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# Preliminary Study on Health-Related Lipid Components of Bakery Products

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## ABSTRACT

The purpose of this study was to evaluate the presence of health-related lipid components, in particular *trans* fatty acids and sterol oxidation products, in four bakery products. Both types of components are known for their adverse biological effects, especially the increase of atherogenic risk, and therefore it is advisable to monitor their presence in food products. *Trans* fatty acids were determined by silver-ion thin-layer chromatography–gas chromatography, whereas sterol oxidation was assessed by gas chromatography and gas chromatography–mass spectrometry determination of 7-keto derivatives (tracers of sterol oxidation). The amount of *trans* fatty acids (0.02 to 3.13 g/100 g of product), sterols (34.9 to 128.3 mg/100 g of product), and 7-keto derivatives of sterols (1.88 to 3.14 mg/kg of product) varied considerably among samples. The supply of phytosterols (22.5 to 64.0 mg/100 g of product) was not significant, and the extent of oxidation of most phytosterols to its corresponding 7-keto derivative was low (0.29 to 0.84%), except for that of brassicasterol (2.01 to 3.11%). The quality of ingredients and raw materials seems to have greatly influenced the fatty acid profile, stability, safety, and quality of the final product; these ingredients should be chosen with extreme care to decrease their potential negative health effects and to increase safety of these products.

Bakery products are usually consumed for breakfast or as snacks. Because of their fat content, they supply a large amount of calories, which makes bakery products unsuitable from the nutritional standpoint. The main negative effect of bakery products, however, is related to the possibility of raising blood lipids and thus increasing the cardiovascular risk as a consequence of the presence of saturated fatty acids (SFA) and *trans* fatty acids (TFA) *(31).* In fact, Judd et al. *(34)* reported that a diet high in vegetable TFA, in comparison with a *cis*-unsaturated diet, increases the plasma concentrations of total cholesterol, low-density lipoproteins (LDL) cholesterol, and triglycerides and decreases high-density lipoproteins (HDL) cholesterol; similar findings were reported by other research groups *(4, 33, 41, 47, 69),* confirming that SFA and TFA consumption increases LDL cholesterol concentrations. Furthermore, a dose-dependent relationship between TFA intake and LDL/HDL ratio has been found, the magnitude of which is greater for TFA compared with SFA *(5).* In addition, TFA can inhibit the activity of hepatic enzymes *(46)* and modify the fluidity of cell membranes *(43),* which leads to a higher atherogenic risk *(44).*

Considering the adverse effects of TFA and SFA, the World Health Organization/Food and Agriculture Organization (WHO/FAO) recommendations on fat and oil intake include the reduction of both supplies *(78).* Recently, the U.S. Food and Drug Administration (FDA) has developed new regulations on food labeling to require that TFA be declared on the nutrition label of conventional foods and dietary supplements *(72).* Although the European Food Safety Authority is at present discussing which strategy to follow concerning TFA in food, there is no European legislation that limits the level of TFA in food, except for the TFA content in infant formula and follow-on formula *(20).*

On the other hand, the intense thermal treatment to which bakery products are subjected, as well as the previous processing steps for preparation of some ingredients (such as dried eggs and milk powder) *(8),* lead to oxidation of the lipid fraction, in particular cholesterol, of bakery products. Oxidation of cholesterol is of great concern because it generates a series of products that are known to have a wide range of adverse biological effects, including cytotoxicity, mutagenesis, carcinogenesis, and atherogenesis *(24, 49, 64)*; it must be noticed that the type and extent of the various biological effects vary according to the type of cholesterol oxidation products (COP) present *(64).* In fact, COP have been found in early and advanced stages of atherosclerotic lesions *(10, 25, 68),* and, even under normocholesterolemic conditions, they can cause endothelial dysfunction, increased macromolecular permeability, and cholesterol accumulation *(59),* all of which are factors involved in the development of early atherosclerotic lesions. Several studies performed with animals have proven that COP present in the diet can be absorbed from the intestine, and they had been found in chylomicrons and other lipoproteins *(6, 30, 50, 55, 56, 75).* Emanuel et al. *(18)* and Linseisen et al. *(40)* concluded that humans can absorb COP from food.

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Bakery products also supply a large amount of lipids from vegetable origin, which can degrade during the different technological steps and storage. In particular, phytosterols can oxidize in a similar way as cholesterol and give rise to analogous oxidation products. In fact, over the past few years, oxidation of phytosterols has been evaluated in model systems as well as in some oils and food products *(7, 15, 36)*; however, several aspects of this oxidation process and the possible toxicity effects of phytosterols *(1, 38, 42)* are still to be elucidated. In fact, Maguire et al. *(42)* and Adcox et al. *(1)* reported that β-sitosterol oxides exhibit less severe but similar toxicity patterns to those observed for COP. Lea et al. *(38)*, on the contrary, concluded that phytosterol oxides do not display a genotoxic potential and no evidence of subchronic toxicity was found. Hiroko et al. *(29)* indicated that phytosterol oxidation products (POP) are absorbed but that they do not promote the development of atherosclerosis in apo E–deficient mice. Recently, the biological effects of COP and POP were compared, and it was concluded that POP have qualitatively similar toxic effects to COP, but higher concentrations of POP are required to achieve comparable levels of toxicity *(60)*.

In general, the extent of sterol oxidation in food depends on the composition of the food itself as well as on the production technologies that are utilized. In fact, processing temperatures, heating times, and storage are known to enhance the formation of COP in food *(63, 71)*. The evaluation of sterol oxidation in food containing lipids from both animal and vegetable sources is particularly difficult because of the great number of compounds that are generated as well as the similarity of their chemical structure. Several gas chromatographic (GC) methods have been set up for the separation and identification of sterol oxidation products (SOP) *(3, 13, 32, 37)*; however, no complete separation of the oxidation products has been achieved, and many peak overlappings are still to be resolved. Despite this fact, most of these GC methods have been able to separate the 7-keto derivatives of sterols without having major interferences from other SOP *(13)*. This analytical result is important because the determination of the 7-keto derivatives can be useful for monitoring sterol oxidation in food (such as bakery products). In fact, many studies have reported that 7-ketocholesterol is one of the most representative compounds among COP from a quantitative standpoint and that it can be considered a helpful marker of cholesterol oxidation in food *(2, 39, 54, 79)*.

The objective of this study was to analyze the lipid fraction of different bakery products, with special emphasis on the compounds with potential health implications, such as TFA and SOP; the latter were evaluated by using 7-keto derivatives as tracers of sterol oxidation. Bakery products that contained lipids from both animal (dairy fat, lard, eggs) and vegetable (hydrogenated and nonhydrogenated oils) sources were chosen to simultaneously evaluate their single contribution to sterol oxidation in these types of products. Inexpensive bakery goods were also selected because low costs are usually related to the use of low-quality raw materials.

## MATERIALS AND METHODS

**Materials and reagents.** Chloroform, *n*-hexane, diethyl ether, methanol, potassium hydroxide, and anhydrous sodium sulfate were purchased from J. T. Baker (Deventer, The Netherlands). Silylating agents (pyridine, hexamethyldisilazane, and trimethylchlorosilane) and large silica gel thin-layer chromatography (TLC) plates (20 by 20 cm; film thickness, 0.25 mm) were supplied by Merck (Darmstadt, Germany). (24*R*)-ethylcholest-5-en-3β-ol (β-sitosterol) (purity: 60% β-sitosterol and 30% [24*R*]-methylcholest-5-en-3β-ol [campesterol]) was purchased from Research Plus (Bayonne, N.J.). Cholest-5-en-3β,19-diol (19-hydroxycholesterol) (purity: 99%), (24*S*)-methylcholest-5,22-dien-3β-ol (brassicasterol) (purity: 99%), and (24*S*)-ethylcholest-5,22-dien-3β-ol-7-one (7-ketostigmasterol) (purity: 99%) were supplied by Steraloids (Newport, R.I.). (24*S*)-ethylcholest-5,22-dien-3β-ol (stigmasterol) (purity: 95%), cholest-5-en-3β-ol-7-one (7-ketocholesterol) (purity: 99%), cholest-5-en-3β-ol (cholesterol) (purity: 99%), and tridecanoic acid methyl ester were purchased from Sigma (St. Louis, Mo.). 5α-cholestane (purity: 96%) was supplied by Fluka (Buchs, Switzerland). The purity of the standards was controlled by GC. A standard mixture of fatty acid methyl esters (FAME) (GLC 463) was purchased from Nu-Chek (Elysian, Minn.). Silica solid-phase extraction cartridges (Strata SI; 1,500 mg/3 ml) from Phenomenex (Torrance, Calif.) were used for sterol oxides purification. 2′,7′-dichlorofluorescein (sodium salt) and silver nitrate were purchased from Carlo Erba (Milan, Italy).

**Samples.** Commercial samples of four bakery products with different ingredient formulas were purchased from different European supermarkets. The main sampling criteria was to choose different types of bakery products (biscuit [sample 1], cake [sample 2], roll [sample 3], and pastry [sample 4]) that contained lipids from both animal (dairy, lard, eggs) and vegetable (hydrogenated and nonhydrogenated oils) sources. A secondary sampling criterion was the product cost; in fact, inexpensive bakery goods were chosen because low costs are usually related to the use of low-quality raw materials. The ingredient labels of three products (samples 1, 2, and 3) declared the presence of fat from both animal and vegetable origin, whereas the label of the other bakery good (sample 4) only claimed vegetable fat sources among the ingredients.

**Synthesis of 7-keto derivatives of phytosterols.** The synthesis and purification procedures were similar to those suggested by Conchillo et al. *(13)*. Eight milliliters of a β-sitosterol-campesterol solution (1 mg/ml of *n*-hexane–isopropanol [3:2, vol/vol]) was placed in a 200-ml open vial, and the solvent was evaporated under nitrogen stream. The phytosterols were then thermo-oxidized at 150°C for 2 h in an oven, and the resulting thermo-oxidized mixture (mixture A) was dissolved in 8 ml of *n*-hexane–isopropanol (3:2, vol/vol). This process was repeated with a solution of brassicasterol (1 mg/ml of *n*-hexane–isopropanol [3:2, vol/vol]) (mixture B).

To purify the 7-keto derivatives from the other phytosterol oxides, about 250 μl of mixture A was loaded on a TLC plate (20 by 20 cm). A spot of 7-ketocholesterol was also loaded on the same TLC plate as reference for identification of the 7-keto-sitosterol and 7-ketocampesterol. The mobile phase was a mixture of diethyl ether–*n*-hexane–ethanol (70:30:3, vol/vol/vol). The TLC band of the 7-keto derivatives was visualized by spraying with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein sodium salt under UV light (254 nm). The TLC band of the 7-ketoderivatives had a retention factor equal to 0.32. The band was then scraped off, and extraction of pure 7-keto derivatives from the

silica was carried out twice with diethyl ether. The solvent was finally evaporated under nitrogen flow at room temperature, and pure 7-keto derivatives were then dissolved in *n*-hexane–isopropanol (3:2, vol/vol). The same procedure was followed for the purification of 7-ketobrassicasterol from mixture B.

The 7-keto derivatives fractions were then subjected to silylation according to Sweeley et al. *(70),* dissolved in *n*-hexane, and injected into a GC and GC–mass spectrometer (MS) for identification.

**Extraction of lipids.** Lipids were extracted according to a modified version *(5)* of the method described by Folch et al. *(23).*

**Preparation and GC analysis of total FAME.** About 5 mg of lipids extracted from each sample was methylated twice with 100 μl of diazomethane *(22)* and then transmethylated with 2 N KOH in methanol *(21).* Samples were then added to 0.2 mg of tridecanoic acid methyl ester (as internal standard) and 400 μl of *n*-hexane, centrifuged at 395 × *g* for 3 min, and injected into the GC (1 μl of the supernatant).

The GC instrument was a Carlo Erba HRGC Fractovap 4160 (Carlo Erba, Milan, Italy), which was coupled to RTX 2330 column (inside diameter [i.d.], 30 m by 0.25 mm; film thickness, 0.20 μm) (Restek, Bellefonte, Pa.) coated with 90% biscyanopropyl–10% phenylcyanopropyl-polysiloxane. Oven temperature was programmed from 60°C (maintained for 1 min) to 240°C at a rate of 4°C/min; the final temperature was maintained for 10 min. The injector and detector temperatures were both set at 240°C. Helium was used as carrier gas at 1.25 ml/min. The split ratio was 1:40.

FAME identification was performed by comparing their retention time with those of the GLC 463 FAME standard mixture. Further confirmation of the FAME identification was obtained by silver-ion TLC (see below for experimental details). The GC response factors of FAME were calculated by using the GLC 463 FAME standard mixture. The limit of quantitation was 0.01 g/100 g of fatty acids.

**Silver-ion TLC–GC analysis of TFA.** To accurately quantify the *trans* isomers of the monounsaturated fatty acids, silver-ion TLC–GC was performed. Silica gel TLC plates were dipped in a 10% $AgNO_3$ aqueous solution (wt/wt) for 30 s and then activated at 70°C for 20 min. One hundred microliters of the transmethylated sample solution (about 20 mg/ml) was loaded along the TLC plate and put into a development chamber (with *n*-hexane–diethyl ether [90:10, vol/vol] as mobile phase), which had previously been equilibrated in the refrigerator for 45 min. Once the sample had run along 10 cm of the TLC plate, the plate was dried, sprayed with a 0.2% solution of 2′,7′-dichlorofluorescein sodium salt in ethanol, and visualized at 254 nm under a UV lamp. The bands corresponding to the saturated FAME and the *trans* isomers of the monounsaturated FAME were scraped off, put together, and extracted twice with 4 ml of chloroform (each extraction). All the extracts were joined, dried under nitrogen stream, and dissolved in 1 ml of *n*-hexane. One microliter of this solution was reinjected in the GC under the same conditions described above.

To verify the correct identification and quantitation of the TFA, the total FAME and the extracted TLC bands were also injected into a GC instrument coupled to a CP-Sil 88 (i.d., 100 m by 0.25 mm; film thickness, 0.20 μm) capillary column (Chrompack-Varian, Middelburg, The Netherlands) coated with 100% cyanopropyl-polysiloxane. The analyses were run under isothermal conditions at 179°C. Injector and detector temperatures were both set at 250°C. Helium was used as carrier gas at a flow rate of 1.32 ml/min. Split ratio was 1:50.

The total C18:1 *trans* isomers percentage was recalculated using the percentage of C18:0 obtained before and after argentation as reference.

**Extraction of sterols and SOP.** The extraction procedure was similar to that suggested by Conchillo et al. *(13).* About 250 mg of the Folch extract was added to 0.025 mg of 19-hydroxycholesterol and 0.5 mg of 5α-cholestane (as internal standards) for the determination of 7-keto derivatives and total sterols, respectively. Ten milliliters of 1 N KOH solution in methanol was then added to perform a cold saponification at room temperature for 18 h in darkness and under continuous agitation *(61).* The unsaponifiable material was extracted with diethyl ether.

For the determination of sterols, one-tenth of the unsaponifiable matter was subjected to silylation *(70),* dried under nitrogen stream, and dissolved in 100 μl of *n*-hexane; 1 μl of the silylated sterols was injected into the GC and GC-MS.

Regarding the determination of the 7-keto derivatives of sterols, nine-tenths of the unsaponifiable matter was purified by silica solid-phase extraction according to Guardiola et al. *(27).* SOP were eluted with acetone. The purified fraction was then silylated, dried under nitrogen stream, and dissolved in 100 μl of *n*-hexane. Two replicates were prepared per each sample. One microliter of the silylated sterols was injected into the GC and GC-MS under the same analytical conditions used for the determination of sterols.

To further confirm that there were no overlappings with peaks due to matrix interferences, the SOP purified fraction was separated by TLC under the aforementioned analytical conditions. The TLC band of the 7-ketoderivatives was scraped off, extracted with diethyl ether, silylated *(70),* and injected into the GC and GC-MS.

**GC and GC-MS analysis of sterols and SOP.** A Fisons Instruments GC 8000 series model 8160 (Milan, Italy) was equipped with a split-splitless injector and a flame ionization detector. A fused silica capillary column (i.d., 50 m by 0.25 mm; film thickness, 0.25 μm) coated with 5% phenyl–95% dimethylpolysiloxane (CP-Sil 8CB, Chrompack-Varian) was used. The oven temperature was programmed from 280°C (maintained for 20 min) to 290°C at a rate of 0.2°C/min and held for 2 min; the oven temperature was then raised to 320°C at a rate of 30°C/min and held for 2 min. The injector and detector temperatures were both set at 325°C. Helium was used as a carrier gas at a flow rate of 2.5 ml/min; the split ratio was 1:30.

Identification of sterols and 7-keto derivatives was performed by GC-MS. A Hewlett-Packard 6890 GC instrument (Palo Alto, Calif.) coupled to a 5973 mass selective detector (Agilent Technologies, Calif.) was used. The system was fitted with a capillary column (i.d., 30 m by 0.25 mm; film thickness, 0.25 μm) coated with 5% phenyl–95% dimethyl-polysiloxane (SPB-5, Supelco, Bellefonte, Pa.), and helium was used as carrier gas at a flow rate of 1 ml/min. The oven temperature was programmed from 250 to 310°C at 0.8°C/min. The injector and transfer line temperatures were set at 310 and 280°C, respectively. Manual injection of 1 μl of the standard solution of 7-keto derivatives and those obtained from the bakery products was performed in the split mode at a 1:10 split ratio. The filament emission current was 70 eV. A mass range from 40 to 650 *m/z* was scanned at a rate of 1,500 atomic mass units per s.

Identification of sterols was performed by comparing the retention time and mass spectra with those of the purchased sterol standards. The 7-keto derivatives of sterols were identified by comparing their retention time and mass spectra with those of the synthesized 7-keto standards and the purchased 7-ketocholesterol and 7-ketostigmasterol standards. The mass spectra of the 7-keto

TABLE 1. *Fatty acid composition (g/100 g of fatty acids) of samples of four bakery products*[a]

| Fatty acid | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| C4:0 | ND[b] A | 0.33 (±0.02) B | ND A | ND A |
| C6:0 | 0.12 (±0.01) B | 0.19 (±0.01) C | ND A | ND A |
| C7:0 | 0.09 (±0.01) B | 0.11 (±0.01) C | ND A | ND A |
| C8:0 | 0.08 (±0.03) B | 0.14 (±0.01) C | 0.06 (±0.02) A | 0.08 (±0.01) B |
| C10:0 | 0.17 (±0.03) C | 0.26 (±0.04) D | 0.04 (±0.01) A | 0.07 (±0.01) B |
| C12:0 | 0.25 (±0.05) B | 0.64 (±0.16) D | 0.12 (±0.03) A | 0.35 (±0.06) C |
| C14:0 | 0.89 (±0.05) B | 1.43 (±0.02) D | 0.49 (±0.02) A | 1.08 (±0.08) C |
| C14:1 | 0.11 (±0.05) B | 0.13 (±0.02) C | ND A | ND A |
| C15:0 | 0.10 (±0.03) B | 0.11 (±0.04) C | 0.05 (±0.02) A | 0.06 (±0.03) A |
| C16:0 | 27.05 (±0.28) B | 31.50 (±0.44) C | 25.32 (±0.43) A | 45.50 (±0.19) D |
| ΣC16:1[c] | 0.66 (±0.05) D | 0.34 (±0.08) C | 0.18 (±0.02) B | 0.12 (±0.02) A |
| C17:0 | 0.18 (±0.03) D | 0.17 (±0.02) C | 0.07 (±0.02) A | 0.10 (±0.05) B |
| C17:1 | 0.04 (±0.01) C | 0.05 (±0.02) C | 0.05 (±0.01) B | ND A |
| C18:0 | 21.73 (±0.18) D | 17.01 (±0.11) C | 5.57 (±0.53) B | 4.89 (±0.34) A |
| Σ*C18:1t*[d] | 0.15 (±0.03) A | 0.53 (±0.05) B | 3.23 (±0.21) C | 8.21 (±0.36) D |
| C18:1 | 34.49 (±0.72) B | 36.35 (±0.16) C | 44.16 (±0.13) D | 33.41 (±0.11) A |
| C18:1vac[e] | 0.75 (±0.08) A | 0.74 (±0.02) A | 1.88 (±0.05) B | 0.78 (±0.03) A |
| Σ*C18:2t*[f] | 0.12 (±0.03) A | 0.20 (±0.03) B | 0.17 (±0.02) B | 1.04 (±0.05) C |
| C18:2 | 11.43 (±0.31) C | 7.78 (±0.63) B | 14.20 (±0.39) D | 7.98 (±0.11) A |
| C20:0 | 0.67 (±0.04) D | 0.59 (±0.01) C | 0.48 (±0.02) B | 0.33 (±0.05) A |
| C18:3*t*[g] | 0.01 (±0.01) A | 0.01 (±0.01) A | 0.05 (±0.01) B | 0.05 (±0.01) B |
| C20:1 | 0.13 (±0.01) A | 0.25 (±0.02) B | 0.70 (±0.03) C | 0.13 (±0.05) A |
| C18:3 | 0.61 (±0.09) B | 1.28 (±0.03) C | 4.07 (±0.03) D | 0.30 (±0.05) A |
| C20:2 | 0.03 (±0.02) B | ND A | 0.04 (±0.01) B | ND A |
| C22:0 | 0.17 (±0.02) C | 0.13 (±0.05) B | 0.23 (±0.02) D | 0.09 (±0.03) A |
| C22:1 | ND A | ND A | 0.16 (±0.02) B | ND A |
| C22:3 | 0.08 ± 0.03 A | 0.07 (±0.02) A | 0.10 (±0.04) B | 0.07 (±0.03) A |
| C22:4 | ND A | ND A | 0.09 (±0.01) B | ND A |
| C22:5 | ND A | ND A | 0.26 (±0.05) B | ND A |

[a] Data are reported as means of four replicates (± standard deviation). Different letters (A through D) denote significant differences among different products ($P \leq 0.05$).

[b] ND, not determined. Below the limit of quantitation (LOQ = 0.01 g/100 g of fatty acids).

[c] ΣC16:1 is the sum of C16:1ω-9 and C16:1ω-7.

[d] Σ*C18:1t* is the sum of all positional *trans* isomers (*t*4 C18:1 to *t*16 C18:1).

[e] C18:1vac is the vaccenic acid.

[f] Σ*C18:2t* is the sum of all positional *trans* isomers (*t*9,*t*12 C18:2; *c*9,*t*13 C18:2 + *t*8,*t*12 C18:2; *c*9,*t*12 C18:2 and *t*9,*c*12 C18:2).

[g] C18:3*t* is the *c*9,*c*12,*t*15 C18:3 isomer.



derivatives were also compared with those reported in literature *(16).* The GC response factors of sterols and SOP with respect to their corresponding internal standards were considered equal to one. The limit of quantitation of the GC analysis of sterols and SOP were 0.2 mg/100 g of sample and 0.05 mg/kg of sample, respectively.

**Data analysis.** Two lipid extractions were carried out per sample. Each parameter was determined four times per each lipid extraction. GC data were stored and processed with a Chrom-Card acquisition system (version 2.3.1, Thermo Electron Corporation, Rodano [MI], Italy). Mean and standard deviation data of the different analytical parameters determined in each sample are shown in Tables 1 through 4. The limit of quantitation of the different GC determinations was calculated as a signal-to-noise ratio equal to 6:1. One-way analysis of variance with a posteriori Tukey b test was carried out to determine statistical differences among samples ($P \leq 0.05$). Statistical analysis of the data was carried out with a SPSS 11.0 program (SPSS Inc., Chicago, Ill.).

## RESULTS AND DISCUSSION

The analyzed bakery products showed a wide range of fat content (8.9, 18.1, 18.5, and 39.7% for samples 1, 2, 3, and 4, respectively). Significant differences were also observed in the profile of both saponifiable and unsaponifiable lipid fractions.

Table 1 shows the fatty acid (FA) composition (including 30 fatty acids) of the four bakery products. Low-molecular-weight FA were present in higher amounts in products that included milk derivatives as ingredients (samples 1 and 2). The most abundant FA in the bakery goods (except for sample 4) was oleic acid (C18:1), followed by palmitic acid (C16:0); the latter was the major FA in sample 4. Palmitic acid is characteristic of animal fats and some vegetable fats, such as palm oil, which may have been used as vegetable fat in sample 4. The essential FA linoleic acid (C18:2) showed significant levels that ranged from 7.98 to 14.20%; α-linolenic acid (C18:3) exhibited lower amounts, even though it reached 4.07% in sample 3. FA with more than 20 carbons were scarce.

The TFA fraction was characterized by a wide range of positional isomers of C18:1*t* and positional and configurational isomers of C18:2*t*, which are typical of hydro-

TABLE 2. *Fatty acid classes (g/100 g of product) and ratios of samples of four bakery products*[a]

| Fatty acid class[b] | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| SFA | 3.57 (±0.24) A | 7.59 (±0.05) C | 4.60 (±0.04) B | 16.55 (±0.10) D |
| MUFA | 2.51 (±0.09) A | 5.47 (±0.04) B | 6.68 (±0.09) C | 10.82 (±0.11) D |
| PUFA | 0.84 (±0.04) A | 1.32 (±0.08) B | 2.65 (±0.11) C | 2.63 (±0.07) C |
| TFA | 0.02 (±0.01) A | 0.11 (±0.08) A | 0.49 (±0.06) B | 3.13 (±0.04) C |
| P/S | 0.24 | 0.17 | 0.58 | 0.16 |
| U/S | 0.94 | 0.90 | 2.03 | 0.81 |

[a] Data are reported as means of four replicates (± standard deviation). Different letters (A through D) denote significant differences among different products ($P \leq 0.05$).

[b] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, *trans* fatty acids; P/S, ratio PUFA/SFA; U/S, ratio (MUFA + PUFA)/SFA.



genated fats. In fact, partial hydrogenation of the diunsaturated FA leads to the formation of positional isomers of monoenoic TFA, which can only be produced by this technological processing step. On the other hand, during physical refining of vegetable oils, the naturally occurring *cis* olefinic bonds of unsaturated FA are converted to a varying degree into the *trans* form, while bond migration leading to positional isomers of the parent FA is negligible *(66).* The formation rate of geometrical isomers of unsaturated FA depends on the degree of unsaturation of the FA and the applied temperature-time conditions during deodorization *(77).* In the bakery products here analyzed, the C18:1*t* isomers were the predominant group, reaching high levels in sample 4 (8.21%). Low amounts of C18:2*t* isomers and only one C18:3*t* isomer (*c*9,*c*12,*t*15 C18:3) were present in all samples. The overall amount of TFA detected in these bakery goods is high, according to the WHO/FAO and FDA recommendations *(72, 78).* As mentioned before, TFA represent a high atherogenic factor *(4, 33, 34, 41, 44, 47, 69),* displaying a dose-dependent relationship with the LDL/HDL ratio *(5).* Besides the inhibition of the activity of hepatic enzymes *(46)* and the modification of the fluidity of cell membranes *(43),* some *trans* isomers exhibit particular biological effects, such as *t*9,*t*12 C18:2 which adversely affects the metabolism of children *(14).*

Table 2 shows the sums of the different FA fractions (expressed as percentages in 100 g of product) as well as the polyunsaturated fatty acids (PUFA)/SFA ratio (P/S) and the monounsaturated fatty acids (MUFA)+PUFA/SFA ratio. The supply of unsaturated FA ranged from 3.35 to 13.45 g/100 g of product, which corresponded to 42.65 and 65.11% of total FA. In addition, large differences in the total SFA (3.57 to 16.55 g/100 g of product, equivalent to 32.46 to 52.61% of total FA) and total TFA (0.02 to 3.13 g/100 g of product, equivalent to 0.28 to 9.29% of total FA) contents were observed. Vicario et al. *(74)* reported a similar FA profile (49.43% SFA, 32.87% MUFA, 12.48% PUFA, and 5.20% TFA) for commercial Spanish cookies and bakery products having an average lipid content equal to 22.70%. SFA are known to increase the cardiovascular risk *(31),* but their effect on the LDL/HDL ratio is lower than that of TFA *(5).*

In particular, it must be noted that sample 4 exhibited the lowest P/S ratio (0.16) and the highest TFA content (3.13 g/100 g of product) among all bakery goods. Considering these data, the FA composition, the compounds of its unsaponifiable fraction (see below), and the product label, which declares 100% vegetable margarine as the only fat source, it can be deduced that this product was most probably prepared in part with hydrogenated palm and palm kernel oil. Sample 2 exhibited a similar P/S ratio to that of sample 4, but its TFA content was about 28.5 times lower. The lowest TFA level (0.02 g/100 g of product), however, was detected in sample 1, whose P/S ratio was slightly higher than those of samples 2 and 4. Sample 3, on the contrary, showed a relatively high P/S (0.58) and a significant amount of MUFA (6.68 g/100 g of product), but the amount of TFA was also significantly elevated (0.49 g/100 g of product).

The large differences found among the FA profiles of the analyzed bakery products are very relevant from both nutritional and health standpoints, especially the TFA content. In fact, the European Food Safety Authority is at present discussing which strategy to follow regarding TFA in food, because 30% of TFA intake derives from baked goods, fast foods, and other prepared foods *(73).* Other studies from Western Europe also reported a high amount of TFA in bakery products and cookies *(19, 53, 73).* Elias and Innis *(17)* stated that bakery goods are the major dietary sources of TFA among pregnant women with diets providing 30% energy from fat.

Table 3 shows the sterol composition of the analyzed bakery products. In general, cholesterol was the most representative sterol, ranging from 25.4 to 71.4 mg/100 g of product. Cholesterol mainly came from egg and milk derivatives used in the product formulations. The amount of cholesterol in samples 1 and 3 was similar to that found in some foods from animal origin, such as meat or fish *(45).* Sample 4, on the contrary, was elaborated only with vegetable fat and, thus, it showed a relatively low cholesterol content (1.1 mg/100 g of product) compared with the other bakery products; however, this cholesterol level corresponded to about 3% of total sterols, which is considered high for most vegetable oils. The occurrence of such a cholesterol level can be attributed to the presence of palm or palm-kernel oil (cholesterol content = 4 to 7%) *(35),* which can be deduced from the analysis of total sterol and fatty acid compositions. On the other hand, the phytosterol con-

TABLE 3. *Main sterols (mg/100 g of product) in samples of four bakery products*[a]

| Sterol | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| Cholesterol | 71.4 (±0.13) D | 25.4 (±0.23) C | 64.2 (±0.07) B | 1.1 (±0.05) A |
| Brassicasterol | 0.4 (±0.05) B | 0.9 (±0.14) C | 4.9 (±0.36) D | ND[b] A |
| Campesterol | 4.0 (±0.62) A | 7.4 (±0.52) B | 23.6 (±1.21) D | 7.6 (±0.56) C |
| Stigmasterol | 3.4 (±0.35) B | 5.1 (±0.30) C | 0.8 (±0.24) A | 3.5 (±0.69) B |
| β-Sitosterol | 14.6 (±0.29) A | 20.5 (±0.45) B | 34.8 (±0.39) D | 22.8 (±0.11) C |
| Total phytosterols | 22.5 (±0.17) A | 33.9 (±0.10) B | 64.0 (±0.08) C | 33.9 (±0.13) B |
| Total sterols | 93.9 (±0.28) C | 59.2 (±0.21) B | 128.3 (±0.14) D | 34.9 (±0.14) A |

[a] Data are reported as means of four replicates (± standard deviation). Different letters (A through D) denote significant differences among different products ($P \leq 0.05$).

[b] ND, not determined. Below the limit of quantitation (LOQ = 0.2 mg/100 g of product).



tent varied between 22.5 and 64.0 mg/100 g of product; such amounts of phytosterols are far below the intake needed to obtain health benefits (2 to 3 g/day) *(28, 51, 76)*. Normen et al. *(48)* found that the phytosterol content of different cereal foods (such as biscuits, cakes, cookies, crackers, and sweet breads) ranged from 27 to 112 mg/100 g of product. Quilez et al. *(58)* reported a total phytosterol content of 77.1 to 94.9 mg/100 g for commercial bakery products with 25.3 to 25.7% lipids.

β-Sitosterol was the most abundant phytosterol (14.6 to 34.8 mg/100 g of product), followed by campesterol (4.0 to 23.6 mg/100 g of product), stigmasterol (0.8 to 5.1 mg/100 g of product), and brassicasterol (0 to 5.0 mg/100 g of product). These data are in agreement with those obtained by Normen et al. *(48)* for the same category of products.

Regarding sterol oxidation, the literature only reports studies performed on cholesterol oxidation of bakery products *(52, 57)*. However, as shown in Table 3, phytosterols constitute from 24% up to 97% of total sterols, so it is therefore important to evaluate their oxidation degree. As mentioned before, 7-ketocholesterol has been used as a marker to provide a simple and reliable evaluation of the extent of cholesterol oxidation in foods *(39, 54, 65, 79)*. Because cholesterol and phytosterols are chemically similar, they actually display analogous oxidation paths, and, therefore, 7-keto derivatives of phytosterols can be also utilized for monitoring their extent of oxidation in bakery products.

Table 4 reports the amount of the 7-keto derivatives of sterols found in the analyzed bakery products. Total 7-ketophytosterols ranged between 1.66 and 2.86 mg/kg of product, which corresponded to 83 to 96% of the total 7-keto derivatives (1.88 to 3.14 mg/kg of product) detected in the bakery products. The total amounts of 7-keto derivatives of sterols were 18.7, 9.5, 10.2, and 7.2 mg/kg of lipids in samples 1, 2, 3, and 4, respectively.

In samples 1, 2, and 4, the most abundant 7-keto derivative was 7-ketositosterol, followed by 7-ketocampesterol. In this case, the relative amount of the single 7-keto derivatives reflected the sterol composition of the samples. However, in sample 3, this behavior was not observed; in fact, 7-ketobrassicasterol was the most abundant oxidation product, followed by 7-ketositosterol and 7-ketocampesterol. Lambelet et al. *(36)* reported that 7-ketobrassicasterol was the main oxyphytosterol in refined rapeseed oil, so it is highly possible that refined rapeseed oil was used in sample 3. On the other hand, 7-ketocholesterol ranged from 0.07 to 0.32 mg/kg of product; these data are lower than those reported by Pie et al. *(57)* (0.45 to 1.68 mg/kg of butter cakes and croissant) and Paniangvait et al. *(52)* (0.46 to 1.29 mg/kg of commercial bakery products).

The extent of oxidation of each phytosterol to its corresponding 7-keto derivative ranged from 0.29 to 0.84%, except for brassicasterol, which exhibited a particularly high oxidation rate (2.01 to 3.11%) in all samples. Grandgirard et al. *(26)* and Conchillo et al. *(13)* reported a total POP oxidation rate equal to 0.08% for commercial spreads enriched with phytosterol esters. The extent of cholesterol

TABLE 4. *7-Keto derivatives of sterols (mg/kg of sample) in samples of four bakery products*[a]

| 7-Keto derivatives | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---|---|---|---|---|
| 7-Ketocholesterol | 0.32 (±0.03) D | 0.15 (±0.08) B | 0.07 (±0.02) A | 0.28 (±0.07) C |
| 7-Ketobrassicasterol | 0.11 (±0.02) B | 0.28 (±0.05) C | 0.98 (±0.07) D | ND[b] A |
| 7-Ketocampesterol | 0.37 (±0.02) B | 0.39 (±0.03) B | 0.28 (±0.01) A | 0.73 (±0.07) C |
| 7-Ketostigmasterol | 0.13 (±0.01) B | 0.18 (±0.03) C | 0.06 (±0.01) A | 0.32 (±0.05) D |
| 7-Ketositosterol | 1.05 (±0.21) C | 0.88 (±0.15) B | 0.57 (±0.09) A | 1.81 (±0.11) D |
| Total 7-Ketophytosterol oxides | 1.66 (±0.02) A | 1.73 (±0.09) B | 1.89 (±0.08) B | 2.86 (±0.06) C |
| Total 7-Ketosterol oxides | 1.98 (±0.06) B | 1.88 (±0.04) A | 1.96 (±0.03) B | 3.14 (±0.05) C |

[a] Data are reported as means of four replicates (± standard deviation). Different letters (A through D) denote significant differences among different products ($P \leq 0.05$).

[b] ND, not determined. Below the limit of quantitation (LOQ = 0.05 mg/kg of sample).

oxidation varied from 0.01 to 0.06%, except for sample 4 (2.54%).

Considering these results, it can be stated that part of the SOP were already present in some ingredients of the bakery products (i.e., vegetable oils or animal products—egg or milk derivatives), as already reported by Grandgirard et al. *(26)* and Conchillo et al. *(13)* for spreads. The elevated extent of cholesterol oxidation found in sample 4 does not contradict this hypothesis, though. Cholesterol present in sample 4 derives from vegetables sources; this can be attributed to the presence of palm or palm-kernel oils, as confirmed by the sterol and fatty acid compositions (high content of low-molecular-weight SFA [C8:0 to C16:0]). In fact, palm and palm-kernel oils are usually subjected to refining, which leads to the formation of different types and amounts of SOP depending on the sterol oxides chemical structure and the type of refining process applied to the oils *(7)*. It must be noticed that the initial level of sterol oxidation of the raw materials could have been further enhanced during processing and storage of bakery products *(2, 11, 12, 62)*, but apparently there is a minor effect of such technological steps on the SOP content of the samples here analyzed. In any case, the total 7-ketocholesterol content of the bakery products ranged between 0.07 and 0.32 mg/kg of product, which could not represent an appreciable risk from the toxicological standpoint, because the minimum amount of 7-ketocholesterol equivalent to a minimum biological activity of the other COP (about one-fifth of 7-ketocholesterol) *(9)* is equal to $10^{-7}$ molar basis *(67)*. Although the total content of 7-keto derivates of phytosterols of these bakery products varied from 1.66 to 2.86 mg/kg of product, it must be pointed out that POP have qualitatively similar toxic effects to COP, but higher concentrations of POP are required to achieve comparable levels of toxicity *(60)*.

In conclusion, this preliminary study evinces that the amount of sterols, 7-keto derivatives of sterols, and TFA can vary considerably among the bakery products category. The supply of phytosterols is not significant in these types of products, and the extent of oxidation of most phytosterols is low but not negligible. It might be possible that the majority of SOP were already present in the raw materials and that oil refining could have influenced the type and content of SOP. Furthermore, the quality of ingredients and raw materials seems to have also greatly influenced the fatty acid profile, stability, safety, and quality of the final product, and, therefore, these ingredients should be chosen with extreme care to decrease their potential negative health effects. This is particularly true for TFA, because most baked goods are still made with partially hydrogenated fat and thus exhibit a high TFA content. Replacement of TFA in such products by healthier fats may be more difficult than in margarines, but it can be achieved. Nevertheless, it is unlikely that this situation can change without the proposal of strong federal regulations.

Considering the results here obtained, it is necessary to evaluate more in depth the TFA and SOP content of a larger number of samples so as to be able to provide a general statement about the health effects of bakery products.

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