J Food Sci Technol (March 2018) 55(3):858–867
https://doi.org/10.1007/s13197-017-2888-x

ORIGINAL ARTICLE

Animal fat replacement by vegetable oils in formulations of breads with flour mixes

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Revised: 17 September 2017 / Accepted: 22 September 2017 / Published online: 27 January 2018

Abstract The improvement of fatty acids (FA) profile of bread made with bovine fat (BF) and a mixture of flours completely replacing fat with canola oil (CO), or olive oil (OO) was evaluated. Technological and sensory characteristics and overall acceptability of the fortified breads were also studied. The results showed a decrease in saturated FA and a relative increase in monounsaturated and polyunsaturated FA compared to bread made with BF. Regarding CO, this caused the higher increase in n3 FA. This effect was maximized in bread made from the mixture of wheat flour (WF) + flaxseed flour (FF) + soybean flour (SF). OO caused a rise of n9 and n6 FA, mainly in bread made with WF + FF + wheat bran (WB). The breads with WF + FF + SF + CO and WF + FF + WB + OO presented higher specific volume, softer crumb and colour similar to those from base formulations. Furthermore, they had a very good sensory acceptance.

Keywords Functional bread · Fatty acids · Canola oil · Olive oil · Flour mixes

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Abbreviation

AACC	American Association of Cereal Chemists
BI	Brownness index
BF	Bovine fat
ALA	Alpha-linolenic acid
CAA	Argentine Food Code
FA	Fatty acids
FF	Whole flaxseed flour
HDL	High density lipoprotein
LDL	Low density lipoprotein
MUFA	Monounsaturated fatty acids
n3	Fatty acids of the n3 family
n6	Fatty acids of the n6 family
n9	Fatty acids of the n9 family
n6/n3	Relationship between fatty acids of series n6 and n3
PUFA	Polyunsaturated fatty acids
SF	Whole soybean flour
SFA	Saturated fatty acids
SV	Specific volume
PUFA/SFA	Relationship of polyunsaturated fatty acid and saturated fatty acids
WB	Wheat bran
WF	Wheat flour

Introduction

In recent decades, studies of the role of fats and oils in human nutrition have emphasized the importance of the intake of n3 fatty acid, the reduction of saturated fatty acids (SFA) and, more recently, the control of intake of trans fatty acids (Gebauer et al. 2006; Chang et al. 2014).

Functionally, bovine fat (BF) is one of the most important ingredients in the preparation of bread due to its influence in the mixing, handling and fermentation of the dough, as well as in bread volume and the sensory qualities of baked bread (Pavlovich-Abril et al. 2009).

The Argentine Food Code (CAA 2014) establishes that bread with fat is a product prepared in the same way as French bread with the addition of no less than 4% of edible fats, of which bovine fat is the most used. This bread is the most consumed bread in the Argentine northeast region and is manufactured in traditional bakeries, with an average daily consumption of 190 g per capita (Lezcano 2011; Osuna et al. 2014). Therefore, bakery products are associated with the presence of SFA and trans fatty acids. It is well known that these fatty acids cause an increase in plasma cholesterol, mainly of the low density lipoprotein (LDL) cholesterol, and in the total ratio of high density lipoprotein (HDL) cholesterol, with a subsequent increase in cardiovascular risk (Mensink et al. 2014). Moreover, excessive amounts of n6 polyunsaturated fatty acids, and a very high n6/n3 ratio (16:1 or higher), as is currently found in Western diets, have been suggested to promote the pathogenesis of many diseases, such as cardiovascular diseases, autoimmune diseases and some types of cancer. However, the same increased levels of n3 polyunsaturated fatty acids (a low n6/n3 ratio) have been shown to exert suppressive effects (Simopoulos 2008; Liu and Ma 2014).

Vegetable oils are cholesterol free and have a higher ratio of unsaturated to saturated fatty acids than animal fats (Liu et al. 1991). Thus, the use of natural vegetable oils, such as extra virgin olive oil and canola oil, could be an alternative to replace the animal fat. In fact, the consumption of extra virgin olive oil (OO) has been linked with a reduced incidence of coronary heart disease, arterial hypertension and diabetes. Furthermore, OO is characterized by high amounts of monounsaturated fatty acids (MUFA) as well as its high content of antioxidant agents, which are able to scavenge free radicals and afford an adequate protection against peroxidation (Caponio et al. 2013).

Regarding canola oil (CO), it is characterized by a low level of SFA (7%); substantial amounts of MUFA, and polyunsaturated fatty acids (PUFA), including 61% oleic acid, 21% linoleic acid, and 11% alpha-linolenic acid (ALA); plant sterols (0.53–0.97%); and tocopherols (700–1200 ppm). With regard to the high MUFA content of CO, Gillingham et al. (2011) have provided evidence supporting positive effects of MUFA compared with SFA on cardiovascular health through the regulation of plasma lipids and lipoproteins, susceptibility of LDL oxidation, and insulin sensitivity (Gebauer et al. 2006). Hence, growing scientific evidence supports the use of CO, beyond its beneficial actions on circulating lipid levels, as a health-promoting component of the diet (Lin et al. 2013).

Moreover, fats play a key role in bakery products. The choice of fat for the preparation of bakery products is driven not only by nutritional needs of consumers, but also by the need to obtain the desired rheological properties. Moreover, fats contribute specific sensory properties (aroma, flavour, softness, volume, palatability, bright appearance), and stabilize the products toward oxidation reactions, staling, and moisture migration (Pagani et al. 2010). Despite the negative effects on technological and sensory characteristics of bread and/or its baked goods, research continues focusing on the replacement of saturated fats with vegetable oils or mixtures in order to produce healthier foods (Pavlovich-Abril et al. 2009; Santos Calderelli et al. 2008). Nevertheless, there is still little scientific evidence on the effects provided by the incorporation of these oils (olive or canola) into the multigrain breads matrix. The objective of this study are to improve the lipid profile of breads made with bovine fat and mixture of flours by totally replacing the fat matter either by canola or olive oil, to see how this influences on technological and sensory characteristics (moisture, specific volume, texture and colour), and to verify if this modification maintains the overall acceptability.

Materials and methods

Materials

Commercial wheat flour (WF) (Florencia®, Argentina), used for all experiments was provided by local stores and wheat flour parameters were: deformation energy (W) = 334×10^{-4} J, tenacity (P) = 135 mm, extensibility (L) = 61 mm, P/L = 2.21 (AACC International 2000), moisture = $13.6 \pm 0.2\%$ and protein = $11 \pm 0.3\%$ (Kjeldahl method, N $\times$ 5.7). Whole soybean flour (SF) (Ricedal Alimentos®, Argentina), whole flaxseed flour (FF) (Vicentin®, Argentina), wheat bran (WB) (Avecon®, Argentina), canola oil (Alimentos Fundación Favaloro®, Argentina), extra virgin olive oil (Mazza®, Argentina), bovine fat (BF) (Friar®, Argentina) and sodium chloride (Celusal®, Argentina) were used for the preparation of the breads. Compressed yeast (*Saccharomyces cerevisiae*, Calsa®, Argentina) was used as leavening agent. The moisture content of yeast was 71.5%.

Bread dough preparation

The base formulations with BF were obtained in previous studies by Osuna et al. (2016), who reported that samples with 16 g/kg FF + 16 g/kg WB and with 16 g/Kg FF + 8 g/Kg SF were the optimum formulations of mixtures of flours to obtain breads with some n3 fatty acids content and good technological characteristics.

Base formulation 1 Total Flours (100 g with 97.6 g WF and 1.6 g FF, and 0.8 g SF), commercial compressed yeast (4% flour basis), BF (4% flour basis), sodium chloride (2% flour basis) and tap water up to optimum absorption (55% flour basis).

Base formulation 2 Total Flours (100 g with 96.4 g WF and 1.6 g FF and 1.6 g WB), commercial compressed yeast (4% flour basis), BF (4% flour basis), sodium chloride (2% flour basis) and tap water up to optimum absorption (55% flour basis).

BF of each base formulation (1 and 2) was totally replaced by OO or CO, resulting in six different combinations.

A straight dough method was used for the preparation of bread. The different steps were made at laboratory scale. The ingredients were mixed and kneaded to an optimum consistency in a rapid mixer (Zonda®, Buenos Aires, Argentina) during 7 min, and then they were put to rest for 15 min. After this period, the dough was laminated into the laminator (Rd®, Buenos Aires, Argentina) and allowed to stand for a15 min period. Then, the dough was divided into portions of 400 g, rounded manually, and allowed to stand for another15 min period. They were cut in portions of 200 g, and the loaves were formed and placed into aluminium pans (24.5 × 6.5 cm). The pans were placed in a proving cabinet at 35 °C and 85% Relative Humidity (RH) for 90 min. The fermented loaves were baked in an electric oven (Zonda®, Buenos Aires, Argentina) for 15 min at 180 ± 5 °C. After baking, the breads were removed from the pans, cooled at room temperature (25 ± 1 °C), and packed into polyethylene bags for subsequent analysis. The experiment was replicated twice.

Bread analyses

Fatty acids composition

Total lipids were extracted using the Bligh and Dyer method (1959). Fatty acid composition was determined after methylation (AOAC 1995) using an Agilent gas chromatograph (model 6850A HP, Agilent Technologies Inc, CA, USA), equipped with a 60 m Supelco 2340 capillary column. The temperature of the injector and the detector was kept at 250 °C. The injected volume was 1.0 µl. The carrier gas was helium at 0.6 µl min^{-1}. The Split ratio used was 1:100. The temperature of the column was kept at 140 °C for 5 min, raised to 240 °C at 4 °C/min, and maintained at 240 °C for 10 min. Fatty acids (FA) were identified by comparing their retention times with international standards (Supelco 37 Components FAME Mixture, Bellefonte, PA), and reported as g 100 g^{-1} of total fatty acids. Results were expressed as relative quantities of SFA, MUFA, and PUFA; PUFA/SFA ratio; and n6/n3 ratio.

Technological parameters

Technological parameters were carried out according to the American Association of Cereal Chemists AACC (2000). Moisture contents of bread were determined by measuring their weight loss after drying in an oven at 130° C until reaching constant weight. Samples of bread were analyzed and results were expressed in grams of water per 100 g of wet product (AACC 2000). The specific volume (SV) of the bread was determined by the flaxseed volume displacement, according to method 10-05 (AACC 2000) and divided by the sample weight (cm^3 g^{-1}), width/height ratio of the central slice and crumb water activity.

Bread crumb texture profile analysis

Texture measurements were performed on cylinder (20 mm diameter and 20 mm height) and samples were cut from the central part of three loaf slices for each formulation, 15 h after baking. On average, six measurements per sample were made. The bread cylinders were compressed using a Textural Analyzer (CT3, Brookfield, EE.UU.) equipped with a TA25/1000 acrylic cylinder probe (50.8 mm diameter). For texture profile analysis (TPA), samples were compressed to 50% of their original height. The uniaxial compression test was performed in two successive cycles, using 0.5 mm s^{-1} as speed test (Osuna et al. 2014). The typical texture profile analysis parameters were determined from the Force-Distances curves and calculated by the software: hardness (N), cohesiveness (dimensionless), springiness (mm) and chewiness (mJ).

Colour analysis

The colour of crumb and crust of breads was determined with a Thermo Scientific Spectrophotometer with an integrating sphere (Hunter Associates Laboratory, Inc., Reston, VA) and the results were expressed in accordance with the CIELab system with reference to illuminate D65 and a visual angle of 10°. The parameters determined were L* (lightness), a* (redness), and b* (yellowness); and colour differences (ΔE*) were estimated by using the following formula:

$$\Delta E^* = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2} \quad (1)$$

Also, the brownness index (BI) was calculated according to Komlenić et al. (2010):

Table 1 Composition of fatty acids, total contents of SFA, MU, PUFA, n3, n6, n9 (g 100 g^{-1} of total fatty acids) and relationship of PUFA/SFA and n6/n3 of breads made from WF, FF, WB and SF using BF, OO and CO

Fatty acids	WF + FF + WB + BF	WF + FF + WB + CO	WF + FF + WB + OO	WF + FF + SF + BF	WF + FF + SF + CO	WF + FF + SF + OO
(14:0)	2.26 ± 0.00^{c}	nd^{a}	nd^{a}	2.18 ± 0.00^{b}	nd^{a}	nd^{a}
(14:1)	0.45 ± 0.01^{c}	nd^{a}	nd^{a}	0.43 ± 0.00^{ab}	nd^{a}	nd^{a}
(15:0)	0.52 ± 0.02^{ab}	nd^{a}	nd^{a}	0.52 ± 0.03^{ab}	nd^{a}	nd^{a}
(16:0)	20.23 ± 0.038^{f}	6.59 ± 0.039^{b}	14 ± 0.04^{d}	19.84 ± 0.028^{e}	6.38 ± 0.03^{a}	13.7 ± 0.04^{c}
(16:1)	2.29 ± 0.007^{e}	0.31 ± 0.005^{b}	0.98 ± 0.08^{c}	2.3 ± 0.005^{e}	0.21 ± 0.007^{a}	1.09 ± 0.006^{d}
(17:0)	1.23 ± 0.00^{c}	nd	0.00 ± 0.00^{a}	1.18 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
(18:0)	16.16 ± 0.005^{f}	2.31 ± 0.004^{b}	2.57 ± 0.005^{d}	16.09 ± 0.004^{e}	2.14 ± 0.005^{a}	2.4 ± 0.006^{c}
(18:1) n9	29.21 ± 0.081^{a}	42.42 ± 0.09^{d}	47.55 ± 0.07^{e}	29.58 ± 0.057^{b}	42.14 ± 0.08^{c}	48.45 ± 0.09^{f}
(18:2 c) n6	14.78 ± 0.008^{b}	23.19 ± 0.07^{e}	22.29 ± 0.009^{d}	14.41 ± 0.006^{a}	24.19 ± 0.008^{f}	21.03 ± 0.06^{c}
(20:0)	0.88 ± 0.015^{a}	1.78 ± 0.01^{d}	1.18 ± 0.014^{c}	0.92 ± 0.011^{b}	1.77 ± 0.015^{d}	1.16 ± 0.016^{c}
(18:3) n3	10.47 ± 0.01^{a}	22.95 ± 0.015^{f}	11.23 ± 0.015^{c}	11.02 ± 0.01^{b}	22.68 ± 0.02^{e}	12.18 ± 0.011^{d}
CLA (18: 2) 9c, 11t	0.58 ± 0.00^{b}	nd^{a}	nd^{a}	0.61 ± 0.00^{c}	nd^{a}	nd^{a}
(20:3) n6	nd^{a}	0.46 ± 0.00^{b}	nd^{a}	nd^{a}	0.5 ± 0.00^{c}	nd^{a}
(20:4) n6	nd^{a}	nd^{a}	0.21 ± 0.00^{b}	nd^{a}	nd^{a}	nd^{a}
SFA	41.29 ± 0.06^{f}	10.68 ± 0.05^{b}	17.69 ± 0.06^{d}	40.73 ± 0.05^{e}	10.29 ± 0.07^{a}	17.26 ± 0.06^{c}
MUFA	32.08 ± 0.1^{a}	42.73 ± 0.09^{d}	48.60 ± 0.09^{e}	32.49 ± 0.08^{b}	42.35 ± 0.09^{c}	49.53 ± 0.1^{f}
PUFA	25.25 ± 0.01^{a}	46.6 ± 0.02^{e}	33.73 ± 0.02^{d}	25.43 ± 0.02^{b}	47.36 ± 0.01^{f}	33.21 ± 0.01^{c}
n3	10.47 ± 0.01^{a}	22.95 ± 0.05^{e}	11.22 ± 0.02^{c}	11.02 ± 0.01^{b}	22.68 ± 0.02^{f}	12.18 ± 0.01^{d}
n6	14.78 ± 0.01^{b}	23.65 ± 0.03^{e}	22.49 ± 0.04^{d}	14.41 ± 0.01^{a}	24.69 ± 0.01^{f}	21.03 ± 0.03^{c}
n9	29.21 ± 0.08^{a}	42.42 ± 0.1^{d}	47.55 ± 0.07^{e}	29.58 ± 0.07^{b}	42.14 ± 0.06^{c}	48.45 ± 0.09^{f}
n6/n3	1.41 ± 0.003^{d}	1.03 ± 0.0002^{a}	1.99 ± 0.0017^{f}	1.31 ± 0.0084^{c}	1.09 ± 0.012^{b}	1.73 ± 0.026^{e}
PUFA/SFA	0.61 ± 0.01^{a}	4.36 ± 0.02^{e}	1.9 ± 0.01^{c}	0.62 ± 0.0031^{b}	4.6 ± 0.02^{f}	1.92 ± 0.01^{d}

Values are mean ± SD (n = 3); values sharing same superscript in row are not statistically significant at $p < 0.05$, determined by ANOVA and Turkey's test for multiple comparisons

nd not detected, *SFA* summation of saturated fatty acids, *MUFA* summation of monounsaturated fatty acids, *PUFA* summation of polyunsaturated fatty acid, *PUFA/SFA* relationship of polyunsaturated fatty acid and saturated fatty acids, *n3* fatty acids of the n3 family, *n6* fatty acids of the n6 family, *n9* fatty acids of the n9 family, *n6/n3* relationship between fatty acids of series n6 and n3, *WF* wheat flour, *FF* flaxseed flour, *SF* soybean flour, *WB* wheat bran., *BF* bovine fat, *CO* canola oil and *OO* olive oil

Table 2 Technological characteristics of breads made from WF, FF, WB and SF using BF, OO and CO

Samples	Specific volume (cm^3/g)	Width/height ratio	Moisture (%)	Water activity
WF + FF + WB + BF	4.41 ± 0.24^b	1.17 ± 0.01^b	29.52 ± 0.25^c	0.924 ± 0.03^a
WF + FF + WB + CO	3.74 ± 0.09^a	1.55 ± 0.02^d	28.99 ± 0.09^{bc}	0.926 ± 0.05^{ab}
WF + FF + WB + OO	4.34 ± 0.25^b	1.26 ± 0.02^{bc}	30.73 ± 0.2^d	0.927 ± 0.05^b
WF + FF + SF + BF	4.19 ± 0.09^{ab}	1.06 ± 0.04^a	28.86 ± 0.15^b	0.928 ± 0.08^{bc}
WF + FF + SF + CO	4.26 ± 0.07^b	1.31 ± 0.06^c	29.45 ± 0.34^c	0.93 ± 0.04^c
WF + FF + SF + OO	4.17 ± 0.15^{ab}	1.19 ± 0.04^b	27.45 ± 0.09^a	0.927 ± 0.02^b

Values are mean ± SD (n = 3); values sharing same superscript in a column are not statistically significant at $p < 0.05$, determined by ANOVA and Turkey's test for multiple comparisons

WF wheat flour, *SF* soybean flour, *FF* flaxseed flour, *WB* wheat bran., *BF* bovine fat, *OO* olive oil and *CO* canola oil

$$BI = \frac{100 \times (x - 0.31)}{0.17} \quad (2)$$

where *BI* is brownness index and *x* is a calculated variable for Eq. 3.

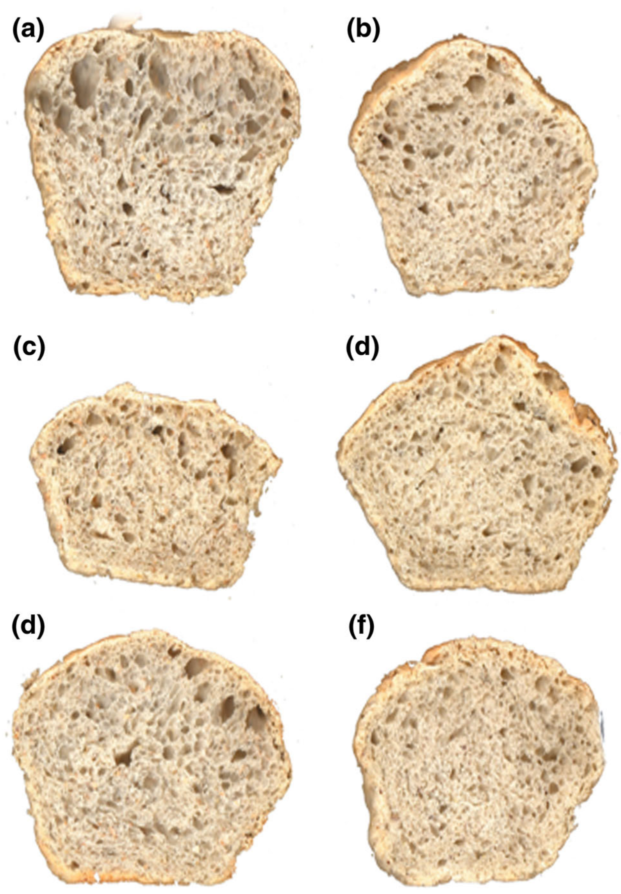

Fig. 1 Crumb slices images of breads made with mixes of flours and different fat matters: **a** WF + FF + WB + BF, **b** WF + FF + WB + CO, **c** WF + FF + WB + OO, **d** WF + FF + SF + BF, **e** WF + FF + SF + CO and **f** WF + FF + SF + OO. *WF* wheat flour, *SF* soybean flour, *FF* flaxseed flour, *WB* wheat bran., *BF* beef fat, *OO* olive oil and *CO* canola oil

$$x = \frac{(a^* + 1.75 \times L^*)}{5.645 \times L^* + a^* - 3.012 \times b^*} \quad (3)$$

where a^* is redness, b^* is yellowness and L^* is lightness. It has been found that the *BI* parameter shows a linear correlation with the concentration of brown pigment. This has been useful to evaluate colour changes in food by enzymatic and non-enzymatic reactions. In particular, in bakery, it has allowed to observe variations in the colour of the bread due to changes in the formulations used.

Sensory analysis

A sensory panel of 90 untrained panellists from 18 to 60 years old, who like bread or are regular consumers of bread, was set up to evaluate the acceptance of the substituted breads. Samples were randomly coded and presented to the panel member in trays with instructions for the evaluation. The analysed attributes were appearance, colour, texture, taste and overall acceptability, using a nine-point hedonic scale for each parameter. The panellists were asked to indicate the score of each sensory descriptor and a score for overall acceptability, on a scale ranging from 1 (extremely dislike) to 9 (extremely like). A five score was used as a minimum threshold for acceptability. For each of these attributes, the relative frequency of the panellist responses was reported.

Statistical analysis

INFOSTAT statistical software (Facultad de Ciencias Agropecuarias, UNC, Argentina) was used to perform the statistical analysis. Determinations were done at least three times. A Tukey's test was made in order to evaluate differences among samples (significant level at $p < 0.05$).

Table 3 Color parameters (L*, a* and b*) the calculated ΔE* and the BI of breads made with made from WF, FF, WB and SF, using BF, OO and CO

Samples	Crumb color				Crust color				
	L*	a*	b*	ΔE_{crumb}	L*	a*	b*	BI	ΔE_{crust}
WF + FF + WB + BF	60.63 ± 0.64^{a}	3.83 ± 0.12^{ab}	10.5 ± 0.4^{a}	–	62.97 ± 0.58^{d}	10.57 ± 0.21^{ab}	21.5 ± 0.35^{b}	52.83 ± 1.63^{ab}	–
WF + FF + WB + CO	67.2 ± 1.08^{bc}	4.33 ± 0.15^{c}	12.47 ± 0.49^{b}	6.89 ± 0.79	54.33 ± 0.61^{b}	8.77 ± 0.76^{a}	17.07 ± 0.87^{a}	48.41 ± 0.93^{a}	9.93 ± 0.64
WF + FF + WB + OO	61.27 ± 1.4^{a}	4.38 ± 0.08^{c}	12.27 ± 0.57^{b}	2.10 ± 0.57	58.17 ± 0.29^{c}	9.9 ± 0.36^{ab}	18.6 ± 0.17^{a}	49.11 ± 1.87^{a}	5.23 ± 1.30
WF + FF + SF + BF	69.07 ± 0.57^{cd}	3.83 ± 0.06^{ab}	11.63 ± 0.21^{b}	–	64.67 ± 0.84^{e}	11.83 ± 0.21^{b}	24.93 ± 0.15^{c}	60.59 ± 2.89^{bc}	–
WF + FF + SF + CO	64.93 ± 0.71^{b}	3.63 ± 0.06^{a}	11.8 ± 0.17^{b}	4.15 ± 0.40	59.4 ± 0.26^{c}	11.43 ± 1.52^{ab}	23.67 ± 0.81^{bc}	63.29 ± 3.74^{c}	5.83 ± 1.89
WF + FF + SF + OO	69.97 ± 0.15^{d}	3.97 ± 0.06^{b}	11.97 ± 0.23^{b}	0.99 ± 0.55	51.37 ± 0.6^{a}	11.1 ± 1.95^{ab}	17.33 ± 1.53^{a}	55.71 ± 5.71^{abc}	12.52 ± 0.68

Values are mean ± SD (n = 5); values sharing same superscript in a column are not statistically significant at $p < 0.05$. determined by ANOVA and Turkey's test for multiple comparisons

WF wheat flour, *SF* soybean flour, *FF* flaxseed flour, *WB* wheat bran., *BF* bovine fat, *OO* olive oil and *CO* canola oil

Results and discussion

Fatty acid composition

Table 1 shows the relative quantities of the identified fatty acids, as well as the total contents of SFA, MUFA, PUFA, n3, n6, n9 (g 100 g^{-1} of total fatty acids), and the PUFA/SFA and n6/n3 relationship of loaves made with flour mixes and different oils. The results showed that replacement of BF completely with vegetable oils (either canola or olive oil) in different formulations of bread produced significant differences in the composition of FA ($p < 0.05$). This replacement decreased SFA and a relative percentage of MUFA and PUFA increased compared to bread without any replacement. The addition of CO in breads produced the highest increase in n3 fatty acids lowering n6/n3 ratio, and increasing PUFA/SFA ratio. The linoleic fatty acid showed an increase of 56.9% in formulation with WB, and 67.86% in formulation with SF while the α-linolenic fatty acid content (18:3 n3) increased by 120% and by 105% respectively in the same formulations.

The addition of OO in formulated breads produced a significant increase in n9 and n6 fatty acids content. Therefore, it caused a small increase in the n6/n3 ratio, and a moderate increase in the PUFA/SFA ratio, the latter being lower than in CO replaced formulation. The oleic acid (18:1n9) was the major component of the n9 family, and increased approximately 63% for both formulations. For samples enriched with OO, the rise of linoleic fatty acid was 50.81% for the WB formulation, and 45.94% for the SF formulation.

These results were consistent with Santos Calderelli et al. (2008), who reported an increased PUFA content and n6 and n3 fatty acids when substituted by hydrogenated fat with soybean oil in the flaxseed bread preparation. Moreover, Simopoulos (2008) studied the importance of n6/n3 ratio in Western diets and concluded that low ratios (1–2) are associated with a reduced risk of several chronic diseases, such as cardiovascular diseases, inflammatory diseases, and cancer. Therefore, the n6/n3 relationship can be used to assess the nutritional quality of the lipid fraction of foods. The n6/n3 ratio calculated in this study for the formulations with CO or OO were consistent with the values previously reported. Thus, the consumption of this fortified breads could contribute to restoring the FA balance of the diet.

Concerning the content of trans fatty acids CLA (18:2) 9c,11t was detected only in samples with BF, and the presence of this conjugated FA is consistent with its origin, since it is a characteristic component of fats from ruminants. Natural trans fatty acids are produced in the rumens of cows and sheep. They arise through partial hydrogenation and/or isomerisation of cis-unsaturated FA from the feed by hydrogen produced during oxidation of substrates with bacterial enzymes as catalysts. As a result, the fat in milk, butter, cheese and beef contains 2–9% trans fatty acids (Brouwer et al. 2010).

Technological characteristics

Table 2 shows the values of specific volume (SV), width/height ratio, moisture and water activity of bread substituted with mixtures of two flours and with the addition of vegetable oils. BF replacement by vegetable oils produced significant differences in SV ($p < 0.05$) only in the formulation bread WF + FF + WB + CO, presenting the lowest volume (see Fig. 1). This behaviour could be due to the combination of the negative effects that the presence of wheat bran fibre exerts, and the physical state of fatty acids and canola oil. This is consistent with studies made by Smith and Johansson (2004). While Wang et al. (2002) reported that higher fibre content adversely interfere with the technological quality of bread, especially in reducing SV. Smith and Johansson (2004) found an increase in bread volume with increase in proportions of solid fat. These increases in bread volume demonstrated the importance of the quantity of solid fat in the shortening. The increase of bread volume indicated that either fat crystals or the saturated fat content play an important role in the development of air cells, and increasing amount of fat crystals to a greater bread volume.

Moreover, Watanabe et al. (2002, 2003) reported not improvement in bread volume with addition of liquid oil and breads showed the porous structure of the gluten network, with large size of gas cells. The addition of vegetable oil produced a negative influence on gluten network, making it thinner and less extensible than the dough containing saturated fats. These authors stated that the liquid oil was adsorbed to the gluten structure causing aggregation thereof.

The width/height ratio of the bread analysed also showed significant differences ($p < 0.05$) among different formulations. WF + FF + WB + CO formulation presented a higher W/H, giving flattened bread and a smaller volume than the other samples.

On the other hand, the moisture content of all formulations was lower than that established by international regulations (Max. 40%). WF + FF + WB + OO formulation showed higher values for moisture content, and the highest values were observed for wheat bran formulations. This could be due to the presence of a large number of hydroxyl groups in the fiber structure, involved interaction with water through hydrogen bonding (Chaplin 2003).

Table 4 Textural characteristics of bread made with WF, FF, WB and SF, and different fat matters

Samples	Hardness (N)	Adhesiveness (J)	Cohesiveness	Springiness (mm)	Chewiness (mJ)
WF + FF + WB + BF	2.45 ± 0.3[ab]	0.06 ± 0.05[a]	0.47 ± 0.02[a]	9.23 ± 0.64[a]	10.72 ± 0.32[a]
WF + FF + WB + CO	3.05 ± 0.31[b]	0.07 ± 0.05[a]	0.49 ± 0.02[ab]	9.66 ± 0.38[a]	13.99 ± 0.62[b]
WF + FF + WB + OO	2.54 ± 0.21[ab]	0.07 ± 0.05[a]	0.49 ± 0.02[ab]	9.37 ± 0.48[a]	10.97 ± 0.15[a]
WF + FF + SF + BF	3 ± 0.39[b]	0.06 ± 0.04[a]	0.48 ± 0.03[ab]	9.95 ± [a]	14.36 ± 0.39[b]
WF + FF + SF + CO	2.18 ± 0.28[a]	0.06 ± 0.04[a]	0.46 ± 0.03[a]	10.06 ± 0.57[a]	10.78 ± 0.74[a]
WF + FF + SF + OO	2.64 ± 0.38[ab]	0.07 ± 0.01[a]	0.52 ± 0.01[b]	10.27 ± 0.69[a]	13.47 ± 0.45[b]

Values are mean ± SD (n = 5); values sharing same superscript in a column are not statistically significant at $p < 0.05$, determined by ANOVA and Turkey's test for multiple comparisons

WF wheat flour, *SF* soybean flour, *FF* flaxseed flour, *WB* wheat bran., *BF* bovine fat, *OO* olive oil and *CO* canola oil

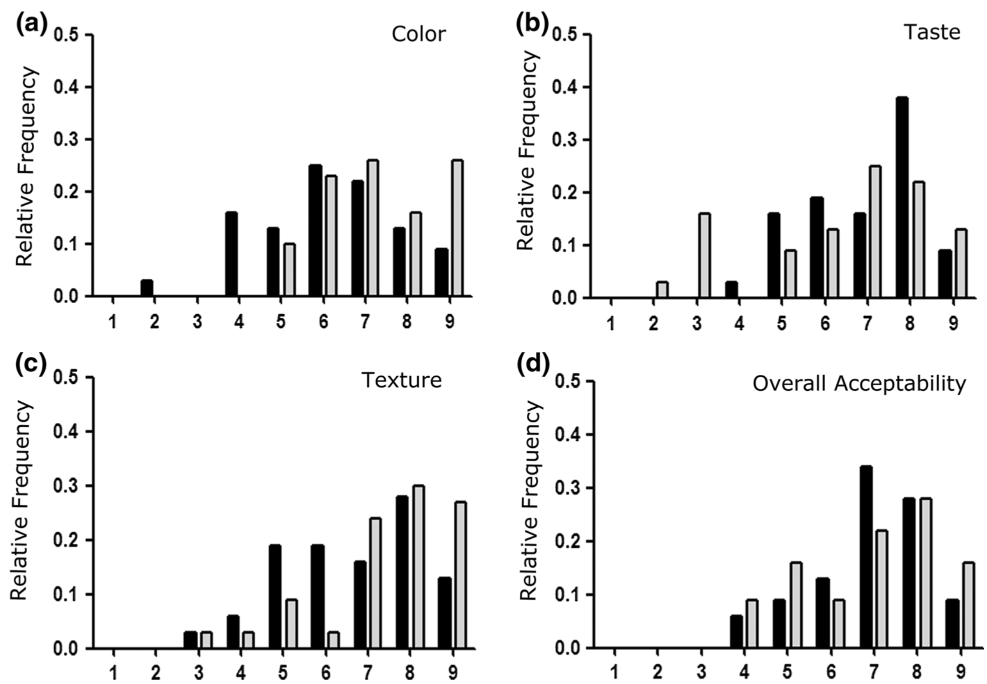

Fig. 2 Sensory evaluation of breads made with WF + FF + WB + OO (black bars) or WF + FF + SF + CO (grey bars) for the following attributes: **a** colour, **b** taste, **c** texture, **d** overall acceptability. Frequency: number of panellists that choose a category/total number of panellists (90). Category: from 1 (extremely dislike) to 9 (extremely like). *WF* wheat flour, *FF* flaxseed flour, *SF* soybean flour, *WB* wheat bran., *CO* canola oil and *OO* olive oil

These results agreed with Santos Calderelli et al. (2010), who reported that the bread with flaxseed flour and soybean oil had similar moisture content.

The values of water activity showed the fraction of total free water content of samples, and consequently, available for growth of microorganisms and various chemical reactions. The data analysis indicated that all samples showed significant differences ($p < 0.05$), and the WF + FF + SF + CO formulation was the most vulnerable sample.

Crumb and crust colour

Table 3 shows the colour parameters (L*, a*, b*), the ΔE*, and BI of crumb and crust of bread made with flour mixtures and vegetable oils, and their base formulations. The effect of BF replacement with either CO or OO on crumb colour of fortified bread was analysed, was observed that, BF replacement with CO in SF and WB formulations caused significant differences ($p < 0.05$), in the L*, ΔE* presented values above 5 (indicating evident colour differences by visual observation) only for WF + FF + WB + CO (6.89 ± 0.79).

The addition of both oils in fortified breads also caused significant differences in crust colour particularly in L* for all assayed formulations, producing a less luminous crust, whereas WF + FF + SF + CO formulation (higher in n3 fatty acid ntent) did not have significant differences either in parameter a* or parameter b*, where their ΔE* value was around 5.

On the other hand, BI did not show a significant change in the assayed formulations.

These results agree with those of Santos Calderelli et al., (2008), who reported that replacement of fat by hydrogenated soybean oil did not affect the crust colour parameters.

Texture profile analysis of crumb

In Table 4, the textural characteristics (hardness, adhesiveness, cohesiveness, springiness and chewiness) of breads made with flour mixes and other oils can be observed.

Regarding the hardness of the crumb, when the formulation had WB, the replacement of fat by oils did not significantly affect this parameter. However, when the formulation possessed soybean flour, the replacement of fat by canola oil resulted in softer bread, which was directly related to the highest water activity (0.93).

These results agree with Santos Calderelli et al. (2008), who found that the hardness of flax bread with hydrogenated fat showed significant differences with flax bread with soybean oil, presenting a softer texture.

Sensory analysis

The fortified breads that presented the highest n3 fatty acids content (WF + FF + SF + CO) and the highest n9 fatty acid content (WF + FF + WB + OO), with technological characteristics similar to samples without BF replacement, were selected for the sensory analysis.

The effect of BF replacement with vegetable oils on overall acceptability, texture, colour and taste in formulated breads with mixtures of flours was evaluated. Figure 2 shows the relative frequency, which corresponds to the number of panellists that chose a category over the total number of panellists (90), being the categories from 1 (extremely dislike) to 9 (extremely like). Panellists did not reject any sample; in all cases, scores were higher than five (considered as the minimum acceptable value in this work) and in the majority of the cases, they were close to six or seven. Therefore, the addition of vegetable oils to fortified breads presented a good overall acceptability for both breads, giving (6.97 ± 1.33) average score for the bread with bran and olive oil, and a (6.91 ± 1.59) for the bread with soybean flour and canola oil. Gimeno Montoya (2013) found that the breads made with 4 and 10% virgin OO had acceptable sensory scores. De Aguiar et al. (2011) studied the enrichment of whole wheat flaxseed rolls with omega-3 by partial substitution of soybean oil with flaxseed oil and reported that in the sensory analysis of the breads, all the evaluated attributes had an average score of 7.6, showing the mild approval of the developed formulations. Our results were consistent with this study.

Conclusion

The replacement of BF by CO and OO produced an improvement of fatty acids composition. The addition of CO in breads caused the highest increase in the content of n3 fatty acids, resulting in a lowering n6/n3 ratio and increasing PUFA/SFA ratio. This increase was greater in the SF formulation. Furthermore, the addition of OO in formulated breads led to a significant increase in n9 and n6 fatty acids, a small increase in n6/n3 ratio and moderate increase of PUFA/SFA ratio. This effect was maximized in formulations with WB. This improvement of the fatty acid profile produced changes in the colour, texture and technological characteristics, but the breads with WF + FF + SF + CO and WF + FF + WB + OO presented higher specific volume, softer crumb, less colour alteration and better sensory acceptance.

The results show that the development of a good quality nutritional product with sensory acceptance is possible through the addition of flours mixture and vegetable oils in breads formulation.

Acknowledgements The authors acknowledge Chaco Austral University and the CONICET for financial support.

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