prevent moisture loss during the NMR experiment. The free induction decay (FID), T_2 (transverse relaxation time, CPMG pulse sequence) and T_1 (longitudinal relaxation time, Inversion Recovery pulse sequence) experiments were carried out. FIDs were acquired using a single 90° pulse, followed by dwell time of 7 μs and a recycle delay of 0.8 s. T_2 was obtained with a recycle delay of 0.8 s ($\geq 5 T_1$) and interpulse spacing of 0.04 ms. T_1 experiment was acquired with an inter pulse spacing ranging from 1 ms to 600 ms, depending on sample's relaxation time, and a recycle delay of 0.8 s ($\geq 5 T_1$). T_2 and T_1 curves were analyzed as quasi-continuous distributions of

relaxation times using a UPEN software (Borgia et al, 1998, Borgia et al., 2000). Duplicated analyses on two pasta samples for each different day of pasta production were carried out for a total of 12 NMR determinations for each pasta sample.

Statistical analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 13.0, SPSS Inc., Chicago, IL, USA). SPSS was used to verify significant differences of evaluated parameters among pasta samples produced with different mixers at the same storage time by one-way-analysis of variance (ANOVA) followed by least significant difference test (LSD) at $p < 0.05$.

4. Results

Fresh pasta produced with all considered mixers had a good appearance at a visual and tactile observation and was, therefore, characterized for the quality parameters perceived by the consumers (colour, texture and cooking loss). A thorough physicochemical characterization (macromolecular and molecular properties) of the produces was also carried out in an attempt to better understand the effect of the different mixing processes on extruded and laminated fresh pasta.

Macroscopic properties

Pasta colour is the first important quality characteristic that greatly influences consumer acceptance and it is highly related to semolina properties (genetic and agronomic origin, carotenoids content, degree of

milling, Borrelli et al., 1999, Dexter et al., 1978) and the conditions set in the pastification process (Stefanis and Sgrulletta, 1990; Acquistucci et al., 1992). All mixer used in this study produced pasta with an uniform colour without white spot indicating good hydration of semolina and the development of a homogeneous product in all processing conditions.

The colour of the extruded pasta samples considered in this study was characterized, by lightness of $\sim 68-70$ (L^*), redness $\sim 1.2-1.6$ (a^*) and yellowness $\sim 17-19$ (b^*) and the overall colour of extruded pasta produced with the continuous mixers was only slightly different from *Std* sample as ΔE value was always ≤ 3 for all samples (Table 1). All laminated products were found to have colour coordinates higher than the extruded products (L^* : 74-79, a^* : 1.5-1.7, b^* : 21.2 – 22-4, Table 1), as previously reported (Zardetto and Dalla Rosa, 2006) but also in this case minor differences in the overall colour ($\Delta E \leq 5$, Table 1) were due to the mixing process when comparing the laminated products.

The amount of residue in the cooking water is widely used as an indicator of pasta quality: low amounts of residue indicate high pasta cooking quality. Significant differences were found in the amount of solids released during cooking between pasta produced with a traditional or innovative mixers both in the extruded and laminated samples (Figure 2). Pastas produced with the traditional mixers were found to loose a lower amount of solids in the cooking water, as compared to the pasta mixed with the innovative mixers. Lamination induced a lower solid loss ($\sim 1.6-1.8$ g solids released / 100 g product) as compared to the extrusion process ($\sim 2.3 - 2.7$ g solids released / 100 g product), as previously reported (Carini et al., submitted). It is interesting to point out that a significant difference in cooking loss was found in the extruded *Pre* and *Bak* samples while they were comparable (and markedly more similar to the *Std*) in the laminated products. The

harsher conditions of the extrusion process did likely affect the formation of the gluten network that was probably “broken” in some domains.

Pasta sheets had a moisture content of about 29-30 % (g water/g pasta) in all samples considered with no significant differences induced by the mixer used (Table 2). Water activity ranged from 0.990 to 0.991 in the laminated products and from 0.978 to 0.984 in the extruded pasta. A slight reduction of pasta water activity was induced by the extrusion process as compared to the lamination process. The mixing process did not affect the water activity of the laminated products, while little, but significant differences were observed in the extruded pasta. *Std* and *Pre* were characterized by the highest water activity (0.983 ± 0.003 and 0.984 ± 0.003 , respectively) while the pasta obtained with the *Bak* mixer had the lowest water activity (0.978 ± 0.005). The water activity values of the samples are higher than the legal limit for fresh pasta (0.97 aw, DPR n. 187, February 9th 2001, Art. 9) but it must be taken into consideration that the product object of this study did not undergo pasteurization and light drying that is used in industrial pasta production processing that can induce a reduction of fresh pasta's water activity (Zardetto et al., 2005).

Textural attributes of fresh pasta were measured and summarized in Figure 3. Extruded samples were harder than the laminated samples as previously reported (Carini et al., submitted) for all mixers used. Force at rupture of the laminated products were comparable and about 9 N while a mixer dependence was found in the extruded products. In particular, the extruded *Std* sample was found to have a force at rupture to 14.21 ± 0.38 N while produced with the innovative mixers (*Pre* and *Bak*) were significantly harder (Figure 3A). Extensibility of both laminated and extruded pasta was found dependent upon the mixer used and it was higher in the pasta samples produced with the standard mixer than with the innovative mixers (Figure 3B). The presence of a low pressure twin extrusion step after the

Premix[®] (i.e. the Bakmix[®] mixer) significantly affected the textural properties of fresh pasta.

Macromolecular properties: Thermal analysis

The interaction of solids and water molecules at macromolecular level was studied by means of the thermal properties of the pasta samples by differential scanning calorimetry. Characteristic thermograms of the pasta produced with different mixer processes are reported in Figure 4A (extruded products). The thermograms indicated the presence of a major endothermic event around 0 °C in all the samples analysed that was primarily attributed to ice melting (Li et al. 1996; Vodovotz et al. 1996).

The ice melting peak had a comparable line-shape in all samples studied (both extruded and laminated). Ton of the ice melting peaks was comparable in all samples (~ -10 °C), while, T_{off} was comparable in the laminated (~ 6 °C, Figure 4B) samples and significantly affected by different mixers used in the extruded products with T_{off} of the extruded *Std* sample higher (~ 18 °C) than T_{off} of extruded *Pre* and *Bak* (~ 10 °C, Figure 4B, comparable among themselves). The narrower ice melting peak in the laminated pasta suggested a more homogeneous water-water interactions in these products as compared to the extruded samples. An effect of the mixer was on observed among the extruded samples with *Pre* and *Bak* more homogeneous than the pasta produced with the traditional mixer.

Frozen water content (at the selected experimental conditions) were found to be significantly affected by the innovative mixers in the case of extruded products for both *Pre* and *Bak*, while smaller differences were detected among the laminated products but still significant in the case of *Pre* (that had lower FW) than *Std* and *Bak*. FW of extruded *Pre* and *Bak* samples was significantly lower than FW of extruded *Std* sample (Figure 4C) suggesting

a stronger water-solids interaction induced by the innovative mixers in the extruded products and in laminated *Pre*.

Molecular properties: ^1H NMR mobility

^1H molecular mobility of pasta samples was studied with FID, T_2 and T_1 experiments in attempt to cover a large range of molecular mobility parameters. The characteristic FIDs of pasta samples obtained with the different mixers were reported in Figure 5 in the 0 - 100 μs experimental time range where the NMR signal is not affected by field inhomogeneity. The fast decay characteristic of all pastas indicated the presence of solid-like protons. A slightly slower decay was found in the extruded *Std* and *Pre* while extruded *Bak* was slightly faster suggesting a more rigid structure (Figure 5A). No significant differences were found among the laminated products (Figure 5B)

T_2 and T_1 relaxation decays were analyzed as quasi-continuous distributions of relaxation times and the results were summarized in Figure 6. Characteristic ^1H T_2 distributions indicated the presence of two well resolved ^1H population in all samples (Figure 6A and 6B). The faster relaxing ^1H population was named pop A, and relaxed in the ~ 0.09 - ~ 0.17 ms range (peaked at ~ 0.14 ms) in all samples. Pop A was found significantly more represented in extruded pasta produced with the innovative mixers if compared with standard mixer (~ 20 - 22 % in *Pre* and *Bak* [comparable among themselves], and ~ 16 % in *Std*, Insert, Figure 6A) indicating the presence in this samples of a larger amount of protons relaxing at ~ 0.14 ms. In the laminated products pop A represented ~ 18 % of the total protons in all pastas considered. The prevalent ^1H T_2 population (named Pop B), relaxed in the ~ 2 - ~ 20 ms range (peak at 3.5 / 5 ms) in all the extruded and laminated and was comparable in *Std* and *Pre* samples

while in extruded *Bak* the population B was shifted towards shorter relaxation times (Figure 6) indicating a slightly lower mobility.

The ^1H T_1 characteristic distributions are represented in Figure 6C and 6D for extruded and laminated products, respectively. All samples had a predominant ^1H T_1 population that peaked at a comparable relaxation time (~ 74 ms) and developed over similar ranges of relaxation times ($\sim 30 - 140$ ms). A second minor faster relaxing ^1H T_1 population relaxing at ~ 4 ms (~ 4 % to total detectable protons) was also found in the extruded *Bak* sample suggesting that the Bakmix[®] mixer induced the formation of a more heterogeneous molecular structure.

Summarizing, the mixing process did not have an effect on the molecular properties of the laminated products while the extruded *Pre* and *Bak* were characterized by a “more rigid” molecular structure.

5. Discussion

The thorough characterization of fresh pasta carried out in this work allowed to identify significant differences for different attributes among pasta samples produced with different mixers more evidently in extruded than in laminated products.

The most common pasta quality indicators measured indicated minor and negligible colour differences of *Pre* and *Bak* from *Std* (both for extruded and laminated pastas), while pasta produced with innovative mixers was less extensible and exhibited higher solids loss during cooking. Moreover, extruded *Pre* and *Bak* products were found to be also harder than the extruded *Std*. These experimental evidences suggest that the innovative mixers may have not allowed the formation of a well developed gluten matrix able to prevent solids loss during cooking and to result in a plastic and extensible texture. It is noteworthy that the shaping processing step

had a largely more drastic effect on pasta properties than the mixing phase. Moreover, the harsher processing conditions of the extrusion step seem to enhance the effect of the mixing process: extruded *Bak* was less extensible and had higher cooking loss than the extruded *Pre*.

The water status of fresh pasta was not affected by the mixing process in the laminated products (with the exception of the lower FW content of *Pre*) indicating that the laminated pasta had comparable macromolecular and molecular dynamics. On the contrary, a stronger water-solids interaction measured (both in the macromolecular [FW] and the molecular [lower ^1H mobility of FID, larger % pop A, bimodal T_1 dispersion] scales) was induced by the innovative mixers in the extruded products. It is here speculated that the stronger water-solid interaction found in extruded *Pre* and *Bak* pastas may have hindered the formation of a proper, plastic and well developed gluten network in these products. Low resolution ^1H NMR literature reports on cereal based food products (Doona and Baik, 2007; Wang et al., 2004; Engelsens et al., 2001) may lead to hypothesize a possible assignment of ^1H T_2 population A to the protons of the gluten-water phase of the pasta matrix.

6. List of Tables

Table 1: Brightness (L^*), redness (a^*) and yellowness (b^*) and ΔE referred to the *Std* sample of samples produced with different mixers for both extruded and laminated pastas.

Table 2: Moisture content, water activity and “frozen water” content of samples produced with different mixers for both extruded and laminated pastas.

Table 1: Brightness (L^*), redness (a^*) and yellowness (b^*) and ΔE referred to the *Std* sample (at the same resting time) of extruded fresh pasta samples.

	L^*		a^*		b^*		ΔE from <i>Std</i>	
EXTRUDED PASTA								
	average	st.dev.	average	st.dev.	average	st.dev.	average	st.dev.
<i>Std</i>	70.3 a	1.1	1.2 b	0.1	18.5 a	0.7	Ref	Ref
<i>Pre</i>	70.3 a	1.7	1.3 b	0.2	19.1 a	1.1	1.9	0.9
<i>Bak</i>	69.7 a	1.8	1.6 a	0.1	16.5 b	1.1	3.0	0.9
LAMINATED PASTA								
	average	st.dev.	average	st.dev.	average	st.dev.	average	st.dev.
<i>Std</i>	74.1 c	2.1	1.7 a	0.2	22.3 ab	1.1	Ref	Ref
<i>Pre</i>	79.3 a	1.6	1.5 b	0.2	21.2 b	1.2	5.2	1.6
<i>Bak</i>	77.8 b	0.9	1.6 ab	0.2	22.4 a	0.9	3.6	0.6

Table 2: Moisture content, water activity and “frozen water” content of samples produced with different mixers for both extruded and laminated pastas

	Moisture content (g H ₂ O/100g sample)		Water activity		FW (g frozen H ₂ O /100 g H ₂ O)	
	average	st. dev.	average	st. dev.	average	st. dev.
EXT						
Std	29.8 a	1.2	0.983 a	0.002	37.9 a	7.6
Pre	29.9 a	1.0	0.983 a	0.003	21.3 b	3.3
Bak	28.7 a	0.2	0.978 b	0.004	24.0 b	4.0
LAM						
Std	27.8 a	1.1	0.990 a	0.002	34.6 a	0.5
Pre	28.6 a	0.4	0.990 a	0.001	26.6 b	0.5
Bak	28.3 a	0.4	0.991 a	0.001	33.6 a	3.5

7. List of Figures

Figure 1: Schematic representation of Premix[®] and Bakmix[®].

[1] motor, [2] semolina feeding, [3] volumetric semolina doser, [4], inlet of liquid ingredients, [5] mixing chamber, [6] outlet, [7] twin-screw, [8] outlet die, [9] extruder.

Figure 2: Cooking loss of extruded and laminated fresh pasta.

Different letters above histogram bars indicate significant differences among pasta produced with different mixers and subjected to the same shaping process ($p \leq 0.05$).

Figure 3: Force at rupture (circle) and extensibility (triangle) of extruded (solid symbols) and laminated (open symbols) fresh pasta.

Different letters (small case letters for extruded and italic capital letter for laminated) next to the solid symbols indicate significant differences among pasta produced with different mixers ($p \leq 0.05$).

Figure 4: A-Characteristic DSC thermograms for extruded *Std*, *Pre* and *Bak* samples in the $-30\text{ }^{\circ}\text{C} - 60\text{ }^{\circ}\text{C}$ range.

B- T_{on} and T_{off} of the ice melting transition for extruded and laminated fresh pasta produced with the different mixers.

C- “Frozen” water content (g frozen water / 100 g water) of extruded and laminated fresh pasta produced with the different mixers.

Different letters (small case letters for extruded and italic capital letter for laminated) next to the solid symbols indicate significant differences among pasta produced with different mixers ($p \leq 0.05$).

Figure 5: Proton Free Induction Decay (0.08 – 0.1 ms range) of extruded (A) and laminated (B) fresh pasta produced with different mixers.

Figure 6: Characteristics ^1H T_2 (A and B) and ^1H T_1 (C and D) distributions of extruded (upper part) and laminated (lower part) fresh pasta produced with different mixers. The percent total area represented by population A for extruded pasta is also reported.

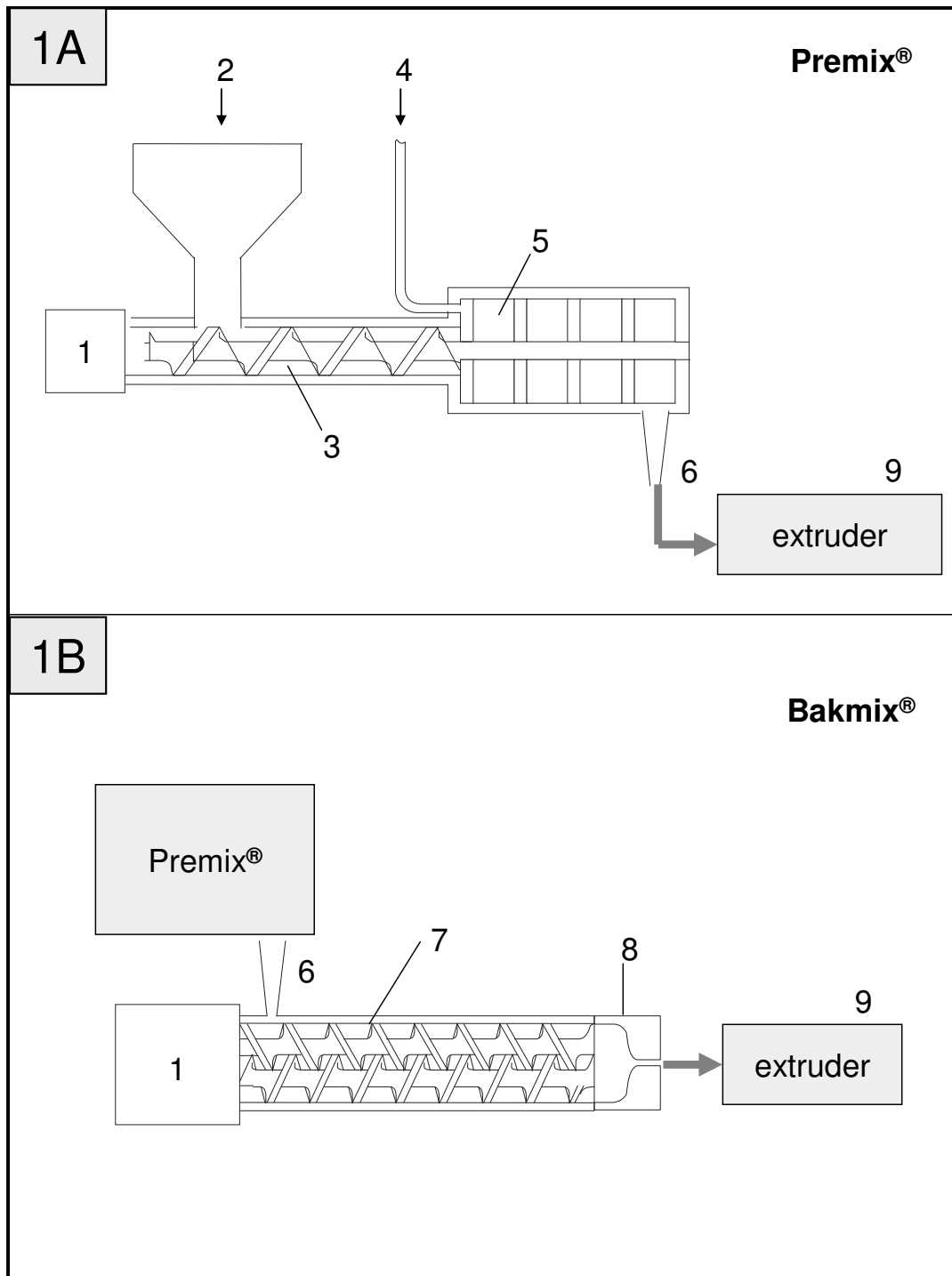


Figure 1: Schematic representation of Premix® and Bakmix®.

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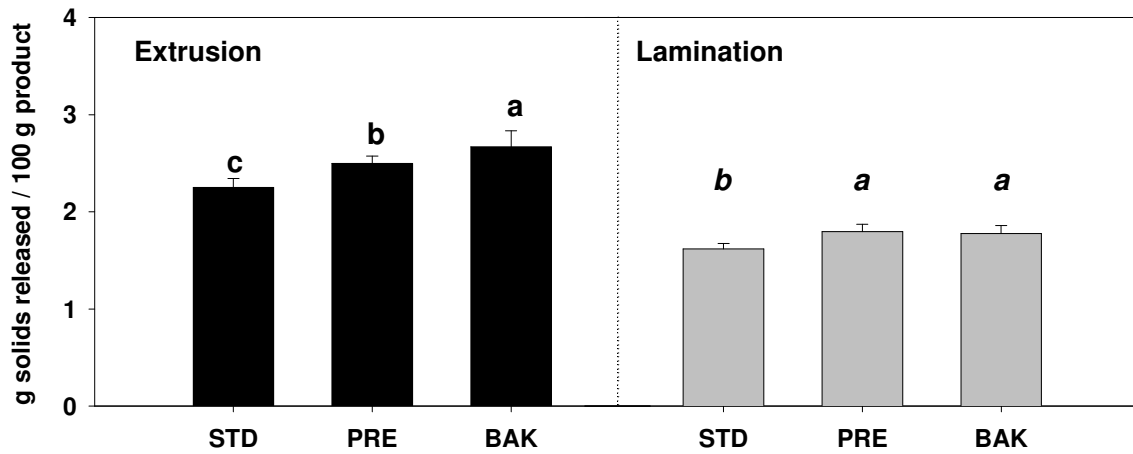


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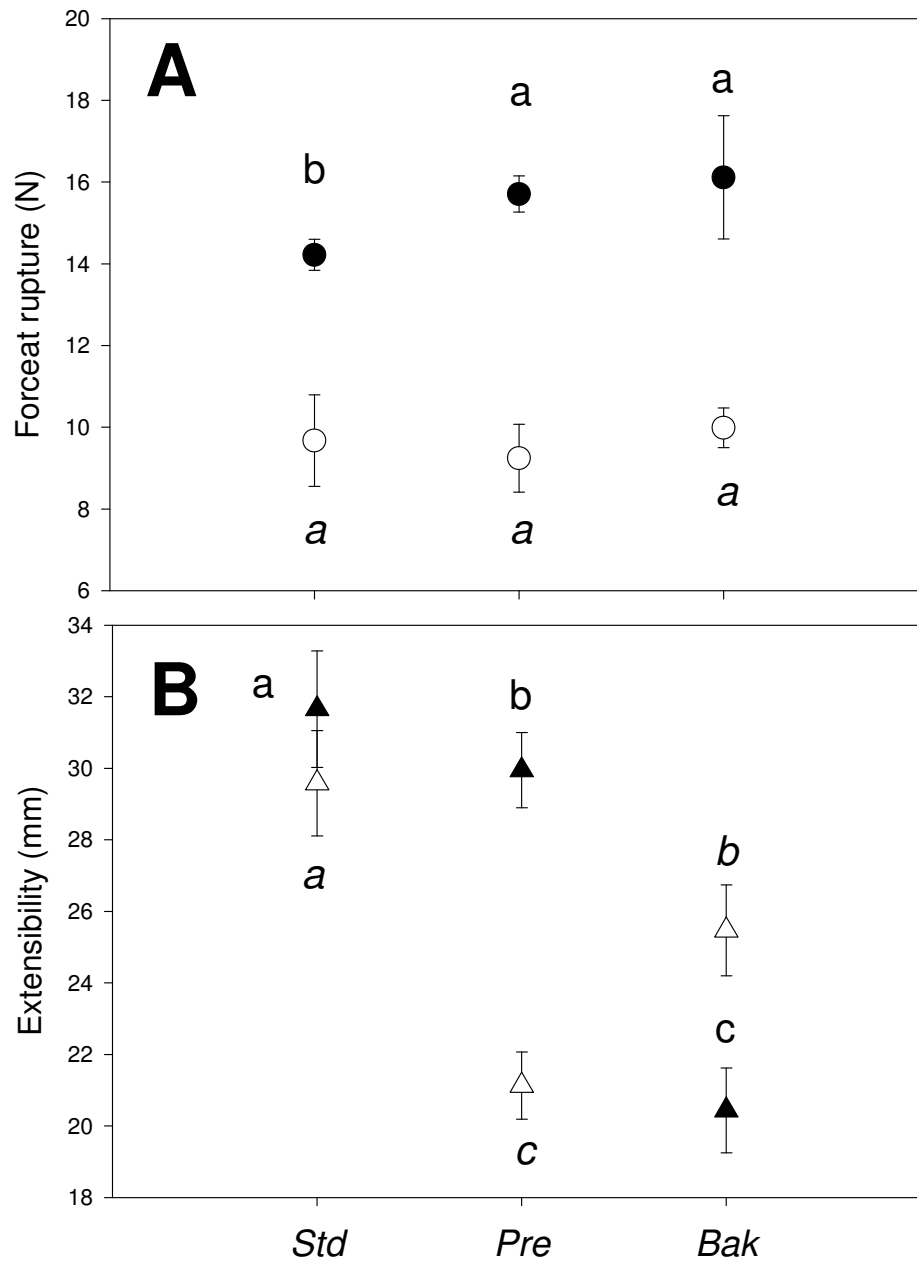


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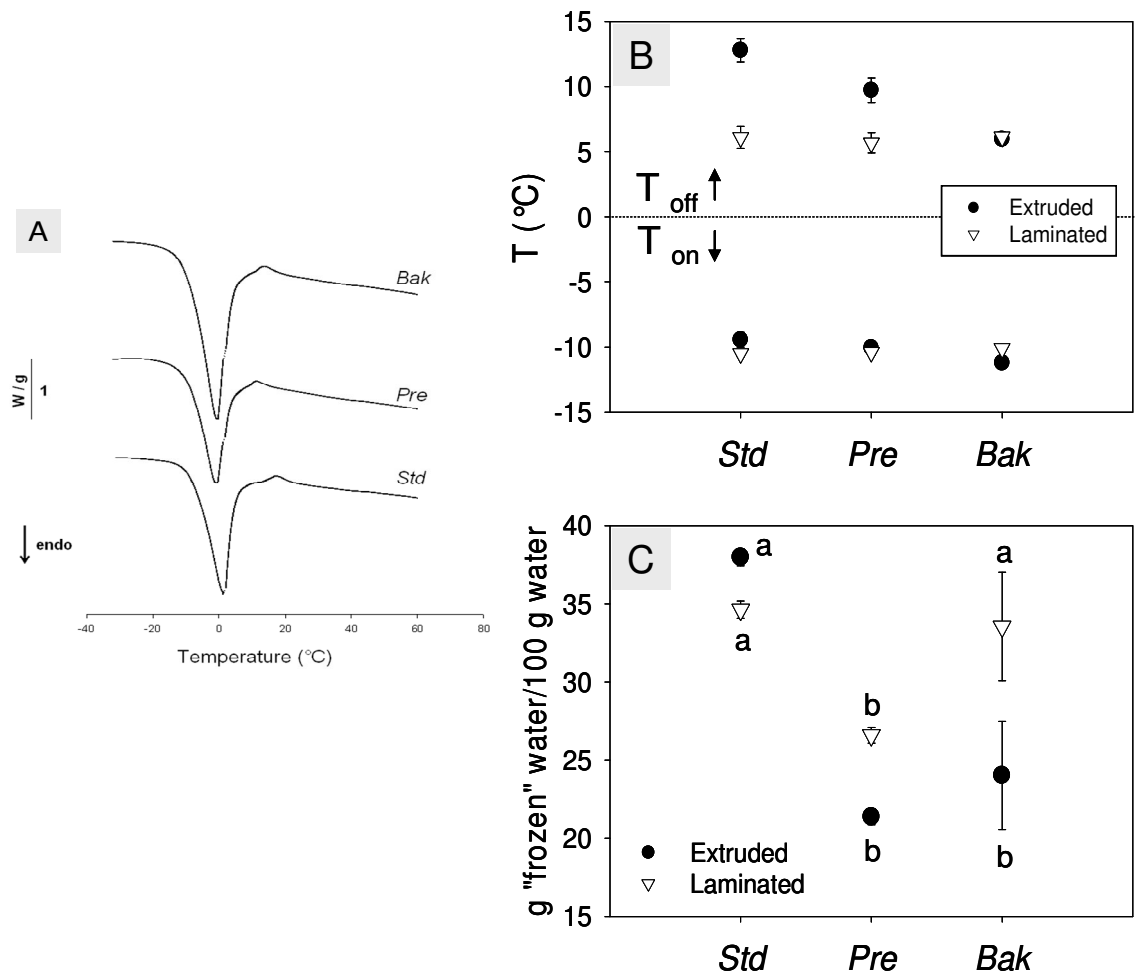


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B- T_{on} and T_{off} of the ice melting transition for extruded and laminated fresh pasta produced with the different mixers.

C- "Frozen" water content (g frozen water / 100 g water) of extruded and laminated fresh pasta produced with the different mixers.

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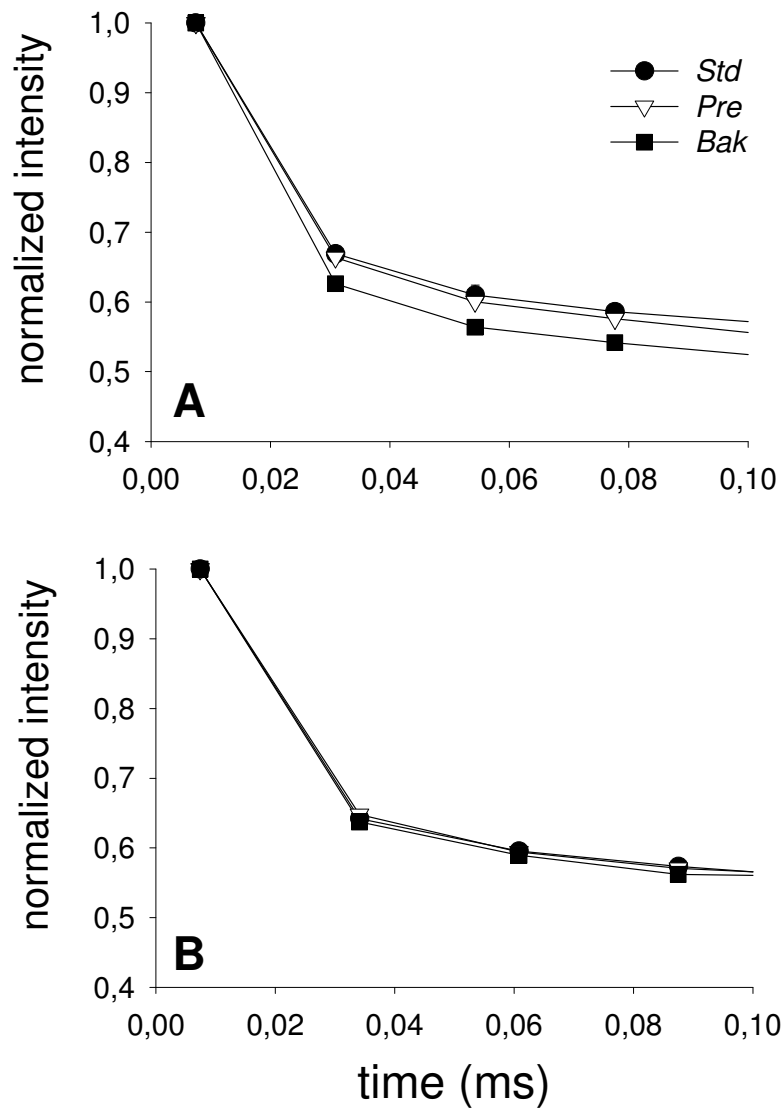


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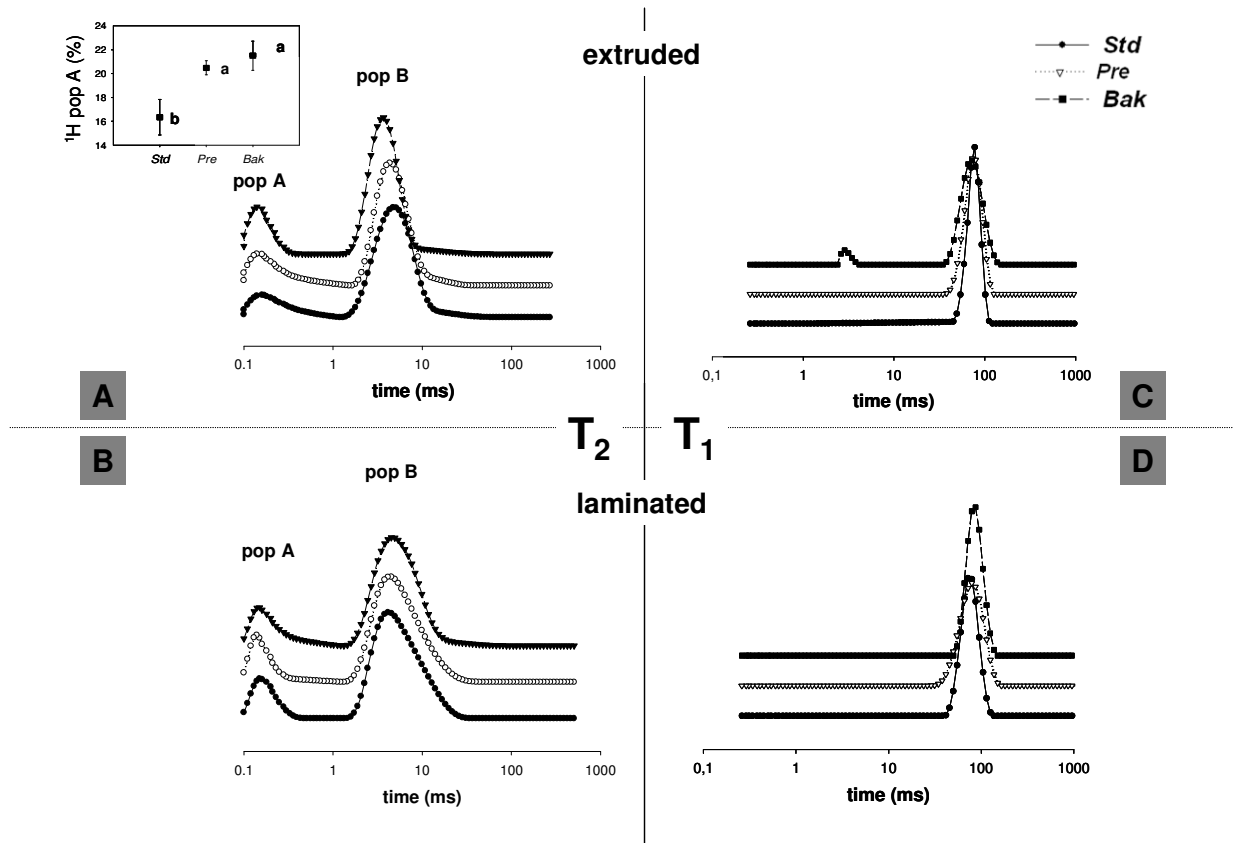


Figure 6: Characteristics ^1H T_2 (A and B) and ^1H T_1 (C and D) distributions of extruded (upper part) and laminated (lower part) fresh pasta produced with different mixers. The percent total area represented by population A for extruded pasta is also reported.

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SECTION B

EFFECT OF FORMULATION
IN FRESH PASTA
AND
NUTRITIONALLY ENHANCED
TORTILLAS

FRESH PASTA

Effect of formulation on physicochemical properties and water status of fresh pasta

Eleonora Carini, Elena Vittadini, Elena Curti and Elisabetta Spotti

1. Abstract

A standard fresh pasta (STD, the control) formulation was modified by introducing ingredients (soy [flour and milk] and carrot [flour and juice]) with documented functional properties to obtain eight enriched fresh pasta samples and the effect of formulation on physicochemical properties of uncooked and cooked fresh pasta were evaluated. Colour, texture and cooking loss were significantly affected by the formulation: the presence of whole soy flour and carrot flour decrease the force at rupture and extensibility of uncooked pasta and increase the solids loss during cooking. A not proper gluten network development was hypothesized in soy flour and carrot flour-containing products. Fresh pasta samples in which were added soy milk and carrot juice did not change these properties.

Water status was significant affected at every level investigated: all samples were lower in water activity, the presence of sugars originated from carrot flour drastically decrease the “frozen water” content and every sample exhibited a different ^1H NMR mobility than the control.

This altered water redistribution could affect the stability of the fresh pasta, however, further studies have to be carried out to optimize the formulations to improve the textural and cooking loss properties that were significantly affected in these experimental conditions.

2. Introduction

Durum wheat semolina and water are the two basic ingredients of traditional pasta. These ingredients are mixed into a crumble-dough before being formed and cut into proper shapes; the product obtained is then either sold as “fresh pasta” (moisture content >24 % and water activity = 0.92-0.97) or can be dried to 12.5 % moisture and sold as “Pasta” (DPR 187, 2001).

Large interest has recently risen in the development of “functional” foods, products that affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way relevant to improved state of health and well-being, reduction of risk of diseases, or both (Riccardi et al., 2005). It could be, therefore, possible to enhance the nutritional value of pasta by adding ingredients with well recognized nutritional functionality in the standard pasta formulation.

The inclusion of other ingredients in pasta formulation has been associated with an alteration to the cooking characteristics and textural properties. Edwards et al. (1995) reported an increase in pasta firmness when xanthan gum was added at levels of 1 % and 2 %; Fardet et al. (1999b) found a decreasing of cooking loss and increasing in firmness with fibre addition, a deterioration of the cooked pasta texture with the addition of oat or pea fibre was also reported by Dougherty et al. (1988). Addition of soybean flour, which has a well documented nutritional functionality, must be carefully carried out since many research evidenced that the soy protein impacts on pasta texture making it less firm and less resilient (Kim et al., 1989; Kobs, 2000).

Cooked pasta textural properties are strongly dependent on protein content and protein quality (Autran et al., 1986; D’Egidio et al., 1990; Matsuo et al., 1982) and are the most important factor in determining consumer

acceptance. Gluten strength (related to protein composition and processing conditions) is universally acknowledged as an important condition for making good-quality pasta (Ames et al., 1999). Zweifel and co-workers (2003), in particular, related the textural characteristics of cooked spaghetti to the continuity and strength of the protein network.

The objective of this work was therefore, to produce enriched fresh pasta by incorporating of ingredients with well documented functional properties in a standard pasta formulation and to study the effect of formulation on physicochemical properties of fresh pasta. The “functional” ingredients selected were soy (flour and milk) for their benefit effects on health (Sacks et al., 2006) and carrot (flour and juice) to increase the carotenoids content.

3. Materials and Methods

Pasta formulation

A standard fresh pasta (STD) formulation was taken as control and was then modified by introducing ingredients with documented functional properties to obtain eight enriched fresh pasta samples (soy flour enriched - SF; soy milk enriched - SM; soy ingredients enriched - S-M; carrot flour enriched - CF; carrot juice enriched - CJ; carrot ingredients enriched - C-J; soy flour and carrot juice enriched - SF-CJ and carrot flour and soy milk enriched - CF-SM). The formulation of the fresh pastas considered in this study are reported in Table 1.

Carrot flour (was provided by Macor Mia Prada company (Milan, Italy) and all other ingredients were obtained from a local supermarket.

Fresh pasta samples were produced with the following process: dry ingredients were mixed for 20 seconds (Kitchen Aid, St. Joseph, Michigan) and then added of liquid ingredients, total mixing time was 15 minutes at speed 4. The product was then allowed to rest for 5 minutes at 25 °C and

was successively laminated eight times (Unika Storci, Italy) to obtain a sheet to 1.5 ± 0.02 mm thick.

Two productions of each fresh pasta type were carried out in different days. Physicochemical characterization of pasta sheets was done both on fresh and cooked pasta. Cooking of fresh pasta was carried out by inserting fresh pasta sheets into boiling water (1:10 solid:water ration) for 5 minutes. Pasta sheets were then drained and allowed to cool on a rack at room temperature for 15 minutes.

Pasta characterization

Colour: L^* (Brightness), a^* (redness), b^* (yellowness) and the overall colour difference, (ΔE) from control (STD sample) of each pasta type were measured (CIE, 1978).

The ΔE parameter was calculated according the following equations:

$$\Delta E = \sqrt{(L^*_{\text{sample}} - L^*_{\text{control}})^2 + (a^*_{\text{sample}} - a^*_{\text{control}})^2 + (b^*_{\text{sample}} - b^*_{\text{control}})^2}$$

The colour parameters were obtained using a colorimeter (CM 2600d, Minolta Co., Osaka Japan) equipped with a standard illuminate D65 using a 2 degree position of the standard observer. Ten punctual colour determinations were taken for each pasta type. The colour characterization was done only for uncooked fresh pasta.

Texture: force at rupture (maximum force [N] required to shear the sample) and extensibility (deformation at breakage [mm]) were obtained using a TA.XT2 Texture Analyzer (Stable Micro Systems, Goldalming, U.K.) with a two-dimensional extensibility test (Bejosano et al., 2005). The test was carried out using a TA-108 Texture fixture that was attached to the texture

analyzer platform and an acrylic probe (2.54 cm diameter at edges) attached to the analyzer arm. The test was conducted in compression mode at a constant speed of 3 mm/s. Textural properties of ten samples of each pasta type were analysed.

Cooking loss: cooking loss (the amount of solid substance lost to cooking water) was determined according to the AACC Method (1999). The analysis was performed in triplicate for each fresh pasta type for each fresh pasta production.

Water status

Water activity

Water activity (aw) of pasta samples was measured at 25 °C using a Decagon Aqualab meter TE8255 (Pullman, WA). Pasta was broken into small pieces immediately before water activity measurement. Water activity of two samples of each pasta type was analyzed in duplicate for each fresh pasta production. Water activity measurement was done only for uncooked fresh pasta.

Moisture content

Moisture content (MC, g water / g product) of pasta was determined from weight loss by drying in a forced-air oven at 105 °C. Moisture content of two samples of each fresh pasta type for each fresh pasta production was analyzed in duplicate.

Thermal properties of ice melting peak

Thermal properties were measured using a differential scanning calorimeter (DSC Q 100 TA Instruments, New Castle, DE, USA), calibrated with n-dodecane. Pasta samples (5 -8 mg) were placed into aluminium pans

(Perkin Elmer, Somerset, NJ, USA), equilibrated at -50 °C and heated to 40 °C with a heating rate of 5 °C/min. The Universal Analysis Software, Version 3.9A (TA Instruments, New Castle, DE) was used to analyze the thermograms obtained. The thermal event observed (endothermic peak at about 0 °C) was characterized for enthalpy (ΔH , J/g), onset (T_{on}) and offset (T_{off}) temperatures of the transitions.

“Frozen” water (FW) content was calculated using the following equation (Baik and Chinachoti, 2001, Vittadini et al., 2004):

$$FW = \text{Enthalpy Ice Fusion} \times \left(\frac{1}{\text{latent heat ice fusion}} \right) \times \left(\frac{1}{MC} \right) \times 100$$

FW = Frozen Water [%, g frozen water / g water]

Enthalpy Ice Fusion [J / g product]

Latent heat of ice fusion = 334 J / g ice

Three DSC scans of two samples of each fresh pasta type for each fresh pasta production were analyzed.

1H NMR mobility

¹H NMR mobility was measured by low resolution (20 MHz) ¹H NMR spectrometer (the miniSpec, Bruker Biospin, Milano, Italy) operating at 25.0 ± 0.1 °C. Approximately 3 g of sample were placed into a 10 mm NMR tube that was then sealed with parafilm to prevent moisture loss during the NMR experiment.

FID, (free induction decay) and the T_2 (transverse relaxation time using a CPMG sequence, Carr and Purcell, 1954, Meiboom and Gill, 1958). FIDs were acquired using a single 90° pulse, followed by dwell time of 7 μs and a recycle delay of 0.8 s. T_2 was obtained with a recycle delay of 1.5 s ($\geq 5 T_1$) and interpulse spacing to 0.04 ms. T_2 curves were analyzed as quasi-continuous distributions of relaxation times using a UPEN software (Borgia et al, 1998, Borgia et al., 2000).

Duplicated analyses on two pasta samples for each fresh pasta type for each pasta production were carried out for a total of 8 NMR analyses for each NMR parameter.

Statistical analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 13.0, SPSS Inc., Chicago, IL, USA). SPSS was used to verify significant differences to the parameters among uncooked fresh pasta samples produced with different formulation by one-way-analysis of variance (ANOVA) followed by least significant difference test (LSD) at $p < 0.05$. A paired student's t-test analysis was used to identify differences between uncooked and cooked fresh pasta (for the same pasta type).

4. Results and Discussion

Standard fresh pasta formulations were added of soy and/or carrot products (ingredients with well recognized nutritional functionality). The maximum amount of “functional ingredients” that could be incorporated and still allowing to obtain palatable and acceptable products were determined. The final formulation are showed in Table 1.

Colour, texture and cooking loss

Consumer considers the pasta colour as an important indicator of pasta quality. The colour of the pasta samples produced for this study are reported in Table 2 and the observable differences are ascribable only to the different ingredients used in the formulations since the processing steps (mixing and lamination) were consistent among all samples. All the colours measured (L^* , a^* and b^* , Table 2), were significantly affected by the formulations. STD had $L^* \sim 73$, $a^* \sim 0.5$ and $b^* \sim 23$, colour of the soy-

enriched fresh pasta samples (SF, SM and S-M) decreased in L^* and increased in a^* and b^* probably for dark fractions originated in whole soy flour. Addition of carrot based ingredients (CF, CJ and C-J) decreased L^* and increased drastically a^* and b^* because the presence of carotenoids in those samples. ΔE indicated that all fresh pasta samples had a different colour if compared with STD samples different colour.

Textural properties were also found to be formulation dependent as shown in Figure 1A. Force at rupture measured with a two dimensional test was ~ 5.3 N for STD sample and all modified formulations resulted in significantly lower force at rupture indicative of the development of weaker macroscopic matrixes. It is noticeable that the macroscopic structure of the final product is strongly related to the continuous phase properties (i.e. gluten matrix) of the product. The presence of whole soy flour in the formulation induced the lowest force at rupture (SF and S-M samples) because of, probably, a not proper gluten network development due to the inability to soy proteins to form gluten. Besides, soy proteins are known to have a great affinity for water, and therefore, they could have interacted with water molecules altering the water distribution in the forming dough and, possibly, preventing appropriate gluten hydration that is essential for the development of a proper gluten network. Similarly, when carrot ingredients were used (CF, CJ) the force at rupture decreased, because, probably, to an altered gluten matrix development due to the large amount of sugars in the recipes. Sugars are known to have high affinity for water that was, possibly, no longer available for proper gluten network development. In the SF-CJ and CF-SM samples the force at rupture reflected the behaviour observed when the single ingredient was added.

Extensibility (results showed in Figure 1B) of the STD sample was ~ 28 mm, comparable to fresh pasta containing soy milk (SM), while it was significantly increased in the presence of carrot juice (CJ, ~ 31 mm). The

presence of whole soy flour and dehydrated carrot decreased the extensibility of the respective products.

Scazzina et al. (2008) investigated the textural properties of enriched tortillas with whole soy flour and carrot juice in comparison with a standard tortilla. Authors reported a hardness increase and a comparable extensibility with the addition of whole soy flour (17 % to whole soy flour) and a hardness and extensibility increase with the addition of carrot juice (17.4 % to carrot juice). The higher amount of these ingredients in the fresh pasta formulations may have greatly altered the textural properties.

The effect of cooking on textural properties of fresh pasta samples are reported in Figure 1A (force at rupture) and 1B (extensibility). Alteration during cooking could be associated to multiple complex processes simultaneously occurring: hydration of the matrix, formation of an amorphous gel like structure of part of the starch components and thermal denaturation of the protein fraction to quote the most important. Cooking induced an overall force at rupture increase in all samples but the relative increase from the raw sample was found formulation dependent: SM and CJ samples had a force at rupture increase comparable to STD while others formulations had a lower increase. This was possibly partially related to the lower starch amount in this formulation. Extensibility decreased in all samples (with the exception of CF and CF-SM samples) and has been observed greater decrease in the case of CJ sample while others pastas had a comparable extensibility decrease.

An indicator of pasta quality is the amount of residue in the cooking water and low amounts of residues indicate high pasta cooking quality.

The cooking loss was drastically affected by different formulations, STD sample resulted to have 3.3 g solids/100 g sample and all fresh pasta types, ad exception to SM, showed a significant higher solids release during cooking. The possible weak gluten network developed in samples

containing whole soy flour and dehydrate carrot was probably responsible of the higher solids loss observed.

Water status

Water macroscopic status of fresh pasta samples was studied by means of water activity and moisture content. Both parameters were significantly affected by different formulations (Figure 3A and 3B, respectively) due, primarily, to the different water amount required in the recipes to obtain good quality products and, possibly, to different water-solids interaction.

STD had water activity equal to 0.975 ± 0.001 , (with the legal limit for fresh pasta to $0.97 a_w$, DPR, 2001) and moisture content to 31.9 ± 0.3 % (g water/100 g sample). The inclusion in the recipe of whole soy flour increased the amount of water needed in the formulations, due to the high capacity of soy proteins to “bind” water molecules, as many authors have reported previously (Traynham et al., 2007, Doxastakis et al., 2002) and that was reflected on moisture content products’ (~ 37 , ~ 36 and ~ 33 % [g water/100 g sample] for SF, S-M and SF-CJ, respectively). Although water was present in larger amounts, the a_w of SF, SM and S-M was significantly lower than in the STD possibly because of a “stronger” water-soy proteins interactions.

The presence of sugars and fibre originated from dehydrate carrot and from carrot juice in the formulations did not change the moisture content of the samples with the exception of C-J sample, where the presence of both the ingredients probably induced a synergistic effect for solids-water interaction development. However, carrot ingredients significantly reduced the water activity in CF, CJ, C-J and CF-SM samples. A greater amount of sugars and fibre in samples containing carrot flour had a stronger water activity reducing effect, in particular the C-J sample resulted the lowest in water activity (~ 0.920).

Moisture content of samples after the cooking process increased, as expected (Figure 3B). Samples showed a different ability to absorb water during cooking, STD, SF, SM and CJ absorbed ~22 % of water while others were found to adsorb from ~26 to ~32 %. Matrixes probably adsorbed water at different levels because ingredients used had different interaction with water inducing the formation of altered microstructures.

The interaction of solids and water molecules at macromolecular level was studied by means of the thermal properties of the fresh pasta samples by differential scanning calorimetry (DSC). Thermograms of all samples exhibited one major endothermic transition as the samples were heated from -50 °C to 40 °C. Characteristic thermograms of the major observed transition in the -30-10 °C (uncooked fresh pasta) and -20-15 °C (cooked fresh pasta) ranges were reported in Figure 4A and Figure 4C, respectively. The endothermic transition observed was primarily attributed to ice melting and the peaks shapes were similar but not identical. STD sample showed an ice melting peak shape comparable to SF, SM and S-M samples while formulations containing dehydrate carrot and carrot juice presented a more broader peak indicating the presence of more heterogeneous solids-water interactions. Ice melting peak transition temperatures (T_{on} , T_{off} and T_{range}) for all uncooked fresh pasta types were shown in Figure 5B and were found to be formulation dependent. T_{on} , T_{off} and T_{range} of STD sample resulted ~-9, ~-2 and ~-17 °C, respectively. The presence of whole soy flour in the formulation (SF and SM) slightly shifted the transition toward lower temperatures and increased the T_{range} indicating the presence of more heterogeneous and stronger water-solids interaction in these samples, while the SM sample temperature transitions were found comparable with STD. A slight decrease of the temperature onset in soy-containing breads was previously reported also by Vittadini and Vodovots 2003. Sugars (and possibly fiber) contained specially in dehydrate carrot significantly shifted

the ice melting peak decreasing T_{on} and T_{off} and increasing T_{range} because of the freezing point depression induced by sugars. The frozen water content (FW) of the uncooked fresh pasta samples (Figure 5A) was formulation dependent (at the given experimental conditions). STD sample had a FW to ~38.1 g frozen water/100 g water and the presence of soy proteins in the formulations was reflected in this way: SF resulted significantly higher than STD while SM lower, the inclusion to both the ingredients made FW comparable with STD. On the contrary, when carrot based ingredients (CF, CJ and C-J samples) were used in the formulation a significant decrease in FW (~21, ~29 and ~23 g frozen water/100 g water for CF, CJ and C-J, respectively) was observed. This was likely due to the solubilization of sugars in water that increased the viscosity of the hydrophilic phase resulting in a decrease in motion of the water molecules that could form ice crystals detectable by DSC (Chinachoti, 1993, Vittadini et al., 2004). The very low frozen water content found in CF and C-J if compared with CJ sample was attributed to the higher amount of sugars expected to be present in these formulations. FW of SF-CJ was comparable with STD due probably, to the synergistic effect of soy proteins (FW increase) and carrot juice (FW decrease). On the contrary, in CF-SM sample FW was found significantly lower than STD indicating a major contribution to carrot dehydrate than soy milk that decrease FW.

The effect of cooking on thermal properties of fresh pasta produced with different formulation was reported in Figure 4B e 4C. All cooked fresh pasta samples had a larger ice melting peak that was also shifted towards higher temperatures (if compared with uncooked fresh pasta) consequent to water adsorption, starch gelatinization and partial protein denaturation. All samples exhibited another endothermic minor transition in the -18-0 °C range and this transition was more evident in STD, SM and CJ samples where a larger amount of semolina was present in the formulation.

After cooking, FW of fresh pasta samples significantly increased but the FW % increase was not the same in all samples due, first of all, to the different moisture adsorption during cooking.

Molecular mobility was studied by low resolution ^1H NMR and multiple experimental techniques were used in an attempt to cover a large range of molecular mobility.

It must be emphasized that the ^1H NMR analysis is not specific for water as the signal detected may arise from any proton present in the sample relaxing in the time frame characteristic of the experiment (Halle and Wennerstroem, 1981; Schmidt and Lai, 1991; Colquhoun and Goodfellow, 1994; Ruan and Chen, 2001).

Mobility of the least mobile ^1H fractions of fresh pasta was analyzed with a FID experiment while the more mobile proton fractions were characterized in terms of T_2 and T_1 relaxation times. The translational motion of protons was measured by the diffusion coefficient.

Characteristics representative FID curves (only the first points) for all uncooked fresh pasta samples were reported in Figure 6. STD, SM and CJ samples showed a comparable decay indicating the presence of a similar fast relaxing solid-like ^1H population and were found to have the less mobile ^1H fraction. SF, S-M, CF and C-J samples resulted to have the more mobile ^1H population and were comparable themselves, other samples showed an intermediate ^1H rigidity. The fast relaxing ^1H population observed with the FID might arise from protons in solid-like components, such as starch and proteins and water molecules tightly associated with those solids (Kim and Cornillon, 2001). After cooking, all pasta types were found to have higher ^1H FID mobility (data not showed) with no significant difference linked to the formulation.

T_2 relaxation decays were analyzed as quasi-continuous distributions of relaxation. The effect of formulation on ^1H T_2 distribution in uncooked

fresh pasta samples was reported in Figure 7. STD characteristic ^1H T_2 distribution indicated the presence of two well resolved ^1H population, as previously reported. The faster population relaxed in the $\sim 0.1 - \sim 1.5$ ms range (peak centred at ~ 0.17 ms) and represented by 20 % of the total protons detectable in the T_2 time frame while the slower and prevalent population relaxed in the $\sim 1.6 - \sim 87$ ms range (peaked at 6.4 ms). Similar results were previously reported by Carini et al., submitted. Several products like bread, tortillas, dough and model systems containing gluten and starch has been studied in recent years by low resolution NMR but no studies regarding fresh pasta have been yet published.

Two ^1H T_2 populations in relaxation times ranges comparable to our results were found by Doona and Baik (2007) in uncooked wheat dough (33.1 % moisture content) and they suggested that the faster population would represent the water molecules closely associated with solids in wheat flour dough; model systems of starch gels, gluten gels and starch-gluten gels and also bread samples were studied by Wang et al. (2004) and they found two proton populations, peaking at ~ 0.1 ms and ~ 3.0 ms, they attributed the last population to water associated with starch. Engelsen et al. (2004), found three proton T_2 populations in bread that were attributed to water associated to protein, water associated to gelatinized starch (and pentosans) and diffusive exchange water between starch and protein, respectively. Based on these information, the authors attempt to explain the results in reference to the formulation of fresh pasta samples.

SM and CJ samples were the only samples that showed a comparable ^1H T_2 distribution with STD (Figure 7) and these samples were systems in which proteins (gluten) and starch contents were roughly the same than in STD. The slower ^1H population was found to cover a larger relaxation times range than STD trough a tail at the end of the peak was observed probably due to some exchangeable protons between the two ^1H populations. Serventi et al.

(2009) reported in whole soy flour enriched tortillas, the presence of a third ^1H population relaxing in the $\sim 6 - \sim 300$ ms and they attributed it mainly to the presence of soy proteins in the formulation. The peak corresponding to the prevalent ^1H population of CJ sample was found broader than STD indicating a major heterogeneity to protons relaxing in this populations.

The presence of whole soy flours in the formulations (SF, S-M and SF-CJ samples) was reflected by the presence of a unique ^1H T_2 population originated, probably, from three not resolved and strongly exchanging ^1H T_2 population (Figure 7). The prevalent population was shifted towards slower relaxation times (~ 10 ms) and overlapped with another minor ^1H population that may be associated to soy proteins contribution (Serventi et al., 2009).

Similarly to whole soy flours containing pastas, CF and C-J fresh pasta presented one ^1H T_2 population whose characteristic peak suggested the presence at least two overlapping (but not resolved) ^1H populations exchanging with in the NMR T_2 time frame.

As reported before, the fastest ^1H population was attributed by some authors to protons belonging to the gluten network, it might be speculated that the presence of a not resolved peak in the soy-flour and carrot-flour containing pastas could be related to the lack of a proper gluten network development and it could therefore bear out the attribution of these protons to those associated to gluten.

The effect of cooking on ^1H T_2 STD spectrum was reported in Figure 8. After cooking, one ^1H population shifted towards higher relaxation times was observed in all samples. A more mobile and exchanging molecular structure was observed as expected as consequence of the water adsorption and phase change of the solids towards an amorphous matrix. The characteristic relaxation times range of this population was found to be formulation dependent. Relaxation spectrum showed a signal characterized by a very

broad relaxation times range (from ~0.1 to ~ 35 ms – ~380 ms). STD, SM and CJ samples showed the 1H population centred at ~22 - ~29 ms while in other samples the population was found more mobile because centred at ~35 - ~44 ms. In this more mobile samples the relaxation times was found to occur in a wider range. The higher temperature and the greater amount of water involved during the cooking process as well as the alteration to the water dynamics induced to the formulation were responsible for the complex relaxation times spectrums.

5. Conclusions

Fresh pastas samples enriched with ingredients of well documented nutritional functionality (soy from flour and milk and carrot from flour and juice) were developed and the effect of formulation on physicochemical properties and water status were studied. Different formulation significantly affected the most important indicators of pasta quality such as colour, texture and cooking loss, water status (at different levels) was also significantly affected by the formulation. The formulations in which semolina was partially substituted with both whole soy flour and carrot flour, if compared with STD, decreased the force at rupture and the extensibility properties and increase the solids released during cooking probably induced to a not proper gluten network development due to a lower amount of proteins able to structure the gluten network. Since also fresh pastas that contained soy milk and carrot juice were negatively affected in texture and cooking loss, it could be hypothesize that components of these ingredients (e.g. soy proteins and sugars) altered the water distribution during the dough formation and consequently hinder a proper gluten hydration.

Formulation affected water status at different levels indicating that ingredients used to produce fresh pasta altered the water redistribution during the dough formation. Formulations with a similar ^1H molecular mobility (products with soy milk and carrot juice contained) to the control were found to have a different macromolecular (frozen water content) and macroscopic (water activity) state while the products in which were added whole soy flour and carrot flour showed a very different ^1H mobility (^1H FID and ^1H T_2). This altered water redistribution could affect the stability of the fresh pasta, however, further studies have to be carried out with the intend to optimize the soy and carrot based formulations to improve the textural and cooking loss properties that significantly affected in these experimental conditions.

6. List of Tables

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Table 2: Brightness (L^*), redness (a^*), yellowness (b^*) and ΔE of fresh pasta samples.

7. List of Figures

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Figure 2: Cooking loss for fresh pasta samples. Different letters above the bars indicate significant difference among fresh pasta samples (LSD test, $p \leq 0.05$).

Figure 3: Water activity and moisture content for fresh pasta samples. Different letters above the circles (water activity) and bars (moisture content, uncooked sample) indicates significant difference among fresh pasta samples (LSD test, $p \leq 0.05$).

Figure 4: Ice melting peak of uncooked fresh pasta samples (A); Effect of cooking on thermal properties of ice melting peak of STD pasta (B); Ice melting peak of cooked fresh pasta samples.

Figure 5: A- Frozen water content for fresh pasta samples; different letters above the bars indicate significant difference among fresh uncooked pasta samples (LSD test, $p \leq 0.05$).

B- Temperatures transition of ice melting peak for fresh uncooked (black circles) and cooked (white triangles) pasta samples.

Figure 6: ^1H FID for fresh uncooked pasta samples.

Figure 7: ^1H T_2 distribution for uncooked fresh pasta samples.

Figure 8: Effect of cooking on ^1H T_2 distribution for STD sample.

Figure 9: ^1H T_2 distribution for cooked fresh pasta samples.

Ingredient (%)	STD	SF	SM	S-M	CF	CJ	C-J	SF-CJ	CF-SM
Semolina (durum)	75.0	41.5	72.3	39.4	48.6	71.9	48.1	44.2	44.5
Whole Soy flour	-	24.6	-	25.2	-	-	-	22.1	-
Carrot dehydrate	-	-	-	-	20.8	-	20.6	-	22.3
Wheat gluten	-	3.1	-	3.2	2.8	-	2.7	3.0	3.0
Distilled water	25.0	30.8	-	-	27.8	-	-	-	-
Soy milk	-	-	27.7	32.2	-	-	-	-	30.2
Carrot juice	-	-	-	-	-	28.1	28.6	30.7	-

Table 1: Fresh pasta samples formulation

	STD	SF	SM	S-M	CF	CJ	C-J	SF-CJ	CF-SM
L*	73.3±0.6	63.2±1.2	71.4±0.6	64.3±1.0	63.2±1.7	67.3±0.8	62.6±1.4	60.5±1.7	62.3±1.1
a*	0.5±0.1	4.3±0.6	0.7±0.1	4.4±0.5	15.0±1.4	13.6±0.5	18.5±0.8	10.7±0.5	11.2±0.8
b*	23.0±0.7	24.0±1.2	24.2±1.0	23.5±1.3	32.0±1.7	42.2±1.8	32.6±1.4	33.8±2.5	31.1±1.2
ΔE	Ref	11.0±1.4	2.5±0.8	9.9±1.2	20.0±2.0	24.3±1.5	23.1±1.6	19.8±2.1	17.5±1.2

Table 2: Brightness (L*), redness (a*), yellowness (b*) and ΔE of fresh pasta samples.

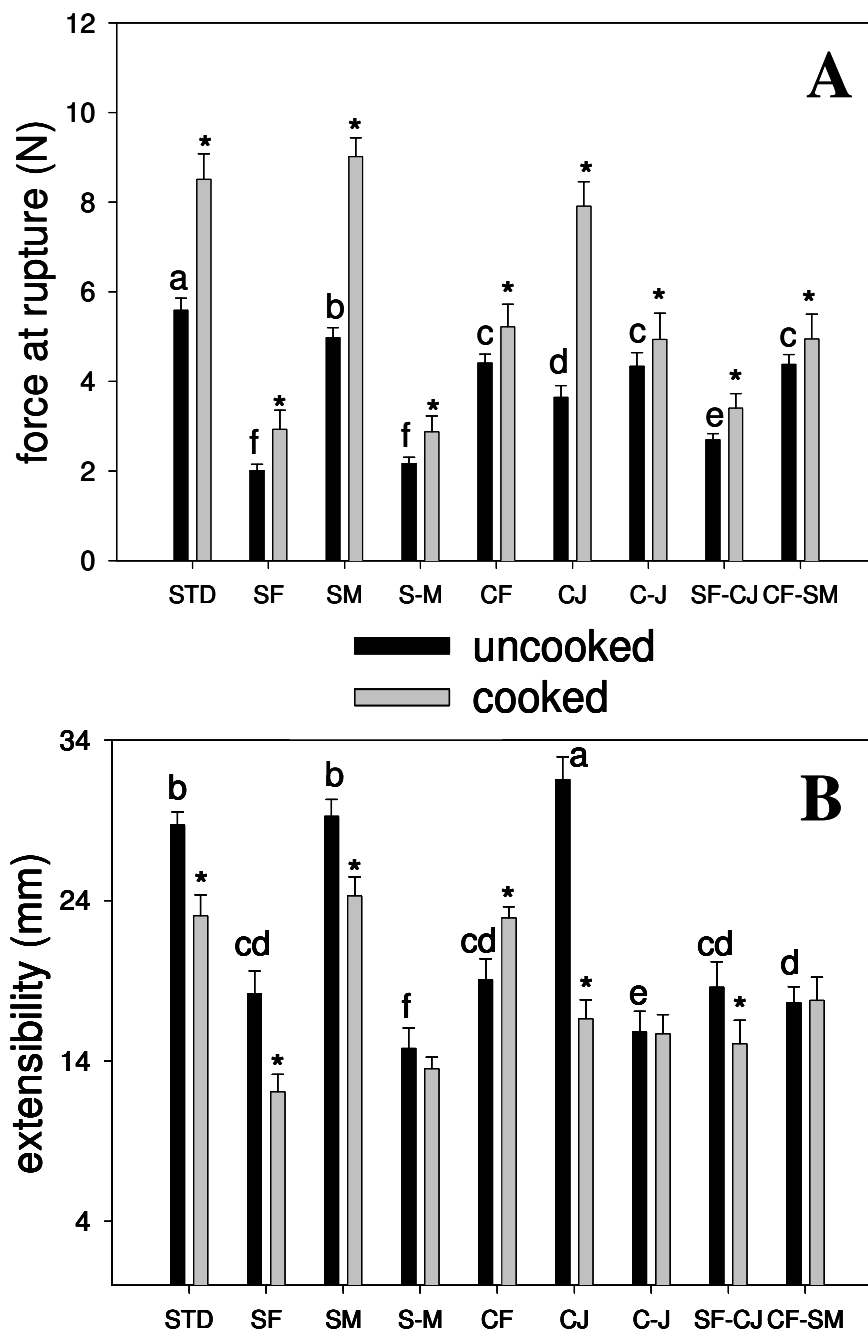


Figure 1: Force at rupture (A) and Extensibility (B) properties for fresh pasta samples.

Different letters above the bars indicate significant difference among fresh uncooked pasta samples (LSD test, $p \leq 0.05$).

An asterisk above the bars indicate a significant difference between fresh uncooked and cooked pasta samples.

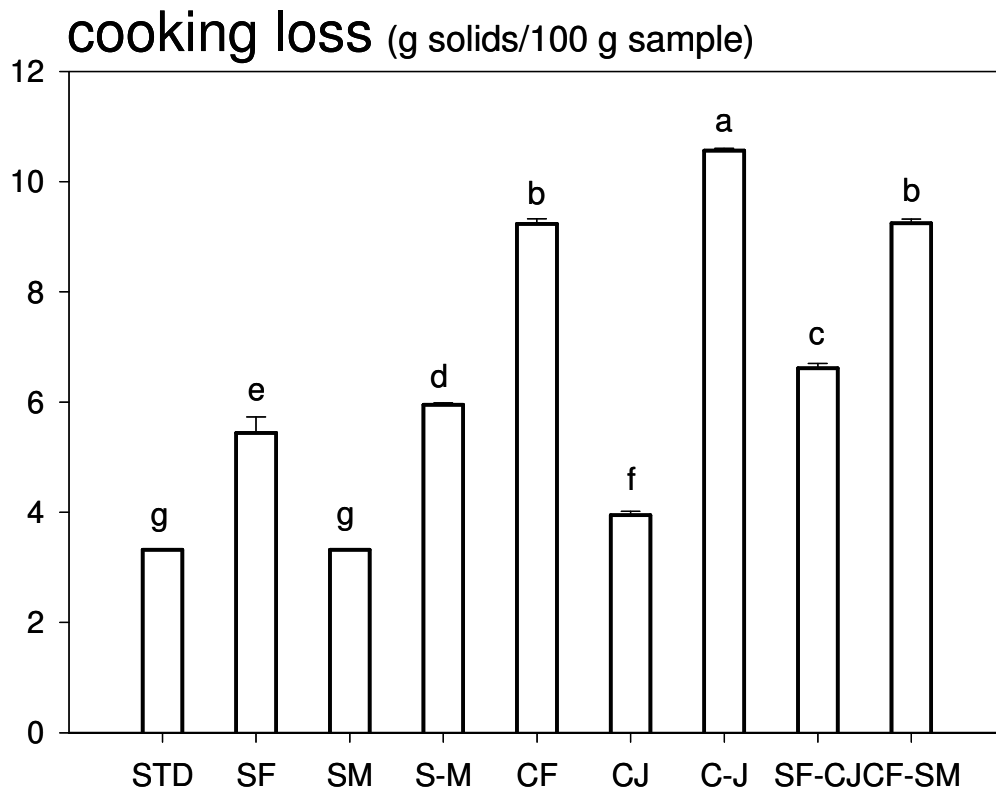


Figure 2: Cooking loss for fresh pasta samples.

Different letters above the bars indicate significant difference among fresh pasta samples (LSD test, $p \leq 0.05$).

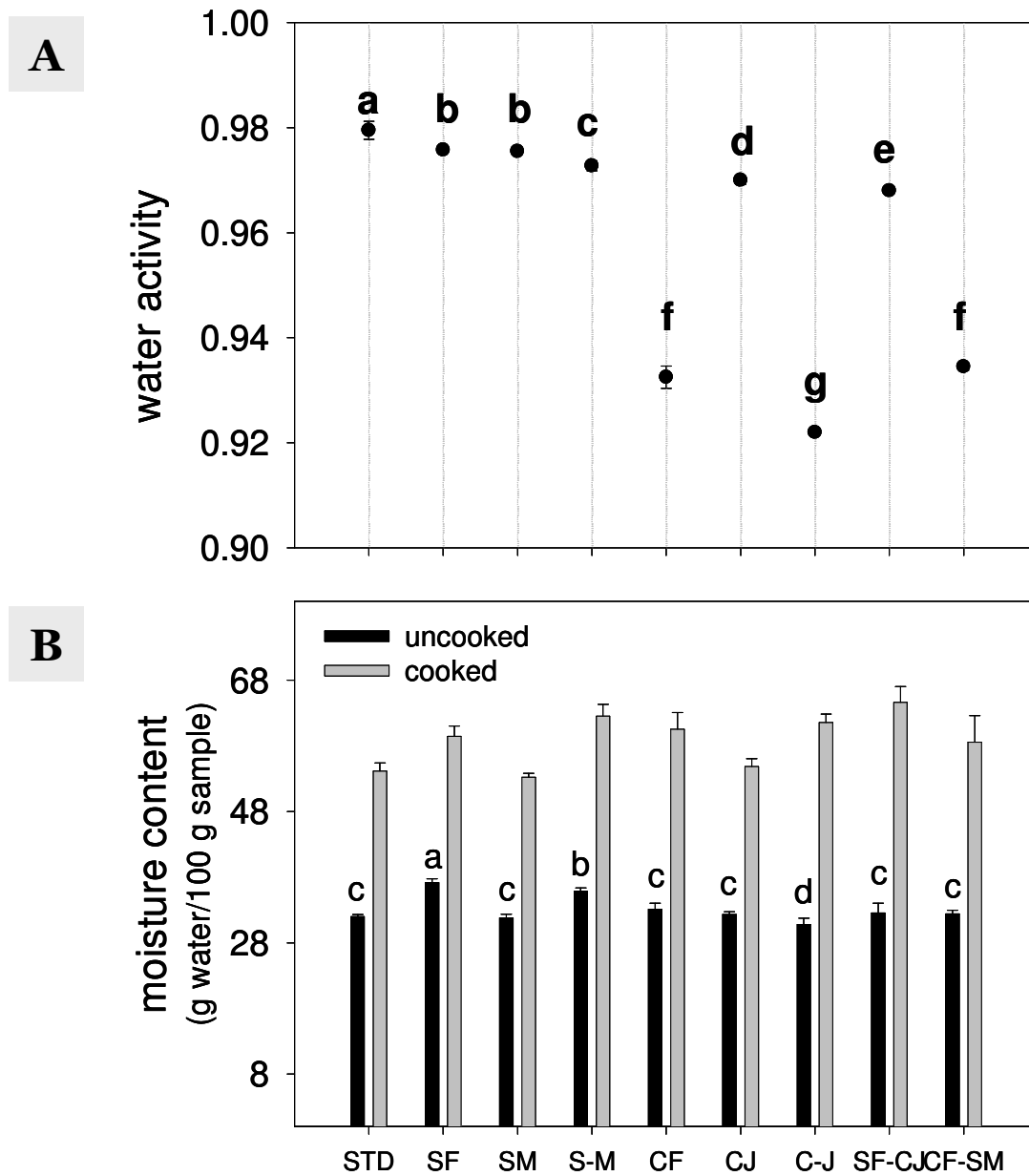


Figure 3: Water activity of uncooked (A) and moisture content (B) for uncooked and cooked fresh pasta samples.

Different letters above the circles (water activity) and bars (moisture content, uncooked sample) indicates significant difference among fresh pasta samples (LSD test, $p \leq 0.05$).

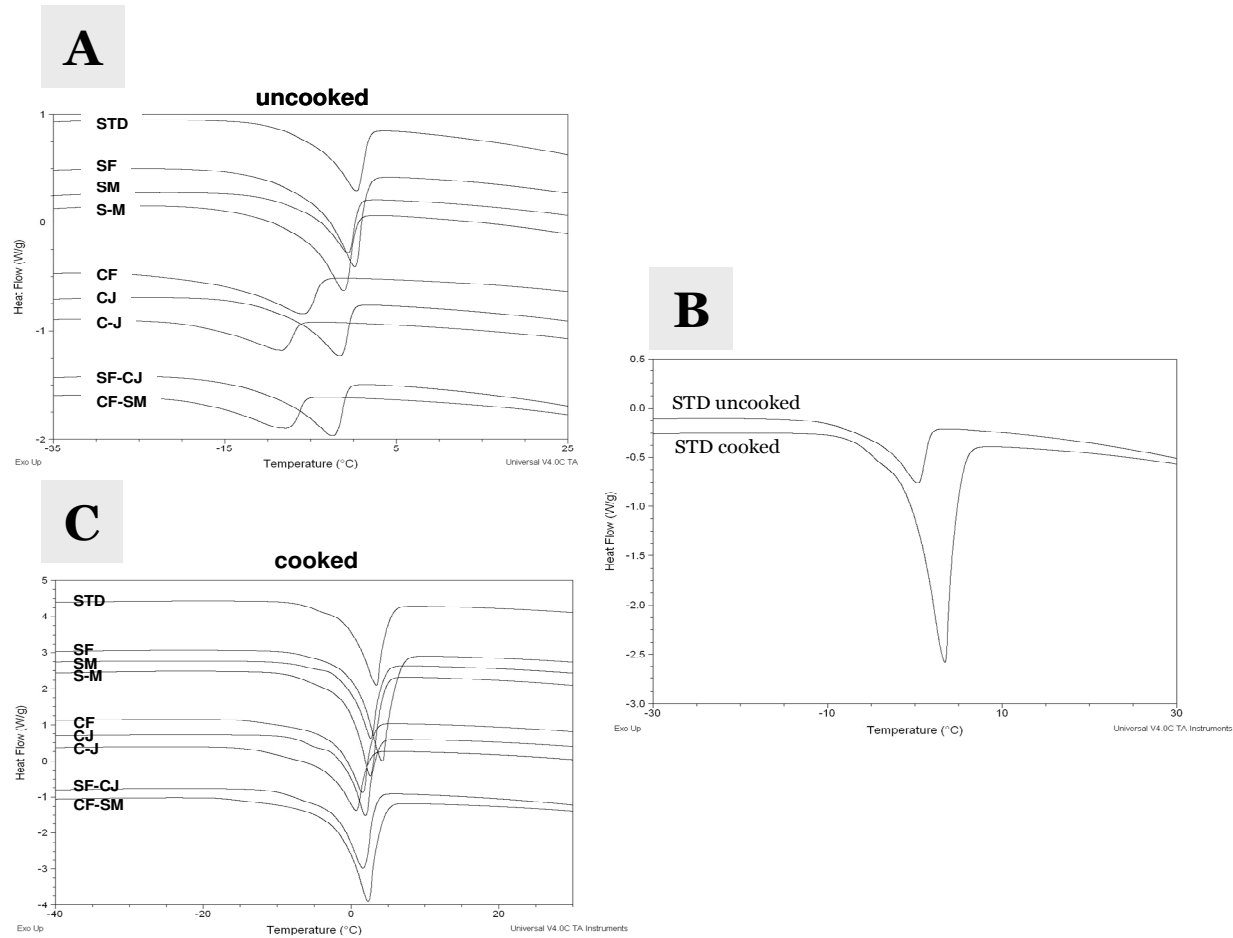


Figure 4: Ice melting peak of uncooked fresh pasta samples (A);
Effect of cooking on thermal properties of ice melting peak of STD pasta (B);
Ice melting peak of cooked fresh pasta samples (C).

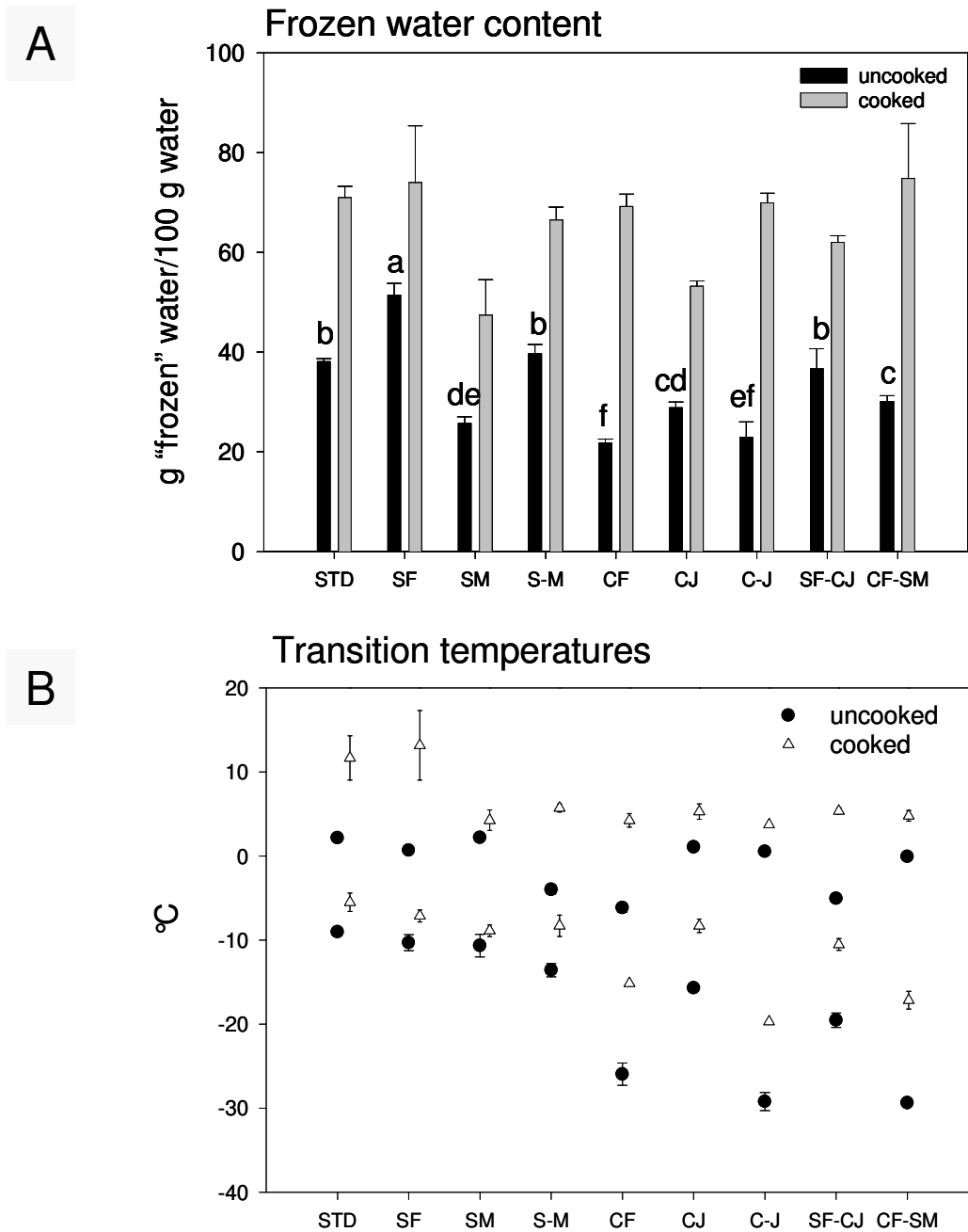


Figure 5: A- Frozen water content for fresh pasta samples; different letters above the bars indicate significant difference among fresh uncooked pasta samples (LSD test, $p \leq 0.05$).

B- Temperatures transition of ice melting peak for fresh uncooked (black circles) and cooked (white triangles) pasta samples.

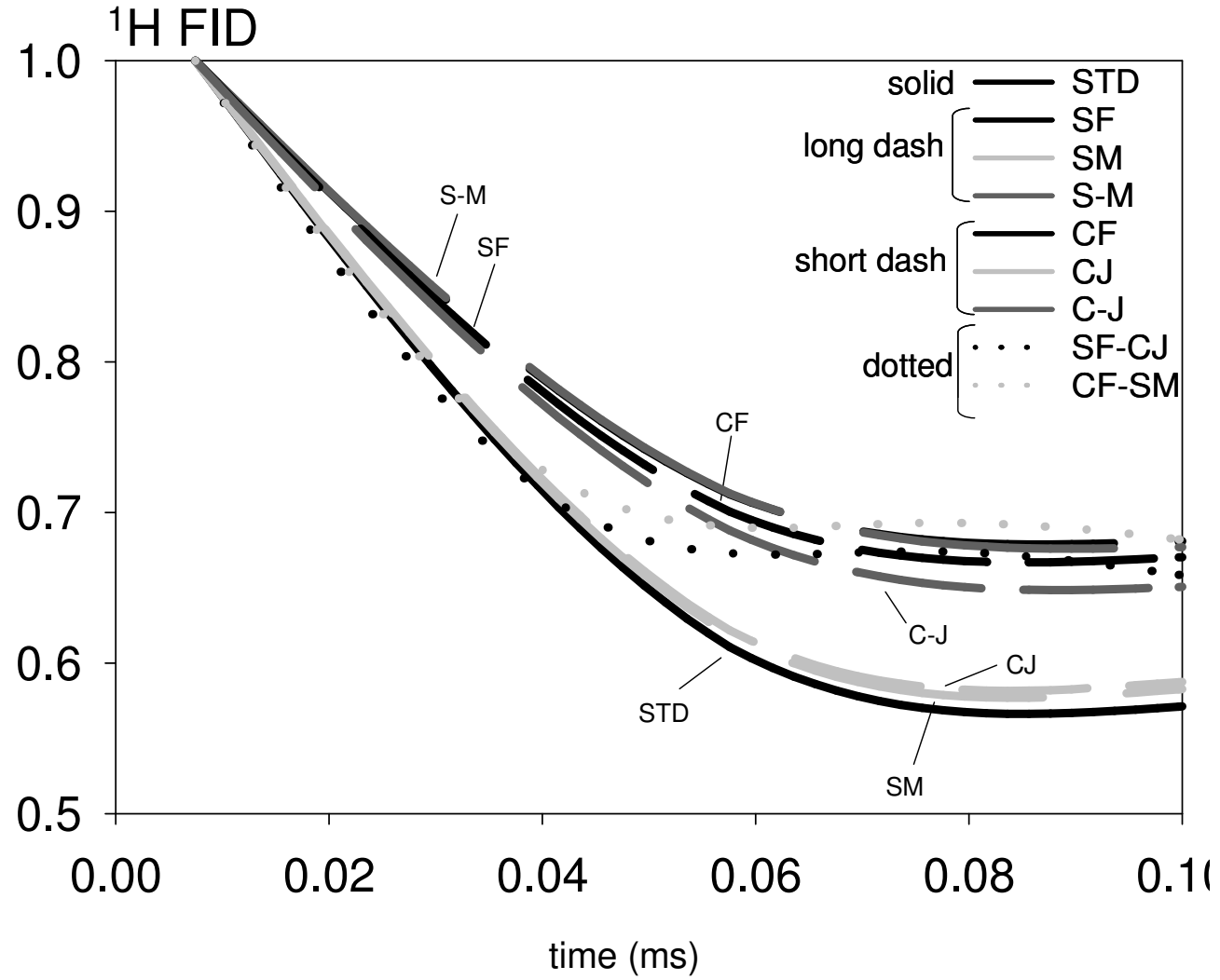


Figure 6: $^1\text{H FID}$ for uncooked fresh pasta samples.

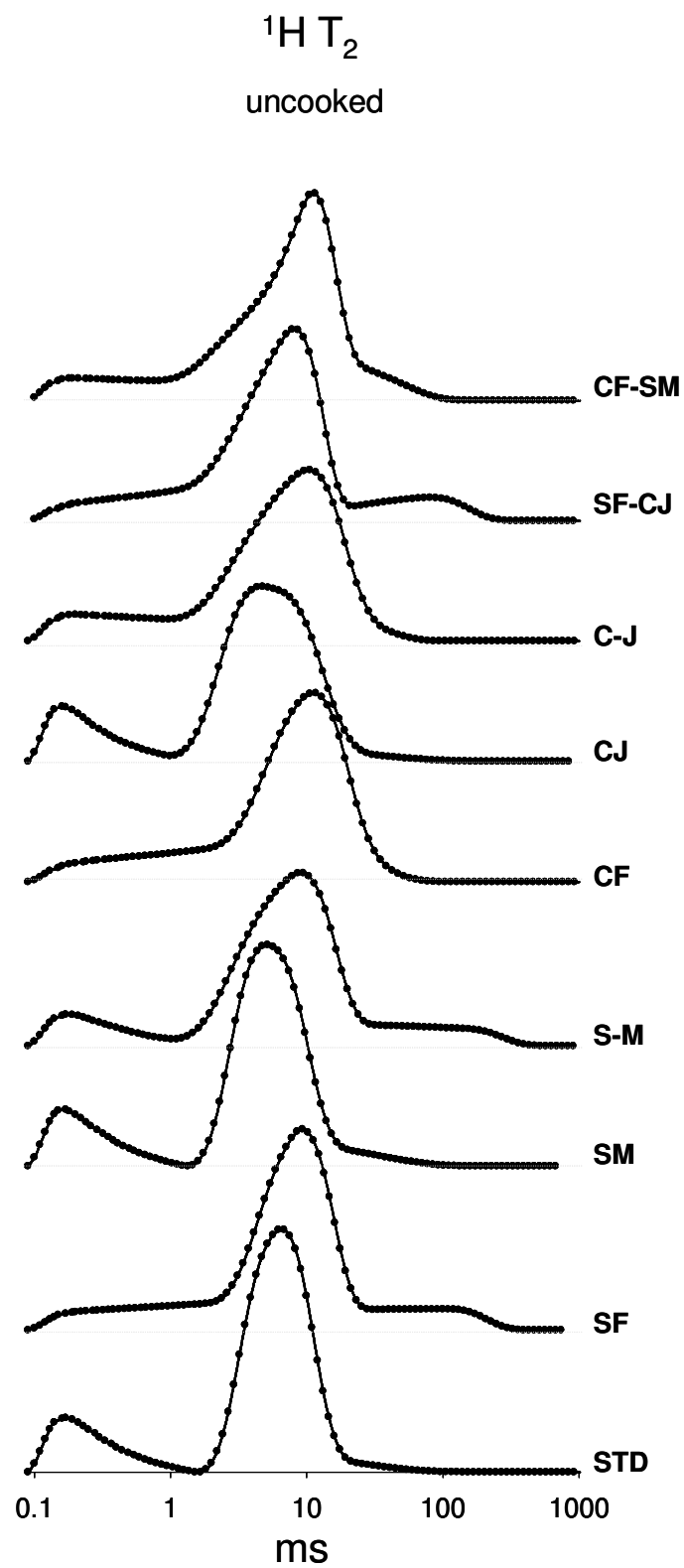


Figure 7: $^1\text{H } T_2$ distribution for uncooked fresh pasta samples.

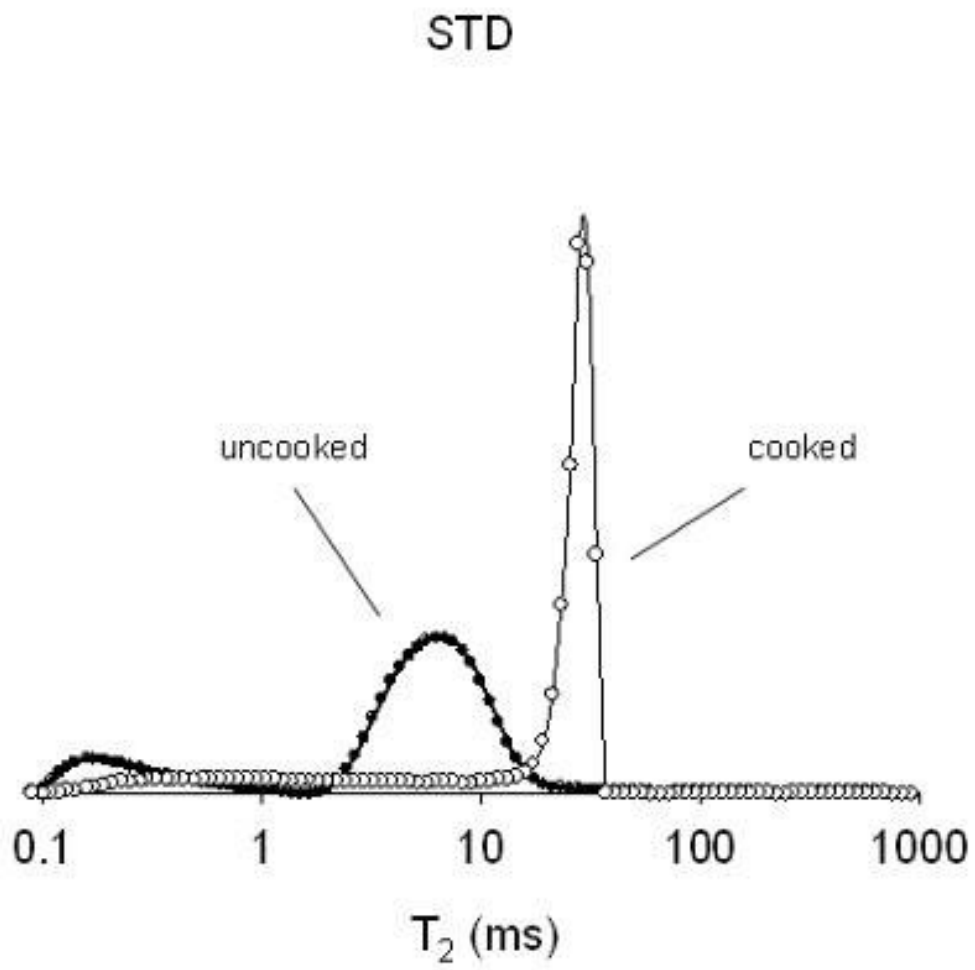


Figure 8: Effect of cooking on ^1H T_2 distribution for STD sample

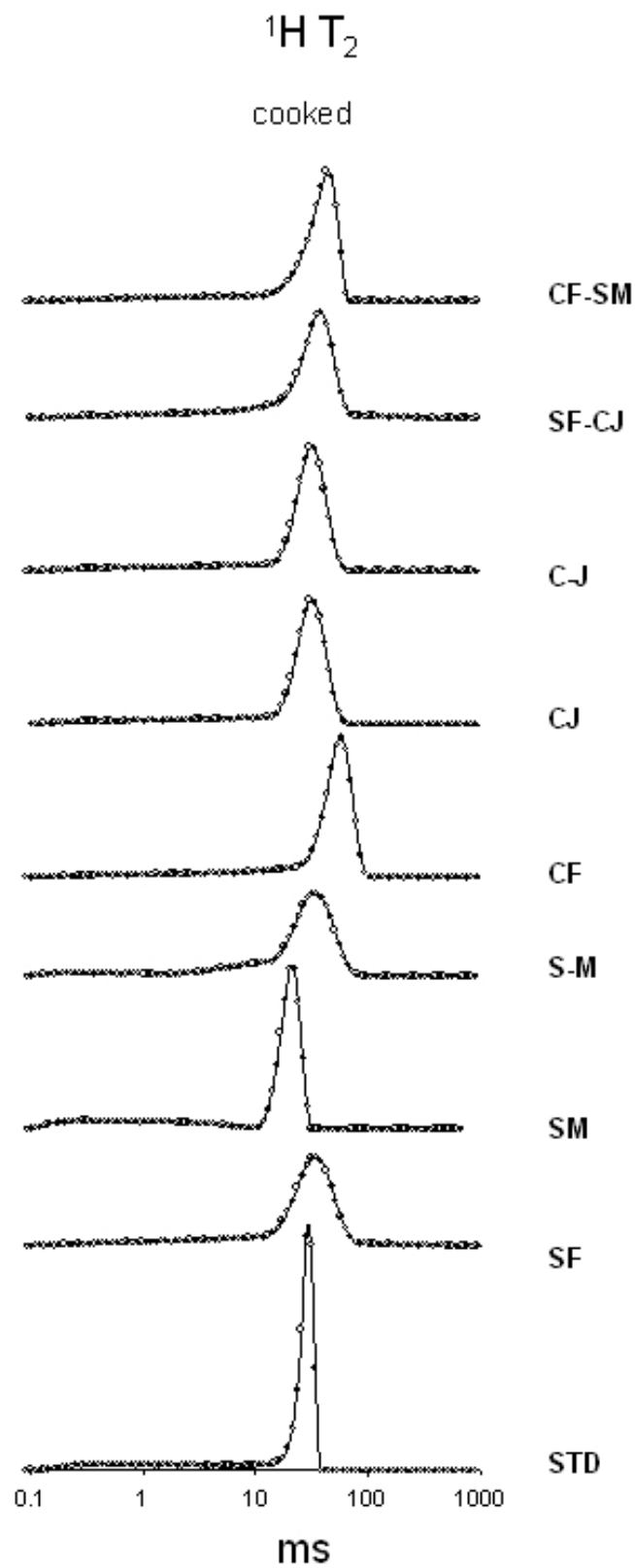


Figure 9: $^1\text{H } T_2$ distribution for cooked fresh pasta samples.

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NUTRITIONALLY ENHANCED TORTILLAS

Nutritionally enhanced tortillas were developed starting from a standard wheat tortillas production procedure and the formulation was changed by incorporating ingredients with well documented nutritional functionality. The additional ingredients able to confer to the product its nutritional function were carrots, soy and kamut.

Carrots, included into the tortillas formulation by substituting part of water with carrot juice, are a well known source of carotenoids, in particular α - and β -carotene. Consumption of this vegetable has been shown to positively influence antioxidant status in healthy human subjects (Bub et al., 2000). In general, fruits and vegetables mediate their beneficial effects via several mechanisms that include metabolism, action on immune system and induction of hormonal signaling. However, in recent years oxidative stress, induced by reactive oxygen species that are generated by normal metabolic activity and by lifestyle factors, have been implicated in the causation and progression of several chronic diseases. Carotenoids, in view of their antioxidant properties, can mitigate oxidative stress (Rao and Rao, 2007).

Soy is a subtropical plant, native to southeastern Asia. Components of soy called isoflavones have raised the interest of nutritional research in recent years. Recent experimental and epidemiological studies have provided convincing evidence for a variety of health benefits derived from the consumption of soy and soy food products (Valachovicova et al, 2004). As an additional benefit, based on the most recent literature, both animal and human studies demonstrate phytoestrogenic soy isoflavones favorably impact bone health (Brynin, 2002). Moreover, soy protein is characterised by a very good nutritional value (amino-acid composition and digestibility) (Mariotti et al., 1999) and may lower LDL cholesterol when it replaces dairy protein or a mixture of animal proteins (Hoie et al, 2007).

Kamut is an ancient type of wheat related to the durum variety used in modern bread making. Compared to common wheat, Kamut is richer in protein (by between 15% and 40%), minerals such as magnesium and zinc, Vitamin Bs and Vitamin E and unsaturated fatty acids, but contains a little less dietary fibre (Gauthier et al., 2006). To increase dietary fiber, wholemeal spelt was used as flour during the preparation of tortillas. Spelt has been reported to be characterized by high dietary fiber content (Bonafaccia et al., 2000). High fiber intakes are associated with lower serum cholesterol concentrations, lower risk of coronary heart disease, reduced blood pressure, enhanced weight control, better glycemic control, reduced risk of certain forms of cancer, and improved gastrointestinal function (Marlett et al., 2002).

Five prototypes of tortillas were, therefore, created based on a standard tortillas production procedure. The standard prototype was modified by substituting part of the water with carrot juice, or some of wheat flour with soy flour, or wheat flour with wholemeal kamut flour. Moreover a prototype containing simultaneously carrot, soy flour and wholemeal kamut flour was also produced.

Tortillas were characterized for their physico-chemical and nutritional properties, sensory acceptability. Nutritional properties were analyzed in terms of total antioxidant capacity (TAC; Pellegrini et al., 2003) and glycemic index (GI; Jenkins et al., 1981) in all products. TAC takes into account the antioxidant activity of single compounds present in food or biological samples as well as their potential synergistic and redox interactions. In recent studies, TAC intake was inversely related to systemic inflammation in healthy subjects (Brighenti et al., 2005) and an independent predictor of plasma beta-carotene (Valtuena et al., 2007). GI is a ranking of carbohydrates on a scale from 0 to 100 according to the extent to which they raise blood sugar levels after eating. Food products with

equivalent amounts of available carbohydrates produce different glycemic responses (Jenkins et al., 1981). This depends on the nature of the food and type and extent of food processing. The slower is the rate of carbohydrate absorption the lower will be the rise of blood glucose and the lower the GI value. Many health benefits are related to reducing the rate of carbohydrate absorption by means of a low GI diet. Among these, strong evidence has been reported for reduced insulin request, improved glucose control, and reduced blood lipid levels (Prosky, 2000). All these constitute independent risk factors for several chronic Western diseases including diabetes, coronary heart disease and possibly certain cancers.

The simultaneous combination of carrot juice, soy and wholemeal kamut resulted in a very interesting product that was not only the most acceptable by the consumers but also had the highest total antioxidant capacity and lowest glycemic index. Physico-chemical characterization indicated that the product was the hardest and that the state of water was largely affected by the presence of soy (lower “freezable water” and higher ^1H NMR molecular mobility) suggesting that this product may possibly be more stable.

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Effect of formulation on physicochemical properties and water status of nutritionally enhanced tortillas

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1. Abstract

BACKGROUND: Nutritionally enhanced tortillas were developed by incorporating ingredients with well documented nutritional functionality (carrot, soy, whole kamut and their combination) in a standard wheat tortillas formulation and the effect of formulation changes on physicochemical properties and water status was evaluated. Tortillas were characterized for their moisture content, water activity, thermal properties and ^1H NMR molecular mobility.

RESULTS: The substitution of part of the water with carrot juice in the tortillas formulation altered slightly the macroscopic and significantly the thermal properties (lower FW content) but only marginally the ^1H molecular mobility (faster ^1H FID decay). Substitution of wheat flour with whole kamut flour did not alter the properties considered. Inclusion of soy flour in tortillas formulation significantly altered all the properties studied (lower water activity, moisture content and FW, higher ^1H NMR molecular mobility). The simultaneous presence of carrot juice, whole meal kamut flour and soy flour in the formulation reflected the distinctive contribution of each specific ingredient.

CONCLUSIONS: The changes in formulation used in this study to produce tortillas with enhanced nutritional value affected the water status of the products in a very interesting manner: the different ingredients altered the water status at different levels (e.g. macroscopic, macromolecular, and molecular).

2. Introduction

Large interest has recently risen in the development of “functional” foods, products that affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way relevant to improved state of health and well-being, reduction of risk of diseases, or both. ¹ Foods rich in antioxidants and low glycemic index (GI) effect can reduce in combination the risk of increased post-prandial oxidative stress (constituent of the onset of several chronic diseases). ^{2,3} Fiber-rich diets are associated with lower serum cholesterol concentrations, lower risk of coronary heart disease and certain forms of cancer, reduced blood pressure, enhanced weight and glycemic control, and improved gastrointestinal function. ^{4,5} Addition/substitution of functional ingredients to/in food formulations are expected to affect physicochemical properties and water status of the product and, consequently, its stability. ⁶ For example, addition of polyols in wheat tortillas formulation was reported to reduce water activity and increase shelf-life; addition of a glycerol-salt combination in corn tortillas was studied in respect to its effect on mechanical properties, water status and stability of the product. ^{7,8} Addition of soy flour in bakery products (bread) was shown to reduce amylopectin retrogradation, significantly modify the water status and to delay firming in baked products. ^{6,9,10} Fiber is known to alter viscoelastic properties and water absorption in bakery doughs and final products. ^{11,12} Addition of fiber in wheat tortillas was reported to increase the water absorption in the dough to facilitate processing and to reduce textural stability (rollability) of the product. ¹³ Simultaneous addition of vital gluten improved machinability and storage stability of fiber-enriched wheat tortillas. ^{13, 12}

Development of functional foods must be carried out by selecting ingredients able increase the nutritional properties of the food while

preserving organoleptic quality of the product. Nutritionally enhanced tortillas were developed in our laboratory by incorporating ingredients with well documented nutritional functionality (carrots, soy, wholemeal kamut and their combination) in a standard wheat tortillas formulation in an attempt to create low GI and antioxidant rich products while preserving/improving sensory acceptability. ¹⁴ The ingredients used to enhance tortillas nutritional value may have altered products properties. The objective of this work was, therefore, to verify the effect of changes of formulation on the physico-chemical properties and water status of nutritionally enhanced tortillas.

3. Experimental

Tortilla formulation and production

Five prototypes of tortillas, wheat based (standard, STD), carrot enriched (CAR), soy enriched (SOY), with kamut (KAM) and one containing simultaneously carrot, soy and kamut (CSK) were produced as according to the formulation shown in Table 1, as previously described. ¹⁴ Two batches of 6 tortillas were produced on different days for each prototype.

Tortillas characterization

Samples used for product characterization were extracted from the central part (3 cm diameter) of each tortilla. The upper and lower tortillas' surfaces were removed and only the central core of the product was used for analyses.

Moisture content

Moisture content of tortillas was determined from weight loss by oven drying at 105 °C to constant weight. Duplicated analyses on three tortillas for each batch were carried out for a total of 12 moisture content determinations for each tortilla prototype.

Water activity

Water activity of tortilla samples was measured at 25 °C using Decagon Aqualab Meter Series 3TE (Pullman, WA). Tortillas were broken into small pieces immediately before water activity measurement. Duplicated analyses on two tortillas for each batch were carried out for a total of 8 water activity determinations for each tortilla prototype.

Thermal properties

Thermal properties were measured using a differential scanning calorimeter (DSC Q 100 TA Instruments, New Castle, DE, USA). Indium and mercury were used to calibrate the instrument and an empty pan was used as reference. Tortilla samples (4-5 mg) were placed into hermetically sealed stainless steel pans (Perkin Elmer, Somerset, NJ, USA), equilibrated at -50.00 °C and heated to 120 °C with a heating rate of 5 °C/min. Thermograms were analyzed with a Universal Analysis Software, Version 3.9A (TA Instruments, New Castle, DE) and enthalpy (ΔH , J/g), onset (T_{on}), peak (T_p) and offset (T_{off}) temperatures of the transitions were obtained.

“Frozen” water (at the given conditions; FW) was calculated from the endothermic peak in the -15 – 15 °C range using the following equation:

$$FW = 100 * [\Delta H (-15 - 15 \text{ } ^\circ\text{C}) - (\Delta H_{Mg} * MgC)] / (\Delta H_{H_2O} * MC)$$

where:

- FW = Frozen Water [g frozen H₂O Kg⁻¹ H₂O]

- $[\Delta H (-15 - 15\text{ }^\circ\text{C}) = \text{Enthalpy endothermic peak } -15 - 15^\circ\text{C}) \text{ [J / g product]}$
- $\Delta H_{\text{Mg}} = \text{latent heat of margarine fusion} = 60 \text{ J / g solid margarine (from DSC analysis)}$
- $\text{MgC} = \text{Margarine Content [g margarine Kg}^{-1} \text{ product]}$
- $\Delta H_{\text{H}_2\text{O}} = \text{latent heat of ice fusion} = 334 \text{ J / g ice}$
- $\text{MC} = \text{Moisture Content [g H}_2\text{O Kg}^{-1} \text{ product]}$

Duplicated analyses on two tortillas for each batch were carried out for a total of 8 DSC determinations for each tortilla prototype.

Recrystallized amylopectin. The melting peak at around 60 °C was assumed to correspond to recrystallized amylopectin.¹⁵ The enthalpy of this peak was measured (W/g) by integration of the thermograms between 56 °C and the flat baseline in all samples. The 56°C reference was selected to be just above the endset temperature of margarine melting (55°C, experimentally determined) and the endset temperature of the endothermic peak was measured.

Proton nuclear magnetic resonance (¹H NMR)

A low resolution (20 MHz) ¹H NMR spectrometer (the miniSpec, Bruker Biospin, Milano, Italy) was used to study proton molecular mobility by measuring the free induction decay (FID), transverse (T_2) and longitudinal (T_1) relaxation times. Three g of tortilla (10 mm high) were placed into a 10 mm NMR tube that was then sealed with parafilm to prevent moisture loss during the NMR experiment. All measurements were made at $25.0 \pm 0.1\text{ }^\circ\text{C}$. FIDs were acquired using a single 90° pulse, followed by dwell time of 7 μs and a recycle delay of 0.3 s. T_2 (transverse relaxation times) were obtained with a Carr Purcell Meiboom Gill (CPMG) pulse sequence with a recycle delay of 0.3 s ($\geq 5 T_1$) and interpulse spacing to 0.04 ms.^{16,17} T_1

(longitudinal lattice relaxation times) were determined by the inversion recovery pulse sequence with an inter pulse spacing ranging from 1 ms to 600 ms depending on the sample relaxation time and a recycle delay of 0.3 s ($\geq 5 T_1$).¹⁸ T_2 and T_1 curves were analyzed as quasi-continuous distributions of relaxation times using a UPEN software.^{19,20}

Duplicated analyses on two tortillas for each batch were carried out for a total of 8 NMR determinations for each tortilla prototype.

Statistical analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 12.0, SPSS Inc., Chicago, Illinois, USA). SPSS was used to perform one-way-analysis of variance (ANOVA) and Least Significant Difference test (LSD) at a 95% confidence level ($p < 0.05$) to identify differences of evaluated parameters.

4. Results and discussion

Macroscopic characterization

The tortillas produced with the modified formulations were previously reported to be either equally (CAR, KAM and SOY) or more (CSK) acceptable by a consumers panel and to have enhanced nutritional properties.¹⁴ The effect of formulation changes on the physicochemical properties and water status of nutritionally enhanced tortillas are reported in this study.

Moisture content and water activity

Moisture content and water activity parameters have been significantly affected by different formulations (Table 2) due, first of all, to the different water amount required in the recipes to obtain palatable products. Tortilla

STD had moisture content (MC) of 2.88 ± 0.04 g water kg⁻¹ sample and water activity (aw) of 0.94 ± 0.01 (Table 2), comparable to literature data. 21 The substitution of wheat flour with whole kamut flour (KAM) did not affect either the moisture content (2.94 ± 0.04 g water kg⁻¹ sample, Table 2), or the water activity of the sample (0.93 , Table 2).

The CAR prototype had a moisture content of 2.69 ± 0.03 (g water kg⁻¹ sample), lower than the STD, due, likely, to the presence of solutes in carrot juice used to replace part of the water. The solutes present had high affinity for water and significantly reduced the water activity of the sample (0.91 ± 0.01). A significantly lower moisture content was found in SOY (2.36 ± 0.03 g water kg⁻¹ sample) and in CSK (2.34 ± 0.03 g water kg⁻¹ sample), due, primarily, to the smaller amount of water added in the formulation, and, possibly, to a stronger interaction of the solids (e.g. proteins) with water that may have reduced water removal during the drying process. Soy proteins are known to have a very strong affinity for water.^{22, 10} This strong interaction of soy solids with water was reflected by a significantly lower water activity in SOY (0.88 ± 0.03) and, even more markedly, in CSK (0.84 ± 0.03). In the CSK prototype a synergistic effect of carrot solutes (e.g. sugars), soy solids (e.g. proteins) and fiber from kamut flour (e.g. 1.01 ± 0.03 g fiber kg⁻¹ sample¹⁴) is likely responsible for the very low water activity of the product. The different formulations may have also resulted in diverse molecular interactions and water partitioning among ingredients leading to the development of heterogeneous microscopic structures that may have also influenced other physico-chemical properties of tortillas (e.g. density, porosity, ...) and lead to different moisture contents and water activities at the end of the cooking process (cooking time constant and formulation dependent).

Thermal properties

Thermal properties of tortillas prototypes were measured by differential scanning calorimetry (DSC). Thermograms of all samples had similar, but not identical, lineshapes and exhibited two major endothermic transitions as the samples were heated from $-50\text{ }^{\circ}\text{C}$ to $120\text{ }^{\circ}\text{C}$. Characteristic thermograms of the major observed transitions in the $-35 - 20\text{ }^{\circ}\text{C}$ and $25 - 90\text{ }^{\circ}\text{C}$ ranges were reported in Figure 1 and Figure 2, respectively. A first major endothermic event (Figure 1) originated at $\sim -15\text{ }^{\circ}\text{C}$ and ended at $\sim 15\text{ }^{\circ}\text{C}$ (Figure 1) while a second endothermic event (Figure 2) occurred at higher temperatures ($40 - 70\text{ }^{\circ}\text{C}$ range). A third thermal event was observed as a slight baseline shift (prior to the endothermic peak at $-15 - 15\text{ }^{\circ}\text{C}$) in SOY and CSK prototypes (Figure 1A) and it was probably induced by the presence of a higher amount of margarine in these samples ($0.8\text{ g margarine Kg}^{-1}\text{ product}$) as compared to the other prototypes ($0.4\text{ g margarine Kg}^{-1}\text{ product}$). A similar transition (although sharper and more defined) was also observed in pure margarine samples, as shown in Figure 1A and it was also noticeable in the DSC heating thermograms of margarine reported by Aktas and Kaya, 2001,²³; a similar transition was also observed in quinoa embryos and seeds and it was related to lipid components.²⁴ A characteristic thermogram for margarine in the $-35 - 20\text{ }^{\circ}\text{C}$ temperature range was also shown in Figure 1 and it can be noticed that of its fractions melted in the $\sim -10 - 15\text{ }^{\circ}\text{C}$ range. The presence of margarine in tortillas' formulation affected, at least partially, the endothermic melting process in the $-15 - 15\text{ }^{\circ}\text{C}$ range where ice melting is also expected to occur. The measured peak ($-15 - 15\text{ }^{\circ}\text{C}$) enthalpy was, therefore, the sum of the two contributions: water and the margarine melting; the FW content was, therefore, accordingly calculated as described in the material and methods section. The FW content of the samples was significantly influenced by tortilla formulation, and it was found to represent $\sim 1.7\text{ g FW kg}^{-1}\text{ water}$

of the total water present in the STD prototype and, ~ 0.8, 1.1, 2.9 and 1.9 g FW kg⁻¹ water in CAR, SOY, KAM and CSK, respectively (Table 2). The very low FW content in CAR (~8 % total water basis, Table 2) was probably related to an increase in viscosity of the hydrophilic phase of the product (consequent to sugar solubilization in water) resulting in a decrease in motion of the water molecules that could form ice crystals detectable by DSC.^{25,26} On the contrary, the amount of FW in the SOY prototype represented ~ 12 % of the water of the sample (Table 2) indicating a different water-solids interaction as compared to that found in CAR. The lower FW content found in the SOY tortillas may result from both the lower water percentage of the recipe and the higher affinity of soy solids for water. Soy proteins are known to be highly hydrophilic^{22,10} and to “bind” a large amount of water and, possibly, induced a decrease of the FW present in the sample. It was previously reported that substitution of wheat flour with defatted soy flour in wheat bread required increased water in the formulation to obtain an acceptable product and that the FW was comparable to the control at 20% soy flour substitution and higher than the control when soy substitution had reached 30 – 40% of the wheat flour.⁶ The FW (total water basis) content in KAM was found higher if compared with STD (~ 2.9 g FW kg⁻¹ water), possibly due to a “weak-interaction” of the water with the fibre present in KAM. The FW content of CSK was found to be ~ 19 % comparable to STD even though the total moisture content and water activity of this sample were the lowest (2.34 ± 0.03 g water kg⁻¹ to product and 0.84 ± 0.03 , respectively). The very high complexity of its formulation may cause a competition for water among the different types of solids (including gluten, amorphous starch, sugars of carrot juice, soy proteins, fiber) resulting in a very interesting and unique macroscopic water status in the product.

The temperature range of the melting endothermic peak around 0°C was comparable in all tortillas but the line shape of the peaks was sharper and more defined in the STD and KAM samples. On the contrary, CAR, SOY and CSK showed a flatter and less evident peak that was also, shifted towards lower temperatures (as shown by the lower T_p , Figure 1, insert B). This may be attributed, at least partially, to more heterogenous water crystallization process in the samples containing solids with affinity for water (e.g. sugars present in the carrot juice and soy proteins in soy flour). The interaction of water with the solids may have induced some changes in the amount of water that could crystallize under the experimental conditions.

The second endothermic event observed in the DSC thermograms of tortilla samples occurred primarily between 60°C and 70°C (Figure 2) and it was mainly attributed to melting of pseudo-crystalline/crystalline amylopectin, suggesting either incomplete starch gelatinization during baking (due to either short cooking time and/or to limited water availability) and/or very fast amylopectin retrogradation after cooling, as previously reported in wheat tortillas.²⁷ An additional endothermic event could be detected as a shoulder at lower temperatures (onset at ~35°C) in SOY and CSK (Figure 2 and 2B). This shoulder was likely associated to lipid (from margarine) melting and it was only visible in these two tortillas prototypes because of their higher margarine content as compared to the other samples (Table 1). Melting of some fraction of pure margarine occurred in a comparable temperature range as shown in Figure 2. The amylopectin enthalpy measured excluding the margarine melting contribution (i.e. in the 56°C – end set range) was found to be 1.8 ± 0.6 , 1.2 ± 0.1 , 1.2 ± 0.1 , 1.5 ± 0.4 and 0.9 ± 0.2 J/g for STD, CAR, SOY, KAM and CSK, respectively, (Figure 2A), with no significant differences among tortillas indicating that the changes in formulation did not affect the amylopectin melting peak. Tortillas samples resulted have a comparable amylopectin melting temperature

range (T_{on} and T_{off} comparable, Figure 2 insert B) with the exception of CAR that showed a slightly narrower peak (higher T_{on} and lower T_{off}) if compared with others prototypes.

Proton nuclear magnetic resonance (^1H NMR)

Molecular mobility was studied by low resolution ^1H NMR and multiple experimental techniques were used in an attempt to cover a large range of molecular mobility. Mobility of the most rigid ^1H components of tortillas was analyzed with a FID experiment while the more mobile proton fractions were characterized in terms of T_2 and T_1 relaxation times.

Characteristic FID decays for the five tortilla prototypes are shown in Figure 3 and they indicate the presence of a fast relaxing ^1H population in the tortillas object of this study. A particularly high molecular rigidity was found in CSK and CAR as suggested by the faster decay (Figure 3). SOY tortilla's FID demonstrated an intermediate mobility of the least mobile detectable (under the selected experimental conditions) ^1H fraction while STD and KAM resulted the most mobile. The fast relaxing ^1H population observed with the FID might arise from protons in solid-like components, such as starch and proteins and water molecules tightly associated with those solids.²⁸

T_2 and T_1 relaxation decays were analyzed as quasi-continuous distributions of relaxation times and the results were summarized in Figure 4. The T_2 distribution spectra were analyzed for $T_2 \geq 0.089$ ms (2 interpulse spacing + instrument dead time) i.e. no T_2 values shorter than the measured point on the CPMG was attempted. Therefore, the first minor and incomplete "peak" observed at very fast relaxation times (~ 0.1 ms) was not considered in the discussion as it fell only partially within the experimental time window. Two T_2 ^1H populations were found in all tortilla samples and were named starting from the lowest to the highest relaxation time A and B,

respectively. T_{2A} represented a population of protons characterized by relaxation times in the $\sim 0.8 - 6/8$ ms range and peaked at ~ 2 ms in all samples. Similar results were previously found in uncooked wheat dough (33.1% moisture content) by Doona and Baik (2007) who reported the presence of a ^1H population relaxing at ~ 3 ms that shifted to 10 ms as moisture content increased to 47.2 %, [wb] and it was attributed related to a variation of the chemical and physical states of water molecules in the dough. ²⁹ Similarly, in flour-water mixtures (22 – 40 % water [wb], manually blended) Assifaoui and co-workers, indicated the presence of a ^1H T_2 population (peak at 3.2 ms) that shifted to longer relaxation times with increasing moisture content and related this increased mobility to an increase in free volume (higher mobility of starch) and to the proton population (gluten, starch and sucrose) likely associated with water. ³⁰

The second ^1H population observed (B population, Figure 3) represented a population of protons whose relaxation time and relative abundance were found to be formulation dependent. STD showed a peak characterized by relaxation time in the 12 – 180 ms range, comparable to T_{2B} of KAM and slightly slower than in CAR (15 – 170 ms range). T_{2B} of SOY and CSK started at relaxation times slightly lower than that of the other samples (~ 6 ms) and developed over a larger range of relaxation times (up to ~ 300 ms) indicating a higher degree of heterogeneity in these two samples. ³¹ T_{2B} in SOY and CSK represented also a larger amount of protons detected in the samples (~ 30 % of the total ^1H) as compared to STD, CAR and KAM (~ 10 % of the total ^1H), indicating the presence of a larger ^1H population of higher mobility. This population (^1H $T_2 \sim 10 - 300$ ms) may possibly be attributed to lipids present in tortillas, as previously reported in uncooked biscuit dough. ³⁰ Contribution of other proton populations, originating from different chemical species and/or physical states could not be ruled out. In particular, the larger ^1H B population in SOY and CSK could be related both

to the larger amount of margarine in the formulation (Table 1) and/or the presence of soy proteins. The margarine contribution was verified by increasing the margarine content in STD tortillas that resulted in an increase of the total detectable protons from 10% (0.4 g margarine Kg⁻¹ product) to 12% (0.8 g margarine Kg⁻¹ product). The soy contribution was hypothesized since the B population represented ~ 30% of the total protons in SOY and CSK (that could not be accounted for the higher margarine content) and it has been previously reported that soy in bread formulation increased molecular mobility.³²

T₁ distribution curves indicated the presence of two ¹H T₁ populations in STD, CAR and KAM and only one in SOY and CSK. The first population observed in STD, CAR and KAM represented a small amount of the total detectable protons (~ 4%) and it was characterized by short relaxation times (1-5 ms), while the second population (significantly predominant) had relaxation times in the 50 – 200 ms range. The single ¹H T₁ population observed in SOY and CSK developed over a 40 – 400 ms range, indicating that the protons were in a “fast exchange” regime in the T₁ experimental time window and confirming the higher ¹H molecular mobility of these samples.

Physicochemical properties and water status were found to be dependent on tortillas formulation. The changes in formulation used in this study to produce tortillas with enhanced nutritional value affected the water status of the products in a very interesting manner: the different ingredients altered the water status at different levels (e.g. macroscopic, macromolecular, and molecular). The substitution of part of the water with carrot juice in the tortillas CAR formulation reduced significantly the macroscopic (water activity and moisture content), and the thermal properties (halved FW content), and only marginally the ¹H molecular mobility (faster ¹H FID decay) as compared to the STD. The substitution of

wheat flour with wholemeal kamut increased the amount of frozen water (under the selected experimental conditions) in this tortilla prototype therefore suggesting that possibly water redistribution among ingredients induced by the fibre present. The inclusion of whole soy flour in tortillas formulation resulted in a higher ^1H molecular mobility (T_{2B} relaxation rate - % ^1H T_{2B} population and single T_1) that was probably induced by the soy proteins that loosely interact with water molecules as previously reported in soy enriched breads 9,32 and/or by the higher margarine content. The higher ^1H molecular mobility was not reflected in the macroscopic water properties (moisture content and water activity). The water status in the CSK tortilla reflected the contribution that each ingredient had in the respective prototype: a fast ^1H FID decay (CAR), high FW (as in KAM), high ^1H molecular mobility and low moisture content and water activity (as in SOY).

The tortillas produced provide an interesting set of products to verify the role of the different water status indicators on chemical, physical and microbiological product stability. The CSK product is expected to be the most s and STD and KAM the least stable products according to the conventional water activity theory. But CSK was shown to have a significantly higher ^1H molecular mobility than STD and KAM that may favour a variation of the chemical and physical states of the CSK product and, possibly, reduce its chemical, physical and microbiological stability. The analysis of storage stability of nutritionally enhanced tortillas is currently undergoing to better understand and evaluate the role of ^1H molecular mobility parameters as product stability indicators.

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Table 1. Nutritionally enhanced tortilla formulations (as previously reported in Scazzina et al, 2008).

Table 2. Water parameters: moisture content (MC) aw, and FW of nutritionally enhanced tortillas. Same letters within each column do not significantly differ ($p \leq 0.05$).

Table 1: Nutritionally enhanced tortilla formulations (as previously reported in Scazzina et al, 2008).

Ingredient (g kg⁻¹)	STD	CAR	SOY	KAM	CSK
Wheat flour	6.00	6.00	4.10	-	-
Whole soy flour	-	-	1.70	-	1.70
Whole kamut flour	-	-	-	6.00	4.10
Mono and diglycerides	0.25	0.25	0.25	0.25	0.25
Salt	0.09	0.09	0.09	0.09	0.09
Leavening agent	0.09	0.09	0.09	0.09	0.09
Margarine	0.40	0.40	0.80	0.40	0.80
Wheat gluten	-	-	0.16	-	0.16
Distilled water	3.17	1.43	2.83	3.17	0.92
Carrot juice	-	1.74	-	-	1.89

Table 2: Water parameters: moisture content (MC), a_w , and FW of nutritionally enhanced tortillas. Same letters within each column do not significantly differ ($p \leq 0.05$).

Prototype	MC (g water kg ⁻¹ sample)	a_w	FW (g “frozen” water / kg ⁻¹ water)
STD	2.88 ± 0.04 (a)	0,94 ± 0.01 (a)	1.67 ± 0.24 (bc)
CAR	2.69 ± 0.03 (b)	0,91 ± 0.01 (b)	0.84 ± 0.13 (d)
SOY	2.36 ± 0.03 (c)	0,88 ± 0.03 (c)	1.10 ± 0.42 (cd)
KAM	2.94 ± 0.04 (a)	0,93 ± 0.01 (a)	2.86 ± 0.20 (a)
CSK	2.34 ± 0.03 (c)	0,84 ± 0.03 (d)	1.88 ± 0.08 (b)

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Figure 1: Characteristic DSC thermogram of nutritionally enhanced tortillas.

Insert A: Detail of the thermograms for SOY, CSK tortillas and margarine.

Figure 2: Typical DSC thermogram of nutritionally enhanced tortillas and margarine in the 25 – 90 °C range.

Insert A: Enthalpy of endothermic transition of nutritionally enhanced tortillas.

Figure 3: ^1H FID decays of nutritionally enhanced tortillas.

Figure 4: ^1H T_2 and T_1 distributions of nutritionally enhanced tortillas.

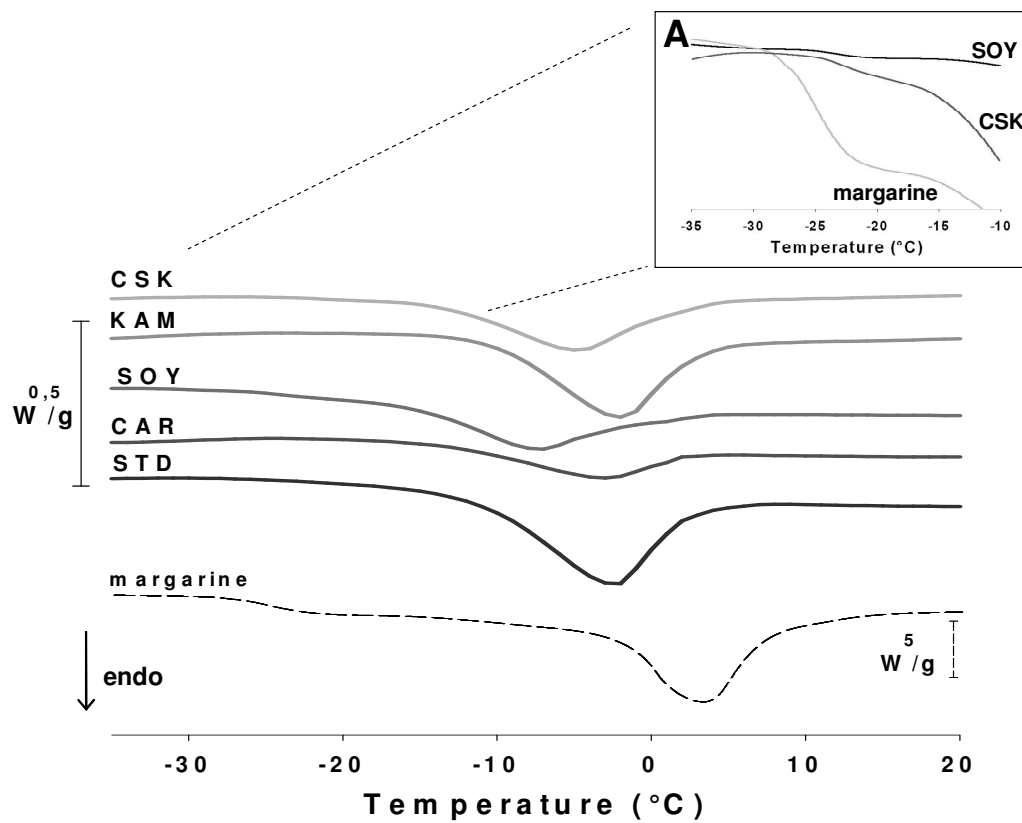


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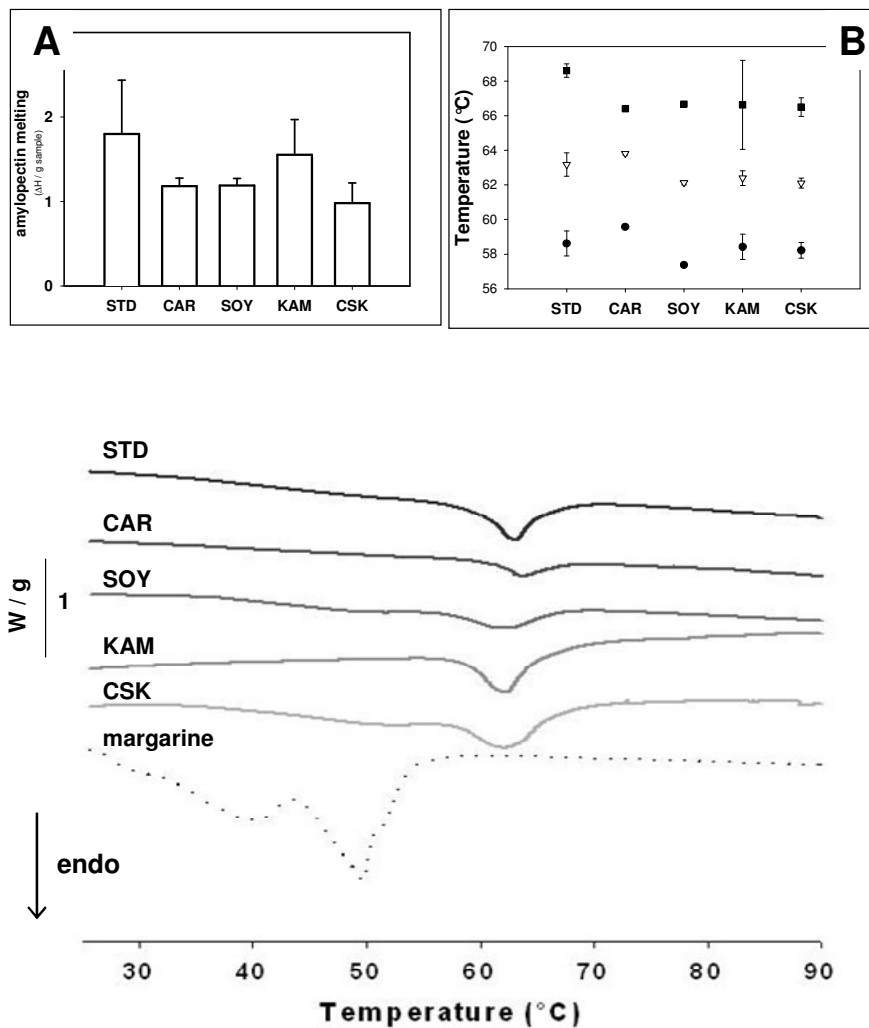


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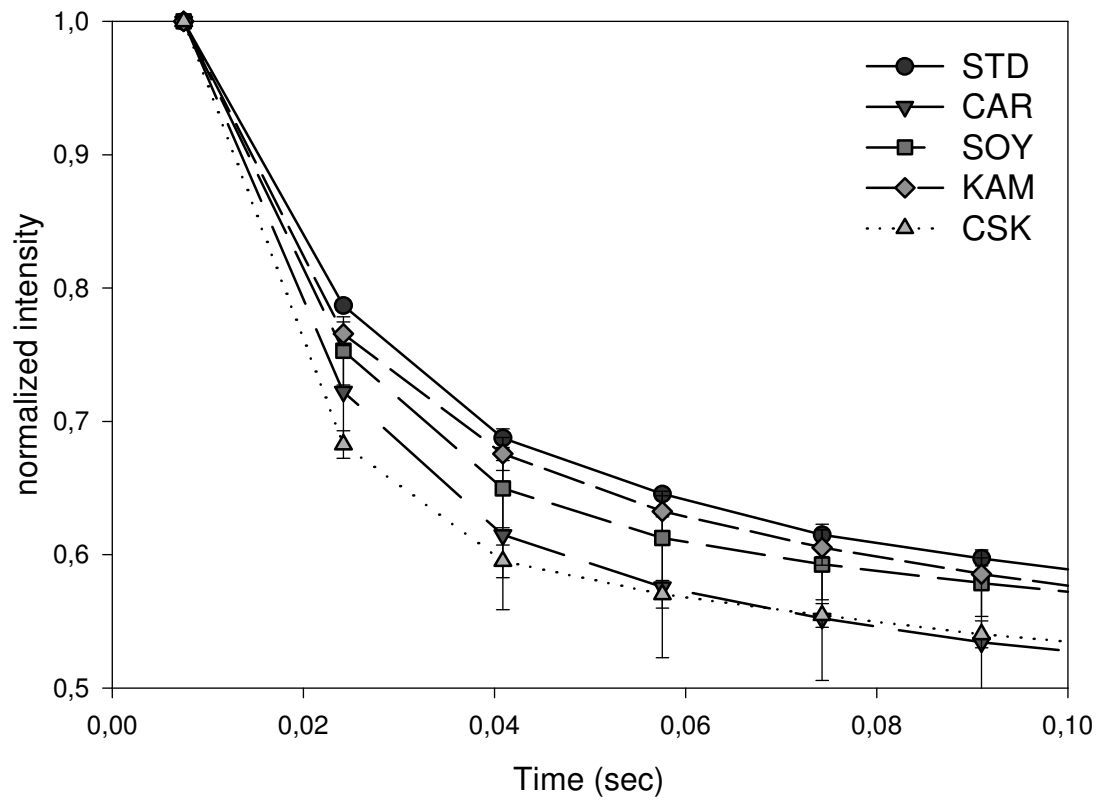


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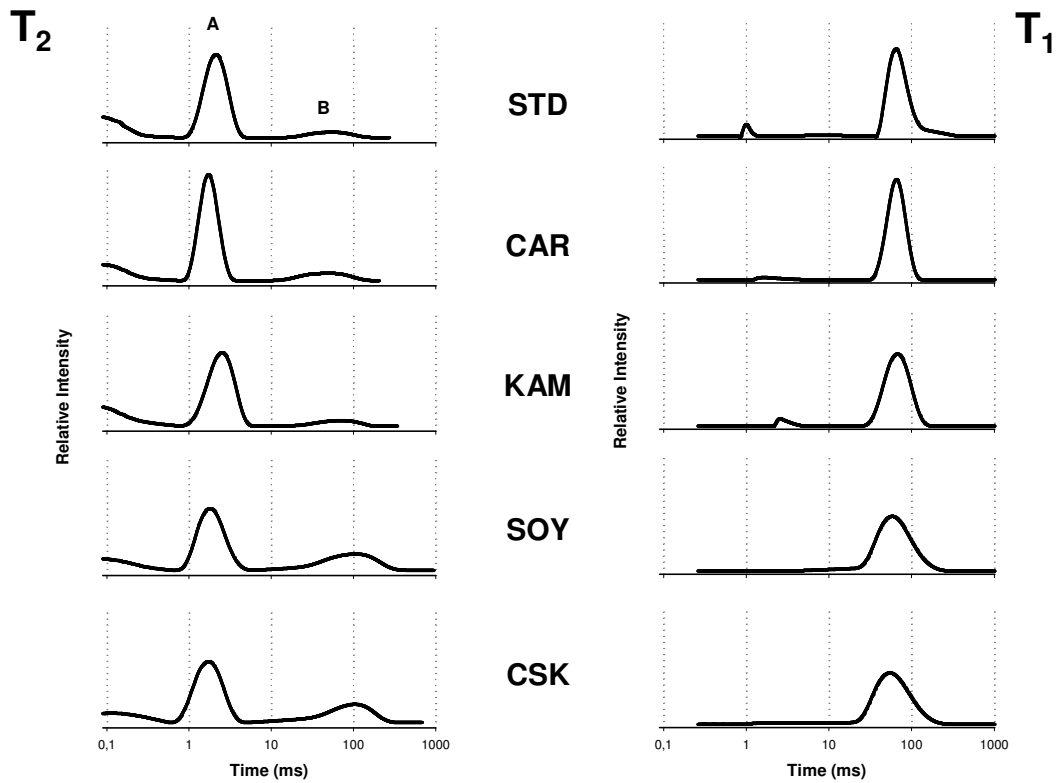


Figure 4: ^1H T_2 and T_1 distributions of nutritionally enhanced tortillas.

Effect of storage on physicochemical properties and water status of nutritionally enhanced tortillas

Eleonora Carini, Elena Vittadini and Elena Curti

1. Abstract

The effect of storage on physicochemical properties (texture and amylopectin recrystallization) and water status (moisture content, water activity, ice melting peak thermal properties and ^1H NMR mobility) in nutritionally enhanced tortillas (carrot juice, whole soy flour and whole kamut flour enrichment) was studied.

Formulations changed significantly modified the water status and enhanced water redistribution during storage resulting in larger changes than in the standard formulation. In particular, the major modifications (decrease of water activity, moisture content and “frozen water” content and ^1H NMR mobility changes) were observed in soy containing products (SOY and CSK tortillas) that had very low water activity during all storage time (if compared with other samples) but presented a higher and more altered ^1H NMR molecular mobility.

2. Introduction

Tortillas represent one of the fastest growing segments of the baking industry in the North America (Cornell, 1998; Tortilla Industry Association, 2007) their formulation could be revisited with the intent to increase their nutritional value without significantly alter their physicochemical properties and sensory acceptability.

One of the major problems in tortillas quality is the deterioration of texture with time due to staling (Waniska, 1999) and therefore, the phenomena implicated/influenced in the textural changes during storage need to be better understood in order to extend their shelf-life.

The mechanism of staling in bakery products has not completely been understood yet it is nowadays widely accepted that water redistribution among components during storage plays a significant role. Gray and Bemiller (2003) suggested that food additives, acting as plasticizers, and/or retarding the redistribution of water between components could play a crucial role in controlling staling.

Tortillas staling has been characterized not only by textural changes (decreased rollability and sensory acceptance (Bejosano et al., 2005), also using DSC analysis focusing on water properties (“frozen water” decrease) and starch retrogradation (amylopectin recrystallization increase), (Clubbs et al., 2008). Moreover, nuclear magnetic resonance (NMR) is one available analytical technique to measure changes in the product occurring at molecular level (water mobility) that has been extensively applied to the study of bread staling but that has also been applied to characterize tortillas (nuclear ^1H cross-relaxation and ^1H T_1 and T_2 relaxation times, Vittadini et al., 2004).

Nutritionally enhanced tortillas were developed in our laboratory starting from a standard wheat tortillas production procedure by incorporating

ingredients with well documented nutritional functionality such as carrots, soy and whole meal kamut (Scazzina et al., 2008). Tortillas were characterized for their physico-chemical and nutritional properties and sensory acceptability (Serventi et al., 2009, Scazzina et al., 2008). The simultaneous combination of carrot juice, soy and wholemeal kamut resulted in a very interesting product that was not only the most acceptable by the consumers but also had the highest total antioxidant capacity and lowest glycemic index. The substitution of part of the water with carrot juice in the tortillas formulation altered slightly the macroscopic, significantly the thermal properties (lower “frozen water” content) but only marginally the ^1H molecular mobility while the substitution of wheat flour with whole kamut flour did not alter the properties considered. Inclusion of soy flour in tortillas formulation significantly altered all the water status indicators studied (lower moisture content, water activity and “frozen water” content, higher ^1H T_2 mobility). The simultaneous presence of carrot juice, whole meal kamut flour and soy flour in the formulation reflected the distinctive contribution of each specific ingredient.

The objective of this work was therefore to study how the functionally ingredients added to the standard tortilla formulation altered the physicochemical properties and water state during long term storage (180 days) of tortillas.

3. Materials and Methods

Please refer to Material and Methods section in the section “Effect of formulation on physicochemical properties and water status of nutritionally enhanced tortillas” for the methodologies used to study the physicochemical properties.

Tortillas were vacuum packed immediately after production and then stored at room temperature up to 180 days and analysed at day 1, 7, 14, 30, 90 and 180 days.

Statistical analysis

Means and standard deviations (SD) were calculated with SPSS statistical software (Version 12.0, SPSS Inc., Chicago, Illinois, USA). The effect of formulation (at the same storage time) and the effect of storage (for the same formulation) were identity with one-way-analysis of variance (ANOVA) and Least Significant Difference test (LSD) at a 95% confidence level ($p < 0.05$). The results obtained were reported in Table 1: small letters indicate significant differences among samples with different formulations; capital letters significant differences among samples with different storage time.

4. Results and Discussion

Effect of storage on tortillas textural properties

Hardness and extensibility of all tortillas samples during storage were reported in Figure 1A and 1B while results of statistical analysis can be found in Table 1.

Hardness increased during storage more markedly during the first 14 days of storage in all samples and slower at longer storage times. STD formulation did not change in hardness between 14 and 90 days indicating that all phenomenon contributing the firmness process occurred in the first two weeks of storage.

Changes in hardness during storage were affected by the formulation: CAR was the hardest up to 90 days of storage possibly because of the presence of sugars that may have also affected the water status. A stronger water-sugars affinity (measured by means of water activity, moisture content and frozen water content, previously reported by Serventi et al., 2009) could have contributed in an altered water redistribution during storage. The presence of whole kamut flour in the formulation induced a lower hardness at day 14, 30 and 90 but the significance was only for day 14. At day 90 all samples showed a comparable hardness. The SOY and CSK samples were comparable at every storage times with STD ad exception to day 7 that CSK was higher than SOY and STD (comparable themselves).

As well hardness, extensibility decreased drastically in the first 14 days of storage (Figure 1B). Different formulation affected slightly this property during storage: at day 7, 30 and 90 no significant difference were observed, at day 14 KAM was found the lowest with no significant difference among other samples, at day 180 SOY had the highest extensibility.

Effect of storage on tortillas amylopectin recrystallization

All DSC thermograms of stored tortillas exhibited an endothermic transition around 60°C (data not showed) mainly attributed to amylopectin recrystallization (AR). In order to minimize the error associated to the peak integration due to other endothermic events (e.g. margarine melting) the peak was integrated from 56 °C to the flat baseline as discussed in Serventi et al., 2009. Figure 2 showed the amylopectin recrystallization during storage for all tortillas and the statistical significance was reported in Table 1. Recrystallized amylopectin increased in all samples during storage, as expected although the rate of amylopectin recrystallization was different among samples with different formulations. Amylopectin recrystallization of STD sample progressively increased with storage time increase; AR of CAR sample significantly increased between 0 and 7 days of storage, was found constant until 30 days, at 90 days increased and at 180 days significantly decreased. AR of SOY in the first 14 days of storage, then remained unchanged was found not change until 90 days and at day 180, like CAR, significantly decreased; a similar trend was observed also in the case of CSK sample. KAM increased at day 7 and then was not found to change for all storage time.

Looking at the effect of formulation on amylopectin recrystallization, no formulation showed significant differences along all storage. The values obtained probably were the result of different factors first of all the different amounts and types of starches originated from different flours used to produce nutritionally enhanced tortillas, besides the presence of multiple overlapping transitions was not excluded.

Effect of storage on tortillas water status

Water is known to play a key role in quality and stability of food products as it can interact with other molecules through hydrogen bonds, hydrophobic

interactions and it can affect their conformation, mobility, plasticity and functionality.

Water status of tortillas stored was investigated at different scales in terms of water activity, moisture content, thermal properties of ice melting peak by DSC and ^1H NMR mobility to better understand the water redistribution among components during storage.

The effect of storage on water activity for all tortillas samples was reported in Figure 3A. Water activity of STD tortilla at day 0 was ~ 0.94 and no significant changes during storage were observed. KAM sample was the formulation that showed a similar trend as STD: until day 30, a_w was almost constant and then significantly decreased at longer storage times. The presence of sugars in the formulation reduced water activity at day 0 (Serventi et al., 2009) that was found slightly increased at day 7, until 90 days was found constant, at day 180 water activity was still significantly decreased (Table 1). Water activity of SOY sample resulted to have a no constant trend showing an alternating in increase/decrease along all storage. CSK resulted to have a water activity increasing until day 90, then drastically decreased (from ~ 0.88 at day 90 to ~ 0.78 at day 180).

The differences in water activity found in fresh tortillas (Serventi et al., 2009) were still present although less marked for the storage duration.

The effect of storage on moisture content for all tortilla samples was summarized in Figure 3B and found to decrease during storage.

Opposite to our results, Clubbs et al., 2008 reported no significant changes in corn tortillas (52.3 ± 0.2 % wb) in moisture content during 14 days of storage, but it must be taken in consideration that composition, process and packaging conditions of tortillas were different than those of our study.

Major changes during storage in moisture content were in first 7 day and between day 90 and 180 while in the intermediate storage time moisture content remained constant. The moisture content decreasing was mainly

attributed to an evaporation phenomenon occurring because the packaging material used was not high-barrier. In SOY and CSK moisture content had, at day 7, an higher decrease than other samples: from ~25 (day 0) to ~19 (day 7) in the case of SOY sample and from ~23 (day 0) to ~17 (day 7) g water/100 g sample for CSK.

The significant differences observed at day 0 (Serventi et al., 2009) has been kept during storage.

From day 90 and 180 both water activity and moisture content significantly decreased indicating that after 90 days of storage, distribution of water significantly changed.

The investigation of water dynamics at macromolecular level was obtained by DSC through the ice melting peak analysis. At day 0, the frozen water content was calculated taken in consideration the margarine contribution since the ice melting and margarine transition overlapped at least partially, as previously reported in Serventi et al., 2009. The effect of storage on margarine in a complex food like tortilla was difficult to estimate during a long term storage as the multiple phase transitions are expected to shift and/or change shape during storage, the authors decided to discuss the effect of storage only in qualitative terms focusing on the shape changes of the peak around 0 °C, attributed to ice melting.

Formulation of nutritionally enhanced tortillas significantly affected the frozen water content at day 0 as extensively reported in Serventi et al., 2009 and the representative thermograms for all tortillas during storage are reported in Figure 4. The area of the ice melting peak progressively decreased during storage in all tortillas. Clubbs and coworkers (2008) reported a decrease in freezable water content in the first 7 days of storage in corn tortillas and, as discussed previously by Baik and Chinachoti (2001) in bread, it was associated to a water portion migration to the more rigid amorphous and crystalline domains that so became unfreezable. STD

tortillas was the only sample that exhibited a clear ice melting peak during all storage indicating that after 180 days, part of the water within the sample was still phase separable from the matrix. The transition was not more observable after 180 days in CAR and KAM samples and after 7 days in SOY. In the above mentioned sample, the peak was still observable at day 14 and 30, after 90 and 180 days was missed. A similar trend was observed also in CSK indicating that this behavior could be possibly induced to the presence of soy proteins in the formulation. The great amount of “frozen water” (estimated) lost in the first seven days in SOY and CSK samples could partial explain the significantly moisture content decrease in the same storage time.

The molecular characterization was carried out with a ^1H NMR mobility study through a ^1H FID (to study the less mobile protons fraction) and ^1H T_2 (to study the more mobile detectable protons fraction) experiments. The effect of storage on FID in tortillas produced with different formulation is shown in Figure 5A. In all tortillas a molecular rigidity increase was observed with increasing storage time indicating a mobility loss to the less mobile protons. This protons relaxed in a approximate range to $\sim 8\text{--}15\ \mu\text{s}$ and might arise from protons in solid-like components, such as starch and proteins and water molecules tightly associated with those solids (Kim and Cornillon, 2001). CAR tortilla had a larger ^1H rigidity increase gap between day 0 and 180 while CSK was found to have the smallest ^1H rigidity increase gap. In Figure 5B the effect of formulation on FID at day 7, 90 and 180 have been reported and it could be observed no pronounced differences among FID decays at day 7, on the contrary, at longer storage time, CAR and SOY at day 90 and CAR at day 180 showed a higher rigidity to the less mobile protons fraction than others tortillas.

The effect of formulation and storage on T_2 distribution obtained from quasi-continuous distributions of relaxation times was reported in Figure 6.

At day 0, tortillas exhibited three ^1H T_2 population and the slower ^1H population was found formulation dependent as previously discussed in Serventi et al., 2009. The three T_2 ^1H populations found in all tortillas were named starting from the lowest to the highest relaxation time A, B and C, respectively. In Figure 6, it also reported the effect of storage on characteristics relaxation times and relative abundance of the three ^1H populations detected for all tortillas. The T_2 distribution spectra were analyzed for $T_2 \geq 0.089$ ms (2 interpulse spacing + instrument dead time) i.e. no T_2 values shorter than the measured point on the CPMG was attempted. The peak of the population A did not fall completely within the limits in all samples. For example, peak A of the distribution spectra of STD was only partially (up to 14 days of storage) and fully within the useful range at storage times ≥ 30 days. A shift of peak A from shorter to longer relaxation times caused this peak to move from out of range to within range values. CAR and KAM followed the same trend described for STD but at different times: e.g. 14 days and 90 days for CAR and KAM, respectively. On the contrary, when soy proteins was part to the tortilla formulation (SOY and CSK), this peak was always completely inside the relaxation spectrum, suggesting that soy proteins could have possibly induced a greater molecular mobility and, possibly, a plasticization effect of this protons fraction.

During storage, the maximum of the peak A was always found at $\sim 0.13 - 0.16$ ms (Figure 6B) but a significant change of peak shape was observed: Pop A became broader and more tailored towards slower relaxation times (Figure 6a). The relative abundance of peak A decreased also during storage. This peak modifications were more evident in STD, CAR and KAM samples indicating a mobility increase due to the effect of storage and/or formulation. In SOY and CSK tortillas, this peak was more consistent until day 90 (both for width and relative abundance) but at day 180 it seemed to

disappear and to overlap with the pop B peak. It could be concluded that this faster ^1H T_2 population was more “stable” during storage in soy-containing products up to 90 days.

Also pop B, characterized by relaxation times in the $\sim 0.8 - 6/8$ ms range and peaked at ~ 2 ms in all samples at day 0 (Serventi et al., 2009), during storage was found shift towards faster relaxation times in all samples (Figure 6a and 6b) indicating a decrease of mobility of this ^1H population. Pop B was less represented in SOY and CSK samples because this samples had the slower population (pop C) more represented (as previously discussed in Serventi et al., 2009). During storage this population an increase in relative abundance in all samples with the exception of CSK sample that was nearly constant. The fastest relaxing population (pop C) was centred at ~ 70 ms (representing $\sim 10\%$ of the total detectable protons) in STD, CAR and KAM samples and at ~ 100 ms ($\sim 28\%$ of the total detectable protons) in SOY and CSK products. This larger abundance of pop C in SOY and CSK was attributed primarily to the presence of soy proteins, besides, although a margarine contribution was not excluded (Serventi et al., 2009). An increase in the width of the pop C towards faster relaxation times was observed in all samples during storage indicating heterogeneity increase of the protons belonging this population during storage.

In the literature, several studies applied ^1H NMR (low resolution) mobility in bread, tortillas, dough and model systems (containing gluten and starch) but only few authors tentatively attributed the signals of the NMR spectra to the relative components. At the authors best knowledge, no scientific paper reporting the effect of storage probed with low resolution ^1H NMR in tortillas has been published. However, the results reported in this study are consistent with some previous works relating to similar but not identical products (bread, dough, model systems).

Engelsen et al. (2001), found three proton T_2 populations peaking at ~ 0.5 ms, $\sim 9-10$ ms and $\sim 21-30$ ms in bread that were attributed to water associated to protein, water associated to gelatinized starch (and pentosans) and diffusive exchange water between starch and protein, respectively. Wang et al. (2004) studied some model systems (starch gels, gluten gels and starch-gluten gels) and also bread samples to evaluate the effect of moisture content and gluten on their proton mobility. They found two proton populations, peaking at ~ 0.1 ms and ~ 3.0 ms and attributed this last population to water associated with starch. Sereno et al. (2007) found in bread one ^1H T_2 population peaking at ~ 9 ms representative of the fast proton exchange between water and starch and the restricted water mobility within the polymers matrix. Chen et al. (1997) found in bread three proton populations, peaking at $8-12 \mu\text{s}$, $320 \mu\text{s}$ and $2.0-2.6$ ms respectively and they attributed the shortest T_2 component to water associated to starch and gluten by hydrogen bonding. Also Ruan et al. (1996), observed the presence of two proton populations in sweet rolls, peaking in the microseconds range and a second one peaking in the milliseconds range. The three ^1H T_2 population found in tortillas were, therefore, tentatively attributed to protons associated with gluten (pop A), protons associated with the starch amorphous phase (pop B) and protons in diffusive exchange between components (pop C), respectively. The changes to the three protons populations occurred during storage could be due to protons (mainly water) that have been exchanged between the gluten matrix and the starch phase; it is hypothesized and tentatively speculated that protons related to gluten have moved towards the starch phase, as evidenced by the protons NMR distribution changes (relaxation times and relative abundance) during storage.

5. Conclusions

The effect of storage on physicochemical properties and water status of nutritionally enhanced tortillas was evaluated.

All properties investigated at different levels (macroscopic, macromolecular and molecular) and followed during storage, indicated that changes in formulation significantly modified the water status and enhanced water redistribution during storage resulting in larger changes than in the control. In particular, the major modifications (decrease of water activity, moisture content and “frozen water” content and ^1H NMR mobility changes) were observed in soy containing products (SOY and CSK tortillas) that had very low water activity during all storage time (if compared with other samples) but presented a higher and more altered ^1H NMR molecular mobility. The formulations of nutritionally enhanced tortillas should be improved to reduced and minimize the changes observed during storage.

6. List of Tables

Table 1: Statistical letters (LSD Test, $p < 0.05$) for hardness, extensibility, Amylopectin recrystallization, water activity and moisture content during storage.

Small letters indicate significant differences among samples with different formulations at the same storage time; capital letters indicate significant differences among samples with different storage times for the same formulation.

Storage (days)	STD	CAR	SOY	KAM	CSK
0	c/C	b/D	b/D	c/D	a/D
7	c/B	a/C	bc/C	bc/BC	ab/C
14	b/A	a/AB	b/B	c/C	b/BC
30	b/A	a/AB	b/AB	b/B	ab/B
90	bc/A	a/A	b/B	c/BC	b/B
180	b/A	a/BC	a/A	a/A	a/A

Storage (days)	STD	CAR	SOY	KAM	CSK
0	c/A	b/A	c/A	a/A	c/A
7	a/B	a/B	a/B	a/B	a/B
14	a/B	a/BC	a/B	b/B	a/B
30	ab/C	ab/CD	ab/B	b/B	a/C
90	a/C	a/D	a/B	a/B	a/C
180	b/C	b/D	a/B	b/B	b/C

Storage (days)	STD	CAR	SOY	KAM	CSK
0	a/CD	a/C	a/C	a/B	a/D
7	c/D	ab/B	abc/BC	a/A	bc/D
14	b/CD	b/B	a/A	b/A	b/C
30	b/BC	b/B	b/AB	b/A	a/A
90	a/AB	a/A	a/A	a/A	a/AB
180	a/A	b/BC	a/BC	ab/A	a/B

Storage (days)	STD	CAR	SOY	KAM	CSK
0	a/A	b/B	c/CD	a/B	d/B
7	a/A	a/A	b/BCD	a/AB	c/AB
14	a/AB	ab/A	b/A	a/A	c/AB
30	a/AB	a/A	b/ABCD	a/AB	b/A
90	b/A	a/AB	b/AB	b/C	ab/A
180	a/B	ab/B	b/D	ab/D	c/C

Storage (days)	STD	CAR	SOY	KAM	CSK
0	a/A	b/A	c/A	a/A	c/A
7	a/B	b/B	c/C	a/B	d/C
14	a/B	b/B	c/B	a/B	d/BC
30	a/B	ab/BC	bc/B	ab/C	c/B
90	a/C	ab/C	bc/C	a/C	c/C
180	a/D	b/D	c/D	ab/D	d/D

Table 1: Statistical letters (LSD Test, $p < 0.05$), for hardness, extensibility, Amylopectin recrystallization, water activity and moisture content during storage.

Small letters indicate significant differences among samples with different formulations at the same storage time; capital letters indicate significant differences among samples with different storage times for the same formulation.

7. List of Figures

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Figure 2: Amylopectin recrystallization for all tortillas during storage.

Figure 3: Water activity (3A) and Moisture content (3B) for all tortillas during storage.

Figure 4: Thermal properties of the peak around 0°C for STD, CAR, SOY, KAM and CSK samples during storage.

Figure 5: Effect of storage (5A) on FID for STD, CAR, SOY, KAM and CSK samples; effect of formulation (5B) on FID at day7, 90 and 180 for all tortillas.

Figure 6: Effect of storage on T₂ distribution (6a) for STD, CAR, SOY, KAM and CSK samples; effect of storage on T₂ and protons % (6b) for all tortillas.

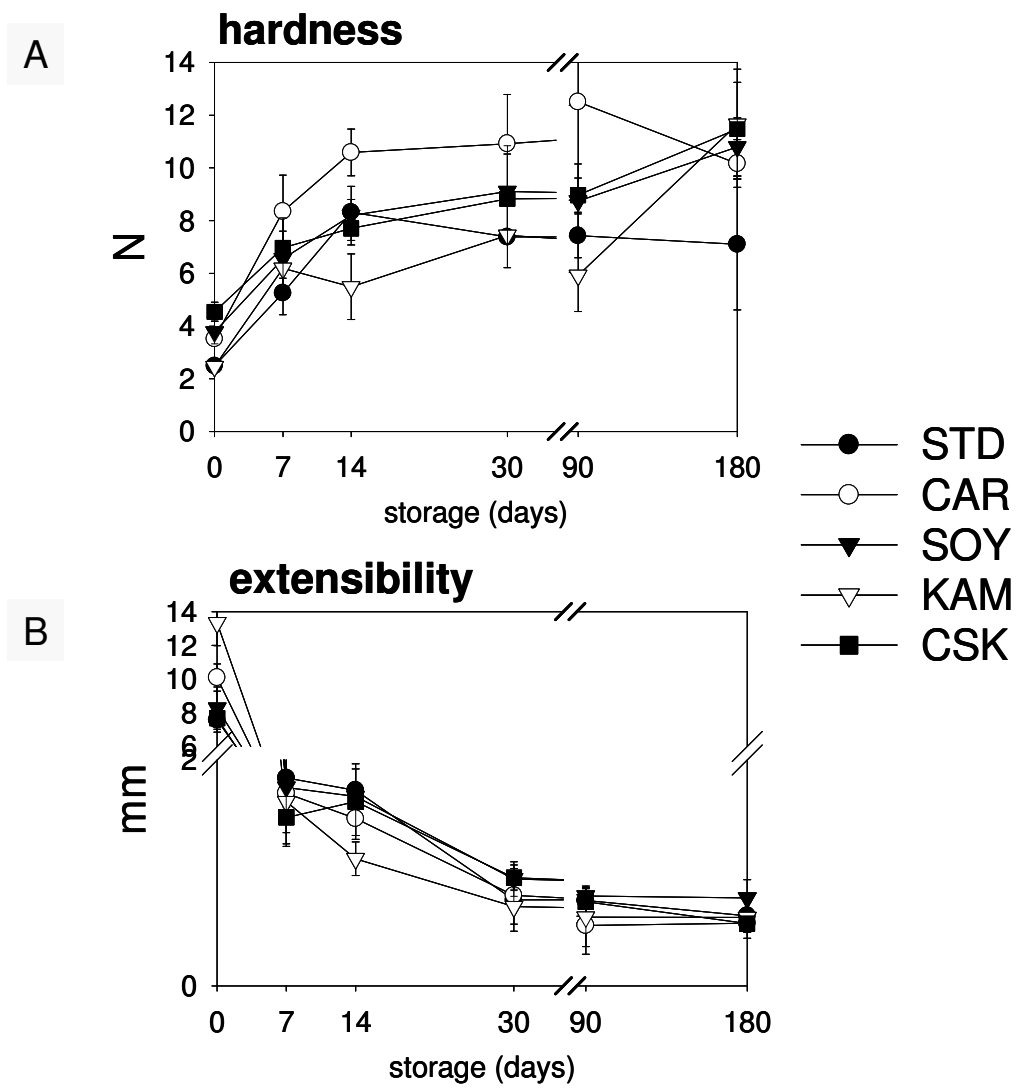


Figure 1: hardness (1A) and extensibility (1B) for all tortillas during storage.

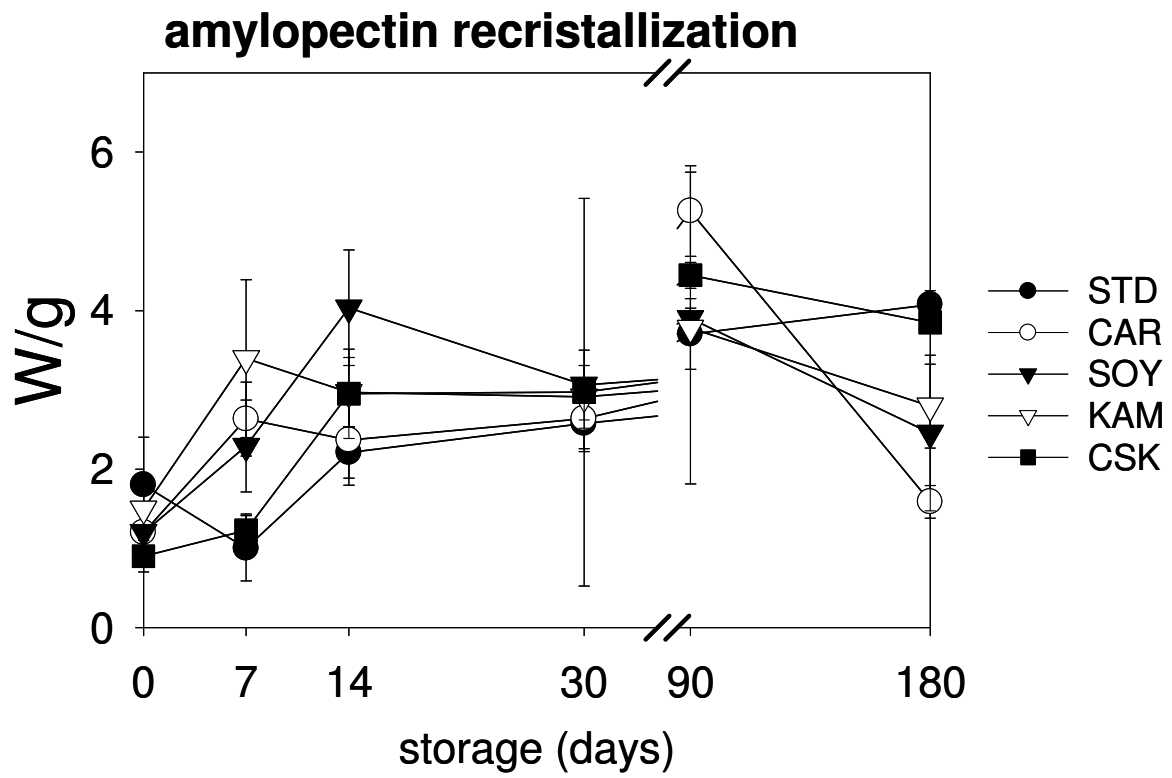


Figure 2: Amylopectin recrystallization for all tortillas during storage.

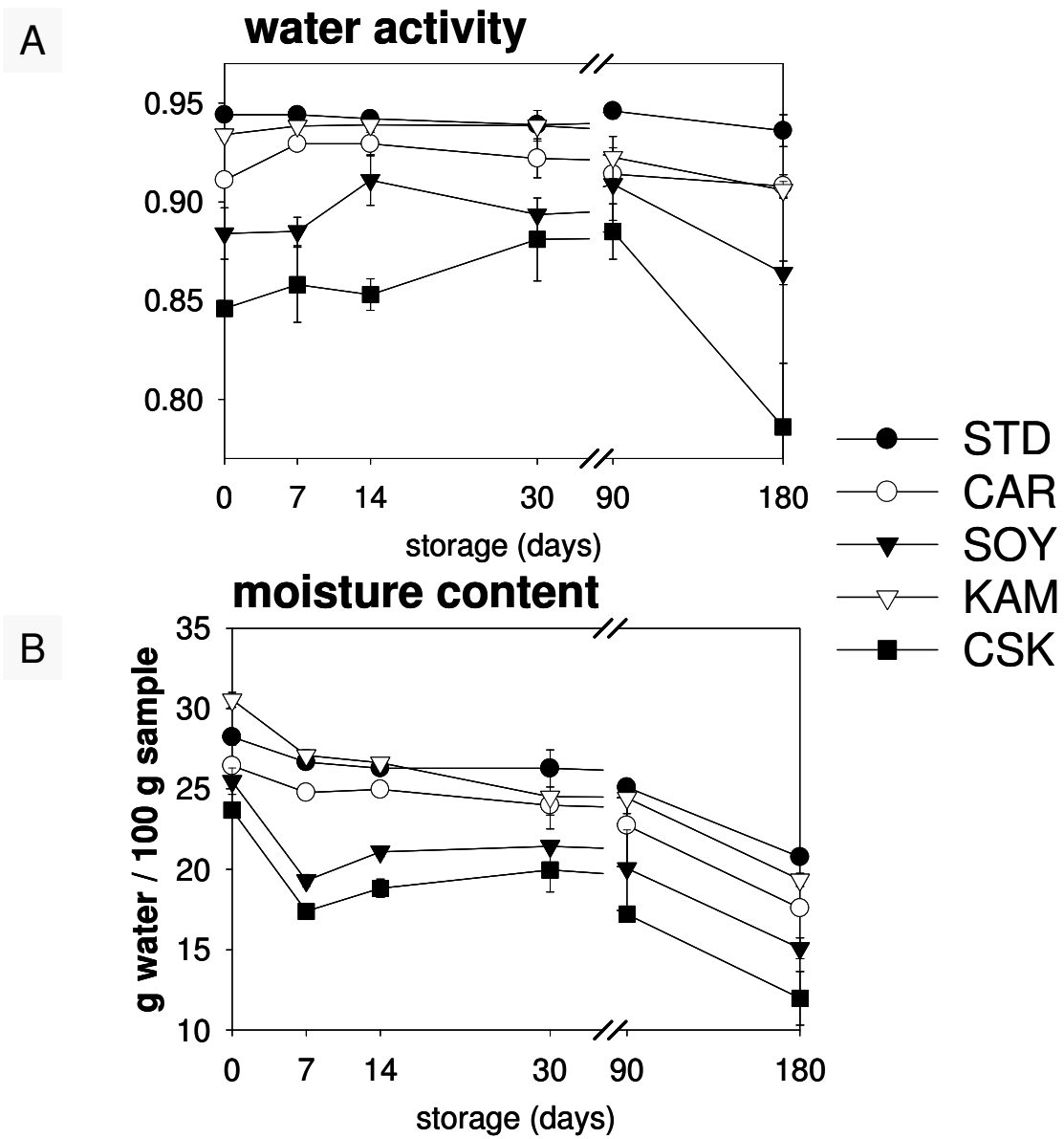


Figure 3: Water activity (3A) and moisture content (3B) for all tortillas during storage.

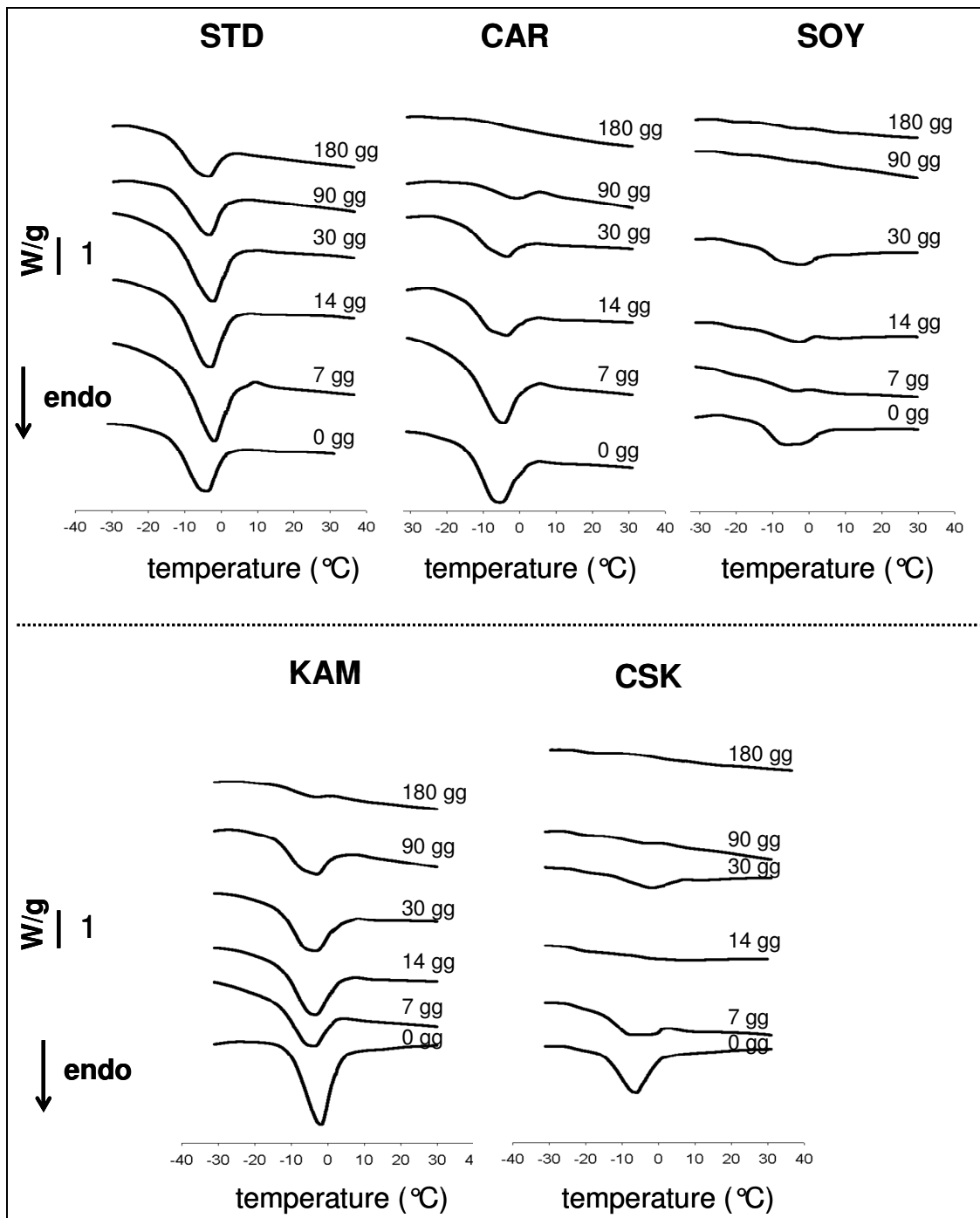


Figure 4: Thermal properties of the peak around 0°C for STD, CAR, SOY, KAM and CSK samples during storage.

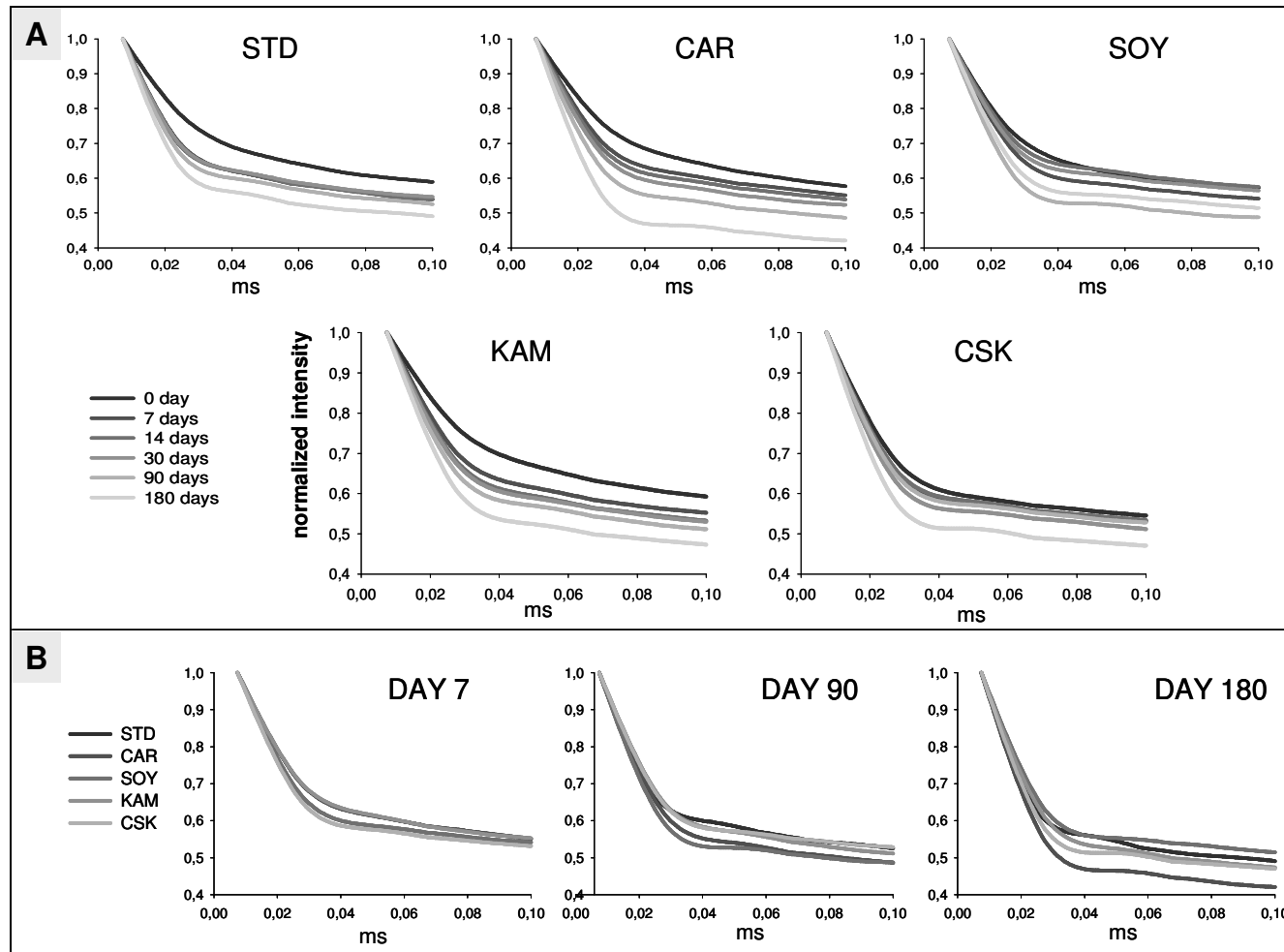


Figure 5: Effect of storage (5A) on FID for STD, CAR, SOY, KAM and CSK samples; effect of formulation (5B) on FID at day7, 90 and 180 for all tortillas.

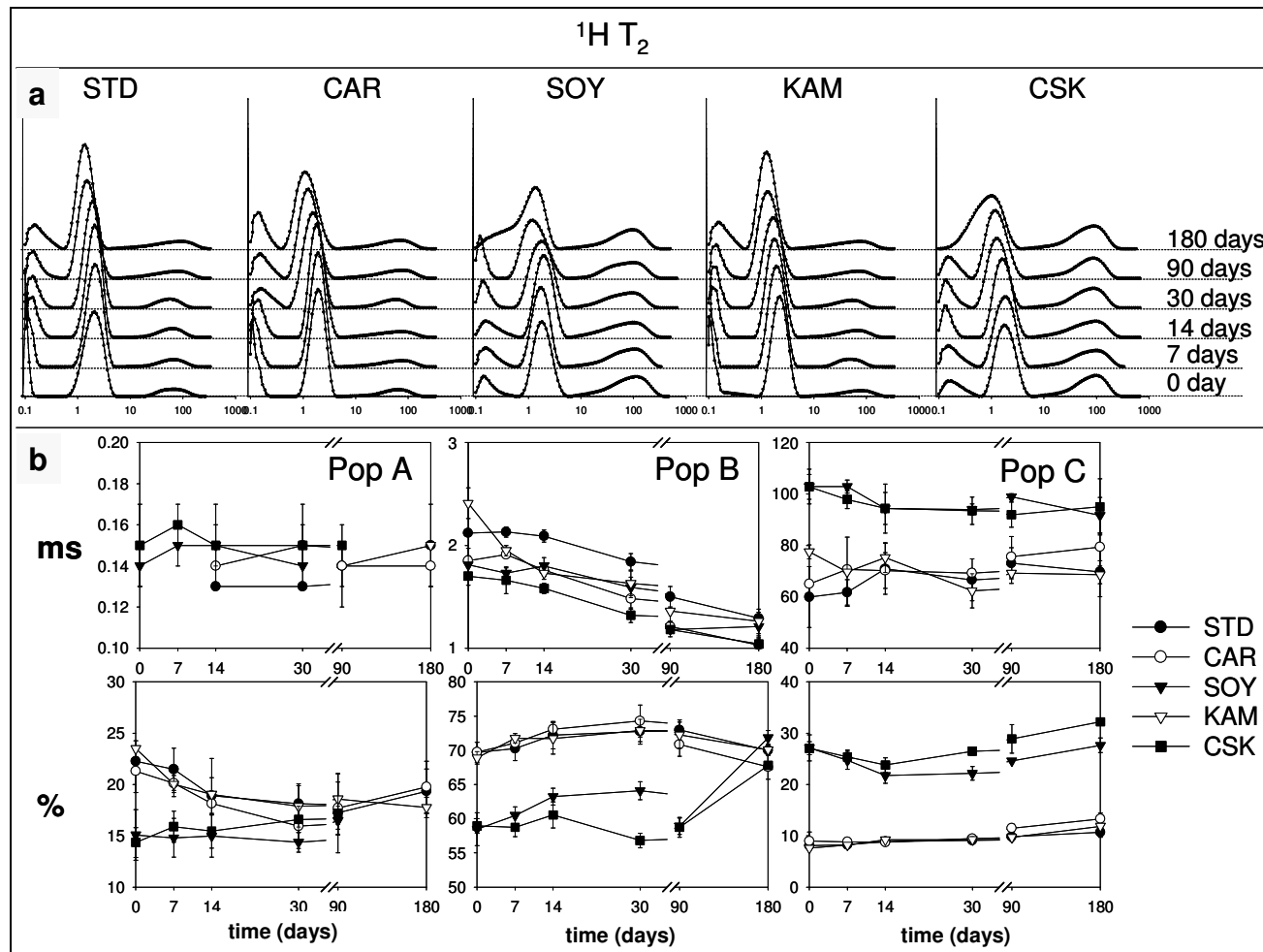


Figure 6: Effect of storage on T_2 distribution (6a) for STD, CAR, SOY, KAM and CSK samples; effect of storage on T_2 and protons % (6b) for all tortillas.

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