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ORIGINAL PAPER

Emulsifiers: Effects on Quality of Fibre-Enriched Wheat Bread

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Abstract Resistant starch (RS) is a nutritional ingredient commonly used in bread products as dietary fibre (DF). This ingredient presents similar physiological functions than those imparted by DF, promoting beneficial effects such as the reduction of cholesterol and/or glucose levels on blood. Quality improvement of bread containing RS, with an optimized combination of emulsifiers, will be useful in the development of new and healthy bakery products. The objective of this research was to analyse the effects of different emulsifiers on several quality parameters of dough and bread prepared with wheat flour partially substituted with resistant starch as a dietary fibre. A blend of wheat flour/maize-resistant starch (MRS; 87.5:12.5) with sodium chloride, ascorbic acid, α-amylase, compressed yeast and water was utilized. Emulsifiers were incorporated to formulations in different levels according to a simplex centroid design. The viscoelastic, textural and extensional properties of dough were analysed. Bread quality was evaluated throughout the gelatinization and retrogradation of starch, specific volume of loaves, and texture and firmness of bread crumb. The incorporation of 12.5% (*w*/*w*) of MRS to wheat flour caused an increase of 5% in water absorption. Stability decreases markedly (from 9.9 to 2.2 min) and the mixing tolerance index increased (from 79 to 35 UF). The sodium stearoyl lactylate (SSL)–diacetyl tartaric acid esters of monoglycerides (DATEM) mixture increased hardness and resistance to extension on dough, whilst dough containing Polysorbate 80 (PS80) was softer; nevertheless, both types of dough retained less CO_2. An optimized concentration of the three emulsifiers (0.24% SSL, 0.18% PS80, 0.08% DATEM, *w*/*w*) was obtained by surface response methodology. The bread prepared with this combination of emulsifiers presented a considerable specific volume with a very soft crumb.

Keywords Resistant starch · Wheat flour · Emulsifiers · Bread making quality

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Introduction

A number of studies have demonstrated that resistant starch has physiological functions similar to those of dietary fibre (ASP 1992; Eerlingen and Delcour 1995; Nugent 2005; Goñi et al. 1997; Åkerberg et al. 1998; Rosin et al. 2002; Queiroz-Monici et al. 2005; Yamada et al. 2005). Maize-resistant starch (MRS) is a white powder which has advantages over other fibres due to its better appearance, taste and texture. The incorporation of MRS to pasta products reduced enzymatic digestibility without affecting the cooking quality. In addition, the sensory properties of pasta are not modified by MRS (Gelencsér et al. 2008). Several studies related to bread with high levels of MRS as a functional food have been performed (Morita et al. 2002; Hung and

Morita 2004; Hung et al. 2005). Technologically, the replacement of wheat flour by MRS affects bread quality due to gluten protein dilution (Michniewicz et al. 1991). A similar effect of decreasing the bread quality is produced by legume flours (Ribotta et al. 2010). These authors found on wheat flour–soy flour systems (90:10) an improvement on dough and bread quality with SSL incorporation. Therefore, whatever the type of fibre added to bread, it is necessary to adjust the dough parameters with the purpose of obtaining good quality products. Several studies have demonstrated that emulsifiers like sodium stearoyl lactylate (SSL), diacetyl tartaric acid esters of monoglycerides (DATEM) and polyethylene sorbitan mono-oleate (Polysorbate 80, PS80) have desirable effects in the bread making process (Stampfli and Nersten 1995; Brandt 1996; Aamodt et al. 2003, 2005a,b; Gómez et al. 2004; Ribotta et al. 2004; Selomulyo and Zhou 2007; Koocheki et al. 2009). During kneading, emulsifiers increase the strength and extensibility of dough. In the fermentation process, these improve gas retention and prevent dough collapse. In the baking process, they enhance texture and reduce water loss, and also interact with amylose and amylopectin molecules, retarding bread ageing (Lakshminarayan et al. 2006).

The rheological behaviour of dough can be determined by two distinct types of measurements, fundamental and empirical tests. The fundamental rheology of dough is assayed at small deformation by dynamic oscillatory measurements, whilst empirical assays, like texture profile analysis, usually use large deformations. In small deformation measurements, the three common types of interactions (protein–protein, starch–starch and starch–protein interactions) cannot be resolved, and the results often do not correlate well with baking quality (Tronsmo et al. 2003). The dynamic oscillatory test is commonly used for testing the viscoelastic properties of gluten and dough once formed. On the other hand, the rheological properties of materials submitted to large deformations are generally associated with bread making quality (Zaidel et al. 2010).

In addition, heating causes changes in gluten protein structure. These changes affect the functionality of wheat proteins and starch and can be determined by differential scanning calorimetry (DSC; Arntfield et al. 1990). The evaluation of thermal behaviour by DSC is one of the most widely used tests for studding starch gelatinization, retrogradation and bread staling (Baik and Chinachoti 2000; Karim et al. 2000; Hassan et al. 2006).

Little information about the influence of emulsifiers on the rheological and thermal characteristics of wheat flour–MRS dough is available. For this reason, the objective of this research was to analyse the effects of three emulsifiers (SSL, PS80 and DATEM) on several quality parameters of dough and bread prepared with wheat flour partially substituted with MRS as a dietary fibre.

Materials and Methods

Materials

Commercial *Triticum aestivum* wheat flour with 14.0% moisture, 9.1% protein, 0.73% ash, 25.8% wet gluten, 8.5% dry gluten and 360 s of falling number was provided by Moinho Pacífico Indústria e Comércio Ltda. (Santos, Brazil). All the composition values, except moisture content, are expressed on dry matter basis. Resistant starch Hi-Maize® 260 was provided by National Starch (São Paulo, Brazil). Dried active yeast (*Saccharomyces cerevisiae*) was obtained from Fleischmann (São Paulo, Brazil).

Three types of emulsifiers—SSL, DATEM and PS80—and also fungal α-amylase, were obtained from Danisco A/S (Copenhagen, Denmark). Ascorbic acid was provided by Casa Americana (São Paulo, Brazil).

Dough Formulation and Preparation

The dough base formulation used comprised a wheat flour–MRS blend (87.5:12.5%, *w/w*), 2.0% sodium chloride, 0.02% ascorbic acid, 0.00125% α-amylase and 62.7% of water (farinographic absorption). The percentages of each compound were expressed on wheat flour–MRS blend basis. The levels of ascorbic acid and α-amylase were selected on the basis of manufacturer-recommended levels. Dough without emulsifiers was prepared as a control sample. Emulsifiers were added to the formulation according to a simplex centroid design, augmented with axial blends with seven points: three vertices of the predefined region, three axial points and one central point. Three replicates were made at the central point of the design to allow the estimation of pure error. The mixture components are the three emulsifiers SSL, PS80 and DATEM. This model can successfully reproduce the response values at the vertices and edges of a triangle, representing the concentrations of pure emulsifiers and their mixtures, respectively. The central point represents the equiproportional ternary mixture of the three emulsifiers (Table 1).

Dough was prepared by mixing raw materials (875 g wheat flour, 125 g MRS, 0.2 g ascorbic acid, 0.0125 g α-amylase, 20 g salt and emulsifiers) for 1 min at 840 rpm in a spiral mixer (SUPREMAX AL-25 IM, Brazil) at 25 °C (Matuda 2008; Matuda et al. 2008). The emulsifiers' quantity was incorporated following the design shown in Table 1. Water was added according to the farinographic absorption of dough prepared with MRS and without emulsifiers (62.7%). Dough was kneaded for 12 min at 1,700 rpm at 25 °C and rested for 15 min. In the uniaxial extension test, a piece of dough was gently rolled into a ball and then was pressed in a Teflon mould with a grooved base and rested for 40 min at a controlled temperature of 30 °C in a

Table 1 Quantities of emulsifiers used in dough formulation

Mixture	Coded variable levels			Decoded variable levels		
	SSL X_1	PS80 X_2	DATEM X_3	SSL C1[a] (g/100 g)	PS80 C2[a] (g/100 g)	DATEM C3[a] (g/100 g)
M0[b]	0	0	0	0	0	0
M1	1	0	0	0.5	0	0
M2	0	1	0	0	0.5	0
M3	0	0	1	0	0	0.5
M4	0.5	0.5	0	0.25	0.25	0
M5	0.5	0	0.5	0.25	0	0.25
M6	0	0.5	0.5	0	0.25	0.25
M7a	0.333	0.333	0.333	0.167	0.167	0.167
M7b	0.333	0.333	0.333	0.167	0.167	0.167
M7c	0.333	0.333	0.333	0.167	0.167	0.167

M wheat flour–MRS–emulsifier mixtures (numbers after M are the coded values of each sample according to the simplex centroid design)

[a]Based on wheat flour–MRS blend

[b]Control sample

fermentation cabinet. For texture profile analysis (TPA), dough was laminated (gap between rods, 10 mm) four times to improve gluten development, turned 90° at each one passage using the rods, cut into pieces with a cylindrical puncher (diameter, 40 mm) and covered with a plastic film until analysis. Cylindrical pieces (diameter, 2.5 cm; height, 0.5 cm) of the different dough samples were cut and submitted to dynamic rheological measurements.

For rheofermentation and baking assays, 2.8% of compressed yeast was incorporated to the blends.

Rheological Properties of Dough

Farinographic Assays

Farinographic assays were conducted on wheat flour and wheat flour–MRS blend, without salt incorporation (AACC 54-21.01). Measurements were performed using a Farinograph equipment (Brabender, Germany), and the water absorption, development time, stability and mixing tolerance index were the parameters evaluated.

Viscoelastic Properties

Rheological measurements were performed on a controlled stress rheometer (Haake RS600 rheometer, Germany) fitted with 35-mm serrated parallel plates. A 1.5-mm gap was used. Excess dough protruding from the edge of the plate was carefully trimmed. Low-viscosity silicone was added around the plate edges to prevent dough dehydration. Before starting the oscillatory measurement, the dough was allowed to rest for 15 min to allow relaxation of residual stresses. Temperature was kept constantly at 25 °C. The equipment was driven through the Haake software (RheoWin Pro Data Manager version 2.94). Stress sweep runs (0.5–200 Pa) were conducted at 1 Hz frequency to determine the linear viscoelastic range. The storage (G') and loss (G'') moduli remain constant within this linear viscoelastic range, but there is a critical value of stress (σ limit) above which both moduli decrease, suggesting disruption of food structure. According to the stress sweep curves, the critical stress value was 10 Pa (data not shown); therefore, the middle stress value of 5 Pa was selected. In the linear viscoelasticity range, storage (G') and loss (G'') moduli were recorded as a function of oscillation frequency (0.005–100 Hz).

Texture Profile Analysis

The TPA of dough was performed using a texture analyzer (TA.XT2i, Stable Micro Systems, UK) with a 25-kg load cell. The discs of dough (diameter, 40 mm; thickness, 10 mm) were placed in a cylindrical cell on the base of the texture analyzer and subjected to a double compression cycle (40% deformation) at a crosshead speed of 0.5 mm s^{-1}. At least 15 dough discs of each formulation were measured. Hardness, consistency, adhesiveness, cohesiveness and springiness were calculated from curves supplied by the software of the texture analyser (Texture Expert 1.22, SMS).

Uniaxial Extension

Measurements were performed with a TA.XT2i texture analyzer (Stable Micro Systems) using the SMS/Kieffer rig for dough extensibility measurements (SMS 1995). The strips were placed on the platform, trimmed and extended until their elasticity was exceeded and the dough was broken. Resistance to extension (R_{max}), maximum extensibility (L) and work needed for dough extension (A) were obtained from load extension curves and calculated using the software Texture Expert 1.22 (Stable Micro Systems). A minimum of ten dough strips of each formulation were measured.

Rheofermentation Assays

The fermentation process was monitored using a rheofermentometer (F3, Chopin, France). A piece of dough (250 g) was placed in the rheofermentometer basket and a piston with 2-kg resistance weight was placed on top. The temperature was maintained at 28.5 °C and the test was conducted for 3 h.

Two curves were obtained from this test. The dough development curve provides information about the maximum height reached by the piston (H_m) and the time taken to reach maximum height (t_1). The gaseous release curve presents the maximum height (H'_m), a measure of the pressure developed by fermentation, the time taken to reach that height (t'_1) and the time when porosity appears (tx, related to CO_2 loss).

Bread Quality

Starch Gelatinization and Retrogradation

Starch gelatinization and retrogradation were analysed using DSC. This assay was carried out to simulate the baking process (León et al. 1997). Analyses were performed in a TA Instrument Mod. Q100 Calorimeter (TA Instrument, USA) fitted with a liquid nitrogen-controlled cooling accessory. The transition temperatures and enthalpies were obtained by the TA Instruments Universal Analysis 2000 software (version 4.2E). The equipment was calibrated with indium, lauric acid and stearic acid. Coated aluminium hermetic pans were used. An empty double pan was used as the reference.

Dough samples, those corresponding to the simplex centroid design (Table 1), and dough belonging to the optimal mixture (Mop), were prepared without yeast incorporation. Dough, once prepared, were freeze-dried, milled and stored at 4 °C until analysis. The samples were reconstituted with 62.7% of water (farinographic absorption of control sample) and heated from 5 to 130 °C at a heating rate of 10 and 5 °C/min for the gelatinization and retrogradation assays, respectively. Starch gelatinization and retrogradation processes and the amylose–lipid complex dissociation of dough (5–10 mg) were analysed through measurements of the endotherm area (in joules per gram). To evaluate starch retrogradation, the gelatinized dough in DSC pans were allowed to cool and stored at 20±2 °C for 7 days. The samples were evaluated at 0, 1 and 7 days of storage. The samples were heated again in the calorimeter and the temperature and enthalpy of the process were determined.

Bread making

The optimum mixture of emulsifiers, i.e. the blend that generated the dough with the highest H_m value, was selected for bread making. Dough (600 g) was separated into two pieces (300 g each one). The pieces were passed through a bread shaping machine (SUPREMAX MC-50, Brazil) and placed in loaf pans (dimensions, 30.0×10.5×10.0 cm) which were previously sprayed with vegetable oil. Pans were placed into the fermenting chamber (Degânia, Italy) at 32 °C for 90 min. Loaves were baked in a Four Turbo Model oven (Degânia) at 180 °C for 25 min with the moisture trap closed and then 5 min with the moisture trap opened. Breads were allowed to cool for 1 h at room temperature prior to the tests.

Specific Volume

Loaf volume was measured by the rapeseed displacement method (Chopin) and the specific volume (in cubic centimetres per gram) of bread was calculated for loaf volume and weight, obtained by direct measure.

Crumb Moisture

Moisture of crumb was determined by oven drying (Estigia, Argentina) for 2 h at 135 °C (AACC Approved Method 44-19).

Crumb Firmness

Bread crumb firmness was determined according to AACC (2000) Approved Method 74-09 using a TA.XT2i Texture Analyser (Stable Micro Systems) fitted with a 36-mm diameter aluminium probe. Slices of bread of 25-mm thickness were cut. Samples were covered with a plastic film until analysis to avoid dehydration. Crumb firmness was measured immediately. The compression rate was set at 100 mm/min. At least six slices per loaf were measured. Firmness was the force required to compress the slices by 40% strain.

Experimental Design and Statistical Analyses

The experimental rheological parameters of dough were analysed using a special cubic mathematical model. This model can successfully reproduce the response values at the vertices and edges of a triangle. The vertices represent the concentrations of pure emulsifiers (1,0,0; 0,1,0; 0,0,1) and the edges correspond to the binary mixtures (1/2,1/2,0; 1/2,0,1/2; 0,1/2,1/2). The central point represents the ternary mixture of the three emulsifiers (1/3,1/3,1/3). This last point differentiates the special cubic model from the quadratic model. Only three replicates of the central point sample were performed. These replicates of the central point sample allow the estimation of the pure error of the model.

The data obtained from dough texture, uniaxial extension and the rheofermentation parameters were analysed using response surface methodology (RSM) using the Statgraphics plus 5.1 software (StatPoint Technologies, Inc., USA).

Parameters were subjected to ANOVA according to the general linear model procedure with least-square mean effects. Significantly different means were determined according to Fisher's least significant differences (LSD, $p<0.05$). The third-order polynomial model of Scheffé (normally used in the modelling of mixtures) proposed for each parameter was:

$$Y_i = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{23} x_2 x_3 + \beta_{13} x_1 x_3 + \beta_{123} x_1 x_2 x_3$$

where Y_i is the response variable of the evaluated parameter i; x_1, x_2 and x_3 are the coded variables which represent the three emulsifiers (SSL, PS80 and DATEM); and β_1, β_2, β_3, β_{12}, β_{23}, β_{13} and β_{123} are the estimated coefficients (Myers and Montgomery 1995).

Results and Discussion

Rheological Properties of Dough

The incorporation of 12.5% of MRS to wheat flour caused an increase of 5% of water absorption (from 59.7% to 62.7%). Matsuda (2007) obtained similar results, with an increase in water absorption with the increment of MRS in the blend. The higher level of substitution (30%) showed a difference of 2% in water absorption. In spite of the quantity of MRS incorporated to the formulations in the present research being lower than that utilized by Matsuda (2007), a higher water absorption value was detected. Probably, the quantity of the damaged starch of flour would also contribute to this phenomenon.

Farinographic stability decreased markedly from 9.9 to 2.2 min, suggesting a low tolerance of flour to kneading. The mixing tolerance index of control sample (79 UF) showed a value higher than that observed for flour without MRS (35 UF). These results indicate that resistant starch conduces to a greater degree of dough softening during kneading.

Viscoelastic Properties

All tested samples showed a linear viscoelastic zone between 0.5 and 10 Pa. Therefore, frequency sweeps were carried out at a constant stress of 5 Pa. In all cases, the storage modulus (G') was higher than the loss modulus (G'') throughout all the frequency ranges assayed, resulting in parallel upwards curves, indicating a predominant elastic behaviour of the system.

When the storage modulus (G') was evaluated at a frequency of 1 Hz, only mixture M3 showed higher values than the control sample (M0), with a value of 23.8×10^3 Pa. Moreover, M1 and M6 mixtures presented the lowest values of G' (13.2×10^3 and 11.0×10^3 Pa, respectively; Fig. 1a). Since mixture M3 had 0.5% of DATEM and sample M5 had 0.25% of this emulsifier plus 0.25% of SSL, the results obtained showed that there was a tendency to increase the G' value with the incorporation of DATEM to the formulation. In the case of M6 sample (0.25% PS80+0.25% DATEM), lower values of G' were obtained, suggesting that PS80 governs the viscoelastic dough behaviour. For all samples, the same tendency for the loss modulus (G'') was observed (Fig. 1b).

On the other hand, values of loss tangent ($\tan\delta=G''/G'$) of M4 and M6 mixtures were significantly higher (0.318±0.017 and 0.313±0.009, respectively) than the control dough (0.284±0.007), suggesting that these dough presented a more viscous behaviour than M0.

According to the viscoelastic properties results, the incorporation of PS80 in combination with SSL or DATEM to wheat dough formulations would produce dough with a more viscous behaviour.

Texture Profile Analysis

A significant increase for all mixtures (except M2) of dough hardness was obtained with respect to M0. Mixtures M1,

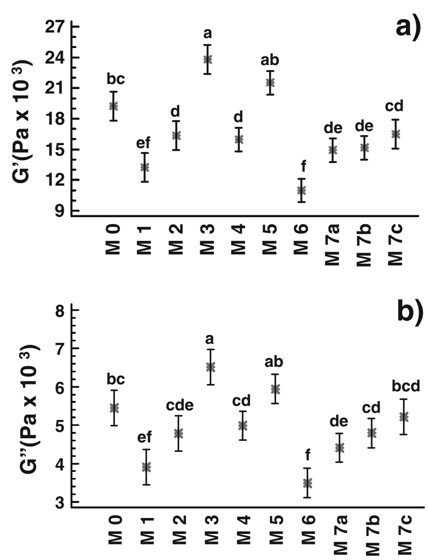

Fig. 1 **a** Storage modulus (G'). **b** Loss modulus (G''). *M* wheat flour–MRS–emulsifier mixtures. Numbers after M are the coded values of each sample according to the simplex centroid design

M3 and M5 were the strongest ones (Fig. 2a). These results suggest that emulsifiers like SSL and DATEM are more effective than PS80 on gluten structuring, even in the presence of resistant starch. The results obtained for consistency showed the same trend as hardness (data not shown).

All samples tested showed a higher adhesiveness in comparison to the control dough. Mixtures containing SSL were the most adhesive ones (M1, M4 and M5; Fig. 2b).

The springiness and cohesiveness of dough presented a similar behaviour (Fig. 2c, d), indicating that the addition of emulsifiers increased these textural parameters with respect to the control dough, mainly in mixtures containing PS80.

The addition of 0.5% of PS80 promoted the formation of softer dough, similar to dough without emulsifiers. In addition, these dough are a little more adhesive and cohesive. Bread prepared with 0.5% of PS80 would be softer and less easily threshed. Dough prepared with 0.5% of SSL and DATEM were stronger and less cohesive, so they would be expected to give bread with a harder but more crumbly crumb.

Uniaxial Extension

Only mixtures M1 (0.5% SSL), M3 (0.5% DATEM) and M5 (0.25% SSL+0.25% DATEM) showed a significant increase on maximum resistance to extension (R_{max}) with respect to the control dough (M0=0.325 N), showing average values of 0.46, 0.38 and 0.43 N, respectively (Table 2). All dough (except M1 and M5) showed a significant decrease of work needed for dough extension (A) with respect to M0. The largest decrease of this parameter was obtained with M2 and M4, which contain PS80 (1.75 and 1.65 mm^2, respectively). The presence of the tested emulsifiers decreased significantly the extensibility (L) of the dough in comparison to the control (Table 2). However, no significant differences between the samples with emulsifiers were found. The incorporation of SSL and DATEM to wheat dough formulations produced less extensible dough with more resistance to extension, whilst dough with PS80 needed less work for extension. These results are in agreement with data found in TPA assays. Differences in the behaviour of the emulsifiers could be attributed to the different chemical nature of PS80 compared with SSL or DATEM.

SSL and DATEM, despite having different hydrophilic–lipophilic balance, have a similar structure with a fatty acid molecule C18:0 in one of the extreme positions of glycerol. On the other hand, PS80 has a higher molecular weight than SSL and DATEM, with a C18:1 and several hydroxyl groups in its chemical formula. These characteristics promote the formation of hydrogen bonds with water and would cause higher water absorption, leading to dough with less resistance to extension.

Some authors studied the uniaxial extension of wheat flour dough. Ravi et al. (2000) evaluated the effect of SSL, DATEM and glycerol monostearate (0.25–1.0%, *w*/*w*) on the uniaxial extension of dough. These authors found that these emulsifiers, at all concentrations tested, caused an increased of R_{max} and a decrease of L in relation to control dough.

On the other hand, Aamodt et al. (2005b) observed, comparing to a control sample, that the R_{max} was higher in dough (without MRS) prepared with DATEM (0.45 and 0.90%, *w*/*w*), whilst the extensibility (L) was lower. Maximum resistance to extension and extensibility increased and decreased with increasing level of emulsifier, respectively. Romeu et al. (2006) found in dough prepared without MRS and with 0.25% of PS80 and 0.25% of DATEM a higher R_{max} than the control sample, whilst the dough extensibility

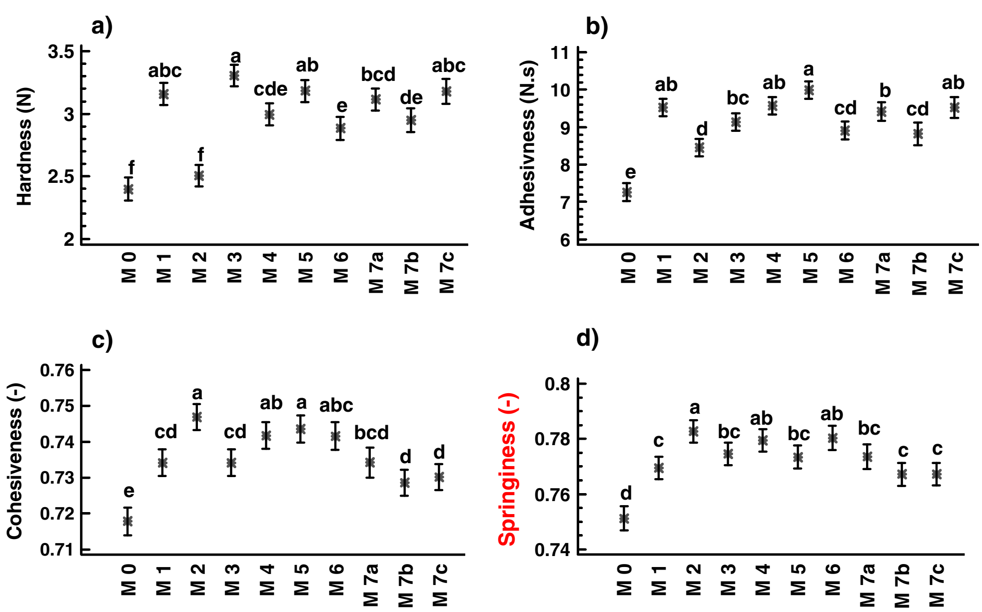

Fig. 2 Textural properties of dough mixtures. Hardness (**a**), adhesiveness (**b**), cohesiveness (**c**) and springiness (**d**). *M* wheat flour–MRS–emulsifier mixtures. Numbers after M are the coded values of each sample according to the simplex centroid design

Table 2 Maximum resistance to extension (R_{max}), work to extension (A) and extensibility (L) obtained from the uniaxial extension of dough

Mixture	R_{max} (N)	A (mm^2)	L (mm)
M0[a]	0.325±0.012 d, e	2.188±0.099 a	18.1±0.5 a
M1	0.464±0.011 a	2.159±0.086 a	15.5±0.4 b, c
M2	0.329±0.011 d, e	1.751±0.086 d, e	15.3±0.4 b, c
M3	0.377±0.012 c	1.926±0.099 b, c, d	15.2±0.5 b, c
M4	0.316±0.011 e	1.648±0.086 e	15.5±0.4 b, c
M5	0.432±0.011 b	2.113±0.086 a, b	15.3±0.4 b, c
M6	0.352±0.011 c, d	1.971±0.086 b, c	16.3±0.4 b
M7a	0.306±0.006 e	1.659±0.050 e	15.4±0.3 b, c
M7b	0.325±0.006 d, e	1.435±0.050 f	15.0±0.3 c
M7c	0.346±0.006 c, d, e	1.892±0.050 c, d	15.3±0.3 b, c
LSD[b]	0.026	0.199	1.007

Different letters in the same column indicate significant differences ($p \le 0.05$)

M wheat flour–MRS–emulsifier mixtures (the numbers after M are the coded values of each sample according to the simplex centroid design)

[a] Control sample

[b] Least significant difference at the 5% level of significance. Difference between two means exceeding this value was significant

(*L*) decreased with 0.5% of SSL, 0.5% of PS80 and with the equiproportional mixture of these three emulsifiers.

Rheofermentation Assays

Table 3 shows the values of parameters obtained from dough development and gaseous release curves of mixtures subjected to the rheofermentation test. High values of maximum height reached (H_m) indicate an optimum dough development; with a greater value of this parameter, greater bread volume would be obtained. These results are expected since the loaf volume is mainly achieved during the fermentation process and progresses during baking. Long development (t_1) and CO_2 retention (t'_1) times are undesirable in industrial processes due to a high energy consumption. High H'_m values indicate that dough is able to retain a great amount of CO_2. Breads obtained in this condition would present high volume and a soft crumb.

M2 (0.5% PS80) and M5 (0.25% SSL+0.25% DATEM) mixtures presented a low dough development with low values of H_m (Table 3). Therefore, bread obtained with these samples would have a small volume. Having taken into account the H_m results obtained for M1 (0.5% SSL) and M3 (0.5% DATEM) mixtures, DATEM might cause a negative effect on M5. M1 the and triple-point mixture (M7a, b and c) reached the maximum values of H_m in the shortest time (t_1).

As observed in dough development, the results obtained from the CO_2 release curves showed that M5 dough presented the lowest value of H'_m (Table 3). This mixture also showed a low value of *tx* (time when dough begins to lose CO_2), which suggests that it not only had little gas retention but also that the dough began to lose CO_2 in less time (73.5 min). However, the triple-point mixture (7a, b and c) showed the highest value of H'_m (67.2±2.0 mm), indicating a positive interaction between the three emulsifiers, as suggested by high the H_m and H'_m values (Table 3). These results showed that the incorporation of SSL, DATEM and PS80 to the dough formulation would develop bread with enhanced rheological properties.

Table 3 Parameters of dough development and CO_2 released from dough

Mixture	Dough development		CO_2 release		
	H_m (mm)	t_1 (min)	H'_m (mm)	t'_1 (min)	tx (min)
M0[a]	42.2	180.0	63.1	126.0	94.5
M1	45.0	160.5	62.4	126.0	93.0
M2	34.5	180.0	62.7	114.0	75.0
M3	40.2	178.5	60.2	127.5	90.0
M4	43.7	171.0	61.3	141.0	103.5
M5	34.4	175.5	58.3	121.5	73.5
M6	37.8	180.0	64.5	126.0	85.5
M7a	45.1	156.0	66.1	130.5	96.0
M7b	44.5	160.0	65.9	133.5	90.0
M7c	44.1	153.5	69.5	128.0	89.0
LSD[b]	2.499	16.291	10.052	13.682	18.811

M wheat flour–MRS–emulsifier mixtures (numbers after M are the coded values of each sample according to the simplex centroid design)

H_m maximum height of dough development (in millimetres), t_1 development time corresponding to H_m (in minutes), H'_m maximum height corresponding to the maximum pressure (in millimetres), t'_1 time corresponding to H'_m (in minutes), *tx* time where dough begins to lose CO_2 (in minutes)

[a] Control sample

[b] Least significant difference at the 5% level of significance. Difference between two means exceeding this value was significant

Rheological Properties of the Optimized Dough

The rheological parameter values obtained from TPA, uniaxial extension and the rheofermentation tests were analysed by ANOVA to evaluate the effect of emulsifiers on these parameters. Mathematical analysis of experimental data was based on the regression of a special cubic model. This model was developed to establish the relationship between the rheological parameter evaluated (Y) and the coded values of independent variables X_1, X_2 and X_3 (emulsifiers) as well as their interactions. In order to simplify the model, it was reformulated by eliminating those terms that did not have a significant effect ($p > 0.05$). The model obtained for each parameter and the respective coefficients of determination are shown in Table 4.

Only for consistency (TPA analysis), extensibility L (uniaxial extension) and maximum height reached by dough H_m (rheofermentation assay) did the predictive models have significance ($p \le 0.05$), with a coefficient of determination (r^2) higher than 90% (Table 4). This last value must be at least 80% for considering that the model has a good degree of adjustment (Guan and Yao 2008).

Consistency is an important parameter due to its relationship with the final bread characteristics. Dough with an excessive hardness and consistency would develop bread with low volume and hard crumb. In such conditions, dough is not able to expand enough during fermentation and will generate bread with low volume. On the other hand, dough with a very low hardness and consistency would generate bread with low volume due to its weak structure; dough will not be able to retain CO_2 during fermentation and will collapse, generating bread with a low volume.

The maximum height of dough development (H_m) is one of the most important parameters to evaluate dough behaviour during the fermentation process. Thus, high H_m values suggest the development of great dough volume reached during fermentation, and consequently, high bread volume can be achieved. For this reason, and according to the results of the predictive model (r^2=99.60%), an optimization of the model considering the H_m parameter was carried out.

The response surface is one of the best ways to visualize the effect of independent variables on the dependent variables. RSM allowed analysing the proposed regression model in order to maximize the H_m variable influenced by different emulsifier concentrations. Figure 3 shows the surface response obtained for the H_m parameter for the proposed mixture of the three emulsifiers (SSL, PS80 and DATEM).

Coded values in the optimum point (H_m=45.5 mm) for SSL, PS80 and DATEM were 0.488, 0.361 and 0.151, respectively. Therefore, decoding these values, the optimum concentrations of each emulsifier obtained were 0.24% of SSL, 0.18% of PS80 and 0.08% of DATEM.

In order to validate the optimum mixture, dough with the quantities of the emulsifiers defined by the model was prepared and rheological tests—TPA, uniaxial extension and rheofermentation analyses—were conducted. Table 5 shows the measured parameters and the comparison of those calculated by the models. The parameters that showed lower percentage relative error were those for the TPA assays. Regarding the H_m, the experimental value was higher than the value calculated mathematically.

Dough prepared with the optimal combination of emulsifiers had a higher development height (H_m) than all the mixtures evaluated (Tables 3 and 5), so the bread prepared with this optimized formulation would have the best physical and textural properties.

Starch Gelatinisation and Retrogradation During Storage

Figure 4 shows a typical gelatinization thermogram obtained for the control dough (M0). In general, all samples tested presented the same thermogram. The gelatinization process showed two endotherms due to water restriction: G peak associated with the starch gelatinization and F peak corresponding to the fusion of the most stable crystallites

Table 4 Predictive models of textural properties (TPA and uniaxial extension assays) adjusted from experimental data from dough prepared with emulsifiers (without yeast) and from the rheofermentation assay (with yeast) according to the simplex centroid design

Assay	Parameter	Predictive equation	p	r^2
TPA	Hardness	H=3.2278×SSL+2.6582×PS80+3.227×DATEM	0.118	50.95
	Consistency	C=15.611×SSL+12.4635×PS80+16.2545×DATEM - 5.237×SSL×PS80 - 3.811×SSL×DATEM+46.2605×SSL×PS80×DATEM	0.003	99.11
	Adhesiveness	A=9.88443×SSL+8.75883×PS80+9.13443×DATEM	0.355	29.21
	Cohesiveness	Cohes=0.734089×SSL+0.745289×PS80+0.733289×DATEM	0.261	36.05
	Elasticity	E=0.768822*SSL+0.779222*PS80+0.775622*DATEM	0.408	25.81
Uniaxial Extension	Maximum resistance	R_{max}=0.446189×SSL+0.352689×PS80+0.344689×DATEM	0.649	13.43
	Extensibility	L=15.5059×SSL+15.2612×PS80+15.2596×DATEM+4.29029×PS80×DATEM - 15.5049×SSL×PS80×DATEM	0.020	91.61
	Work	A=2.10207×SSL+1.85847×PS80+1.81247×DATEM	0.751	9.10
Rheofermentation	H_m	H_m=45.0×SSL+34.65×PS80+40.35×DATEM+15.5×SSL×PS80 - 33.1×SSL ×DATEM+176.102×SSL×PS80×DATEM	0.001	99.60
	t_1	t_1=163.041×SSL+180.656×PS80 – 140.15×DATEM	0.137	63.89
	H'_m	H'_m=61.5035×SSL+62.5162×PS80+59.3162×DATEM+37. 873×PS80×DATEM	0.055	75.55
	t'_1	t'_1=133.0×SSL+118.0×PS80+121.0×DATEM	0.561	17.50
	tx	tx=86.6367×SSL+77.0367×PS80+85.6258×DATEM+85.8904×SSL×PS80	0.167	60.66

*$p \le 0.05$

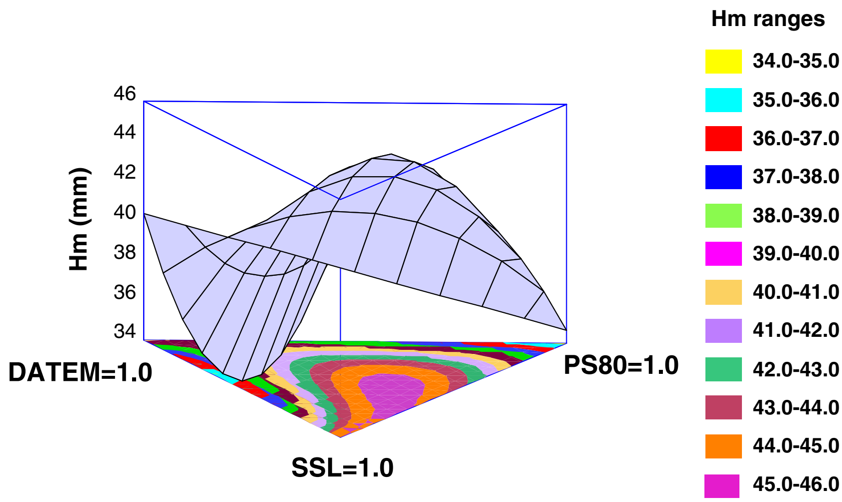

Fig. 3 Response surface of the H_m parameter for the special cubic model of the three-emulsifier (SSL, PS80, DATEM) mixture experiment

(Biliaderis 1980; León et al. 1997; Ribotta et al. 2004). In this study, there was also a third endothermic peak corresponding to the dissociation of the amylose–lipid (AL) complex, widely studied by Jovanovich et al. (1992, 1999).

The gelatinization temperatures of the control dough were 70.2±0.4 °C for the *G* peak and 85.8±2.0 °C for the *F* peak. The gelatinization temperature range was between 62.9±0.5 and 96.8±0.4 °C. The emulsifiers did not change the *G* and *F* peak temperatures. In general, starch retrogradation during storage caused a decrease in the gelatinization temperature between 2 and 4 °C, except for the M1 sample (SSL=0.5%) which increased 10 °C; in the case of *F*, there was an increase of 7–10 °C (data not shown).

During gelatinization, all samples containing emulsifiers showed a decrease in enthalpy values (ΔH) with respect to the control dough (M0). The optimized dough (Mop) was the sample which showed the lowest value (Table 6). This reduction in gelatinization enthalpy could be related to the interaction between the hydrophobic tail of emulsifiers and the amylose helix of starch to form the amylose–emulsifier complex. It could be related as well to the binding of the hydrophilic head of these additives to the amylopectin by hydrogen bridges bonds. Because of this, less water could be absorbed by the starch granules, with a consequent decrease in their gelatinization. Therefore, there would be less gelatinized starch that could be retrograded during storage.

After 1 day of storage, the optimized mixture (Mop) showed the lowest value of retrogradation enthalpy, followed by the triple sample (M7a, b, c) with an average value of 1.72±0.10 J/g dry dough. The same trend was observed after 7 days of storage. At this time of storage, M7 and Mop samples showed the lowest percentages of retrogradation (59% and 63%, respectively). Therefore, the emulsifier's combination in equal (M7) or optimum (Mop) proportions

Table 5 Calculated values of TPA, uniaxial extension and rheofermentation parameters of dough and experimental values of the optimized mixture

Assay	Parameters	Calculated value	Experimental value	Relative error (%)
TPA	Hardness (N)	3.11	3.24±0.26	4.0
	Consistency (N s)	32.1	33.5±3.1	3.3
	Adhesiveness (N s)	9.43	9.41±0.66	−0.2
	Cohesiveness	0.74	0.73±0.01	−1.3
	Elasticity	0.77	0.76±0.01	−1.3
Uniaxial extension	R_{max} (N)	0.37	0.34±0.08	−8.8
	Extensibility (mm)	15.2	14.7±1.4	−3.4
	A (N mm)	1.81	1.66±0.30	−9.0
Rheofermentation	Hm (mm)	45.5	48.2±0.1	5.6
	t_1 (min)	164.6	173.3±5.3	5.0
	H'_m (mm)	63.5	65.8±1.6	3.5
	t'_1 (min)	133.7	111.7±5.3	−19.7
	tx (min)	97.8	85.5±4.2	−14.4

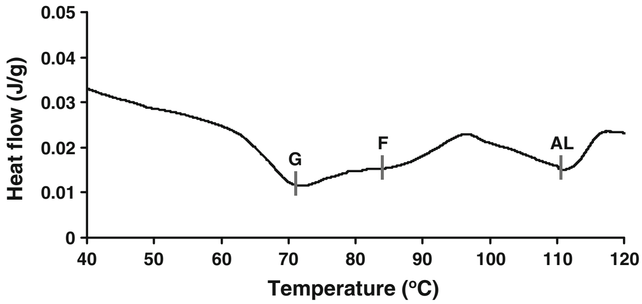

Fig. 4 DSC thermogram of control dough (*M0*). Endotherms: *G* starch gelatinization, *F* fusion of the most stable crystallites, *AL* amylose–lipid complex dissociation

inhibited starch retrogradation more effectively than pure emulsifiers or their binary mixtures did.

All emulsifiers (except M3 sample) caused an increase in the dissociation of amylose–lipid complex enthalpies (Table 6). This increment would be related to the formation of the amylose–emulsifier complex. The optimized mixture (Mop) presented the highest value (1.85 J/g dry dough), showing that this combination of emulsifiers would improve the formation of the amylose–emulsifier complex.

During 1 day storage, in all samples, a decrease in dissociation enthalpy of the amylose–lipid complex was observed (Table 6). After 7 days, the enthalpy increased. This behaviour could be attributed to the manner in which amylose associates with emulsifiers. In the starch gelatinization process (day 0), the amylose dissociates from the lipid during complex melting. After the first day of storage, some of these amylose molecules become available to rejoin the complex. Longer storage time (7 days) favours the formation of high amounts of complex (Table 6).

Bread Quality Properties

Quality evaluation of bread was assessed through bread specific volume and crumb moisture and firmness measurements. An increase in bread volume due to the incorporation of SSL, PS80 and DATEM was observed. Specific volume of the bread prepared with the optimized mixture (6.14 cm^3 g^{-1}) was 68.7% greater than the volume obtained for the control bread (3.64 cm^3 g^{-1}), although both bread crumbs presented the same moisture content, 46.1% for the optimized (Bop) and 46.4% for the control bread (B0). Crumb firmness of Bop was 72.8% lower (0.72 N) than that obtained for B0 (2.65 N). This decrease could be attributed to the emulsifier improvement effect on bread crumb structure that generated a soft crumb texture.

According to the results obtained in this last section, the optimum combination of emulsifiers found by an optimized analysis resulted in bread with enhanced quality.

Conclusions

Resistant starch is a nutritional ingredient commonly used as the dietary fibre in bread making. The partial substitution of wheat flour by maize-resistant starch (MRS) affects the bread quality due to gluten protein dilution and produce changes in water absorption. The results obtained in this work highlight the need of incorporating emulsifiers for improving dough and bread quality. Therefore, in order to obtain a high-quality product, dough formulation was adjusted by adding three different types of emulsifiers (SSL, DATEM and PS80). The SSL–DATEM mixture increased hardness and resistance to extension on dough, whilst dough containing PS80 was softer, an unfavourable condition for bread making; nevertheless, both kinds of dough retained less CO_2.

Only a specific combination of emulsifiers gave proper results, the one that achieved a balance between gluten reinforcement (moderate hardness and consistence, high R_{max}) produced by SSL and/or DATEM and dough softening (low dough hardness) induced by PS80. This optimum combination (0.24% SSL, 0.18% PS80, 0.08% DATEM) not only improved the rheological properties of dough and

Table 6 Gelatinization, retrogradation and amylose–lipid complex dissociation enthalpies

ΔH (J/g dry dough)		M0	M1	M2	M3	M4	M5	M6	M7a	M7b	M7c	Mop	LSD[a]
Gelatinization	Day 0	5.71	4.88	4.94	4.90	4.89	4.85	4.71	4.86	4.65	4.81	4.08	0.54
	Day 1	2.38	2.17	1.81	2.00	2.29	2.24	1.77	1.62	1.76	1.80	1.59	0.47
	Day 7	4.38	3.81	3.47	4.27	3.63	3.73	3.73	2.84	2.82	2.77	2.57	0.16
Amylose–lipid complex	Day 0	1.20	1.58	1.31	1.19	1.25	1.61	1.43	1.50	1.49	1.62	1.85	0.35
	Day 1	0.25	0.66	0.27	0.40	0.42	0.78	0.44	0.36	0.51	0.52	0.47	0.44
	Day 7	0.62	0.69	0.65	0.56	0.46	0.99	0.64	0.52	0.66	0.61	0.60	0.35

M wheat flour–MRS–emulsifier mixtures (numbers after M are the coded values of each sample according to the simplex centroid design)

[a] Least significant difference at the 5% level of significance. Difference between two means exceeding this value was significant

crumb firmness but also increased bread volume and contributed to extend the shelf-life of product pieces.

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References

Aamodt, A., Magnus, E. M., & Færgestad, E. M. (2003). Effect of flour quality, ascorbic acid and DATEM on dough rheological parameters and hearth loaves characteristics. *Journal of Food Science, 68*, 2201–2210.

Aamodt, A., Magnus, E. M., & Færgestad, E. M. (2005). Hearth bread characteristic: Effect of protein quality, protein content, whole meal flour, DATEM, proving time, and their interactions. *Cereal Chemistry, 82*(3), 290–301.

Aamodt, A., Magnus, E. M., Hollung, K., Uhlen, A. K., & Færgestad, E. M. (2005). Dough and hearth bread characteristics influenced by protein composition, protein content, DATEM, and their interactions. *Journal of Food Science, 70*, 214–221.

Åkerberg, A., Liljeberg, H., & Björck, I. (1998). Effect of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. *Journal of Cereal Science, 28*, 71–80.

AACC. (2000). Approved methods of the AACC, 10th ed. Methods 44-19, 54-21, 74-09. American Association of Cereal Chemists: St. Paul, MN.

Arntfield, S., Ismond, M., & Murray, E. (1990). Thermal analysis of food. In V. R. Harwalkar & C. Y. Ma (Eds.), *Thermal analysis of food proteins in relation to processing effects* (pp. 51–91). New York: Elsevier Science.

ASP, N. G. (1992). Preface: Resistant starch. *European Journal of Clinical Nutrition, 46*, 51.

Baik, M. Y., & Chinachoti, P. (2000). Moisture redistribution and phase transition during bread staling. *Cereal Chemistry, 77*, 484–488.

Biliaderis, C., Maurice, T., & Vose, J. (1980). Starch gelatinization phenomena studied by differential scanning calorimetry. *Journal of Food Science, 45*, 1669–1674.

Brandt, L. (1996). Emulsifiers in baked goods—Applications. Retrieved from http://www.foodproductdesign.com/archive/1996/0296AP.html. Accessed 26 Dec 2011.

Eerlingen, R. C., & Delcour, J. A. (1995). Formation, analysis, structure and properties of type III enzyme resistant starch. *Journal of Cereal Science, 22*, 129–138.

Gelencsér, T., Gál, V., Hódsági, M., & Salgó, A. (2008). Evaluation of quality and digestibility characteristics of resistant starch-enriched pasta. *Food and Bioprocess Technology, 1*, 171–179.

Gómez, M., Del Real, S., Rossell, C. M., Ronda, F., Blanco, C. A., & Caballero, C. A. (2004). Functionality of different emulsifiers on the performance of breadmaking and wheat bread quality. *European Food Research and Technology, 219*(2), 145–150.

Goñi, I., García-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*, 427–437.

Guan, X., & Yao, H. (2008). Optimization of viscozyme L-assisted extraction of oat bran protein using response surface methodology. *Food Chemistry, 106*, 345–351.

Hassan, A. G., Azizi, M. H., Barzegar, M., & Ameri, M. A. (2006). Effect of selected hydrocolloids on bread staling as evaluated by DSC and XRD. *Journal of Food Technology, 4*(3), 185–188.

Hung, P. V., & Morita, N. (2004). Dough properties and bread quality of flour supplemented with cross-linked cornstarches. *Food Research International, 37*, 461–467.

Hung, P. V., Yamamori, M., & Morita, N. (2005). Formation of enzyme-resistant starch in bread as affected by high-amylose wheat flour substitutions. *Cereal Chemistry, 82*(6), 690–694.

Jovanovich, G., Zamponi, R. A., Lupano, C. E., & Añón, M. C. (1992). Effect of water content on the formation and dissociation of the amylose–lipid complex in wheat flour. *Journal of Agricultural and Food Chemistry, 40*, 1789–1793.

Jovanovich, G., & Añón, M. C. (1999). Amylose–lipid complex dissociation. A study of the kinetic parameters. *Biopolymers, 49*(1), 81–89.

Karim, A. A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch retrogradation. *Food Chemistry, 71*, 9–36.

Koocheki, A., Mortazavi, S. A., Mahalati, M. N., & Karimi, M. (2009). Effect of emulsifiers and fungal-amylase on rheological characteristics of wheat dough and quality of flat bread. *Journal of Food Process Engineering, 32*(2), 187–205.

Lakshminarayan, S. M., Rathinam, V., & KrishnaRau, L. (2006). Effect of maltodextrin and emulsifiers on the viscosity of cake batter and on the quality of cakes. *Journal of the Science of Food and Agricultural, 86*(5), 706–712.

León, A. E., Duran, E., & Benedito de Barber, C. (1997). A new approach to study starch changes occurring in the dough-baking process and during bread storage. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung A, 204*, 316–320.

Matsuda, L.Y. (2007). Concentração de amido resistente em pão francês pré-assado congelado: Aspectos tecnológicos. MS thesis, Escola Politécnica, Universidade de São Paulo (SP, Brasil), 111 pp.

Matuda, T.G. (2008). Estudo do congelamento da massa de pão: Determinação experimental das propriedades termofísicas e desempenho de panificação. PhD thesis, Food Engineering Laboratory, Escola Politécnica, University of São Paulo, São Paulo, Brazil.

Matuda, T. G., Chevallier, S., de Alcântara Pessoa, P., LeBail, A., & Tadini, C. C. (2008). Impact of guar and xanthan gums on proofing and calorimetric parameters of frozen bread dough. *Journal of Cereal Science, 48*, 741–746.

Michniewicz, J., Biliaderis, C. G., & Bushuk, W. (1991). Effect of added pentosans on some properties of wheat bread. *Food Chemistry, 43*, 251–257.

Morita, N., Maeda, T., Miyazaki, M., Yamamori, M., Miura, H., & Ohtsuka, I. (2002). Dough and baking properties of high-amylose and waxy wheat flours. *Cereal Chemistry, 79*(4), 491–495.

Myers, R. H. & Montgomery, D.C. (1995). Experiments with mixtures. In: *Response surface methodology, process and product optimization using designed experiments* (pp. 535–623). New York: Wiley Interscience.

Nugent, A. P. (2005). Health properties of resistant starch. British Nutrition Foundation. *Nutrition Bulletin, 30*, 27–54.

Queiroz-Monici, K. S., Costa, G. E. A., Silva, N., Reis, S. M. P. M., & Oliveira, A. C. (2005). Bifidogenic effect of dietary fiber and resistant starch from leguminous on the intestinal microbiota of rats. *Nutrition, 21*, 602–608.

Ravi, R., Sai Manohar, R., & Haridas, R. (2000). Influence of additives on the rheological characteristics and baking quality of wheat flours. *European Food Research and Technology, 210*, 202–208.

Ribotta, P., Pérez, G. T., León, A., & Añón, M. C. (2004). Effect of emulsifier and guar gum on micro structural, rheological and baking performance of frozen bread dough. *Food Hydrocolloids, 18*, 305–313.

Ribotta, P. D., Pérez, G. T., Añón, M. C., & León, A. E. (2010). Optimization of additive combination for improved soy–

wheat bread quality. *Food and Bioprocess Technology, 3*, 395–405.

Romeu, C. C., Tadini, C. C., & Matuda, T. G. (2006). Influência do congelamento na estrutura da massa do pão francês. *PIC-EPUSP, 3*, 1816–1845.

Rosin, P. M., Lajolo, F. M., & Menezes, E. W. (2002). Measurement and characterization of dietary starches. *Journal of Food Composition and Analysis, 15*, 367–377.

Selomulyo, V. O., & Zhou, W. (2007). Frozen bread dough: Effect of freezing storage and dough improvers. *Journal of Cereal Science, 45*(1), 1–17.

SMS (Stable Micro Systems). (1995). TA-XT2i application study, extensibility of dough and measure of gluten quality. Texture expert guide contents.

Stampfli, L., & Nersten, B. (1995). Emulsifiers in breadmaking. *Food Chemistry, 52*(4), 353–360.

Tronsmo, K. M., Magnus, E. M., Baardseth, P., Schofield, J. D., Aamodt, A., & Færgestad, E. M. (2003). Comparison of a small and large deformation rheological properties of wheat dough and gluten. *Cereal Chemistry, 80*(5), 587–595.

Yamada, Y., Hosoya, S., Nishimura, S., Tanaka, T., Kajimoto, Y., Nishimura, A., & Kajimoto, O. (2005). Effect of bread resistant starch on postpandrial blood glucose levels in humans. *Bioscience, Biotechnology, and Biochemistry, 69*(3), 559–566.

Zaidel, D. N. A., Chin, N. L., & Yusof, Y. A. (2010). A review on rheological properties and measurements of dough and gluten. *Journal of Applied Science, 10*(20), 2478–2490.