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Fermented milk obtained with kefir grains as an ingredient in breadmaking

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Summary The objectives of this research were to study the effect of the addition of lyophilised kefir milk to premixes for household production of bread and evaluate the quality attributes of them. Four lyophilised samples were obtained from the followings: skim milk, acidified skim milk, fermented skim milk and neutralised fermented skim milk. Breads were prepared with commercial wheat flour, lyophilised milk samples and yeast through a straight dough process. Quality was assessed through loaf volume, crumb porosity and moisture, crumb texture and crust colour. Changes in texture and starch recrystallisation by X-ray diffractometry were determined after 1 and 3 days of storage at room temperature. Breads with acidified milks showed the highest specific volumes and crumbs with the best texture properties. Crystallinity in bread with fermented milks was higher than for skim milk sample. This would indicate that there would be a certain effect of the type of milk processing on the promotion of starch retrogradation.

Keywords Breadmaking, bread quality, fermented milk, kefir grains.

Introduction

Kefir is a unique cultured dairy product due to combined lactic acid and alcoholic fermentation of milk using a mixture of lactic acid bacteria, yeast and fungi that coexist in a symbiotic relationship. These microorganisms are organised in the form of kefir 'granules' or 'grains', surrounded by a matrix of polysaccharide-protein nature (Lopitz-Otsoa *et al.*, 2006; Hugenholtz, 2013).

Due to the claimed health benefits of kefir which include stimulation of the immune system, lowering cholesterol and antimicrobial properties, kefir has become an important functional dairy food, and consequently, research on kefir has increased in the past decade (Liu *et al.*, 2005; Vinderola *et al.*, 2006; Guzel-Seydim *et al.*, 2011). There are several compounds in kefir that may have bioactive properties. The microorganisms themselves (dead or alive), metabolites of the microorganisms formed during fermentation (polysaccharides, bacteriocins), or breakdown products of the food matrix, such as peptides, may be responsible for these beneficial effects (Garrote *et al.*, 2010). The existence of an exopolysaccharide called 'kefiran' confers to this product a certain viscosity that could influence textural attributes in foods where it is incorporated and contributes to their health-promoting properties (Medrano *et al.*, 2008, 2011). Kefir nutritional and functional benefits suggest that it could be used as an ingredient in milk bread formulations (Abraham *et al.*, 2010).

Some authors have evaluated the effect of the incorporation of native and lyophilised kefir grains on wheat bread quality. Plessas *et al.* (2005) studied the leavening activity of kefir grains in lean dough and their efficiency in producing bread with good sensorial characteristics. They found that kefir grains produced bread similar to traditional sourdough bread. Bread produced with kefir retained more moisture, had a firmer texture, lower acidity and retained their freshness for longer time compared with baker's yeast bread. Filipčev *et al.* (2007) found that kefir grains could be used in breadmaking either directly or as a starter in the sourdough breadmaking process for enhancing bread taste and shelf life. In both studies (Plessas *et al.*, 2005; Filipčev *et al.*, 2007), kefir grains were used as a substitute of baker's yeast. The positive impact of kefir grains in the sourdough bread production regarding flavour has been proved through the identification and determination of volatile compounds

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(Plessas *et al.*, 2011a). Besides, it has been reported that the application of kefir grains in straight dough bread delays mould spoilage (macroscopically) to maximum of 6 days (Plessas *et al.*, 2011b).

Nowadays, the use of 'ready-to-cook' premixes is widespread because these formulations can be stored long time at home, and they allow obtaining just baked products. This trend has promoted technological research to optimise these formulations (Bautista & Pérez, 2010; Jha *et al.*, 2013). The incorporation of dehydrated kefir fermented milk to bread formulations would be interesting to formulate premixes that could be hydrated by consumers at household level to obtain milk breads to be consumed in a short term. There is no information about the use of dehydrated kefir fermented milk as an ingredient in breadmaking process. Therefore, the objectives of this research were to study the effect of the addition of lyophilised kefir to premixes for household production of milk bread and evaluate the quality attributes of them.

Materials and methods

Materials

Commercial wheat flour (*Triticum aestivum*) was provided by Moinho Pacífico Indústria e Comércio Ltda. (Santos, Brazil). Wheat flour had 0.73% ash, 28.6% wet gluten, 9.5% dry gluten and 14.8% moisture content. All the composition values, except moisture content, are expressed on dry matter basis. Compressed yeast (*Saccharomyces cerevisiae*) was obtained from Fleischmann (São Paulo, Brazil). An ultra-high-temperature (UHT) low-fat milk obtained from SanCor (Santa Fe, Argentina) was used for preparing dehydrated kefir fermented milk samples. Lyophilised samples were obtained in a FD4 Heto freeze dryer (Heto-Holten A/S, Allerød,Denmark).

Kefir grains were obtained from a household at La Plata, Argentina. The grains were maintained at −20 °C and were reactivated by successive subcultures in ultra-high-temperature (UHT) low-fat milk. Kefir grains, washed with sterile water, were inoculated (10 g) into 100 mL of milk. After incubation at 20 °C for 15 h, the grains were separated from the fermented product by filtration through a plastic sieve (sanitised by immersion in 70% ethanol and then washed with sterile water) and were washed prior to the next culture passage (subculture). Fermented milk obtained had a pH value of 4.5.

Four lyophilised samples were prepared: a skim milk (SM, pH = 6.7), an acidified skim milk (ASM, pH = 4.5), a fermented skim milk (FSM, pH = 4.5) and a neutralised fermented skim milk (NFSM, pH = 6.7). The pH values of ASM and NFSM were adjusted with 2N HCl and 2N NaOH, respectively.

Methods

Baking procedure

A straight dough process was performed using 100.0 g wheat flour, 2.0 g salt, 3.0 g compressed yeast, 8.0 g lyophilised skim milk and 54.0 g water (farinograph absorption). Dough was prepared by mixing raw materials (wheat flour, salt and lyophilised skim milk) for 1 min at 840 r.p.m. in a spiral mixer (SUPREMAX AL-25 IM, Brazil) at 25 °C (Gómez *et al.*, 2013). Water amount was fixed according to farinograph absorption. Compressed yeast was dispersed into water and water was gradually added during mixing. Dough was kneaded at 1700 r.p.m. at 25 °C according to the farinograph development time. Dough was divided into three pieces (420 g each one). The pieces were passed through a bread shaping machine (SUPREMAX MC-50, Brazil) and placed in loaf pans (dimensions, 25.0 × 10.0 × 6.0 cm). Pans were placed into the fermenting chamber (Degânia, Italy) at 32 °C for 60 min. Loves were baked in a Four Turbo Model oven (Degânia, Italy) at 180 °C for 15 min with the moisture trap closed and then 5 min with the moisture trap open. After baking, the loaves were depanned and cooled during 2 h at room temperature.

Bread quality

Bread volume. Loaf volume was measured in CHOPIN-206 rapeseed displacement equipment (Chopin, France). The specific volume of bread was calculated with loaf volume and weight, obtained by direct measure.

Bread crust colour. Crust colour of fresh bread samples was measured using a ColorQuest XE colour measurement spectrophotometer (HunterLab, Reston, VA, USA) using the Hunter L^*, a^* and b^* colour scale. L^* values indicate black or white ($L^* = 0$, Black and $L^* = 100$, White). A greater L^* value indicates a lighter sample colour. Hunter a^* parameter varies from red (positive values) to green (negative values). Hunter b^* parameter varies from yellow (positive values) to blue (negative values). Seven slices of each bread loaf were analysed and at least four readings on crust of each bread slice at different positions were carried out at room temperature.

Crumb structure. Bread crumb structure was analysed on the centre of three bread slices (2.5 cm thick). Each slice was scanned in an HP Scanjet 4070 Photosmart scanner (Hewlett-Packard, Palo Alto, CA, USA). The obtained JPEG images were analysed with an image analyser (ImageJ 1.37v software, National Institutes of Health, Bethesda, MD, USA). An area corresponding to the centre of the slice, which was representative of the total crumb of each slice, was selected. The colour image was converted into 8-bit image and analysed in grey scale (0 black and 255 white). The segmentation of the image (binary image conversion) was performed by the software for automatic selection of the threshold value. The binary image has only two grey levels: 0 for empty areas (black, air alveoli) and 255 for the walls of the alveoli (white bread crumbs). Object category (air cells) was assigned to those image zones that had a grey intensity between 0 and the threshold value. The crumb grain characteristics studied were as follows: cell density (cells cm^{-2}, higher levels denoted finer structure), mean cell area (mm^2) and void fraction (%, computed as the percentage of the total analysed square occupied by detected cells) (Caballero *et al*., 2007).

Texture profile analysis. The texture of bread crumb was evaluated in a TA.XT2i Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 25-kg load cell. An aluminium cylinder probe of 36 mm in diameter was attached to a moving cross head. Two hours after baking, the bread loaves were cut and slices (25 mm thick) were subjected to a double cycle of compression under the following conditions: 100 mm min^{-1} compression rate and 40% maximum deformation. The texture profile parameters were determined using the Texture Expert 1.22 software (Stable Micro Systems). Bread crumb firmness, consistence, cohesiveness, elasticity and chewiness were calculated from a force–distance graph. Bread samples were analysed in duplicate and at least six slices of each bread sample were evaluated.

Crumb moisture. Moisture of crumb was determined by an oven drying (Fanem Orion 515, Brazil) for 2 h at 135 °C (AACC Approved Method 44-19).

Bread pH and total titratable acidity. pH was determined according to the method described by Thompson *et al*. (1998) with modifications. Total titratable acidity (TTA) was performed according to AACC method 02-31 (2000).

Bread composition. Ash, protein and lipid contents were analysed according to AACC methods 08-01, 46-12 and 30-10, respectively. Total dietary fibre (TDF) was tested using the Megazyme kit (Megazyme International Ireland Ltd., Bray, County Wicklow, Ireland) according to 985.29 AOAC method (1990). Carbohydrates different from fibre were determined by difference.

Bread storage
Two hours after baking, breads were stored at 20 ± 2 °C in polyethylene bags to evaluate their stability. Texture and moisture of bread crumb were measured, as mentioned above, at different storage periods (0, 1 and 3 days).

Starch recrystalisation
Recrystalisation of amylose and amylopectin was determined by X-ray diffractometry. Bread samples were stored (0 and 3 days) at 20 ± 2 °C to analyse starch retrogradation. After storage crumb samples were freeze-dried and pulverised before testing. The samples were covered with a Kapton film to prevent dehydration and analysed in an X'Pert Pro diffractometer (PANalytical, Almelo, the Netherlands) with a cooper target X-ray tube set to 40 kV and 40 mA. Diffractograms were obtained using Cu Kα radiation (λ = 0.154 nm), scanning 2θ angles from 4° to 40° at a rate of 1.00° min^{-1} and a step size of 0.025°. OriginPro 7.0 Software (OriginLab Corporation, Northampton, MA, USA) was used to analyse the diffractograms. The total crystallinity degree was calculated by the ratio of crystalline area and the total area (crystalline plus amorphous areas).

Statistical analysis

Results were subjected to a one-way analysis of variance (ANOVA) according to the general linear model procedure with least-square means effects. A multiple range test was applied to determine which means were significantly different ($P < 0.05$) according to Fisher's least significant differences (LSD). Statistical analysis was carried out using Statgraphics Plus 5.1 (Statpoint Technologies, Inc., Warrenton, VA, USA).

Results and discussion

Fresh bread quality

Breads prepared with fermented skim milk (FSM) and acidified skim milk (ASM) showed significantly higher specific volumes (3.78 and 3.87 cm^3 g^{-1}, respectively) than those prepared with skim milk (SM) (3.29 cm^3 g^{-1}). Samples prepared with neutralised fermented skim milk (NFSM) showed the lowest volume (3.07 cm^3 g^{-1}).

Differences between bread sample volumes can be related to different acidities of FSM and ASM samples compared with SM and NFSM. pH values of FSM and ASM were 5.15 and 5.36, respectively, while SM and NFSM had pHs of 5.97 and 6.09, respectively. Total titratable acidity also exhibited differences between samples. For FSM and ASM, values were as follows: 0.45 and 0.47 g lactic acid 100 g^{-1} sample, respectively, and SM and NFSM had lower values (0.27 and 0.24 g lactic acid 100 g^{-1} sample). Other authors (Clarke *et al*., 2002; Crowley *et al*., 2002) have also found higher specific volumes when dough was more acid than control one. Differences in acidity promote changes in protein conformation. Gluten proteins have an isoelectric point ranging from 6 to 9. A low pH in dough would lead to a net positive charge on proteins due to the protonation of some of the carboxyl anions of glutamic and aspartic acids. This net positive charge causes intramolecular and intermolecular repulsions, which produces protein unfolding, generating a somewhat open structure (Galal *et al.*, 1978). Jeckle & Becker (2012) found a local maximum of bread volume at moderate acid pH (close to 5.5) and then a decreasing effect on volume at lower pHs. Thus, a certain degree of unfolding in gluten network could lead to more extensible dough and volume development. However, a more intense acidification and consequently a higher degree of unfolding could excessively soften dough matrix. A soft network is unable to retain gas during fermentation leading to a poor loaf volume.

Along with volume, crust colour is the other quality attribute first perceived by consumers. Breads with fermented milk (FSM) showed a more yellowish, less reddish and more luminous crust (Table 1). These results would indicate a partial inhibition of Maillard browning reaction in FSM mainly due to the acidic pH. Maillard reaction is inhibited at pH lower than 6 because of the protonation of basic groups that are involved in the first steps of the reaction (O'Brien & Morrissey, 1989; Tan & Harris, 1995; Martins *et al.*, 2001). Other possible cause for the decrease in Maillard browning could be the lactose reduction induced by fermentation.

Crumb of breads with SM and NFSM presented the highest cell densities (195 and 190 cells cm^{-2}, respectively). The lowest mean cell area corresponded to SM sample (21.7 10^{-3} cm^2) while ASM showed the highest mean cell area (34.0 10^{-3} cm^2). As shown in Table 1, void fractions of crumbs with FSM and ASM (37.9% and 41.5%, respectively) were the highest ones and void fraction of SM was significantly lower than those of other samples (33.9%).

No significant differences in the crumb parameters were found between ASM and FSM samples, and they exhibited the highest specific volumes. High void fraction, together with specific volume, is usually related to a spongy and open crumb. This best performance of the acidified dough would be related to the structural changes in gluten network as commented above.

Fresh bread prepared with FSM and ASM presented the lowest firmness while NFSM sample showed the highest firmness (Fig. 1). Crumb consistency showed similar trends to those observed for firmness (data not shown). This effect of pH on crumb texture is related to the more spongy structure obtained. In addition, FSM and ASM samples presented more cohesive and springy crumbs, while NFSM showed the lowest values (Fig. 1). Crumb of NFSM bread was the hardest, less cohesive and less springy one. Chewiness (firmness × cohesiveness × springiness) is a parameter related to the energy required to masticate a solid food, and in the case of bread, it is desirable to obtain low values of this attribute. Breads made with acid milks (FSM and ASM) were those with the lowest chewiness, while NFSM sample had the more chewiness crumb (Fig. 1). Therefore, formulations with better volume and crumb porosity were those that also exhibited the best global textural profile.

Crowley *et al.* (2002) also found that breads produced through sourdough methodology (exhibiting lower pH than control one) showed a softer crumb and a lower chewiness than control bread.

These results indicate that milk fermented with kefir, in comparison with skim milk, is an interesting alternative as a nutritional ingredient in breadmaking, leading to breads with improved textural attributes.

Table 1 Specific volume, crust colour and crumb structure of bread samples. SM: skim milk (pH = 6.7), FSM: fermented skim milk (pH = 4.5), ASM: acidified skim milk (pH = 4.5), NFSM: neutralised fermented skim milk (pH = 6.7)

	SM	FSM	ASM	NFSM
Specific volume (cm^3 g^{-1})	3.29 ± 0.02 c	3.78 ± 0.10 b	3.87 ± 0.04 a	3.07 ± 0.01 d
Crust colour				
a*	15.97 ± 0.89 b	15.01 ± 2.00 c	16.73 ± 1.01 a	16.44 ± 0.88 ab
b*	35.18 ± 1.44 b	36.35 ± 1.74 a	35.44 ± 1.54 b	33.09 ± 2.32 c
L*	54.91 ± 3.08 b	58.91 ± 5.15 a	54.35 ± 3.14 b	52.39 ± 3.75 c
Crumb structure				
Cell density (number of cells cm^{-2})	195 ± 16 a	162 ± 21 b	149 ± 9 b	190 ± 31 a
Mean cell area (10^{-3} cm^2)	21.7 ± 3.6 c	29.2 ± 4.6 ab	34.0 ± 3.4 a	24.8 ± 7.4 bc
Void fraction (%)	33.9 ± 3.0 c	37.9 ± 1.3 ab	41.5 ± 3.0 a	36.9 ± 4.8 b

Different letters in the same row indicate significant differences ($P \leq 0.05$).

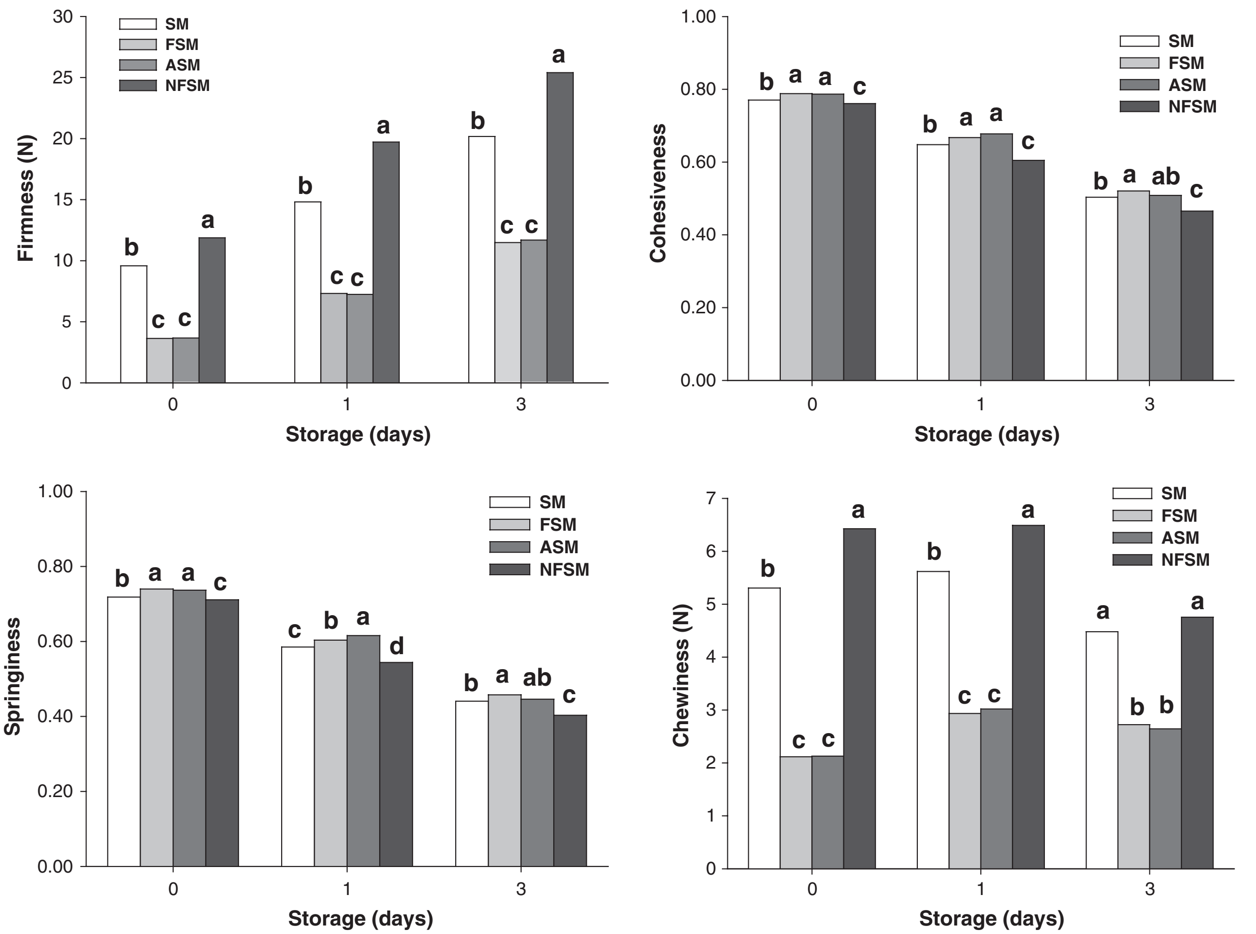

Figure 1 Crumb firmness, cohesiveness, springiness and chewiness. SM: bread with skim milk (pH = 6.7), FSM: bread with fermented skim milk (pH = 4.5), ASM: bread with acidified skim milk (pH = 4.5), NFSM: bread with neutralised fermented skim milk (pH = 6.7). Different letters in the same day indicate significant differences ($P \leq 0.05$).

Bread storage

Changes during storage were evaluated up to the third day, taking into account that breads prepared at home using premixes are intended to be consumed within a short term. Breads were exempt of mould spoilage within this period. Moisture and texture changes in crumb were determined.

Texture

There were no appreciable differences in crumb moisture between fresh breads prepared with the four premixes (mean value ± SD for all samples = 42.6 ± 0.3) and stored ones after 3 days (mean value ± SD for all samples = 42.0 ± 0.6).

During storage period, there was a progressive increase in crumb firmness of all samples, but differences observed at Day 0 among them were maintained (FSM and ASM presented the lowest firmness while NFSM sample showed the highest firmness) (Fig. 1). However, the relative increase in firmness with respect to Day 0 was dissimilar according to the type of sample (Table 2). For acidified samples (ASM and FSM), the firmness increase was more pronounced. At the first day of storage, firmness increased up to 101% (FSM) and 97% (ASM) respect to values obtained at Day 0. After 3 days of storage, the firmness increased up to 216% and 218% for FSM and ASM, respectively. SM and NFSM firmness increased in a lower degree (54–66% at Day 1 and 110–114% at Day 3) (Table 2). However, as acidified samples have a low initial firmness (at Day 0), even at the end of storage period, they maintained softer crumbs than SM and NFSM samples (Fig. 1). Corsetti *et al.* (2000) also found a trend to a more pronounced increase in crumb firmness during storage when they used sourdough or chemical acidification of dough.

Table 2 Percent variation in textural attributes during storage respect to Day 0. SM: skim milk (pH = 6.7), FSM: fermented skim milk (pH = 4.5), ASM: acidified skim milk (pH = 4.5), NFSM: neutralised fermented skim milk (pH = 6.7)

	Firmness		Cohesiveness		Springiness		Chewiness	
	Day 1 %	Day 3 %	Day 1 %	Day 3 %	Day 1 %	Day 3 %	Day 1 %	Day 3 %
SM	54.6	110.5	−15.9	−34.7	−18.5	−38.7	5.90	−15.6
FSM	101.4	216.0	−15.4	−34.0	−18.4	−38.2	38.6	28.6
ASM	97.2	218.4	−13.9	−35.4	−16.4	−39.4	41.9	24.3
NFSM	66.0	113.8	−20.5	−38.9	−23.5	−43.3	0.97	−26.0

Crumbs of breads prepared with FSM and ASM were also the most cohesive and elastic ones. This trend was observed for the whole period of storage, showing a progressive decrease as the days passed (Fig. 1).

Chewiness followed the same trend as firmness. Breads made with FSM and ASM were those with the lowest chewiness (Fig. 1). On the third day, a decrease in chewiness values was observed in all samples; this behaviour would be associated with the low cohesiveness and springiness presented by crumb samples; therefore, bread crumb would be more crumbling and the energy required for the chewing would be smaller. Percentage variations in chewiness (Table 2) were lower at the third day than for the 1st day of storage. This trend was particularly pronounced when nonacid milks (SM and NFSM) were used; in this case, negative variations were observed, indicating less chewiness than in control sample. This could be explained by the different degrees of relative variation in firmness, cohesiveness and springiness among samples. In samples prepared with nonacid milks, the degree of increase on firmness was inferior to that found in acid milk samples. As the loss of cohesiveness and springiness was similar among all them, chewiness was mainly determined by firmness variations.

Starch recrystallisation

Native wheat starch shows a characteristic A-type X-ray diffraction pattern with peaks at 2θ angle close to 15, 17, 18 and 23° (Zobel, 1988; Jovanovich, 1997; Primo-Martín *et al.*, 2007). Thermal treatment causes loss of starch crystallinity, due to gelatinisation phenomenon. Heating and cooling during breadmaking also lead to the formation of V-type and B-type structures (Ribotta *et al.*, 2004). During storage, starch recrystallisation takes place, and it is mainly caused by the self-association of branched amylopectin molecules (Karim *et al.*, 2000; Primo-Martín *et al.*, 2007). The increment in the percentage of crystallinity of starch during bread storage can be assessed by X-ray diffractometry (Ottenhof & Farhat, 2004; Primo-Martín *et al.*, 2007; Ratnayake & Jackson, 2007).

A single reflection at 2θ angle of 20° was the main peak detected in control (fresh) bread crumb of all samples (SM, FSM, ASM, NFSM). As an example, the typical spectrum of SM samples is shown (Fig. 2, curve a). Similar profiles were obtained in the other cases. This spectrum corresponds to a typical V-type pattern related to the presence of amylose-lipid complex formed during the gelatinisation process (Zobel & Kulp, 1996). During bread storage, crystallinity of crumb of all bread increased mainly due to the increment of peaks of 15, 18 and 23° (Fig. 2, curve b). These peaks are associated with B-type structures. This pattern is characteristic of the crystallisation of melted amorphous starch (Ribotta *et al.*, 2004).

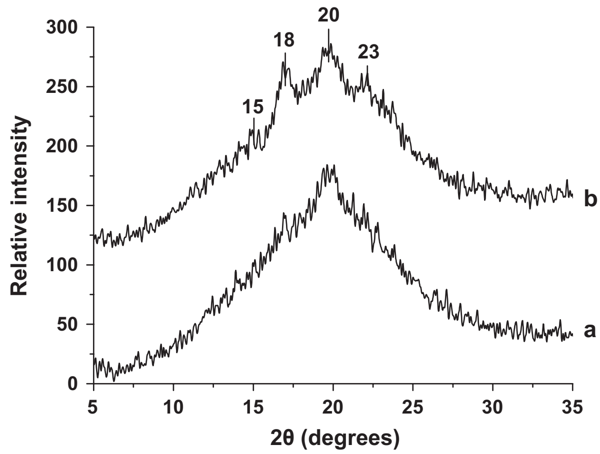

Figure 2 X−ray diffraction of skim milk (SM) bread crumb. (a) Fresh bread (Day 0). (b) Stored bread (Day 3, 20 ± 2 °C).

In Fig. 3, percentage crystallinity corresponding to Days 0 and 3 of storage is shown. In samples where fermented or acidified milk was used for preparation (FSM, ASM and NFSM), crystallinity significantly increased after 3 days at room temperature respect to Day 0. No significant differences were observed at the third day between FSM and NFSM samples. Crystallinity in these samples was significantly higher than for SM sample. This could indicate that there would be a certain effect of milk processing on the promotion of starch retrogradation. Acidification and the presence of higher amounts of salt (NFSM) would be promoting factors. Changes in crystallinity were not

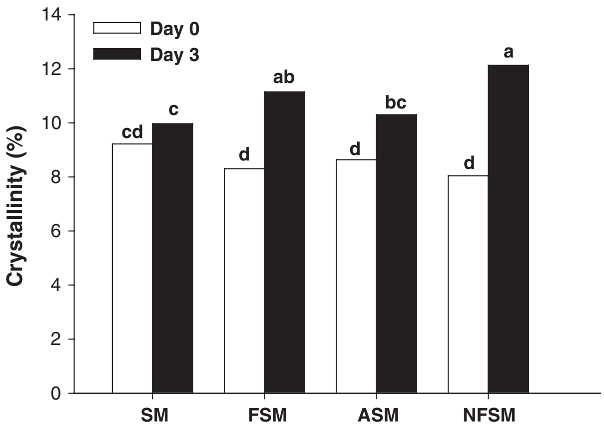

Figure 3 Crumb crystallinity. SM: bread with skim milk (pH = 6.7), FSM: bread with fermented skim milk (pH = 4.5), ASM: bread with acidified skim milk (pH = 4.5), NFSM: bread with neutralised fermented skim milk (pH = 6.7). Different letters indicate significant differences ($P \leq 0.05$).

Table 3 Physicochemical analysis of breads with skim milk (SM) and fermented skim milk (FSM)

	SM	FSM
Moisture (%)	29.7 ± 0.9 a	29.0 ± 1.1 a
pH	5.97 ± 0.02 a	5.16 ± 0.01 b
Total titratable acidity (TTA)[a]	0.27 ± 0.00 b	0.45 ± 0.00 a
Protein ($n \times 5.7$) (g 100 g^{-1} d.b.)	12.14 ± 0.09 a	12.09 ± 0.07 a
Ash (g 100 g^{-1} d.b.)	2.52 ± 0.03 a	2.45 ± 0.08 a
Lipids (g 100 g^{-1} d.b.)	0.81 ± 0.00 b	1.16 ± 0.01 a
Dietary fibre (g 100 g^{-1} d.b.)	4.18 ± 0.12 a	4.48 ± 0.24 a
Energy (kJ 100 g^{-1})[b]	275	280

Different letters in the same row indicate significant differences ($P \leq 0.05$).
[a](g lactic acid 100 g^{-1} d.b.)
[b]Carbohydrates for energy calculations were estimated by difference.

completely reflected on textural evolution of samples during storage.

Physicochemical analysis of breads

In Table 3, the compositional analysis of breads along with other parameters such as pH and energy contribution are shown. Bread with fermented milk (FSM) showed a lower pH and higher acidity; these results were expected taking into account the fermentation process. Besides, significantly higher lipid content was found in bread with fermented milk, a result probably related to the fermentation process by kefir microorganisms. Other compositional features were not significantly different between samples.

Conclusions

Breads prepared with fermented skim milk (FSM) and acidified skim milk (ASM) showed a higher specific volume than breads elaborated with skim milk (SM) and neutralised fermented skim milk (NFSM). Addition of FSM and ASM also reduced the firmness, consistency and chewiness of fresh bread crumb. This trend was also observed during storage. This behaviour can be attributed to the lower pH of FSM and ASM samples (4.5) respect to SM and NFSM (6.7). A lower pH could induce gluten proteins' modification leading to more open and disordered matrices, with more expansion during fermentation and higher specific volumes. Breads with acidified milks remain softer during storage. These results show that the use of acidified milks can improve bread volume and texture as a consequence of protein modification. However, the acidification through milk fermented with kefir grains would have potential advantages respect to the chemical acidification because other valuable nutrients are produced by kefir microorganisms during fermentation. This probiotic effect would be an additional advantage of these breads. Further studies on the nutritional value of bread with milk fermented with kefir can give a scope about this point.

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