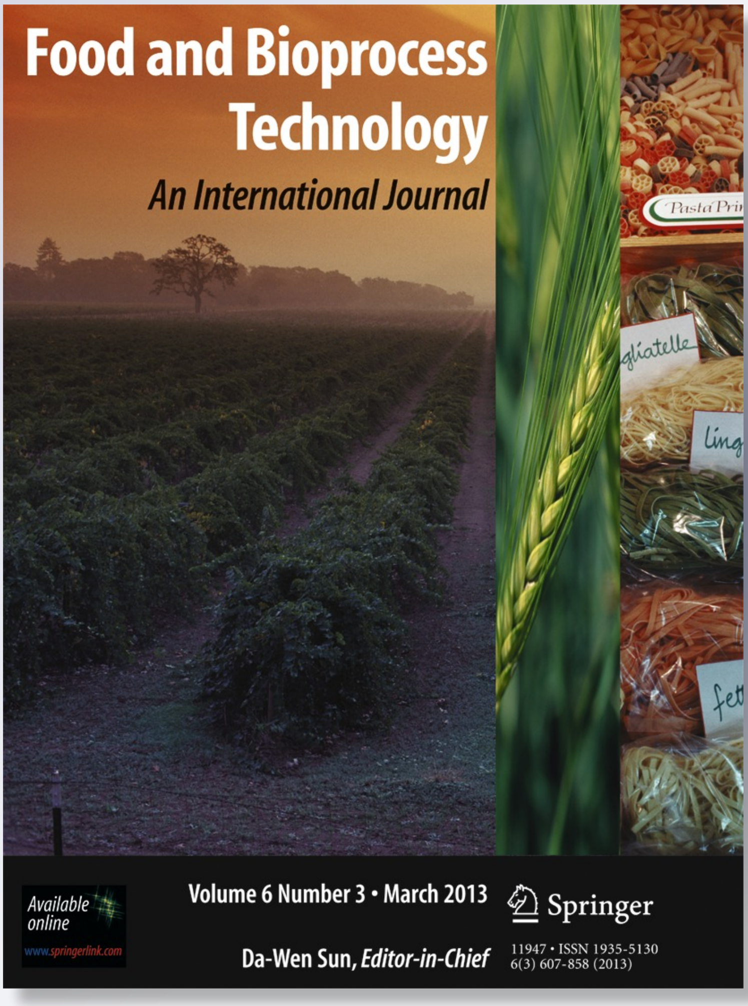
Effect of Butternut (Cucurbita moschata Duchesne ex Poiret) Fibres on Bread Making, Quality and Staling

Marina de Escalada Pla, Ana María Rojas & Lía Noemí Gerschenson

Food and Bioprocess Technology
An International Journal

ISSN 1935-5130
Volume 6
Number 3

Food Bioprocess Technol (2013)
6:828-838
DOI 10.1007/s11947-011-0744-y



Food Bioprocess Technol (2013) 6:828–838
DOI 10.1007/s11947-011-0744-y

ORIGINAL PAPER

Effect of Butternut (*Cucurbita moschata* Duchesne ex Poiret) Fibres on Bread Making, Quality and Staling

Marina de Escalada Pla · Ana María Rojas ·
Lía Noemí Gerschenson

Received: 13 August 2011 / Accepted: 18 November 2011 / Published online: 7 December 2011

Abstract The effect of different enriched fibre products obtained from butternut (*Cucurbita moschata* Duchesne ex Poiret) on bread making and bread quality was evaluated through the study of bread yield, quality parameters (specific volume, crumb firmness, crumb and crust colour) and bread shelf life. Fractions tested were obtained from butternut mesocarp through ethanolic treatment (fraction AIR) or through dehydration (fraction S) or from the ethanolic treatment of peel (fraction C). These fractions were incorporated in a bread formula, at levels of 5, 10 and 15 g of fibre fraction per kilogram of wheat flour. The study of crumb through digital imaging and thermal analysis was also performed in order to better understand the effects observed. An important influence of water absorption kinetics and chemical composition of the fibre fractions studied was observed in the results obtained. Lower bread firmness was determined 24 h after baking when 10 g of C or either 10 or 15 g of S was present per kilogram of wheat flour used. Breads made with flour containing 10–15 g of S or 5 g of C per kilogram of wheat flour tended to be softer, while 10 g of C per kilogram of flour produced significantly softer breads along 9 days storage.

Keywords Dietary fibre · Butternut · Bread · Quality · Staling

---

Marina de Escalada Pla, Ana María Rojas and Lía Noemí Gerschenson are members of the National Council of Scientific and Technical Research, Argentina (CONICET).

M. de Escalada Pla · A. M. Rojas · L. N. Gerschenson (✉)
Industry Department, Natural and Exact Sciences School,
University of Buenos Aires (UBA),
1428 Buenos Aires, Argentina
e-mail: lia@di.fcen.uba.ar

Introduction

Nowadays, there is a growing demand for a new generation of healthier food products, which, at the same time, are required to have excellent sensory quality. Dietary fibre is a common and important ingredient of these healthy food products (Gómez et al. 2003). On the other hand, modern methods of food elaboration and processing frequently reduce the content of dietary fibre.

Moreira et al. (2011) explored the technological possibilities of non-traditional flours; they studied chestnut flour doughs using Mixolab® apparatus and established a comparison with gluten and gluten-free flour doughs as well as with corn starch pastry- and bread making formulations. Other researchers have studied the effect of increasing fibre content in bakery products. Wittig de Penna et al. (1998) used lupinus fibre in muffins. Wheat bran, corn bran, oat bran, soy hulls were used by Jeltema and Zabik (1979) in cakes. Dietary fibre, in general, tends to increase the nutritional value of bread but usually, at the same time, alters rheological properties of dough and, finally, the quality and sensorial properties of bread. High percentages of different kinds of dietary fibres in baking had been reported to produce important detriments to dough handling and bread quality, unless some food additives were used. Gómez et al. (2003) studied the effect of addition of cellulose, cocoa, coffee, pea, orange and wheat fibres up to 50 g/kg flour. They stated that the main problem of dietary fibre addition in baking is the important reduction of loaf volume and the different texture of the breads obtained. Anyhow, Ptitchkina et al. (1998) found that an increase in loaf volume and an enhancement in organoleptic acceptability of wheat bread were obtained when small additions of pumpkin powder were used. Correa et al. (2011) studied the effect of low and high methoxyl pectin on dough and bread characteristics when salt was or was not present in the formulation. Texture profile analysis showed that pectins softened the dough, particularly when salt was added.

Cucurbitas were associated to the origin of agriculture and civilisation. They were found among the first plants to be domesticated (Whitaker and Bemis 1975). Although butternut (*Cucurbita moschata* Duchesne ex Poiret) was native from Mexico and South America, it was well adapted to a wide range of weather conditions and soils (Gwanama et al. 2000). Nowadays, in Argentina, production of *C. moschata* Duchesne ex Poiret duplicates the production of all the other varieties of pumpkins. de Escalada Pla et al. (2005, 2006, 2009) studied extensively the characteristics and uses of butternut. More recently, functionality and composition of dietary fibre fractions obtained from them were also investigated (de Escalada Pla et al. 2007; Fissore et al. 2010; Gerschenson et al. 2009), showing the potential of these fibre fractions to be used as food ingredients or additives to improve food quality.

The aim of the present work was to study the effect on bread making, quality and staling during bread storage of enriched fibre products extracted from mesocarp [alcohol insoluble residue (AIR) or S] or peel (C) of butternut (*C. moschata* Duchesne ex Poiret) when they were used at levels of 5, 10 or 15 g fibre per kilogram of wheat flour.

Materials and Methods

Materials

Fibre was prepared from fresh butternuts (*C. moschata* Duchesne ex Poiret), as previously described by de Escalada Pla et al. (2007). Briefly, butternuts were carefully cleaned. Peel, pulp and seeds were separated. A great part of water content was eliminated with a juice extractor. Then, three fractions were obtained:

(a) "S" fraction: the extracted pulp remaining after juice extraction was dried for 4 h at 50 °C in a convection chamber.

(b) "AIR" fraction: 100 g of extracted pulp were mixed with 350 ml 95% *v*/*v* ethanol and boiled for 30 min under stirring. The residue was boiled again with 350 ml of 80% *v*/*v* ethanol for 15 min and then twice with 250 ml of 80% *v*/*v* of ethanol for 15 min. The alcohol insoluble residue (AIR) was then washed with 100 ml 80% *v*/*v* and 100 ml 95% *v*/*v* ethanol. Between each ethanol treatment, the suspension was filtered, and the supernatants were discarded. The AIR finally obtained was dried into an air convection chamber at 50 °C for 4 h.

(c) "C" fraction: the vegetable was peeled off with a surgical blade. Then, the peel was submitted to the same treatment described in item (b).

"S", "AIR" and "C" were milled and sifted (sieve ASTM-E11 Nr.40). Composition of each fraction was determined previously by de Escalada Pla et al. (2007).

Wheat flour (Molino Central Norte™, Buenos Aires, Argentina) with ash content of 0.65 g/100 g according to the manufacturer, moisture of 13.3 g/100 g determined according to AOAC (1995) and with alveographic parameters $P/L=1.4$ and $W=250\times10^{-4}$ J determined according to ISO 5530-4:1991 was used.

Compressed yeast [*Saccharomyces cerevisiae* (CALSA™, Buenos Aires, Argentina)], NaCl and sodium propionate (Saporiti™, Buenos Aires, Argentina) food grade were used in the formulations.

Bread formulas without fibre addition (called "control": 0 g fibre/kg of flour) or with 5, 10 or 15 g fibre per kilogram of wheat flour were prepared with each fraction studied (S, AIR or C).

Bread-Making Procedure

Fibre was tested in a bread formula in the bakery school of Taxonera S.A. Company (Buenos Aires, Argentina). A basic formula was used comprising 1,000 g of wheat flour (moisture content, 13.3 g/100 g), enough water to reach the same dough development time (8 min total) for all systems studied, 40 g of compressed yeast, 20 g of NaCl and 3.2 g of sodium propionate; no emulsifier was added. This formula without fibre addition (control) or others including 5, 10 or 15 g of each fibre fraction per kilogram of flour were mixed for 2 min at low speed and 6 min at high speed in a spiral mixer (Indupan™, Rosario, Argentina). In all cases, fibre was hydrated with 100 g of the total water included in the formula, at 30 °C for 15 min (de Escalada Pla et al. 2007). Flour, NaCl, sodium propionate and hydrated fibre were added altogether into the mixer. At this point, low speed mixing started, and yeast and the rest of the water were jointly added.

After mixing, dough was divided into 350 g pieces, which were hand moulded and then pieces rested for 1 h at 22 °C. Afterwards, dough pieces were sheeted, punched and shaped, put into tin pans and let develop. Then, they were baked in a convection oven for 30 min at 200 °C. Scanlon and Zghal (2001) reported that, in a breadmaking process, the baker strives to optimise crumb structure in the final loaf. Therefore, punching, sheeting and moulding are carried out to redistribute leavening agents and gas cells so as to improve crumb characteristics. After baking, bread was cooled for 2 h at room temperature (≈23 °C).

The bread making was performed in duplicate, and control breads without fibre addition were also prepared each time that breads with different fibre fractions were processed.

Moisture content

Moisture content of different breads was determined in a convection chamber at 110 °C for 1 h (AOAC 1995).

Bread Yield

Bread yield was calculated for each kilogram of flour used, through evaluation of the weight of bread obtained after discounting the fibre added.

Evaluation of Bread Quality

Specific volume of the bread pieces was calculated from weight and volume evaluation after cooling. The volume was determined by seed displacement.

Breads were cut perpendicular to its axis in 25-mm thick slices, and they were packaged in low density polyethylene bags and stored at room temperature (23 °C). The following measurements were carried out:

Bread Firmness

Twenty-four hours after baking, force–deformation curves were recorded. Compression assays were performed with an Instron Universal Testing Machine model 1011 (Instron Corp, Canton, MA, USA), with a 30 mm diameter aluminium probe. The probe speed during the test was 100 mm/min. For each composition, four different slices of 25 mm thickness, coming from different breads, were evaluated.

Force–deformation curves were also recorded during storage at room temperature (≈23 °C) for times of 2, 3, 5, 6, 8 and 9 days after baking, in order to establish staling kinetics in control and fibre added breads.

Loaf firmness (F = force/deformation) was calculated as the ratio between the force recorded in the compression test at a 25% of crumb deformation and the deformation, according to American Association for Cereal Chemistry (AACC) Method 74.09 (Ponte and Ovadia 1996). Avrami equation (Avrami 1939, 1940, 1941; Cornford et al. 1964; McIver et al. 1968; Willhoft 1971; Ponte and Ovadia 1996) was used to fit the firmness data:

$$\varphi = \frac{(F_\infty - F_t)}{(F_\infty - F_O)} = \exp(-kt^n)$$

where φ is the fraction of non-crystalline material remaining at time t; k is the rate constant, and it is a combined function of nucleation and growth rate constants; n indicates the mechanism of nucleation and growth, and it is a combined function of the number of dimensions in which growth takes place, and the order of the time dependence of the nucleation process (0 or 1). In our case, the expression of φ comprises the crumb limiting firmness, F_∞; the crumb firmness at time t, F_t; and the crumb firmness at zero time or initial firmness, F_0 (McIver et al. 1968).

Crumb and Crust Colour

Colour was measured using a Minolta spectrophotometer CM-508d (Minolta, Japan) as described by Gómez et al. (2003). Results were expressed in the CIE L^* a^* b^* colour space and were obtained using the D65 standard illuminant and the 2° standard observer (CIE, 1931). Each point was measured five times by the equipment, which informed the average.

Crumb and crust colour were evaluated at two different points on three slices of different breads. Averages of the values obtained for the two measurements in the three slices are reported in the tables.

Thermal Analysis

Samples of ≈10 mg were taken from the centre of a bread slice after 7 days of storage. They were placed in 40-μl aluminium pans, and distilled water was added 3 h before performing the study, with a micropipette, to obtain a ratio of water to crumb of 3:1 (*w/w*) (Haros et al. 2002). Pans were hermetically closed, and an empty one was used as reference. Measurements were performed with a differential scanning calorimeter DSC 822^e Mettler Toledo (Schwerzenbach, Switzerland), heating the samples from 0 to 120 °C at a heating rate of 10 °C/min. It was calibrated with the melting points of indium (156.6 °C), lead (327.5 °C) and zinc (419.6 °C), in addition to the DSC periodic calibration performed with a sapphire disk, in the whole temperature range where the equipment is usually employed (Mettler 1997). The temperature and enthalpy of melting of retrograded starch were evaluated from the thermograms using the program Mettler Star^e. The exact dry matter content was determined for each individual pan after the DSC scan by puncturing the pan and drying at 105 °C for 16 h. The enthalpy was expressed in Joules per gram of sample. Control bread and bread with 10 g of C fraction per kilogram of flour were analysed in triplicate.

Digital Image Analysis

Crumb cells (alveolus) were characterised after scanning bread slices (Cannon scanner, Mod. CanoScan N640P ex, New York, USA) by measuring their areas and counting them as particles or objects in a binary and threshold image, using the Scion Image program (Release Beta-3b, Scion Corporation 1998, Maryland, USA). Total area, total particle area and total number of particles were recorded. Control bread and bread with 10 g of C fraction per kilogram of flour were analysed. Three slices from different breads of each composition were analysed.

Statistical Analysis

Results are informed on the basis of their average and standard deviation (α: 0.05). The significant differences among treatments were determined through ANOVA followed by pairwise multiple comparisons evaluated by Tukey's significant difference test (α=0.05). Non-linear regressions, correlations and statistical analysis (Sokal and Rohlf 1980) were performed using the Statgraphics Plus package (V 5.1, 2004, Rockville, MD, USA).

Results and Discussion

The effect on breadmaking of enriched fibre products (AIR, C and S) obtained from butternut (*C. moschata* Duchesne ex Poiret) was evaluated by analysing the bread quality obtained when 5, 10 or 15 g of fibre were added to a kilogram of wheat flour. Their effect on bread staling was also assessed after storage.

Effect of Butternut Fibre Addition on Bread Making and Final Characteristics of Bread

Water absorption by the bread formula during mixing in the spiral-bakery mixer increased with the presence of any fibre fraction (Table 1). As can be observed, water losses after dough baking and cooling at room temperature were not significantly different for each formula when comparing with the corresponding control system.

Bread yield increased with fibre concentration (Fig. 1A) in a linear relationship for C or S breads and in a quadratic dependence for AIR dough. In general, AIR presence produced the highest bread yields. This trend could be explained by the greatest water absorption shown by this fraction that gave origin to breads with the best water absorption/water loss ratio after baking, a fact that led to a higher moisture content (Table 1). It is important to remark that, in general, the moisture content of breads increased with the fibre content for each fibre assayed.

Specific volumes of AIR, C or S breads were not significantly different ($p<0.05$) from control at any fibre concentration assayed (Fig. 1B). This means that the addition of butternut fibre fractions to bread formulas at concentrations ranging from 5 to 15 g/kg did not produce an undesirable diminishing of loaf volume. Rosell et al. (2001) reported that the specific volume was improved by the presence of hydrocolloids [κ-carageenan, xanthan gum and hydroxypropyl methylcellulose (HPMC)] at a concentration of 5 g per kilogram of flour. It was found that the largest specific volume was promoted by κ-carrageenan followed by HPMC, which, as shown in the fermentation analysis performed through a rheofermentometer, improved the dough

Table 1 Water absorption by the mixture of dough components, water loss after baking and moisture content of breads for the different compositions assayed

Composition	Water absorption (g/100 g flour)	Water loss after baking[a] (g/100 g flour)	Bread moisture content[a] (g/100 g dry matter)
Control	62±2	22±1	86.2±0.6A
5 g C/kg flour	65±2	23±2	88.63±0.03B
10 g C/kg flour	68±2	25±1	92.9±0.5C
15 g C/kg flour	68±2	25±1	91.2±0.3C
Control	60±1	19±1	83.6±0.4A
5 g AIR/kg flour	69±2	21±1	89.4±0.5B
10 g AIR/kg flour	69±2	21±2	91±1B,C
15 g AIR/kg flour	71±2	20±1	92.8±0.6C
Control	63±2	26±2	84.9±0.4A
5 g S/kg flour	67±2	25±1	85.9±0.5A
10 g S/kg flour	69±2	27±3	91.2±0.4B
15 g S/kg flour	70±2	26±2	89.5±0.5B

[a] Standard deviations are indicated (n=3). Data followed by different letters show significant differences ($p<0.05$); comparisons were performed within each system (C, AIR or S addition) and column

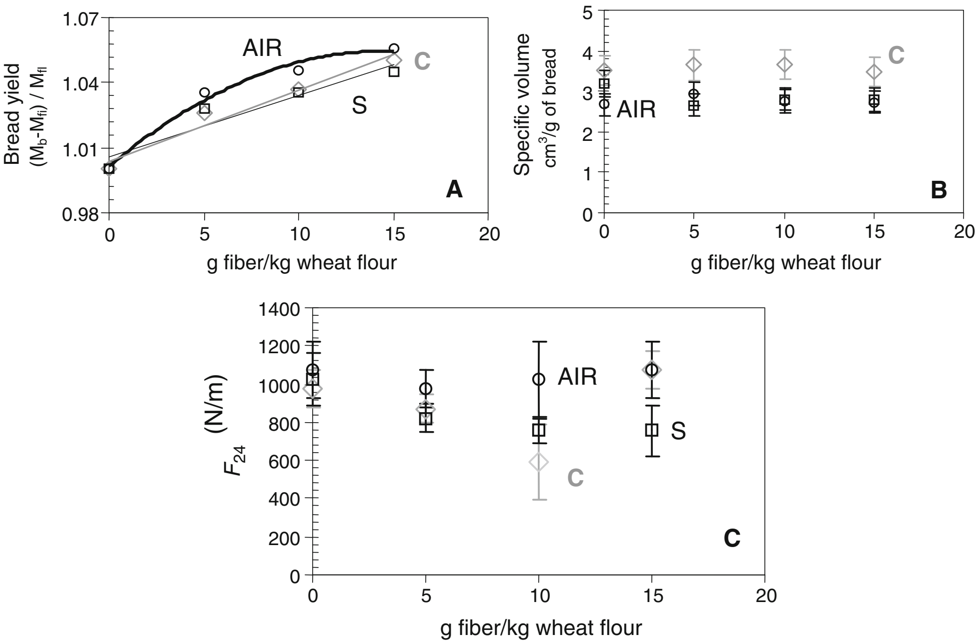


Fig. 1 Bread yield (**A**), specific volume (**B**) and bread firmness determined 24 h after baking (F_{24}) (**C**) vs butternut (*Cucurbita moschata* Duchesne ex Poiret) fibre concentration. ◊ C, ○ AIR, □ S. Control bread corresponds to 0 g of fibre fraction/kg wheat flour. M_b Bread weight, M_{fi} fibre fraction weight, M_{fl} flour weight

development and gas retention. Pomeranz et al. (1977), who used cellulose, wheat bran and oat hulls, reported that none of the fibre materials depressed gassing power; consequently, loaf volume-depressing effects of fibre materials seemed to result from reduced gas retention rather than from reduced gas formation. Gómez et al. (2003), who used seven different types of fibres at levels of 20 and 50 g/kg, reported that specific volume decreased as a consequence of fibre addition. This effect was attributed to the interaction between fibre and gluten, which led to a decrease in the gas retention capacity. Ptitchkina et al. (1998) observed a maximum value of specific volume at 8 g of pumpkin powder added to 1 kg of flour for bread production. They suggested that pumpkin powder acts by improving gas cell stability rather than by increasing the strength of the surrounding matrix, an effect that came from surface activity of pumpkin pectins. See et al. (2007) found significantly higher loaf and specific volumes for the breads obtained with a 5% of pumpkin flour. Sangnark and Noomhorm (2004) determined that volume and specific volume of breads decreased with the increment in the percentage of dietary fibre (from sugarcane bagasse and a commercial fibre) added to the flour.

Loaf firmness (F) was determined following the recommendation of AACC (Ponte and Ovadia 1996). Force–deformation curves obtained by compression of bread loaves were characterised by a first phase where force increased rapidly with deformation until reaching a point from which the force increased at a lower rate. As an example, curves obtained with bread made with flour containing C at 10 g/kg level, when fresh or after 7 days of storage, can be observed in Fig. 2A and B, respectively. An inflexion point could be determined where the slope of the compression curve changed. Extrapolating a straight line from the constant slope of the first compression phase to the X-axis, it was determined the initial point (X=0) for calculation (as an example, see Fig. 2B). From this initial point, the force developed at 25% deformation (6.25 mm) was determined. Hence, firmness parameter (F) is evaluating the resistance of the just deformed crumb, beyond the first compression phase.

In Fig. 1C, it can be observed the firmness of loaves 24 h after baking (F_{24}) plotted vs fibre concentration. It can be concluded that AIR did not modify the control firmness at any concentration, whereas S at 10 and 15 g/kg level gave origin to breads with F_{24} values lower than those calculated from control (0 g fibre/kg of flour). C addition at 10 g/kg of flour produced bread pieces that showed the lowest F_{24} of

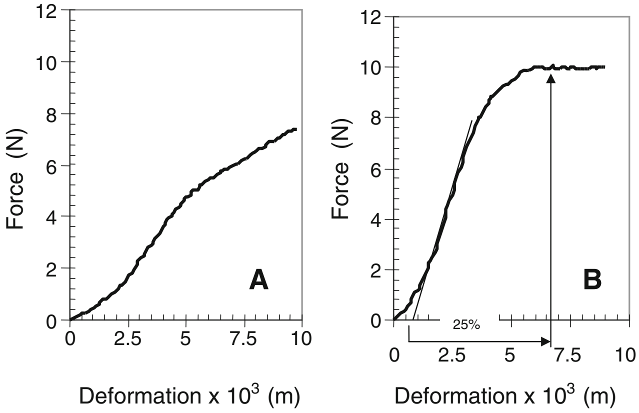


Fig. 2 Force–deformation curves recorded by compression of loaves from recently baked (**A**) and 7-day staled (**B**) breads made with flour containing 10 g of C per kilogram of flour

all bread formulations assayed in this work. According to bibliography, bread softening is related to an increase in loaf specific volume (Cornford et al. 1964) and/or to the greater dough cohesiveness (Collar et al. 1999). In our research, no significant differences in specific volume were observed in loafs when fibres were included in the formulation (Fig. 1B). Jeltema and Zabic (1979) studied the relationship between fibre components and cake quality, and they found that pectin concentration correlated with larger cells and thicker cell walls, while square of the concentration of cellulose correlated only with larger cells. They also reported that lignin showed a positive correlation with increased tenderness, an effect that was also shown by water-insoluble hemicelluloses. Table 2 summarises the chemical composition and hydration properties reported by the authors in a previous work (de Escalada Pla et al. 2007). It is important to remark that C fraction obtained from *C. moschata* had a significant content of lignin (6 g/100 g). AIR presented a higher amount of branched hydrophilic pectins among its dietary fibre content than C. Conversely, C presented more linear pectins in addition to lignin presence and higher protein content. In addition, it can be observed that the different composition of the fibre fractions led to different water binding capacities (Q) and to different rates of water absorption evaluated from the time (B) needed to absorb a quantity of water equal to $Q/2$. The smaller water binding capacity and slower water absorption rate of C may be related to the softening effect produced by this fraction on bread. Probably, C can interfere with water distribution in the gluten–starch environment, leading to softer bread.

Bread is a composite solid; it comprises, at a macroscopic level, crumb cells full of air in a matrix that is a viscoelastic solid. The mechanical behaviour of the cellular bread crumb itself is known to be complex. Jeltema and Zabic (1979) reported that tenderness was positively correlated with cell size and cell wall thickness. Considering the results obtained by us through image analyses (Fig. 3), bread made with flour added with 10 g of C per kilogram showed 55% more air occluded (cell area 100/total area) than the control bread. At the same time, it showed the same number of particles (alveolus or gas cells) per square centimetre than the control and a mean size of 4 mm^2/particle, while in the control bread, the mean-cell size was 2.0 mm^2/particle (Table 3). Thus, air cell distribution into crumb may be, at least, partially responsible for lower F_{24} in bread obtained with flour added with 10 g of C per kilogram.

During baking, lignin could develop different actions: It could hinder water distribution between the amorphous phase and the gluten network, leading to more hydrophobic interactions and/or it could scavenge free radicals (Zobel and Kulp 1996), a characteristic that might promote a higher resistance of the developed gluten network (Morel et al. 2002). These events could lead to higher stabilisation of the gas cell wall produced in the dough during fermentation and to a better gas retention during baking, resulting in greater particle size. However, this lignin effect on firmness quality was only detected at a specific C concentration (10 g/kg flour). Probably, a precise balance would be required between the natural degree of hydrophobicity of the gluten proteins and the hydrophobicity provided by the C fibre fraction through its lignin content, for attaining the low level of firmness (F_{24}) observed.

According to Pomeranz et al. (1977) and Gómez et al. (2003), one of the organoleptic properties much modified by addition of fibres is the colour of both crumb and crust. The

Table 2 Chemical composition and hydration properties of pumpkin-extracted products

	AIR	C	S
Water (g/100 g dry sample)	13.0±0.1	8.74±0.03	8.32±0.03
Ash (g/100 g dry sample)	4.6±0.2	4.5±0.1	2.1±0.4
Protein (g/100 g dry sample)	9.1±0.3	19.3±0.9	6.2±0.2
Total fibre content (g/100 g dry sample)	78.4±0.8	79.6±0.8	44.6±0.5
Lignin[a]	0.2±0.1	6.0±0.3	Nd
Celullose[a]	46±1	45±4	Nd
Total (non cellulosic) carbohydrates[a]	62±3	47±2	Nd
Uronic acids[a]	10±2	11.8±0.2	Nd
Hydration Properties			
Q[b]	25.5±0.2	12.5±0.3	13.7±0.1
B[c]	0.1±0.01	13±1	0.31±0.02

Standard deviations (α, 0.05) are indicated (n=3). Data obtained in a previous work (de Escalada Pla et al. 2007)

Nd Not determined

[a] Gram per 100 g total fibre

[b] Water binding capacity (Q) evaluated by Bauman assay (millilitre of water/gram of dry mass sample)

[c] Time (min) to absorb $Q/2$ ml of water

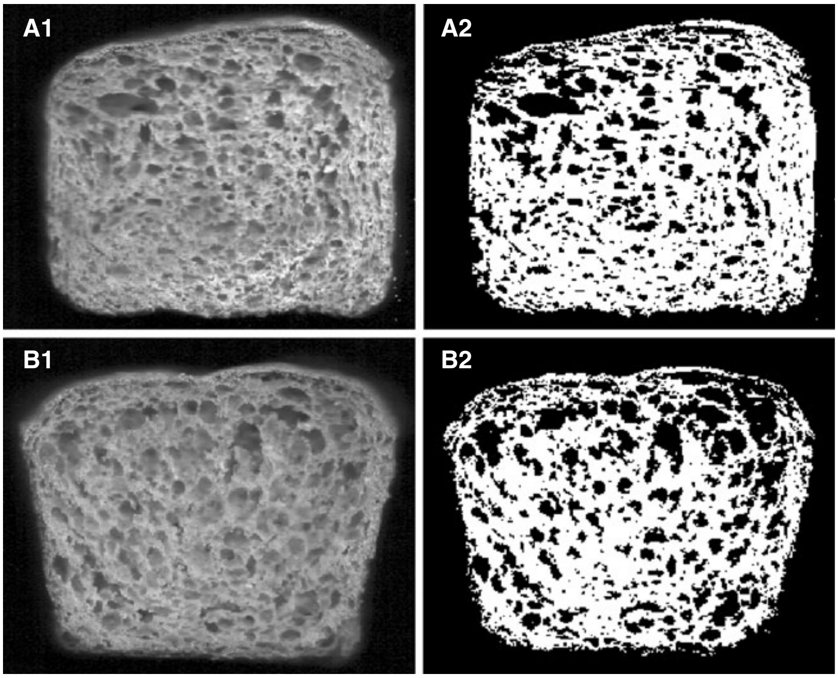


Fig. 3 Crumb images. *A1* Control bread, *A2* threshold of control bread, *B1* bread made with flour containing 10 g of C per kilogram of wheat flour, *B2* threshold of bread made with flour containing 10 g of C per kilogram of wheat flour

effect of butternut (*C. moschata* Duchesne ex Poiret) fibre addition on bread colour is summarised in Table 4. Addition of AIR, S and C at the levels herein assayed did not produced significant differences on luminosity (L*) and chromatic coordinates (a* and b*) neither in crumb nor in crust. This trend is ascribed to the low fibre concentrations used and to the consideration that, in the case of AIR and C, the process carried out to produce these fractions yielded white or clear fibres, which precluded crumb and crust colour modification.

Effect of Butternut Fibre on Bread Staling

Slices obtained from bread pieces were stored during 9 days for aging and were evaluated through compression testing as was previously described.

During storage, bread stales, and it is generally accepted that this is mainly due to starch retrogradation (Cornford et al. 1964; Willhoft 1971; Del Nobile et al. 2003; Goesaert et al. 2005). Young's modulus increases with storage as it was early demonstrated by Cornford et al. (1964). At the same time, the shape of the compression curves changes, reflecting a more solid behaviour. Figure 2 shows the characteristic force–deformation curves obtained from fresh (Fig. 2A) and stale (Fig. 2B) breads made with 10 g of C per kilogram of flour, as an example. It can be seen in Fig. 2A that the bread presented a response typical of soft solid foams with flexible cell walls where force increased with strain in a way that is coherent with an important component of plastic behaviour. The staled bread curve (Fig. 2B) presented a higher slope at the first compression phase. On the other hand, at higher strain values, rigid or brittle cell walls were broken, showing a remarkable inflexion point, and finally, the force–deformation slope tended to zero for staled bread. This is coherent with a solid-like behaviour coming from rigidification of the solid foam (crumb).

Table 3 Crumb characteristics of control bread and bread made with wheat flour containing 10 g of C per kilogram of flour

Composition	Air occluded[a] (%)	Number of particles[a]/cm^2	Mean particle area[a] (mm^2)
Control bread	20±4	9.6±0.2	2.0±0.4
Bread with 10 g C/kg flour	31±6	9±1	4±1

[a] Standard deviations are indicated (n=3)

The firmness (F) evaluated for different periods of time along bread storage was then fitted to the Avrami equation. The function $(F_\infty - F_t)/(F_\infty - F_0)$ involved in that equation indicates the extent of starch changes, irrespective of the initial crumb firmness level, providing a mean for distinguishing the effect of an additive on starch changes, or a true anti-staling effect (Cornford et al. 1964). For that adjustment, it was considered that n=1. This value for the n exponent indicates that the mode of nucleation in starch crystallisation is instantaneous and that the growth of crystallites is only in one dimension (McIver et al. 1968; Russell 1983; Haros et al. 2002). McIver et al. (1968) mentioned that instantaneous nucleation may be not unexpected because when concentrated starch slurry is heated to its gelatinisation temperature, the system obtained is not

Table 4 Bread colour parameters

Composition	Crumb[a]			Crust[a]		
	L^*	a^*	b^*	L^*	a^*	b^*
Control	66±2	−0.9±0.2	15±1	56±2	13±1	33±1
5 g C/kg flour	63±3	−0.6±0.4	16±1	57±3	13±2	33±1
10 g C/kg flour	63±2	−0.8±0.2	16±1	61±4	9±2	30±2
15 g C/kg flour	65±2	−0.5±0.2	17±2	60±2	11±1	32±1
Control	68±2	−0.8±0.1	17.6±0.7	55±3	15±2	33.1±0.8
5 g AIR/kg flour	67±2	−0.9±0.3	15±1	58±4	12±2	33±1
10 g AIR/kg flour	65±2	−0.6±0.3	16.8±0.3	57±1	13±1	34±1
15 g AIR/kg flour	64±5	−0.6±0.8	15±1	61±6	10±4	32±2
Control	68±3	−0.9±0.3	17±1	54±2	13.4±0.9	31.4±0.8
5 g S/kg flour	64±4	−0.8±0.3	17±1	58±2	11.3±0.8	31.8±0.8
10 g S/kg flour	66±2	−1.0±0.3	18±4	54±3	13±1	32±1
15 g S/kg flour	64±5	−1.2±0.1	19±1	54±2	14±1	32.1±0.8

[a] Standard deviations are indicated ($n=6$)

homogeneous and ordered regions probably remain, as it was also pointed out by Avrami (1940) when describing the heterogeneous nature of a system where instantaneous nucleation occurs.

Firmness data as a function of time fitted adequately to Avrami equation (Table 5 and Fig. 4). This shows that the process mainly responsible for firmness increase was an augment in crystallinity of the material. Actually, this general procedure of staling evaluation in breads considers an overall bulk and macroscopic property (firmness) as an indication of a microscopic change, that is, crystallisation of part of the bread structure. It can be seen in Fig. 4 that a clear tendency to firmness (F) diminishing during bread storage for 9 days, after any fibre addition with respect to the control bread (without fibre). This crumb softening is more evident at 5–15 g/kg S concentration and 5–10 g/kg C concentration, which coincides with trends observed for F_{24} of bread loaves. The changes observed with fibre addition were, in general, non-significant ($p<0.05$) for rate constant (k) and F_∞ calculated from Avrami. Anyhow, F_∞ value for C addition at 10 g/kg was significantly lower than the F_∞ for the other systems (Table 5). The F_0 fitted values showed high errors that precluded its utilisation for systems comparison. The k values herein obtained (≈0.25 day^{-1}) coincided with constant rates of staling reported by other authors (Cornford et al. 1964; McIver et al. 1968). Changes in firmness during storage when fibre was added can be ascribed to the same mechanism that led breads to lower firmness after baking and cooling (F_{24}), for example the water distribution in the gluten–starch environment and the crumb cell size. Considering that the adequate packaging herein used prevented from moisture loss (which would have resulted in textural firming and drying during storage), the predominant mechanism of staling in bread crumb was then the time-dependent starch retrogradation. The early stages of this process are dominated by amylose. Crystallisation of amylose lipid is favoured over retrogradation. This phenomenon that occurs during the first 24 h after baking contributes to the setting of the fresh bread, in part, by cementing together the swollen granules (Zobel and Kulp 1996). The later stages of retrogradation and the overall aging of bread are dominated by recrystallisation of amylopectin (Slade and Levine 1994). According to Barcenas and Rosell (2006) the formation of complexes between starch and proteins and the water redistribution among the bread constituents can also explain bread hardening. It can be then hypothesised that C fibre products may restrict this water diffusion

Table 5 Parameters obtained by fitting of the firmness experimental data to the Avrami's equation

Composition	F_∞[a] (N/m)	F_O [a] (N/m)	k[a] (day^{-1})
Control	2,700±200	400±200	0.27±0.08
5 g C/kg flour	2,300±200	400±200	0.28±0.09
10 g C/kg flour	1,700±300	300±200	0.2±0.1
15 g C/kg flour	2,600±300	700±300	0.2±0.1
Control	2,550±250	800±200	0.21±0.08
5 g AIR/kg flour	2,200±200	650±150	0.21±0.07
10 g AIR/kg flour	2,250±100	700±100	0.25±0.05
15 g AIR/kg flour	2,350±350	800±150	0.17±0.09
Control	3,010±210	350±210	0.27±0.07
5 g S/kg flour	2,590±210	280±280	0.28±0.09
10 g S/kg flour	2,450±280	350±210	0.24±0.09
15 g S/kg flour	2,660±350	490±140	0.16±0.05

[a] Standard deviations are indicated ($n=8$)

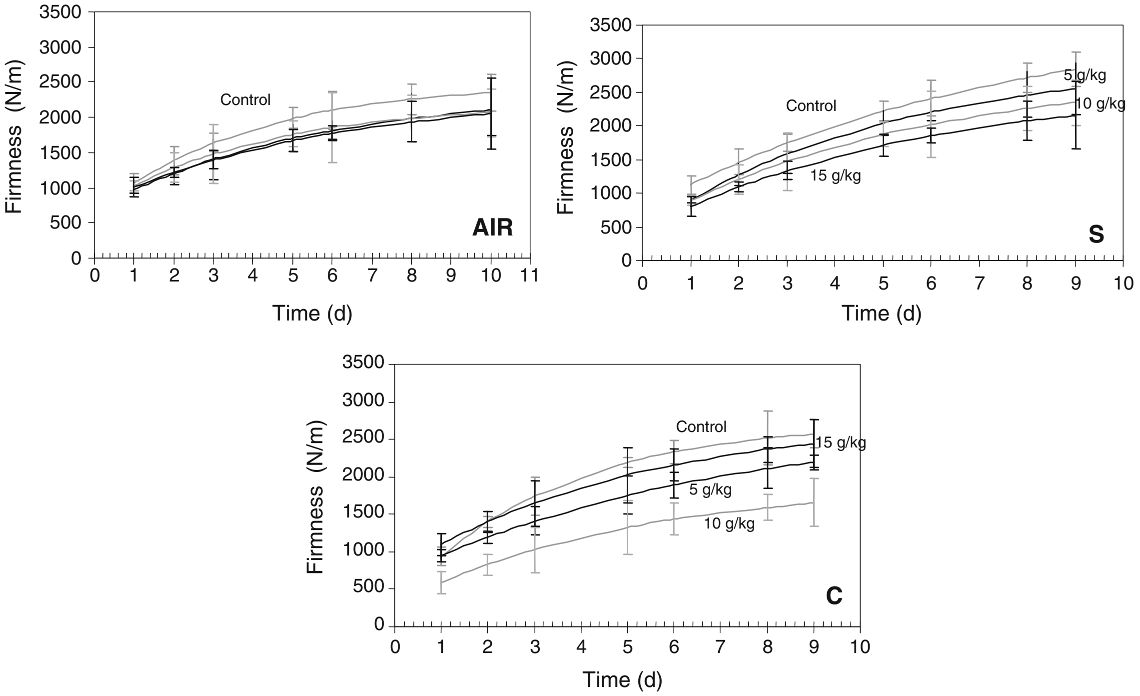
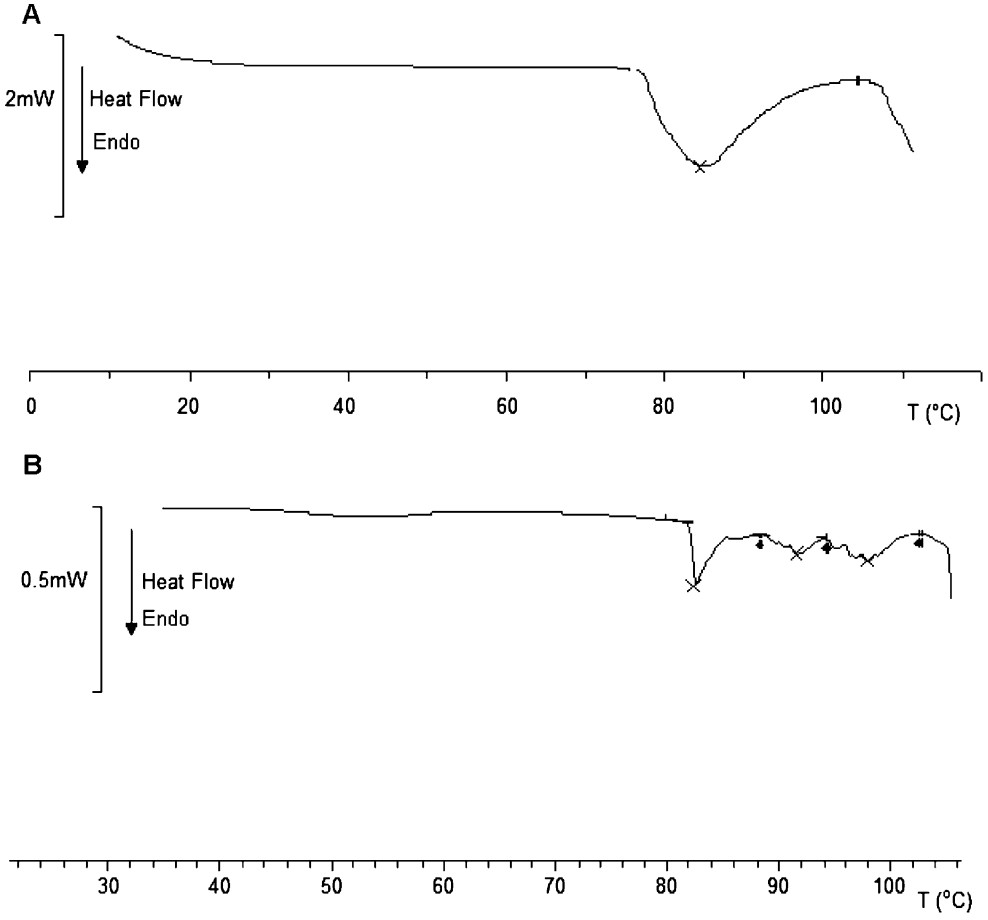


Fig. 4 Firming processes of breads made with wheat flour and either AIR, S or C fraction addition

Fig. 5 Thermal transitions of control bread (**A**) and breads made with wheat flour containing 10 g of C fraction per kilogram (**B**), all of them after storage for 7 days



from gluten and/or across the amorphous matrix due to its chemical composition, being in this way affected the breads containing this fraction.

Control bread and bread made with 10 g of C per kg of wheat flour were studied by DSC. As it can be seen in Fig. 5, an endotherm corresponding to recrystallised amylopectin melting was obtained at 84 °C by scanning the control bread sample stored for 7 days (Slade and Levine 1994). A change in enthalpy (ΔH) of 14.78 J/g (dry matter) was determined (Fig. 5A). Conversely, a total ΔH value of only 1.50 J/g (dry matter) was obtained for bread containing fraction C (Fig. 5B), indicating that amylopectin recrystallisation during bread storage was affected by the addition of C fibre to the wheat flour at 10 g/kg level. In particular, the additional two peaks detected at 91 and 97.58 °C showed a ΔH value of only 0.55 J/g (dry matter). They can be associated to the dissociation of amylose-native lipid complexes formed during baking and crystallising at early stages after baking (Davidou et al. 1996), effect that could be favoured by the presence of C fibre. Zobel and Kulp (1996) suggested that the formation of the lipid complexes prevents part of the amylose from retrograding and from contributing to crumb hardening during storage.

In relation to the effect of fibre on retrogradation, Gómez et al. (2003) reported an important effect of fibre addition on bread life time. This effect was related to the well-known water binding capacity of fibre that avoided water loss during storage and with the possible interaction between fibre and starch that would delay the starch retrogradation. It was determined that the presence of HPMC and pectins decreased the pasting temperature, increased the maximum viscosity and decreased the tendency of bread to retrograde (Rojas et al. 1999). Some authors (Kim and D'Appolonia 1977a, b) have reported that protein retarded bread staling by diluting starch and retarding its changes, regardless of protein quality. It is important to remark that C fibre fraction contained lignin, and its pectin was less branched than AIR one (less hydrophilic groups), in addition of having twice as much protein, as it was previously discussed (Table 2). As a consequence, composition of C and its interactions with wheat flour components in a bread formulation can be the cause not only of the obtention of softer breads after baking but also of the effect of C addition on bread staling.

Conclusions

The effect of enriched fibre products (AIR, C and S) obtained from butternut (*C. moschata* Duchesne ex Poiret) on bread making and bread quality was evaluated through the study of breads produced with no fibre (control) or with 5, 10 or 15 g of fibre per kilogram of wheat flour. Neither specific volume decrease nor colour alterations were observed on bread after butternut fibre addition. Lower bread firmness was observed 24 h after baking when 10 g of C was added per kilogram of flour. Breads made with flour containing 5–15 g of S or 5–10 g of C per kilogram of flour also showed a softer crumb along 9 days storage. However, this bread softening was only significant when flour was mixed with 10 g of C per kilogram. Probably, the effect of C fraction at 10 g/kg on flour basis can be ascribed to its composition: presence of lignin, less branched pectin chains and significant higher protein content, which might affect the water distribution across the amorphous matrix and the size of the air cells in the bread crumb.

It can be concluded that the utilisation of S or C butternut fractions at concentrations ranging from 5 to 15 g fibre per kilogram of wheat flour is promising to improve bread texture after baking and along storage.

Acknowledgements The authors acknowledge the financial assistance from the University of Buenos Aires (UBA, EX-089 and EX-726), National Council of Scientific and Technical Research-Argentina (CONICET, PIP 11220090100531), National Agency of Scientific and Technological Promotion of Argentina (ANPCyT-PICT 38239) and Fundación Antorchas. The authors also want to thank Taxonera S.A. Company that generously allowed them to use their installations and equipment and provided the help of trained bakers and also to Lic. D. F. Vivas for his useful help during the baking process.

References

AOAC (1995) Official methods of analysis of the Association of Official Analytical Chemists, 16th edn, Vol. 2. Method 925.10. USA: AOAC, Inc.

Avrami, M. (1939). Kinetics of phase change. I. *Journal of Chemical Physics, 7*, 1103–1112.

Avrami, M. (1940). Kinetics of phase change. II. *Journal of Chemical Physics, 8*, 212–224.

Avrami, M. (1941). Granulation, phase change, and microstructure. Kinetics of phase change. III. *Journal of Chemical Physics, 9*, 177–184.

Barcenas, M. E., & Rosell, C. M. (2006). Effect of frozen storage time on the bread crumb and aging of par-baked bread. *Food Chemistry, 95*, 438–445.

Collar, C., Andreu, P., Martínez, J., & Armero, E. (1999). Optimization of hydrocolloid addition to improve wheat bread dough functionality: A response surface methodology study. *Food Hydrocolloids, 13*, 467–475.

Cornford, S., Axford, D., & Elton, G. (1964). The elastic modulus of bread crumb in linear compression in relation to staling. *Cereal Chemistry, 41*, 216–229.

Correa M., Pérez G. & Ferrero C. (2011) Pectins as breadmaking additives: Effect on dough rheology and bread quality. Food and Bioprocess Technology, doi:10.1007/s11947-011-0631-6.

Davidou, S., Le Meste, M., Debeve, E., & Bekaert, D. (1996). A contribution to the study of staling of white bread: effect of water and hydrocolloid. *Food Hydrocolloids, 10*(4), 375–383.

de Escalada Pla, M., Ponce, N., Wider, E., Stortz, C., Rojas, A., & Gerschenson, L. (2005). Chemical and biochemical changes of pumpkin (*Cucumis moschata*, Duch.) tissue in relation to osmotic stress. *Journal of the Science of Food and Agriculture, 85*(11), 1852–1860.

de Escalada Pla, M., Delbon, M., Rojas, A., & Gerschenson, L. (2006). Effect of immersion and turgor pressure change on mechanical properties of pumpkin (*Cucumis moschata*, Duch). *Journal of the Science of Food and Agriculture, 86*(15), 2628–2637.

de Escalada Pla, M., Ponce, N., Stortz, C., Gerschenson, L., & Rojas, A. (2007). Composition and functional properties of enriched fibre products obtained from pumpkin (*Cucurbita moschata* Duchesne ex Poiret). *Lebensmittel-Wissenschaft und Technologie, 40*, 1176–1185.

de Escalada Pla, M., Campos, C., Gerschenson, L., & Rojas, A. (2009). Pumpkin (*Cucurbita moschata*, Duchesne ex poiret) mesocarp tissue as a food matrix for supporting iron in a food product. *Journal of Food Engineering, 92*(4), 361–369.

Del Nobile, M., Martoriello, T., Mocci, G., & La Notte, E. (2003). Modeling the starch retrogradation kinetic of durum wheat bread. *Journal of Food Engineering, 59*, 123–128.

Fissore, E., Ponce, N., de Escalada Pla, M., Stortz, C., Rojas, A., & Gerschenson, L. (2010). Characterization of acid-extracted pectin-enriched products obtained from red beet (*Beta vulgaris* L. var. conditiva) and butternut (*Cucurbita moschata* Duch ex Poiret). *Journal of Agricultural and Food Chemistry, 58*, 3793–3800.

Gerschenson, L. N., Rojas, A. M., de Escalada Pla, M. N., & Fissore, E. (2009). Functional properties of dietary fibre isolated from *Cucurbita moschata* Duchesne ex Poiret through different extraction procedures. In J. N. Govil & V. K. Singh (Eds.), *Recent progress in medicinal plants* (pp. 359–370). Houston: Editorial Studium Press LLC.

Goesaert, H., Brijs, K., Veraverbeke, W., Courtin, C., Gebruers, K., & Delcour, J. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology, 16*, 12–30.

Gómez, M., Ronda, F., Blanco, C., Caballero, P., & Apesteguía, A. (2003). Effect of dietary fibre on dough rheology and bread quality. *European Food Research and Technology, 216*, 51–56.

Gwanama, C., Labuschagne, M., & Botha, A. (2000). Análisis of genetic variation in *Cucurbita moschata* by random amplified polymorphic DNA (RAPD) markers. *Euphyrica, 113*, 19–24.

Haros, M., Rosell, C., & Benedito, C. (2002). Effect of different carbohydrases on fresh bread texture and bread staling. *European Food Research and Technology, 215*, 425–430.

Jeltema, M., & Zabic, M. (1979). Fibre components-quantitation and relationship to cake quality. *Journal of Food Science, 44*, 1732–1735.

Kim, S., & D'Appolonia, B. (1977a). Bread staling studies. I. Effect of protein content on staling rate and bread crumb pasting properties. *Cereal Chemistry, 54*, 207–215.

Kim, S., & D'Appolonia, B. (1977b). Bread staling studies. II. Effect of protein content and storage temperature on the role of starch. *Cereal Chemistry, 54*, 216–224.

McIver, R., Axford, D., Colwell, K., & Elton, G. (1968). Kinetic study of the retrogradation of gelatinised starch. *Journal of the Science of Food and Agriculture, 19*, 560–563.

Mettler (1997). Mettler DSC User's Manual, 42–45.

Moreira, R., Chenlo, F., Torres, M. D., & Prieto, D. M. (2011). Technological assessment of chestnut flour doughs regarding to doughs from other commercial flours and formulations. Food and Bioprocess Technology, doi:10.1007/s11947-011-0524-8.

Morel, M., Redl, A., & Guilbert, S. (2002). Mechanism of heat and shear mediated aggregation of wheat gluten protein upon mixing. *Biomacromolecules, 3*, 488–497.

Pomeranz, Y., Shogren, M., Finney, K., & Bechtel, D. (1977). Fibre in breadmaking-effects on functional properties. *Cereal Chemistry, 54*(1), 25–41.

Ponte, J., & Ovadia, D. (1996). Instrumental Methods. In R. E. Hebeda & H. F. Zobel (Eds.), *Baked goods freshness. Technology, evaluation, and inhibition of staling* (pp. 151–170). New York: Marcel Dekker.

Ptitchkina, N., Novokreschonova, L., Piskunova, G., & Morris, E. (1998). Large enhancements in loaf volume and organoleptic acceptability of wheat bread by small additions of pumpkin powder: possible role of acetylated pectin in stabilizing gas-cell structure. *Food Hydrocolloids, 12*, 333–337.

Rojas, J., Rosell, C., & Benedito de Barber, C. (1999). Pasting properties of different wheat flour-hydrocolloid systems. *Food Hydrocolloids, 13*, 27–33.

Rosell, C., Rojas, J., & Benedito de Barber, C. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids, 15*, 75–81.

Russell, P. (1983). A kinetic study of bread staling by differential scanning calorimetry and compressibility measurements. The effect of added monoglyceride. *Journal of Cereal Science, 1*, 297–303.

Sangnark, A., & Noomhorm, A. (2004). Effect of dietary fiber from sugarcane bagasse and sucrose ester on dough and bread properties. *Lebensm.-Wiss. u.-Technol, 37*, 697–704.

Scanlon, M., & Zghal, M. (2001). Bread properties and crumb structure. *Food Research International, 34*, 841–864.

See, E., Wan Nadiah, W., & Noor Aziah, A. (2007). Physico-Chemical and sensory evaluation of breads supplemented with pumpkin flour. *ASEAN Food Journal, 14*(2), 123–130.

Slade, L., & Levine, H. (1994). Structure-function relationships of cookie and cracker ingredients. In H. Faridi (Ed.), *The science of cookie and cracker production* (pp. 23–141). New York: Chapman & Hall.

Sokal, R., & Rohlf, F. (1980). *Biometry*. San Francisco: Freeman.

Whitaker, T., & Bemis, W. (1975). Origin and evolution of the cultivated *Cucurbita*. *Torrey Botanical Club Bulletin, 102*, 362–368.

Willhoft, E. (1971). Bread staling II.—Theoretical study. *Journal of the Science of Food and Agriculture, 22*, 180–183.

Wittig de Penna, E., Bunger, A., Ibieta, A., Soto, D., Hernández, N. & López, L. (1998). Alimentos para el adulto mayor: muffins enriquecidos con fibra de lupino. In Programa Iberoamericano de Ciencia y Tecnología para el desarrollo, CYTED (Eds.), *Temas en Tecnología de Alimentos. Vol.2. Fibra Dietética* (pp. 259–277). Mexico DF: Alfaomega Grupo Editor.

Zobel, H., & Kulp, K. (1996). The staling mechanism. In R. E. Hebeda & H. F. Zobel (Eds.), *Baked goods freshness. Technology, evaluation, and inhibition of staling* (pp. 1–64). New York: Marcel Dekker, Inc.