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Evaluating Effect of Fat, Sugar and Flour Substitutes on Properties of White Slice Dairy Bread

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***Abstract*—**White slice dairy bread (WSDB) is the most important industrial bread consumed in Argentina, it results an interesting target for developing a reduced calorie bread as it contains fat and sugar in its formulation. However, substitutions of ingredients should be carefully studied to keep original properties of the bread. The aim of this study was to evaluate the effects of three commercial products, Toler Fat Less Saladas (TFLS) as fat substitute, Granofiber Sweet (GS) as sugar substitute and Granofiber Sym 200 (GS200) as flour substitute, in order to improve nutritional profile of WSDB. The rheological properties, evaluated by Rheofermentograph and Mixolab, were studied on flour and on white slice dairy bread formulation. Additionally, texture profile analysis was applied on baked bread. TFLS caused a similar effect to fat. GS showed less gas production than sugar during fermentation stage, however baked bread presented similar loaf specific volume. Substitution of flour with GS200 revealed significant decrease of fermentation capacity and dough development. Otherwise, bread loaf specific volume showed satisfactory results when GS200 was applied, allowing to formulate a high fiber bread with similar end-product texture properties to original bread. We conclude that substitutes evaluated in this work can be used for breadmaking to improve nutritious quality of bread for health benefits.

Keywords—White bread, fat substitute, sugar substitute, flour substitute, dough rheology, bread texture.

I. INTRODUCTION

Improving nutritional profile food is a growing area of interest in the food industry, due to there being a raising awareness toward healthy foods. Furthermore, knowledge about the relationship between food, its physiological function and diseases is increasing, particularly in obesity, diabetes, cardiovascular diseases and some types of cancer [1, 2, 3, 4]. Therefore, food industry works with healthcare professionals, scientific community, government, and media in order to ensure that the public has accurate information on healthy food [5]. Accordingly, the improvement of the nutritional quality of bread represents an interesting alternative to accompany the planning of food policies for healthy lifestyles, due bread is one of the most consumed food products in the world, in Argentina 70 it being kg / inhab / year [6]. Particularly white slice dairy bread (WSDB) is the most important industrial bread consumed in Argentina, and it contains fat and sugar in its formulation, resulting in an interesting target to reduce calory content.

Knowing the functions and responses of substitutes in dough, it is very important to establish when reducing or removing it from the baking products. Substitutes result in the induction of different bread properties; hence, these effects study should be considered in order to preserve the original quality parameters required for bread production. Bread may be successfully prepared with a reduction of fat, sugar and flour, this modification may fit into many calorie-restricted diets and the product will be similar than the unmodified original counterpart. Thus, this research aims to evaluate the effect of fat, sugar and flour substitution on fermentative and rheological properties on flour and then on WSDB. Toler Fat Less Saladas (TFLS) as fat substitute, Granofiber Sweet (GS) as sugar substitute, Granofiber Sym 200 (GS200) as flour substitute, were respectively tested in order to validate their replacement capabilities to formulate bread with better nutritional quality than originally formulated.

Manuscript is organized as follows. Section I contains the introduction of the background of this investigation. Section II focuses in the process involved in the bread production and the properties of each stage. Section III provides an explanation on the determination of the fermentative and rheological properties of flour and bread formulations, including preparation of bread and bread baking quality. Section IV discusses the effects of substitutes on flour and bread properties. Section V provides the concluding remarks.

II. RELATED WORK

Several works focused on the development of healthy bread [7, 8, 9, 10, 11]. The bread production process is highly complex and includes a series of parameters that must be regulated. Thus, all steps in the bread production are important to analyse when a substitute is added. Initially, mixing involves hydration of the compounds, alignment and stretching of the proteins, which lead to the formation of a three-dimensional viscoelastic structure stage. Kneading process ensures dough formation and weakening [12]. Then, CO_2 production plays the mean role in the fermentation stage. It is produced biologically by yeast. Gas fills an expands air cell and the gluten structure. Subsequently, baking includes several aspects as weakening proteins, gelatinization and stability of the starch gel when heated [13]. Finally, starch retrogrades when dough temperature is decreased. The measurement of retrogradation can be correlated with the staling phenomena in bread [14]. Rheological parameters are used in the prediction of the behavior of wheat dough during bread production and of the final product quality. Hence, in this study we have focused the effect of substitute in each stage of bread process.

III. METHODOLOGY

Substitute ingredients. In this study three commercial products (Granotec Argentina) were evaluated, Toler Fat Less Saladas (TFLS, blend of modified starch, wheat fibres, soy lecithin) as fat substitute, Granofiber Sweet (GS, bland of polidextrose, inuline, sucralose) as sugar substitute, Granofiber Sym 200 (GS200, blend of wheat fibres) as flour substitute.

Flour characterization. Flour used in this study has been characterized applying the following methods: Humidity (ISO 712) [15], ashes (AOAC 923.03) [16], gluten (AACC 38-12) [17], falling number (AACC 56-81B) [18], alveograph (AACC 54-30A) [19], Mixolab (modified method AACC 54-60.01) [20], damaged starch (AACC 76-33) [21].

Determination of the fermentative and rheological properties of formulations. The effect of fat, sugar or flour substitutes were evaluated in the fermentation stage and doughs rheological behavior, comparing TFLS, GS, GS200 in replacement of fat, sugar, flour, respectively. To do this, first they were analyzed on doughs flour, then on doughs WSDB. Assay conditions are shown in Table 1. Doughs obtained in a bakery mixer (model A-120T, Hobart, USA) were tested in a Reofermentograph F3 (Chopin, France) to study fermentation stage and in Mixolab to determine dough consistency. In case of doughs flour, they were kneaded for 1 min at slow speed and 4 min at medium speed, with 55% hydration, then 315 g of dough were tested in the Reofermentograph, applying 2 kg weights over dough, at 28 ºC for 3 h [22]. In case of the bread formulation, they have kneaded for 1 min at slow speed, 2 min at medium speed and 3 min at fast speed, with 65% hydration, then 200 g of dough were tested in the Reofermentograph, applying 2 kg weights over dough, at 28 ºC for 3 h. Fermentation assays allowed to obtain mass development and gas evolution curves, with their specific parameters. Additionally, 75 g dough was analyzed in Mixolab (Chopin, France) to determine instant dough consistency (C1, Nm) at 100 rpm for all cases [23]. This parameter was obtained in order to verify that the fermentation conditions, in terms of consistency, were similar in all cases and therefore validating the tests.

The rheological characteristics of dough were measured using Mixolab according to modified AACCI Approved Methods 54-60.01 [20]. 50 g Flour (14% moisture basis) was added up to 75 g with distilled water. Mixing speed was 80 r min–1, peak torque was maintained as (1.1±0.09) Nm for dough development and initial mixing was for 22 min. Results were analyzed by Chopin Mixolab software (Version 3.14, Chopin, France).

Table 1. Assay conditions to evaluate the effects of TFLS, GS, GS200 on flour and WSDB.

Control	*Reference*	*Substitution*
Flour (F)	Flour + fat (3%) (F+F)	Flour + TFLS (0.6% + 2.4% water) (F+TFLS)
Bread without fat (WSDB-F)	Bread (WSDB)	Bread +TFLS (0.6% + 2.4% water) (WSDB +TFLS)
Flour (F)	Flour + sugar (7,5%) (F+S)	Flour + GS (7.5%) (F+GS)
Bread without sugar (WSDB -S)	Bread (WSDB)	Bread + GS (7.5%) (WSDB +GS)
Flour (F)		Flour + GS200 (3%) (F+GS200 3)
Flour (F)		Flour + GS200 (6%) (F+GS200 6)
	Bread (WSDB)	Bread + GS200 (3%) (WSDB +GS200 3)
	Bread (WSDB)	Bread + GS200 (6%) (WSDB +GS200 6)

Preparation of bread. Formulation for WSDB was: 1 kg flour, 12 g dry yeast, 20 g salt, 75 g sugar, 30 g of vegetable oil, 20 g milk powder, 10 g wheat gluten, 3.5 g calcium propionate, 15 g Toler Miga Bollo Directo (blend of ascorbic acid and enzymes, Granotec Argentina), 650 ml water. Substitutions were carried out as in Table 1. All ingredients were kneaded for 1 min at slow speed, 2 min at medium speed and 3 min at fast speed in a bakery mixer (model A-120T, Hobart, USA). The dough obtained was divided into 500 g portions of spherical shape which were left to rest for 10 min. Then, doughs were passed through a dough pressing machine (model 0203, Indupan, Argentina). Subsequently, pieces were rolled down like tube shape and placed into pans (20 cm length, 10 cm width, 10 cm height). For each formulation, two sequence of three pans were placed in the fermentation camera at 36 ºC for 90 min, RH = 80%. Three loaves of bread were baked in an oven (RPO4A10-2, Eurofours, France) at 150 ºC with lidded pans for 35 min and another three loaves of bread were baked without the lids for 40 min. Loaves baked without the lids were left to cool to determine specific volume. Otherwise cool loaves baked with the lids were packed and stored at room temperature until texture analysis were performed 5, 10 and 15 days after baking.

Bread baking quality. Loaves volume were measured by rapeseed displacement according to AACC 10-05 method [24], using bread loaf volumeter equipment (Chopin, France). Three loaves of each formulation were tested. Specific volume of the loaves was calculated from the measured volume and weight, obtained by direct measure. Otherwise, texture profile analysis was analyzed in order to study the structure of the crumb. It was carried out using QTS Farnel Texture Analyser (Brookfield). Crumb firmness was determined according to the method AACC 74-09 [25]. Slices (25 mm-thickness) were compressed with a 36 mm diameter cylindrical probe at a speed of 2 mm/s until a deformation, to a total deformation of 10 mm and a trigger force of 4 g were the selected settings. Springiness parameter was determined by texture profile analysis (TPA). Bread slices (50 mm-thickness) were compressed twice using a 25.4 mm diameter cylindrical probe (TA 11) and a test speed of 1.0 mm/s; to a total deformation of 15 mm and a trigger force of 4 g were the selected settings. Bread slices (50 mm-thickness) were compressed twice to give a TPA from which springiness textural parameter was obtained [26]. Crumb firmness and springiness textural parameters were obtained through Textute Pro v. 2.1 software. The test was carried out at different times of storage (5, 10 and 15 days) in order to evaluate bread aging.

Statistical analysis. All assays were performed in triplicate and analyzed by Microsoft Excel 2010 software. Significant differences were determined at $p < 0.05$ by analysis of variance (ANOVA) and Tukey's HSD test. The analyses were performenced using the software Statgraphics Centurion XVII (Statpoint Technologies, USA).

IV. RESULTS AND DISCUSSION

Wheat flour characterization. Argentinian wheat flour used in this study was characterized. The results obtained were humidity 14.22 %, ashes 0.637 %, wet gluten 28.80 %, index gluten 99 %, dry gluten 10.58 %, falling number 415 s, damaged starch 9 %. The alveograph parameters were tenacity/extensibility, P/L: 1.20; and deformation work, W: 307 10-4 J. Departure time and stability of dough were also measured by Mixolab, which were 10.26 min and 14.00 min respectively.

Evaluation of fat substitute. The effect of TFLS was evaluated in comparison with fat on the flour dough, and then on WSDB. The rheological properties studied on flour dough involved Rheofermentograph and Mixolab analysis (Table 2). Rheofermentograph data showed that addition of fat or TFLS to base flour dough significantly increased ($p<0.05$) the amount of gas produced (V_T) during the analysis. Lower amount of gas was lost by the dough (V_L) and higher quantity of gas was retained (VR) in F+F and in F+TFLS. However, coefficients of gas retention (V_R/V_T) and maximum height of gas production curve (H'_m) resulted similar ($p > 0.05$) to F. Besides, F+F and in F+TFLS caused no effect ($p>0.05$) on dough development (H_m, h) comparing to F.

Table 2. Effect of fat (F+F) and TFLS (F+TFLS) on fermentative and rheological attitudes of flour.

	F	*F+F*	*F+TFLS*
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	$(1604±8)^a$	$(1654±12)^b$	$(1679±13)^b$
V_R (volume of gas retained, mL)	$(1256±10)^a$	$(1302±8)^b$	$(1344±7)^c$
V_L (volume of gas lost, mL)	$(348±9)^a$	$(352±8)^a$	$(335±9)^a$
V_R/V_T (coefficient of gas retention, %)	$(78±1)^a$	$(79±2)^a$	$(80±2)^a$
H'_m (maximum height of gas production curve, mm)	$(54±1)^a$	$(52±1)^a$	$(54±1)^a$
T_x (time needed to start losing gas, min)	$(81±1)^a$	$(96±2)^b$	$(90±4)^b$
Curve of dough development			
H_m (maximum dough height, mm)	$(31±1)^a$	$(31±1)^a$	$(33±2)^a$
H (dough height after 3 h, mm)	$(30±1)^a$	$(31±1)^a$	$(33±2)^a$
Dough consistency (Nm)	$(2.87±0.19)^a$	$(2.73±0.10)^a$	$(2.66±0.12)^a$
T_x (time needed to start losing gas, min)	$(81±1)^a$	$(96±2)^b$	$(90±4)^b$
Mixolab			
WA (water absorption, %)	$(58.0±0.1)^a$	$(58.8±0.7)^a$	$(58.0±0.1)^a$
Stability (min)	$(13.5±0.7)^a$	$(12.1±0.7)^a$	$(15.2±0.4)^b$
C2 (protein weakening, Nm)	$(0.45±0.02)^a$	$(0.35±0.02)^b$	$(0.46±0.01)^a$
C3 (starch gelatinization, Nm)	$(1.83±0.03)^a$	$(1.84±0.02)^a$	$(1.93±0.02)^b$
C3-C2 (starch gelatinization range, Nm)	$(1.38±0.02)^a$	$(1.49±0.02)^b$	$(1.47±0.01)^b$
C4 (hot gel stability, Nm)	$(1.76±0.04)^a$	$(1.79±0.02)^b$	$(1.87±0.04)^b$
C4-C3 (cooking stability range, Nm)	$(-0.07±0.04)^a$	$(-0.05±0.02)^a$	$(-0,06±0.04)^a$
C5 (starch retrogradation in the cooling phase, Nm)	$(3.21±0.02)^a$	$(3.23±0.04)^a$	$(3.26±0.04)^a$
C5-C4 (gelling, Nm)	$(1.45±0.02)^a$	$(1.44±0.04)^b$	$(1.39±0.04)^b$

Means with different letters in each row are statistically different (P<0.05).

Mixolab rheological behaviour of dough showed that fat and TFLS did not affect water absorption (WA) (Table 2). Dough stabilities were similar between F and F+F, but F+TFLS registered a significantly increased ($p<0.05$) of stability parameter. Besides, a significant difference ($p<0.05$) was observed in the protein weakening pattern value (C2) between F+F and F+TFLS. The highest values ($p<0.05$) of the starch gelatinization range (C3–C2) were for F+F and F+TFLS. The cooking stability range (C4-C3) and the gelling range (C4-C5) had no significant differences between the treatments.

Table 3. Effect of fat (WSDB) and TFLS (WSDB+TFLS) on fermentative and texture attitudes comparing to bread without fat (WSDB-F).

	WSDB-F	***WSDB***	***WSDB+TFLS***
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	$(662±1)^a$	$(659±2)^a$	$(663±3)^a$
V_R (volume of gas retained, mL)	$(660 ± 2)^a$	$(655 ± 2)^a$	$(660±1)^a$
V_L (volume of gas lost, mL)	$(2 ± 1)^a$	$(4 ± 2)^a$	$(3 ± 2)^a$
V_R/V_T (coefficient of gas retention, %)	$(99±1)^a$	$(99±1)^a$	$(99±1)^a$
H'_m (maximum height of gas production curve, mm)	$(42±1)^a$	$(42±1)^a$	$(40±2)^a$
T_x (time needed to start losing gas, min)	-	-	-
Curve of dough development			
H_m (maximum dough height, mm)	$(39±1)^a$	$(37±2)^a$	$(37±1)^a$
H (dough height after 3 h, mm)	$(37±1)^a$	$(37±2)^a$	$(37±1)^a$
Dough consistency (Nm)	$(1.77±0.10)^a$	$(1.79±0.08)^a$	$(1.80±0.05)^a$
Bread loaf specific volume (g/mL)	$(5.75±0.03)^a$	$(5.80±0.02)^a$	$(5.76±0.02)^a$
Texture profile analysis			
Crumb firmness (g)			
5 (days)	$(464±9)^a$	$(400±6)^b$	$(413±7)^b$
10 (days)	$(902±7)^a$	$(852±6)^b$	$(861±4)^b$
15 (days)	$(1394±17)^a$	$(1358±20)^a$	$(1369±12)^a$
Springiness			
5 (days)	$(0.91±0.01)^a$	$(0.90±0.01)^a$	$(0.92±0.01)^a$
10 (days)	$(0.92±0.01)^a$	$(0.92±0.01)^a$	$(0.92±0.01)^a$
15 (days)	$(0.92±0.01)^a$	$(0.92±0.01)^a$	$(0.93±0.01)^a$

Means with different letters in each row are statistically different ($P<0.05$).

Subsequently, TFLS was studied in the WSDB formulation. In the Rheofermentograph analysis (Table 3), the results obtained from development and gas release curves corresponding to WSDB-F, WSDB and WSDB+TFLS had no significant differences between them. Additionally, bread loaf specific volume values did not show significant differences between the three cases. Through textural parameters of experimental bread, it was noticed that the springiness was very similar in all products, whereas the crumb firmness was lower ($p<0.05$) for WSDB+F and WSDB +TFLS than for B-F (Table 3). These effect of fat and TFLS on crumb firmness was noticed at 5 and 10 days.

Evaluation of sugar substitute. Fermentative and rheological characteristics of doughs were evaluated to study the influence of supplementation with sugar (7.5%) and GS (7.5%) on flour and on WSDB. Rheofermentograph results (Table 4) showed that F+S significantly increased ($p<0.05$) V_T on dough flour.

Table 4. Effect of sugar (F+S) and GS (F+GS) on fermentative and rheological attitudes of flour.

	F	***F+S***	***F+GS***
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	$(1604±8)^a$	$(1759±5)^b$	$(1725±5)^c$
V_R (volume of gas retained, mL)	$(1256±10)^a$	$(1583±5)^b$	$(1535±7)^c$
V_L (volume of gas lost, mL)	$(348±9)^a$	$(176±5)^b$	$(190±6)^b$
V_R/V_T (coefficient of gas retention, %)	$(78±1)^a$	$(90±2)^a$	$(89±2)^a$
H'_m (maximum height of gas production curve, mm)	$(54±1)^a$	$(57±2)^a$	$(55±2)^a$
T_x (time needed to start losing gas, min)	$(81±1)^a$	$(69±2)^b$	$(70±2)^b$
Curve of dough development			
H_m (maximum dough height, mm)	$(31±1)^a$	$(40±1)^b$	$(39±2)^b$
H (dough height after 3 h, mm)	$(30±1)^a$	$(40±1)^b$	$(38±2)^b$
Dough consistency (Nm)	$(2.87±0.19)^a$	$(2.57±0.15)^a$	$(2.48±0.22)^a$
T_x (time needed to start losing gas, min)			
Mixolab			
WA (water absorption, %)	$(58.0±0.1)^a$	$(49.8±0.2)^b$	$(50.0±0.1)^b$
Stability (min)	$(13.5±0.7)^a$	$(12.1±0.8)^a$	$(24.0±0.5)^b$
C2 (protein weakening, Nm)	$(0.45±0.02)^a$	$(0.35±0.02)^b$	$(0.40±0.05)^{ab}$
C3 (starch gelatinization, Nm)	$(1.83±0.03)^a$	$(1.84±0.02)^a$	$(1.78±0.04)^a$
C3-C2 (starch gelatinization range, Nm)	$(1.38±0.02)^a$	$(1.50±0.02)^b$	$(1.38±0.04)^a$
C4 (hot gel stability, Nm)	$(1.76±0.02)^a$	$(1.98±0.04)^b$	$(2.01±0.05)^b$
C4-C3 (cooking stability range, Nm)	$(-0.07±0.04)^a$	$(0.14 ± 0.04)^b$	$(0.23±0.05)^b$

	F	***F+S***	***F+GS***
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	(1604±8)^{a}	(1759±5)^{b}	(1725±5)^{c}
V_R (volume of gas retained, mL)	(1256±10)^{a}	(1583±5)^{b}	(1535±7)^{c}
V_L (volume of gas lost, mL)	(348±9)^{a}	(176±5)^{b}	(190±6)^{b}
V_R/V_T (coefficient of gas retention, %)	(78±1)^{a}	(90±2)^{a}	(89±2)^{a}
H'_m (maximum height of gas production curve, mm)	(54±1)^{a}	(57±2)^{a}	(55±2)^{a}
C5 (starch retrogradation in the cooling phase, Nm)	(3.21±0.02)^{a}	(3.43±0.04)^{b}	(3.42±0.04)^{b}
C5-C4 (gelling, Nm)	(1.45±0.02)^{a}	(1.49±0.04)^{a}	(1.41±0.04)^{a}

Means with different letters in each row are statistically different (P<0.05).

Although F+GS significantly increased ($p < 0.05$) V_T comparing to F, it was lower than F+S. Also it was noticed that three conditions had similar V_R/V_T and H'm. Dough development showed higher values (H_m, h) (p<0.05) for F+S and F+GS comparing to F. The addition of sugar and GS significantly decreased (p<0.05) WA of the flour dough in Mixolab assay (Table 4). Dough stability was significantly prolonged (p<0.05) by the addition GS (F+GS) in relation to F and F+S. Also F+S indicated that C2 value decreased and C3-C2 increased in comparison to the control (p<0.05). Parameter C4-C3 had the highest values for F+S and F+GS. The set back C4-C5 had no significant differences between the treatments.

Table 5. Effect of sugar (WSDB) and GS (WSDB+GS) on fermentative and texture attitudes comparing to bread without sugar (WSDB-S).

	WSDB-S	***WSDB***	***WSDB+GS***
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	(562±1)^{a}	(688±2)^{b}	(698±2)^{b}
V_R (volume of gas retained, mL)	(560±2)^{a}	(582±2)^{b}	(681±1)^{c}
V_L (volume of gas lost, mL)	(2±2)^{a}	(6±2)^{a}	(14±2)^{b}
V_R/V_T (coefficient of gas retention, %)	(99±1)^{a}	(99±1)^{a}	(98±1)^{a}
H'_m (maximum height of gas production curve, mm)	(32±1)^{a}	(40±1)^{b}	(39±1)^{b}
T_x (time needed to start losing gas, min)	-	-	-
Curve of dough development			
H_m (maximum dough height, mm)	(29±1)^{a}	(37±2)^{b}	(35±2)^{b}
H (dough height after 3 h, mm)	(27±1)^{a}	(37±2)^{b}	(35±2)^{b}
Dough consistency	(1.77±0.1	(1.79±0.1	(1.58±0.09

	WSDB-S	***WSDB***	***WSDB+GS***
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	(562±1)^{a}	(688±2)^{b}	(698±2)^{b}
V_R (volume of gas retained, mL)	(560±2)^{a}	(582±2)^{b}	(681±1)^{c}
V_L (volume of gas lost, mL)	(2±2)^{a}	(6±2)^{a}	(14±2)^{b}
V_R/V_T (coefficient of gas retention, %)	(99±1)^{a}	(99±1)^{a}	(98±1)^{a}
H'_m (maximum height of gas production curve, mm)	(32±1)^{a}	(40±1)^{b}	(39±1)^{b}
(Nm)	5)^{a}	6)^{a}	)^{a}
Bread loaf specific volume (g/mL)	(5.60±0.03)^{a}	(5.95±0.02)^{b}	(5.92±0.02)^{b}
Texture profile analysis			
Crumb firmness (g)			
5 (days)	(462±7)^{a}	(471±8)^{a}	(470±5)^{a}
10 (days)	(905±5)^{a}	(898±8)^{a}	(908±4)^{a}
15 (days)	(1410±15)^{a}	(1425±10)^{a}	(1418±11)^{a}
Springiness			
5 (days)	(0.92±0.01)^{a}	(0.92±0.01)^{a}	(0.91±0.01)^{a}
10 (days)	(0.92±0.01)^{a}	(0.91±0.01)^{a}	(0.92±0.01)^{a}
15 (days)	(0.92±0.01)^{a}	(0.92±0.01)^{a}	(0.92±0.02)^{a}

Means with different letters in each row are statistically different (P<0.05).

The replacement of sugar by GS in the WSDB formulation indicated that WSDB and WSDB+GS had significantly (p<0.05) a higher curve of dough development (H_m) and gas produced (H'_m) than WSDB-S during fermentation (Table 5). Bread loaf specific volume measurement showed the maximum values for WSDB and WSDB+GS. Regarding to textural parameters, crumb firmness and springiness, evaluated in baked bread did not show significant differences between treatments.

Evaluation of flour substitute. Flour substitution was assessed by GS200 application on flour and on WSDB. The quantities of substitution were 3 and 6 % of flour. Fermentative properties tested on flour indicated that the addition of GS200 significantly reduced (p<0.05) V_T and H'_m (Table 6). However, F+GS200 3 and F+GS200 6 reflected a significant increase of V_R/V_T comparing to F. For the dough development curve, H_m decreased significantly (p<0.05) with substitute at both concentration, indicating a lower inflation of the dough.

The addition of GS200 significantly (p<0.05) influenced all Mixolab parameters (Table 6). In particular, WA progressively increased as the wheat flour substitution level increased. Regarding dough stability, it was prolonged (p<0.05) by the addition GS200. C2, C3-C2 and

C4-C3 parameter increased meanwhile C4-C5 decreased when GS200 was added.

Properties of WSDB containing GS200 were studied. The fermentative stage revealed that V_T and H'_m values were reduced ($p<0.05$) at both concentrations of GS200 (Table 7). In spite of reduction of gas production in WSDB+GS200 3 and WSDB+GS200 6, dough development curves were similar to WSDB. Additionally, bread loaf specific volume decreased with GS200. Furthermore, WSDB +GS200 3 and WSDB +GS200 6 showed no differences in crumb firmness and springiness values between treatments.

Discussion

All flour parameters measured showed to be according to specification of a good quality flour for WSDB production. In case of falling number, result evidenced that flour had low α-amylase activity, this fact is typical of Argentinian flours. For this reason, flour must be supplemented with commercial enzyme α-amylase. The use of Toler Miga Bollo Directo corrected falling number in our study case.

According to fat substitution assays, fermentation stage revealed that TFLS and fat on flour dough had differences on production, lost and retention of gas comparing to control. However, this fact did not affect dough development. Mixolab rheological behaviour of dough indicated that TFLS significantly increased stability parameter. This may have been a result of the protein–protein networks formed by the emulsifiers [27], due to the presence of TFLS on the dough. Besides, gluten network weakening was reduced when TFLS was added (C2). Both ingredients caused better quality of starch (C3–C2) comparing to F. This point could indicate that the addition of them produce that the interaction of emulsifiers with the starch lead to crumb softening [10].

Table 6. Effect of GS200 at 3 % (F+GS200 3) and 6 % (F+GS200 6) on fermentative and rheological attitudes of flour.

	F	*F+GS200 3*	*F+GS200 6*
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	(1604±8)^a	(1398±6)^b	(1400±5)^b
V_R (volume of gas retained, mL)	(1256±10)^a	(1194±5)^b	(1187±5)^b
V_L (volume of gas lost, mL)	(348±9)^a	(204±6)^b	(213±5)^b
V_R/V_T (coefficient of gas retention, %)	(78±1)^a	(85±2)^a	(84±2)^a
H'_m (maximum height of gas production curve, mm)	(54±1)^a	(55±2)^a	(55±2)^a
T_x (time needed to start losing gas, min)	(81±1)^a	(90±2)^b	(90±2)^b
Curve of dough development			
H_m (maximum dough height, mm)	(31±1)^a	(26±1)^b	(25±1)^b
H (dough height after 3 h, mm)	(30±1)^a	(26±1)^b	(25±1)^b

	F	*F+GS200 3*	*F+GS200 6*
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	(1604±8)^a	(1398±6)^b	(1400±5)^b
V_R (volume of gas retained, mL)	(1256±10)^a	(1194±5)^b	(1187±5)^b
V_L (volume of gas lost, mL)	(348±9)^a	(204±6)^b	(213±5)^b
V_R/V_T (coefficient of gas retention, %)	(78±1)^a	(85±2)^a	(84±2)^a
H'_m (maximum height of gas production curve, mm)	(54±1)^a	(55±2)^a	(55±2)^a
Dough consistency (Nm)	(3.09±0.19)^a	(3.07±0.11)^a	(2.97±0.17)^a
T_x (time needed to start losing gas, min)			
Mixolab	(58.0±0.1)^a	(59.4±0.3)^b	(61.6±0.2)^c
WA (water absorption, %)	(13.5±0.7)^a	(19.3±0.3)^b	(19.2±0.2)^b
Stability (min)	(0.45±0.02)^a	(0.49±0.01)^b	(0.53±0.03)^b
C2 (protein weakening, Nm)	(1.83±0.03)^a	(1.94±0.02)^b	(2.00±0.04)^b
C3 (starch gelatinization, Nm)	(1.38±0.02)^a	(1.45±0.02)^b	(1.47±0.04)^b
C3-C2 (starch gelatinization range, Nm)	(1.76±0.02)^a	(1.80±0.02)^b	(1.85±0.03)^b
C4 (hot gel stability, Nm)	(-0.07±0.04)^a	(-0.14±0.02)^b	(-0.15±0.03)^b
C4-C3 (cooking stability range, Nm)	(3.21±0.02)^a	(3.03±0.05)^b	(3.12±0.05)^b
C5 (starch retrogradation in the cooling phase, Nm)	(1.45±0.02)^a	(1.23±0.04)^b	(1.27±0.04)^b
C5-C4 (gelling, Nm)	(31±1)^a	(26±1)^b	(25±1)^b

Means with different letters in each row are statistically different (P<0.05).

TFLS studied in the WSDB formulation showed similar fermentation parameters and bread loaf specific volume comparing to fat and control. In fact, the effect of fat or substitutes in bread formulation is related to decrease the firmness of crust, produce a brighter crumb structure and help to prevent the staling process of baked products [28], rather than effect on fermentation process. Besides, several other ingredients of WSDB, such as gluten, sugar, oxidizing and enzymatic agents, promote volume increase [29, 30, 31], and all these ingredients were in the three conditions. Textural parameters of experimental bread evidenced that fat and TFLS improve crumb firmness at short shelf life of the breads (5 and 10 days). These results are in agreement with already indicated starch gelatinization range (C3–C2) in Mixolab, which could lead to softening the crumb causing less staling and improving the quality of bread preservation. Studies reported that composite bread produced from wheat exhibited a good crumb structure when emulsifiers were added [7, 8].

WSDB is characterized to present soft crumb, so it is necessary that the firm and elastic character of gluten is compensated by other materials that have a softening effect. Thus, in these cases the fat or TFLS were used to lubricate the gluten chains and to soften the crumb of baked bread.

Table 7. Fermentative and texture attitudes caused by effect of GS200 at 3 % (WSDB +GS200 3) and 6 % (WSDB +GS200 6) comparing to bread (WSDB).

	WSDB-S	***WSDB***	***WSDB+GS***
Rheofermentograph			
Curve of gas			
V_T (volume of gas produced, mL)	$(692±5)^a$	$(660±2)^b$	$(661±2)^b$
V_R (volume of gas retained, mL)	$(681±1)^a$	$(659±1)^b$	$(660±1)^b$
V_L (volume of gas lost, mL)	$(11±2)^a$	$(1±1)^b$	$(1±1)^b$
V_R/V_T (coefficient of gas retention, %)	$(98±1)^a$	$(99±1)^a$	$(99±1)^a$
H'_m (maximum height of gas production curve, mm)	$(38±1)^a$	$(32±1)^b$	$(30±1)^b$
T_x (time needed to start losing gas, min)	-	-	-
Curve of dough development			
H_m (maximum dough height, mm)	$(35±2)^a$	$(32±2)^a$	$(30±3)^b$
H (dough height after 3 h, mm)	$(35±2)^a$	$(32±2)^b$	$(30±3)^b$
Dough consistency (Nm)	$(1.76±0.15)^a$	$(2.01±0.12)^a$	$(2.15±0.25)^a$
Bread loaf specific volume (g/mL)	$(5.93±0.01)^b$	$(5.55±0.02)^b$	$(5.57±0.02)^b$
Texture profile analysis			
Crumb firmness (g)			
5 (days)	$(492±5)^a$	$(481±7)^a$	$(485±5)^a$
10 (days)	$(995±4)^a$	$(988±5)^a$	$(992±5)^a$
15 (days)	$(1427±9)^a$	$(1432±10)^a$	$(1435±10)^a$
Springiness			
5 (days)	$(0.92±0.01)^a$	$0.92±0.01)^a$	$(0.91±0.01)^a$
10 (days)	$(0.91±0.01)^a$	$(0.90±0.02)^a$	$(0.90±0.02)^a$
15 (days)	$(0.91±0.0)^a$	$(0.91±0.02)^a$	$(0.91±0.02)^a$

Means with different letters in each row are statistically different ($P<0.05$).

Sugar substitute allowed to obtained some positive fermentative characteristics of dough in relation to sugar. Sugar increased gas production, as it is indicated to include in yeast bread formulation because it is fermented by yeast to produce CO_2. The higher gas production caused by sugar addition comparing to GS was no evidenced on dough development, as dough development parameters were similar for both conditions. The addition of sugar and GS decreased WA of the flour dough in Mixolab assay. The decrease of WA can be explained by the fact that sugar and GS competitively absorb water, instead of flour proteins and starch. Also, dough stability was prolonged by the addition GS in relation to sugar and control. GS could induce a change of dough network structure, conferring greater structural stabilization compared to sugar. Similar results were reported in substitute sugar [11]. GS showed similar protein behaviour (C2) than sugar and control. As for C3-C2, the highest value for sugar addition indicates better quality of starch. This result was unexpected as sugar in a recipe absorbs water, the competence for water established between starch and sugar would contribute to less water available for starch gelatinization [13]. Parameter C4-C3 had the highest value for sugar and GS doughs, which indicates the stability of the starch gel when heated. This implied that both ingredients reduce the ability of starch to withstand amylolysis. Finally, these ingredients did not affect starch retrogradation (C4-C5).

The replacement of sugar by GS in the WSDB formulation indicated that fermentative properties were as good as sugar. The addition of sugar or GS increased CO_2 production, resulting in higher dough development, thereby increasing loaf volume. This fact was evidenced in bread loaf specific volume measurement, the maximum values were for WSDB and WSDB+GS. Some publications reported that within the properties of the bread, loaf specific volume decreased with increasing substitution of sugar by substitute [32, 9]. However, other studies [33, 34] noticed that sugar substitutes improved bread quality, as loaf specific volume, according to results of this investigation. Consequently, the sugar substitution success would depend on the nature of substitute and the bread recipe to be applied.

Flour substitution by GS200 revealed that this substitute affects fermentative properties. The addition of GS200 significantly reduced gas production, however GS200 improved the gas retention capacity of dough, suggesting that the substitute may enhance the matrix of gas retention. For the dough development curve, H_m decreased with GS200 addition, indicating a lower inflation of the dough. This behaviour is related to a reduction in the amount of gluten due to wheat flour being substituted, which usually produce a deterioration of dough properties. As a percentage of the dry matter was replaced by fibre, which has a larger particle size compared to the refined flour, it leads to a disruption in the gluten network formation [35]. Further, the addition of GS200 on dough flour influenced all Mixolab parameters. Dough stability was prolonged by the addition GS200. This finding could mainly be due to the lower rate of hydration of the components due to an increased competition for water between fibre and gluten proteins [36]. The increase of WA and C2 could be attributed to the presence of fibre in GS200, able to absorb water [37]. Other publication observed similar findings when fibre was added [38, 39]. Also, GS200 improved gelatinization process (C3-C2) involving better quality of starch and reduced the stability of the starch gel when heated. Furthermore, GS200 produced less starch retrogradation (C4-C5) than control.

GS200 applied on WSDB formulation showed less gas production than control during fermentation. This effect was already noticed in the substitution of GS200 into flour. However, dough development was similar to WSDB. This may indicate that when WSDB had flour substitution by GS200 evidenced a mitigating effect on fermentative properties comparing to flour analysis. Additionally, although bread loaf specific volume decreased with GS200, this effect was not so notable (5.93 Vs. 5.55-5.57) yielding satisfactory results. Publications reported that reduction in specific volume of breads occurs when substitutions of flour are made in bread recipes [40, 41], whereas other studies evidenced successful flour substitutions concerning to specific volume of bread [42, 43]. So, the effect of flour substitution on bread would depend on the type of bread and on the source of substitute. In case of GS200, substitution showed good performance when flour was replaced in WSDB.

V. CONCLUSION AND FUTURE SCOPE

The obtained data indicate that several dough and baked bread properties are affected when fat or sugar are added to the dough, even when the flour is replaced by a substitute. In case of fat substitution, TFLS caused a similar effect to fat, showing high starch gelatinization. In agreement, fat and TFLS provided WSDB with similar changes in crumb texture, which led to improve the quality of bread preservation at short shelf life of end-product (5 and 10 days). Otherwise, sugar substitution affected mainly gas production during flour dough fermentation. However, dough development was similar when sugar or GS were evaluated in WSDB, and loaf specific volume was comparable between both ingredients. These results may indicate that GS applied in a complex formulation bread as WSDB, provides a suitable effect like sugar. Concerning flour replacement, several rheology changes and a significant decrease of gas production occurred on GS200 added-dough. However, GS200 contributed considerably in gas retention capacity, influencing in a considerable dough development. Therefore, bread loaf specific volume of WSDB+GS200 3 and WSDB+GS200 6 showed acceptable results, indicating the potential capacity to use GS200 on WSDB recipe to formulate high fiber bread. Based on these results, the WSDB substitutions evaluated in this study led to similar end-product texture properties to original bread. In this way the substitutes tested in this study represent a nutritionally effective strategy and a significant forward to develop reduced calorie bread.

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