

Available online at www.sciencedirect.com



Food Chemistry 94 (2006) 399-405

Food Chemistry

www.elsevier.com/locate/foodchem

# Bakery products enriched with phytosterols, $\alpha$ -tocopherol and $\beta$ -carotene. Sensory evaluation and chemical comparison with market products

Joan Quílez<sup>a</sup>, Joan A. Ruiz<sup>a</sup>, Gemma Brufau<sup>b</sup>, Magda Rafecas<sup>b,\*</sup>

<sup>a</sup> Departament de Tecnologia, Europastry S.A. Ctra, Sarral a Barberà s/n, Sarral 43424, Spain <sup>b</sup> Departament de Nutrició i Bromatologia, Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n, Barcelona 08028, Spain

Received 4 May 2004; received in revised form 19 November 2004; accepted 19 November 2004

### Abstract

A sensory evaluation of croissants and magdalenas (Spanish muffins) has been carried out to compare them with the same products with phytosterol esters,  $\alpha$ -tocopherol and  $\beta$ -carotene added. The subjects were habitual consumers and no differences were detected between the two types of product. Furthermore, the chemical composition (53 parameters) of croissants (n = 9) and muffins (n = 8) marketed in Spain, has been analysed and a nutritional evaluation performed on these products with regard to their suitability as carriers of the functional ingredients mentioned above. The chemical compositions are not similar and the main difference is related to fatty acids. Croissants are characterized by a high percentage of saturated fatty acids (SFA) (44.0 ± 3.8%), and the presence of *trans* fatty acids (TFA) (4.29 ± 1.48%). This contrasted with the high content of polyunsaturated fatty acids (PUFA) (62.1 ± 1.5%) in the muffins and the near absence of TFA (0.16 ± 0.23%). Furthermore, the large differences between the compositions of the commercial croissants and muffins (*magdalenas*) and, therefore, between their nutritional values, makes the use of phytosterols recommendable in muffins and, in general, in all bakery products in which the SFA + TFA represent  $\leq 20\%$  of the fatty acid fraction.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Bakery products; Phytosterols; β-Carotene; Sensory evaluation; Composition

## 1. Introduction

From the point of view of cardiovascular diseases (CVD), the consumption of bakery products is not recommended. These products are associated with the presence of saturated fatty acids (SFA) and trans fatty acids (TFA) and it is well known that these fatty acids provoke an increase of plasma cholesterol, principally of LDL-cholesterol and of the total to HDL-cholesterol ratio, with a consequent increase in cardiovascular risk (Mensik, Zock,

*E-mail addresses:* jquilez@europastry.com (J. Quílez), magdarafecas@ub.edu (M. Rafecas).

Kester, & Katan, 2003). In southern Europe, for example, two important characteristics of the Mediterranean diet are a low intake of SFA and a high intake of carbohydrates; however, blood cholesterol levels are increasing in the population and nowadays are similar to those in the USA (Amorim Cruz, 2000). The consumption of bakery products in Spain in the last few years is near 15 kg/ person/year (Ministerio de Agricultura, Pesca y Alimentación, 2003) and evidence suggests that these figures will remain stable, because they are important food components of breakfast and mid-morning and afternoon snacks and also their sweetness and marked palatability are elements which promote their consumption.

On the other hand, considerable efforts have been made, in recent years, to design functional ingredients

<sup>\*</sup> Corresponding author. Tel.: +34 93 403 48 41; fax: +34 93 402 18 96.

<sup>0308-8146/</sup>\$ - see front matter © 2004 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodchem.2004.11.029

able to reduce LDL-cholesterol; certain types of high viscosity soluble fibres, such as  $\beta$ -glucan or psyllium (Jenkins, Kendall, Axelsen, Augustin, & Vuksan, 2000) and the phytosterols (Ostlund, 2002; Quílez, García-Lorda et al., 2003) are those which have proved most effective in this area. In the case of the fibres, the quantities necessary to achieve a significant effect (10-15 g/d), and their rheological and sensorial characteristics, could be a hindrance to their use in bakery products (Trautwein, Carls, Erbersdobler, & Hisserich, 2000). This is not the case with the phytosterols as, apart from theoretically not altering their characteristics, their effects are achieved with significantly lower quantities (1.5-3 g/d) (Quílez, García-Lorda, & Salas-Salvadó, 2003), with the only side effect, described to date, being the interference they cause in the absorption of the carotenoids, particularly  $\beta$ -carotene (Gylling, Puska, Vartiainen, & Miettinen, 1999). In previous work (Quílez, Rafecas et al., 2003), it was observed that the addition of  $\beta$ -carotene compensated for the deficit in its absorption provoked by the phytosterols. Another important aspect of bakery products, in relation to other carriers, such as fat spreads or salad dressings, is the control of the phytosterol intake. Some authors (Plat, van Onselen, van Heugten, & Mensink, 2000) have reported that the daily dose is effective when it is included in a single portion.

The objective of the present study was to investigate whether there were appreciable sensory differences between standard bakery products (SBP) and phytosterol-enriched bakery products (PBP). Furthermore, due to the incongruence of adding phytosterols to a carrier which has a theoretically incremental effect on plasma cholesterol, a chemical analysis and nutritional evaluation was carried out on this type of product manufactured industrially in Spain, paying particular attention to the lipid content and to the two forms of presentation: (1) trade marked bakery products, baked and packed at the site of origin and sold in food shops or supermarkets (baked), (2) those made using frozen doughs, baked at the point of sale and sold to the public with no trade name (frozen).

## 2. Materials and methods

### 2.1. Samples

Eight samples of baked products (four croissants and four *magdalenas* or Spanish muffins), from the most representative trade names, were acquired in supermarkets. Nine samples of frozen products (five croissants and four *magdalenas* or Spanish muffins), also from the most important trade names in the sector, were acquired from the distributors of these products. The two samples of PBP (one croissant and one muffin) used in this trial were manufactured specifically for this purpose and were frozen (Europastry SA, Sarral, Spain). Products were served individually and they were randomized according to triangular test ISO norm. Frozen products were defrosted for 15 min and baked in a commercial convection oven. Croissants were baked at 190 °C for 15 min after proofing and muffins at 170 °C for 20 min. Samples were cooled at room temperature for 30 min. Then, they were cut into small pieces and frozen with liquid nitrogen and ground with an analytical grinding device (Ika, Labortechnik, Germany) to give a powder, which was stored at -20 °C until it was analysed. Baked goods were ground by the same procedure.

Each sample was analysed in duplicate. All solvents used were of HPLC grade and all reagents were of analytical grade from Panreac (Barcelona, Spain), Scharlau Chemie, SA (Barcelona, Spain) and SDS (Peypin, France). Standards were supplied by Sigma Chemical Co. (Saint Louis, MO).

### 2.2. Sensory evaluation

This was based on the triangular test (International Organization for Standardization, 1983). A total of 30 persons, habitual consumers of bakery products, were asked to detect appearance or taste differences between SBP and PBP, both in croissants and muffins. All the products belonged to the Frozen group, and the only differences between SBP and PBP were the addition of phytosterol esters (1.53 g/serving 68 g in croissant and 1.68 g/serving 54 g in muffins),  $\alpha$ -tocopherol (2.8 and 2.5 mg, respectively) and  $\beta$ -carotene (0.33 and 0.59 mg). The yellow-orange colour of the SBP was made equal to that of the PBP using colorants (Tartrazine and Ponceau 4R). The answer "no difference" was permitted and these replies were considered as incorrect.

#### 2.3. Proximate analysis

Protein was analyzed by the Kjeldahl method. Total lipid was determined by the Soxhlet method. Ash was analysed by burning the sample in a furnace device at 525 °C for 5 h. Salt was determined by the Volhard method. Total dietary fibre was analysed according AOAC Official Method 991.43 (AOAC, 1998). Carbohydrates were calculated by difference (100 – water – protein – total fat – total ash – total dietary fibre). Sugars were determined enzymatically by a UV method with a sucrose/D-glucose/D-fructose commercial kit (Roche-Biopharm, Darmstadt, Germany).

# 2.4. Lipid analysis

#### 2.4.1. Extraction

Total lipids were extracted according to Folch, Lees, and Stanley (1957). The samples (3 g) were extracted with 100 ml chloroform:methanol 2:1 solution for 2 h.

Homogenate was filtered and phases were separated in a funnel with 35 ml of 2% NaCl solution. The organic layer was filtered and evaporated. Extracted fat was dissolved with 10 ml of chloroform.

## 2.4.2. Phytosterols and cholesterol

Sterols were analysed by the method of Toivo, Piironen, Kalo, and Varo (1998). An aliquot of extracted fat solution (0.2 ml) was completely evaporated in a centrifuge tube. Then, 5 ml of 0.5 M ethanolic KOH solution were added and saponification was done at 80 °C for 20 min. The non-saponifiable fraction was extracted three times with cyclohexane. Then, a solid-phase extraction was carried out to separate sterols from more polar lipids and to achieve a cleaner extract. Finally, the sample was trimethylsilyl-derivatized with 50 µl Bis (trimethylsilyl) Trifluoroacetamide with 1% Trimethyl Chlorosilane TMCS and 50 µl pyridine. Samples were injected into a Shimadzu GC-17A gas-liquid chromatograph coupled to a mass spectrometer detector, with a 30 m capillary column (SAC-5; Supelco, Bellefonte, PA). The GC temperature programme was: 250 °C (5 min) – 3 °C/min - 320 °C (1.5 min), the total run time being 30 min. The carrier gas was helium with a column flow of 1.0 ml/min. The injector was at 280 °C in split mode (1:9). Detector temperature was 320 °C. The select iron monitoring mode was used to detect all the compounds, acquiring only the more intense ions m/z of each compound (total *m*/*z* analysed: 41, 43, 55, 57, 69, 73, 75, 81, 83, 217). 5- $\alpha$ -Cholestane was used as internal standard.

## 2.4.3. Fatty acids

Fatty acids were determined by the Slover and Lanza (1979) method. An aliquot of extracted fat solution (0.2 ml) was evaporated completely in a test tube. 0.5 ml 5% MeONa solution was added and the mixture was incubated at 100 °C for 30 min. The sample was cooled for 15 min. Then, 1 ml 14% BF<sub>3</sub> in methanol was added to a test tube and it was methylated at 100 °C for 15 min. The sample was cooled again for 15 min. Finally, 2 ml of *n*-hexane and 4 ml of 10% NaCl solution were added. The test tube was vigorously shaken and the upper phase was extracted and filtered before it was injected. Samples were injected into a Shimadzu model gas-liquid chromatograph coupled to a mass spectrometer detector, with a 50 m capillary column (ID-BPX70; Teknokroma, Barcelona, Spain). The GC temperature programme was: 100 °C (2 min) - 10 °C/  $min - 170 \ ^{\circ}C \ (10 \ min) - 5 \ ^{\circ}C/min - 200 \ ^{\circ}C \ (6 \ min), the$ total run time being 30 min. The carrier gas was helium with a column flow of 2.0 ml/min, The injector was at 250 °C in split mode (1:25). Detector temperature was 230 °C. The SCAN mode (m/z: 33–450) was used to detect all the fatty acids. Tridecanoic acid methyl ester was used as internal standard. Fatty acids were identified by comparison with standard methylated compounds.

## 2.4.4. Tocopherols and carotenoids

Tocopherols and carotenoids were analyzed according the method proposed by Cavina, Gallinella, Porra, Pecora, and Suraci (1988). An aliquot of fat extract was dried under a stream of nitrogen and redissolved with 5 ml of methanol–*n*-hexane (85:15, v/v). Samples were injected into a Shimadzu HPLC system with a variable wavelength UV–visible SPD-10A detector and a Tracer Extrasil ODS-2 column ( $250 \times 4.0 \text{ mm}$  i.d.; 5 µm particle size) protected by an ODS guard cartridge system (Teknokroma, Barcelona, Spain). Samples were isocratically eluted with a methanol–*n*-hexane mixture (85:15, v/v), with the detector at 292 nm, for detecting tocopherols and at 450 nm for carotenes.

#### 2.5. Statistical analyses

Results are presented as means  $\pm$  standard deviation. Statistical analyses were performed with the statistical software SYSTAT 10 for Windows (SPSS Inc., Chicago, USA). Variables between groups (croissants vs. muffins) were compared by the *t* test and differences were considered significant when  $P \leq 0.05$ . Relationships between variables were tested using Pearson's correlation and Bonferroni probabilities. Discriminant analysis, with complete estimation and tolerance = 0.001, was carried out for the main components (protein, carbohydrate and total ash) and fatty acids (SFA, TFA, MUFA and PUFA). Total fat was not considered due to similar values in both products.

# 3. Results

## 3.1. Sensory evaluation

The results of the sensory evaluation are presented in Table 1. On the total of 30 replies, it was observed that the majority response in the two groups was that differences were not found between samples (12 in croissants and 17 in muffins). Correct replies were limited to 11 in croissants (36.7%) and five in muffins (16.7%), and, due to the fact that the minimum number of these replies for a significance level of 0.1% is 19 (International

Table 1

Sensory scores of triangular test for standard (SBP) vs. phytosterolenriched (PBP) croissants and muffins

	Croissants <i>n</i> (% within group)	Muffins <i>n</i> (% within group)
Correct replies <sup>a</sup>	11 (36.7%)	5 (16.7%)
Incorrect replies	7 (23.3%)	8 (26.7%)
No found difference	12 (40.0%)	17 (56.7%)
Total	30 (100%)	30 (100%)

<sup>a</sup> For n = 30, the minimum number of correct replies for a significance level of 0.1% is 19.

Organization for Standardization, 1983), we can conclude that there are no differences of either appearance or taste between SBP and PBP.

## 3.2. Chemical composition

The results of the proximal compositions of the commercial SBP compared with the PBP samples, are presented in Table 2, fatty acids in Table 3, and the compositions of sterols and fat-soluble antioxidants in Table 4. These results show a very large qualitative difference between croissants and muffins. Although the fat content was similar in the two types of products, being about 25%, the differences in protein, salt, dietary fibre and sugars were highly significant (each with P < 0.001), as was also found for the fatty acid fraction and phytosterols. With respect to nutritional relevance, the sum of the SFA + TFA fractions was  $13.2\% \pm 1.8$ in muffins and  $48.3\% \pm 3.6$  in croissants (P < 0.001). With respect to the energetic value (kcal/100 g), there were no significant differences between the two types of product:  $441 \pm 28$  for croissants and  $448 \pm 14$  for muffins. The PBP present similar values to frozen products and obviously shows higher values of phytosterols and lipid soluble antioxidants, as a result of the quantities of these components which were added. The differences between the two groups, apart from the significance for each one of the components analysed, is demonstrated by the discriminant analysis (Fig. 1). According to the canonical discriminant functions, they are associated with Factor 1, with positive values for croissants and negative values for muffins, with the mean distance between the two groups being 17.4 (8.2;

-9.2). Factor 2 separates the groups made up of frozen (positive values) and baked products (negative values), with the mean distance being 13.9 (5.4; -8.5) between the croissants and 5.2 (2.9; -2.3) between the muffins. The TFA were not entered into these functions as the tolerance of the variable was very low (<0.001), since it showed a very close correlation with the SFA (r = 0.83; P < 0.001) and the PUFA (r = -0.86; P < 0.001).

# 4. Discussion

Appearance and taste are highly relevant to the choice of foods, and they are particularly important, in the case of health foods, in those groups most inclined to unhealthy diets (Neumark-Sztainer, Story, Perry, & Casey, 1999). This conclusion may be extrapolated to functional foods and therefore, in our study, an assessment has been made of whether sensory differences exist between bakery products with the same ingredients, except for the addition of phytosterol esters,  $\alpha$ -tocopherol and  $\beta$ -carotene. The result was that the evaluators, habitual consumers of these products, were unable to identify (significantly) the type of product they had tested.

In spite of this, even lack of significance for the triangular test, there are differences between the correct replies between croissants and muffins (11 vs. 5). The enhanced flavour of muffins by essences (lemon, orange, vanilla) and their more homogeneous appearance and texture, due the production process, could explain the favourable score of these products. The sensory test results are similar to those obtained by Niinikoski, Viikari,

Table 2

Proximate analysis of commercial and phytosterol-enriched bakery products (PBP) (%w/w)

	Magdalenas (muffins)				Margarine croissants				
	Frozen $(n = 4)$	Baked $(n = 4)$	Total muffins $(n = 8)$	Sterol added $(n = 1)$	Frozen $(n = 5)$	Baked $(n = 4)$	Total croissants $(n = 9)$	Sterol added $(n = 1)$	
Water	$18.5 \pm 2.2$	$16.0 \pm 1.1$	$17.2 \pm 2.1$	19.8	$19.8 \pm 2.5$	$15.5 \pm 2.4$	$17.9 \pm 3.2$	23.5	
Protein	$5.57 \pm 0.81$	$5.08 \pm 0.34$	$5.32 \pm 0.63$	5.77	$7.62\pm0.60$	$8.03\pm0.49$	$7.80 \pm 0.56^{***}$	7.49	
Total fat	$25.6 \pm 2.0$	$25.1 \pm 1.3$	$25.3 \pm 1.6$	23.1	$27.5 \pm 3.6$	$23.4 \pm 4.5$	$25.7 \pm 4.3$	24.6	
Total ash	$1.36 \pm 0.06$	$1.15 \pm 0.14$	$1.26 \pm 0.15$	1.36	$1.79 \pm 0.18$	$1.25 \pm 0.19$	$1.55 \pm 0.33^{*}$	0.60	
Salt (NaCl)	n.d.	$0.11 \pm 0.14$	$0.06 \pm 0.11$	n.d.	$0.97 \pm 0.24$	$0.38 \pm 0.23$	$0.71 \pm 0.38^{***}$	1.24	
Total dietary fibre	$1.28 \pm 0.06$	$1.09 \pm 0.10$	$1.18 \pm 0.13$	1.31	$2.57\pm0.15$	$1.88 \pm 0.53$	$2.27 \pm 0.50^{***}$	2.48	
Insoluble	$0.69\pm0.09$	$0.60\pm0.18$	$0.65 \pm 0.14$	0.67	$1.64 \pm 0.27$	$0.96\pm0.40$	$1.34 \pm 0.47^{**}$	1.48	
Soluble	$0.59 \pm 0.15$	$0.48\pm0.08$	$0.53 \pm 0.13$	0.73	$0.93 \pm 0.15$	$0.92 \pm 0.14$	$0.93 \pm 0.14^{***}$	1.00	
%Carbohydrates <sup>a</sup>	$47.8 \pm 0.8$	$51.7 \pm 0.4$	$49.7 \pm 2.2$	48.6	$40.7 \pm 1.8$	$50.0 \pm 1.8$	$44.8 \pm 5.2^*$	40.1	
Total sugars	$19.1 \pm 1.1$	$22.4 \pm 1.9$	$20.7 \pm 2.1$	17.7	$3.69\pm0.33$	$9.92 \pm 1.98$	$6.46 \pm 3.51^{***}$	3.52	
Sucrose	$18.5 \pm 1.0$	$21.7 \pm 1.7$	$20.1 \pm 2.2$	16.8	$0.06 \pm 0.13$	$2.82 \pm 2.65$	$1.28 \pm 2.18^{***}$	0.14	
Glucose	$0.46 \pm 0.35$	$0.51\pm0.64$	$0.48\pm0.48$	0.54	$1.47\pm0.13$	$4.40\pm2.94$	$2.77 \pm 2.37^{*}$	1.50	
Fructose	$0.11 \pm 0.21$	$0.17 \pm 0.29$	$0.14 \pm 0.24$	0.37	$2.16 \pm 0.29$	$2.70 \pm 1.10$	$2.40 \pm 0.76^{***}$	1.88	

n.d., not detected.

<sup>a</sup> %Carbohydrates = 100 - water - protein - total fat - total ash - total dietary fibre.

\* Probability < 0.05 of significant difference between total muffins and total croissants (t test).

<sup>\*\*</sup> P < 0.01.

\*\*\* P < 0.01.

Table 3
Fatty acids of commercial and phytosterol-enriched bakery products (PBP) (%w/w of total fatty acids)

	Magdalenas (muffins)				Margarine croissants				
	Frozen $(n = 4)$	Baked $(n = 4)$	Total muffins $(n = 8)$	Sterol added $(n = 1)$	Frozen $(n = 5)$	Baked $(n = 4)$	Total croissants $(n = 9)$	Sterol added $(n = 1)$	
SFA <sup>a</sup>	$12.1 \pm 0.4$	$14.1 \pm 1.8$	$13.1 \pm 1.6$	12.3	$45.4 \pm 4.4$	$42.3 \pm 2.2$	$44.0 \pm 3.8^{***}$	36.1	
C 4:0	$0.02 \pm 0.04$	n.d.	$0.01 \pm 0.03$	n.d.	n.d.	n.d.	n.d.	n.d.	
C 6:0	$0.01 \pm 0.02$	n.d.	$0.01 \pm 0.02$	n.d.	n.d.	n.d.	n.d.	n.d.	
C 8:0	$0.01 \pm 0.02$	n.d.	$0.01 \pm 0.01$	n.d.	$0.12 \pm 0.10$	$0.04 \pm 0.02$	$0.09 \pm 0.08^{*}$	0.04	
C 10:0	$0.01 \pm 0.02$	n.d.	$0.01 \pm 0.01$	n.d.	$0.10 \pm 0.10$	$0.03 \pm 0.01$	$0.07 \pm 0.08^{*}$	0.03	
C 12:0	$0.03 \pm 0.02$	$0.02 \pm 0.01$	$0.03 \pm 0.01$	0.03	$0.72 \pm 0.68$	$0.22 \pm 0.14$	$0.50 \pm 0.56^{*}$	0.25	
C 14:0	$0.11 \pm 0.06$	$0.08 \pm 0.01$	$0.09 \pm 0.04$	0.09	$1.06 \pm 0.37$	$0.69 \pm 0.15$	$0.89 \pm 0.34^{***}$	0.75	
C 15:0	$0.02 \pm 0.00$	$0.02 \pm 0.01$	$0.02 \pm 0.01$	0.03	$0.02\pm0.02$	$0.03 \pm 0.02$	$0.02 \pm 0.02$	0.05	
C 16:0	$6.99 \pm 0.36$	$9.42 \pm 2.05$	$8.20 \pm 1.88$	7.15	$33.8 \pm 2.9$	$32.3 \pm 2.1$	$33.2 \pm 2.6^{***}$	28.8	
C 17:0	$0.03 \pm 0.02$	$0.07 \pm 0.02$	$0.05 \pm 0.03$	0.04	$0.13 \pm 0.04$	$0.10 \pm 0.01$	$0.12 \pm 0.04^{***}$	0.10	
C 18:0	$3.93 \pm 0.23$	$3.60 \pm 0.24$	$3.76 \pm 0.28$	4.10	$8.81 \pm 3.02$	$8.28 \pm 1.44$	$8.53 \pm 2.33^{***}$	5.28	
C 20:0	$0.21 \pm 0.03$	$0.31 \pm 0.07$	$0.26 \pm 0.07$	0.29	$0.42 \pm 0.06$	$0.41 \pm 0.06$	$0.42 \pm 0.06^{***}$	0.47	
C 22:0	$0.58 \pm 0.11$	$0.49 \pm 0.07$	$0.53 \pm 0.10$	0.60	$0.16 \pm 0.08$	$0.18 \pm 0.07$	$0.17 \pm 0.07^{***}$	0.37	
C 24:0	$0.11 \pm 0.08$	$0.13 \pm 0.01$	$0.12 \pm 0.06$	n.d.	n.d.	n.d.	n.d.	n.d.	
MUFA <sup>b</sup>	$25.1 \pm 1.2$	$24.3 \pm 1.4$	$24.7 \pm 1.3$	28.8	$31.8 \pm 3.9$	$35.2 \pm 3.8$	$33.3 \pm 4.1^{***}$	38.1	
C 16:1	$0.19 \pm 0.03$	$0.17 \pm 0.03$	$0.18 \pm 0.03$	0.21	$0.30 \pm 0.39$	$0.18 \pm 0.06$	$0.25 \pm 0.29$	0.17	
C 18:1	$24.7 \pm 1.3$	$23.9 \pm 1.4$	$24.3 \pm 1.3$	28.3	$31.2 \pm 3.7$	$34.8 \pm 3.7$	$32.8 \pm 3.9^{***}$	37.4	
C 20:1	$0.11 \pm 0.04$	$0.19 \pm 0.05$	$0.15 \pm 0.06$	0.24	$0.29 \pm 0.20$	$0.25 \pm 0.09$	$0.27 \pm 0.15^{*}$	0.56	
PUFA <sup>c</sup>	$62.9 \pm 1.5$	$61.3 \pm 1.1$	$62.1 \pm 1.5$	59.0	$18.6 \pm 6.2$	$16.6 \pm 4.0$	$18.4 \pm 5.8^{***}$	21.2	
C 18:2 <sup>e</sup>	$62.8 \pm 1.5$	$57.5 \pm 3.3$	$60.1 \pm 3.7$	58.2	$17.4 \pm 6.3$	$15.6 \pm 4.2$	$17.3 \pm 5.9^{***}$	18.5	
C 18:3	$0.15 \pm 0.07$	$3.82 \pm 2.49$	$1.98 \pm 2.55$	0.74	$1.24 \pm 0.30$	$1.07 \pm 0.59$	$1.16 \pm 0.43$	3.05	
TFA <sup>d</sup>	n.d.	$0.33 \pm 0.22$	$0.16 \pm 0.23$	n.d.	$4.28 \pm 1.91$	$5.08 \pm 1.76$	$4.29 \pm 1.48^{***}$	4.56	
C 18:1 t	n.d.	n.d.	n.d.	n.d.	$3.54 \pm 0.85$	$4.94 \pm 1.70$	$4.17 \pm 1.41^{3***}$	4,08	
C 18:2 c, t	n.d.	n.d.	n.d.	n.d.	$0.61 \pm 1.17$	$0.74 \pm 0.91$	$0.67 \pm 1.00$	0.30	
C 18:3 t	n.d.	$0.33\pm0.22$	$0.16 \pm 0.23$	n.d.	$0.12\pm0.27$	$0.14\pm0.16$	$0.13 \pm 0.22$	0.18	

n.d., not detected.

<sup>a</sup> Saturated fatty acids.
<sup>b</sup> Monounsaturated fatty acids.
<sup>c</sup> Polyunsaturated fatty acids.
<sup>c</sup> Fatty acids.

<sup>d</sup> Trans fatty acids.

<sup>e</sup> Mix of position isomers.

\* Probability < 0.05 of significant difference between total muffins and total croissants (*t* test). \*\*\* P < 0.01.

Table 4	
Sterols and lipid antioxidants of commercial and phytosterol-enriched bakery products (PBP) (mg/kg) <sup>a</sup>	

	Magdalenas (muffins)				Margarine croissants			
	Frozen $(n = 4)$	Baked $(n = 4)$	Total muffins $(n = 8)$	Sterol added $(n = 1)$	Frozen $(n = 5)$	Baked $(n = 4)$	Total croissants $(n = 9)$	Sterol added $(n = 1)$
Cholesterol	$807 \pm 223$	589 ± 147	698 ± 210	1940	$70 \pm 92$	$493 \pm 383$	$258 \pm 330^{**}$	1470
Total phytosterols	$1028 \pm 151$	$869 \pm 95$	$949 \pm 144$	32110	$687 \pm 93$	$876 \pm 221$	$771 \pm 180^{*}$	23210
Campesterol	$100 \pm 16$	$125 \pm 21$	$113 \pm 22$	8740	139 ± 44	$205 \pm 90$	$168 \pm 72$	6140
Stigmasterol	$100 \pm 20$	$153 \pm 35$	$127 \pm 39$	4340	63 ± 25	59 ± 19	$61 \pm 21^{***}$	3140
β-Sitosterol	$513 \pm 84$	$401 \pm 63$	$457 \pm 91$	15120	$338 \pm 40$	$416 \pm 102$	$373 \pm 80$	10500
Sitostanol	$30 \pm 4$	$36 \pm 6$	$33 \pm 6$	1810	48 ± 3	$46 \pm 4$	$47 \pm 3^{***}$	1660
$\Delta^5$ -Avenasterol	$27 \pm 2$	$28 \pm 8$	$27 \pm 6$	n.d.	$21 \pm 4$	$26 \pm 5$	$24 \pm 5$	n.d.
$\Delta^7$ -Stigmasterol	$116 \pm 23$	$50 \pm 38$	$83 \pm 45$	n.d.	n.d.	n.d.	n.d.***	n.d.
$\Delta^7$ -Avenasterol	$52 \pm 4$	$26 \pm 8$	$39 \pm 15$	n.d.	n.d.	n.d.	n.d.***	n.d.
Others	91 ± 15	$50 \pm 37$	$70 \pm 34$	2090	$79 \pm 20$	$124 \pm 29$	99 ± 33	1770
α-Tocopherol	$91.3 \pm 17.3$	$40.4 \pm 21.8$	65.8 ± 32.7	117	$33.2 \pm 7.9$	$44.7 \pm 16.6$	$38.3 \pm 13.1$	81.8
γ-Tocopherol	$8.6 \pm 0.8$	$69.8 \pm 30.3$	$39.2 \pm 38.2$	8.7	$19.1 \pm 13.9$	$25.5 \pm 13.2$	$21.9 \pm 13.1$	19.9
β-Carotene	$0.5 \pm 0.9$	n.d.	$0.3 \pm 0.6$	10.9	$0.8\pm0.6$	$1.6 \pm 2.3$	$1.2 \pm 1.5$	4.8

n.d., not detected.

<sup>a</sup>  $\alpha$ -Carotene and lycopene were not detected in any product. \* Probability < 0.05 of significant difference between total muffins and total croissants (*t* test). \*\*\* P < 0.01. \*\*\* P < 0.01.

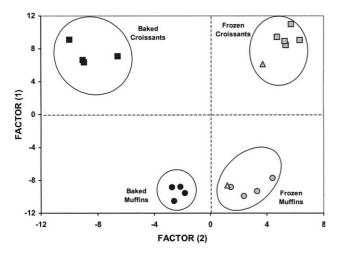


Fig. 1. Discriminant analysis; plot values of the two principal factors. Canonical discriminant functions: Factor 1 = 16.451 + 1.137 protein + 1.451 total ash - 0.128 carbohydrates + 0.054 SFA - 0.298 MFA - 0.330 PSA. Factor 2 = -73.775 + 0.892 protein + 5.646 total ash - 0.892 carbohydrates + 1.133 SFA + 0.807 MFA + 1.157 PSA. Cumulative proportion of total dispersion = 0.991. Symbols: round = muffins; square = croissants; triangles = PBP. Colours: black = baked, grey = frozen.

and Palmu (1997) in fat spreads with added phytosterols, thus confirming the sensorial neutrality of these compounds and their suitability as functional ingredients.

The result of the chemical analysis of commercial croissants and muffins revealed that there were marked differences between the two types of bakery products. This finding, also reported in other studies (Parcerisa, Codony, Boatella, & Rafecas, 1999; Quilez & Rafecas, 2002; Vicario, Griguol, & León-Camacho, 2003), implies that bakery products cannot be discussed in a generic manner, but rather that the type must be specified. The differences in the main components are determined by the different recipes in each type of product. The greater content of flour in the croissants leads to a higher percentage of protein and dietary fibre (both with P < 0.001). The sugar content is also very different (P < 0.001), with sucrose predominating in the muffins and the monosaccharides in croissants, though these are produced by the hydrolysis of sucrose in the fermentation process. Another important difference is in the mineral content as, although the difference in total ash between the two products is slightly significant (P = 0.034), the muffins contain practically no salt whereas this is found in the croissants (P < 0.001). In contrast, muffins include raising agents, to cause the oven-spring directly, whilst the croissants, as has already been stated, must undergo prior fermentation with yeast. Despite this, the greatest differences between the two groups are found in the lipids, principally in the fatty acid fraction.

Apart from the ingredients already mentioned, the different tastes and the technological requirements,

needed to produce each kind of product, necessitate the use of vegetable oils in muffins and of high melting-point fats in croissants. This leads to a higher content of PUFA in muffins (62.1%  $\pm$  1.5) and of SFA in croissants (44.0%  $\pm$  3.8). In recent years, particular attention has been paid to the presence of TFA in foods, principally in margarines and bakery products (Parcerisa et al., 1999; van Erp-baart et al., 1998; Vicario et al., 2003), as these form a significant part of the diet. The data are consistent with what we have stated above, and whilst a very low concentration  $(0.16\% \pm 0.23)$  is found in muffins, related to the oil refining process (León-Camacho, Ruiz-Méndez, & Graciani-Constante, 1999), high values  $(4.29\% \pm 1.48)$  are found in croissants, related to the hydrogenation process of the vegetable oils (as confirmed by their phytosterol content) to obtain hard, plastic margarines, appropriate for the manufacture of laminated doughs (Quilez & Rafecas, 2002).

Apart from phytosterols, the presence of cholesterol in the muffins is principally due to the presence of egg, as milk fat was only detected in one sample by means of the short-chain fatty acid content. The same occurred with croissants, with the exception that the frozen products contained no fat of animal origin, except for one sample. In view of the results concerning  $\beta$ -carotene, its use is very irregular and the pale-yellow to yellow-orange colour, required for these products, is obtained not only from the carotene itself but also from the use of colorants of chemical or natural origin, such as bixin (according to the information on the label). It should be noted that there was one croissant with a  $\beta$ -carotene content of 5.0 mg/kg, higher even than the PBP type (4.8 mg/kg).

The discriminant analysis performed in Fig. 1 not only provides a perfect separation of the croissants group from the muffins (Factor 1), it also separates the baked from the frozen products (Factor 2). However, the distances are smaller with this factor, particularly in the case of the muffins, demonstrating that these behave as a much more homogeneous group with respect to their composition, independently of whether they are baked or frozen. Furthermore, the croissants have another source of variability not contemplated in the study as, although the principal fat with which they are manufactured in Spain (margarine) has been taken into account, there are regions where croissants are consumed which have been manufactured with lard (pig fat) or with butter, as occurs particularly in France and other countries.

In conclusion, it has been demonstrated that the phytosterol esters,  $\alpha$ -tocopherol and  $\beta$ -carotene, are particularly suitable for inclusion in bakery products, as functional ingredients, since the addition of effective doses does not affect the appearance or taste. Furthermore, the large differences in the composition of the commercial croissants and muffins (*magdalenas*) and, therefore, in their nutritional value, makes the use of these and other functional ingredients recommendable in muffins, sponge cakes and, in general, in all the bakery products in which the SFA + TFA represent  $\leq 20\%$  of the fatty acid fraction.

# References

- Amorim Cruz, J. A. (2000). Dietary habits and nutritional status in adolescents over Europe-Southern Europe. *European Journal of Clinical Nutrition*, 54(Suppl. 1), S29–S35.
- AOAC. (1998). *Official methods of analysis* (16th ed.). Arlington, VA: Association of Official Analytical Chemists.
- Cavina, G., Gallinella, B., Porra, R., Pecora, P., & Suraci, C. (1988). Carotenoids, retinoids and alpha-tocopherol in human serum: identification and determination by reversed phase HPLC. *Journal* of Pharmaceutical and Biomedical Analysis, 6, 259–269.
- Folch, J., Lees, M., & Stanley, S. G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Gylling, H., Puska, P., Vartiainen, E., & Miettinen, T. A. (1999). Retinol, vitamin D, carotenes and α-tocopherol in serum of a moderately hypercholesterolemic population consuming sitostanol ester margarine. *Atherosclerosis*, 145, 279–285.
- International Organization for Standardization. (1983). Sensory analysis – methodology – triangular test. *International Standard ISO* 4120.
- Jenkins, D. J. A., Kendall, C. W. C., Axelsen, M., Augustin, L. S. A., & Vuksan, V. (2000). Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. *Current Opinions in Lipidolology*, 11, 49–56.
- León-Camacho, M., Ruiz-Méndez, M. V., & Graciani-Constante, E. (1999). Isomerization of fatty acids during deodorization/physical refining stripping with nitrogen. *Fett*, 101, 290–294.
- Mensik, R. P., Zock, P. L., Kester, A. D., & Katan, M. B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American Journal* of Clinical Nutrition, 77, 1146–1155.
- Ministerio de Agricultura, Pesca y Alimentación. (2003). La alimentación en España 2001. Madrid, Spain: MAPA.
- Neumark-Sztainer, D., Story, M., Perry, C., & Casey, M. A. (1999). Factors influencing food choices of adolescents: findings from

focus-group discussions with adolescents. *Journal of American Dietetic Association*, 99, 929–937.

- Niinikoski, H., Viikari, J., & Palmu, T. (1997). Cholesterol-lowering effect and sensory properties of sitostanol ester margarine in normocholesterolemic adults. *Scandinavian Journal of Nutrition*, 41, 9–12.
- Ostlund, R. E. (2002). Phytosterols in human nutrition. *Annual Reviews of Nutrition*, 22, 533–549.
- Parcerisa, J., Codony, R., Boatella, J., & Rafecas, M. (1999). Fatty acids including trans content of commercial bakery products manufactured in Spain. *Journal of Agricultural and Food Chemistry*, 47, 2040–2043.
- Plat, J., van Onselen, E. N. M., van Heugten, N. M. A., & Mensink, R. P. (2000). Effect on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters. *European Journal of Clinical Nutrition*, 54, 671–677.
- Quílez, J., & Rafecas, M. (2002). Aspectos nutricionales de la bollería. Presente y futuro. *Alimentaria*, 338, 53–62.
- Quílez, J., García-Lorda, P., & Salas-Salvadó, J. (2003). Potential uses and benefits of phytosterols in diet: present situation and future directions. *Clinical Nutrition*, 22, 343–351.
- Quílez, J., Rafecas, M., Brufau, G., García-Lorda, P., Megías, I., Bullò, M., Ruiz, J. A., & Salas-Salvadó, J. (2003). Bakery products enriched with phytosterol esters, α-tocopherol and β-carotene decrease plasma LDL-cholesterol and maintain plasma β-carotene concentrations in normocholesterolemic men and women. *Journal* of Nutrition, 133, 3103–3109.
- Slover, H., & Lanza, E. (1979). Quantitative analysis of food fatty acids by capillary gas chromatography. *Journal of American Oil Chemists Society*, 56, 933–943.
- Toivo, J., Piironen, V., Kalo, P., & Varo, P. (1998). Gas chromatographic determination of major sterols in edible oils and fats using solid-phase extraction in sample preparation. *Chromatographia*, 48, 745–750.
- Trautwein, E. A., Carls, C. R., Erbersdobler, H. F., & Hisserich, D. (2000). Entwicklung von Brotsorten mit Psylliumzusatz als Beitrag zu einer cholesterolbewuβten Ernährungsweise. *Deutsche Lebben-smittel-Rundschau*, 96, 58–64.
- van Erp-baart, M.-A., Couet, C., Cuadrado, C., Kafatos, A., Stanley, J., & van Poppel, G. (1998). Trans fatty acids in bakery products from 14 European countries: the TRANSFAIR study. *Journal of Food Composition and Analysis*, 11, 161–169.
- Vicario, I. M., Griguol, V., & León-Camacho, M. (2003). Multivariate characterization of the fatty acid profile of Spanish cookies and bakery products. *Journal of Agricultural and Food Chemistry*, 51, 134–139.