OCCURRENCE OF LIPID OXIDATION COMPOUNDS IN COMMERCIALIZED FUNCTIONAL DAIRY PRODUCTS

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Abstract

Oxidative stability of bioactive lipid-functional dairy products is essential to ensure their role in the prevention and/or treatment of certain diseases. In this study, the oxidation state of commercial functional dairy products, along with conventional dairy products, was evaluated at the acquisition and expiry dates. Functional dairy products tested containing omega-3 fatty acids (FP ω 3), conjugated linoleic acid (FPCLA) or stanol esters (FPSE) were tested. Quantitation of oxidized triacylglycerols, not applied so far to dairy products, was used as a measurement of primary and secondary oxidation compounds. Complementarily, tocopherols and peroxide values were determined. Results showed the high stability of conventional dairy products and FPSE in contrast with the relatively lower stability of FP ω 3 and FPCLA. FP ω 3 and FPCLA were even significantly oxidized at the initial date, and half of such samples oxidized further during the shelf-life period. However, while hydroperoxides were predominant in FP ω 3, polymer formation was found to be the early oxidation marker of FPCLA oxidation.

Key words:

Functional dairy products – Lipid oxidation – Omega-3 fatty acids – Conjugated linoleic acid – Shelf-life – Stanol esters

Abbreviations:

CLA, conjugated linoleic acid; DG, diacylglycerols; EM, evaporated milk; FM, fermented milk; FPCLA, functional dairy product with conjugated linoleic acid; FPSE, functional dairy product with stanol esters; FP ω 3, functional dairy product with omega-3 polyunsaturated fatty acids; HPSEC, high-performance size-exclusion chromatography; oxTGM, oxidized triacylglycerol monomers; PO, pasteurized milk; POM, powder milk; PUFA, polyunsaturated fatty acids; PV, peroxide value; SM, sterilized milk; SPE, solid-phase extraction; TGD, triacylglycerol dimers; TGP, triacylglycerol polymers; TOC; UHTM, ultra-high-temperature treated milk.

1. Introduction

Lipid oxidation in foods is of great concern because it results in development of undesirable flavours, decrease of shelf-life, losses of nutritional value and formation of compounds that may be detrimental to health (Dobarganes & Márquez-Ruiz, 2003; Kanner, 2007). During the last decade, consumers' demand for dairy-based functional foods has increased considerably and those enriched with functional lipids including omega-3 polyunsaturated fatty acids (ω -3 PUFAs), conjugated linoleic acid (CLA) or plant sterols make up a significant part of them (Gulseven & Wohlgenant, 2014). A large body of scientific research has confirmed the health-promoting properties of ω -3 PUFAs concerning risk reduction of cardiovascular diseases (Shahidi, 2015), while CLA isomers are mainly claimed to prevent tumours growth or to reduce body fat (Yang et al., 2015). Plant sterols, in turn, have consistently shown total and low-density lipoprotein cholesterol-lowering effects (Gylling et al., 2014). Lipid oxidation is not the main deterioration source in dairy products because of the high proportion of stable, saturated fatty acids of milk fat, although oxidation reactions may be promoted by heating treatments with oxygen exposure during processing and storage (Stapelfeldt, Nielsen & Skibsted, 1997; Fenaille et al., 2006; Smet et al., 2009; García-Martínez et al., 2010; Duncan & Wester, 2010; Márquez-Ruiz, García-Martínez & Holgado, 2014). However, ω -3 PUFAs and CLA are polyunsaturated fatty acids, therefore highly susceptible to form oxidized compounds with adverse physiological effects (Dobarganes & Márquez-Ruiz, 2003; García-Martínez & Márquez-Ruiz, 2009). Most studies on the oxidative stability of functional dairy products have focused on those enriched with ω -3 PUFAs, as reported by Jacobsen and co-workers (Let, Jacobsen, Frankel & Meyer, 2003; Let, Jacobsen & Meyer, 2004, 2005; Jacobsen, 2010; Saga et al., 2013). Interest on oxidative stability of functional dairy products enriched with CLA or sterol plants is also increasing, as reflected in recent publications (Martínez-Monteagudo, Leal-Dávila, Curtis, & Saldaña, 2015;

González-Larena, García-Llatas, Clemente, Barberá & Lagarda, 2015; Nieminen, Leaksop, Kuusisto, Niemela, & Laitinen, 2016). However, very little is known on the state of oxidation of commercially available functional dairy products. To the best of our knowledge, the only information published in this regard refers to fatty acid profiles of commercial CLA-fortified dairy products (Rodríguez-Alcalá & Fontecha, 2007) and to sterol oxides in three commercial plant sterol-enriched dairy products (García-Llatas et al., 2013; González-Larena, García-Llatas, Clemente, Barberá, & Lagarda, 2015).

Different methods have been used to evaluate lipid oxidation in dairy products, such as peroxide value, loss of substrate, thiobarbituric acid reactive substances, volatile oxidation compounds, loss of antioxidants and sensory analysis (Garcia-Martinez & Marquez-Ruiz, 2009). However, each of these methods only detects compounds formed during a particular stage of the oxidation process (Frankel & Meyer, 2000; Dobarganes & Márquez-Ruiz, 2003; Barriuso, Astiasaran & Ansorena, 2013). Even when some of these methods are used in combination, the real level of oxidation compounds formed remains unknown. However, application of combined chromatographic techniques provides a complete picture of the oxidation state at any stage of the oxidation progress (Márquez-Ruiz, Jorge, Martín-Polvillo, & Dobarganes, 1996; Dobarganes, Velasco & Dieffenbacher, 2000). This method has reported excellent results in many food samples including vegetable oils, fish oils, fried foods, microencapsulated oils and infant formulas (García-Martínez et al., 2010; Giménez, Gómez-Guillén, Pérez-Mateos, Montero, & Márquez-Ruiz, 2011; Márquez-Ruiz, Velasco & Dobarganes, 2000; Márquez-Ruiz & Dobarganes, 2006; Dobarganes & Márquez-Ruiz, 2013), but it has not been applied yet to conventional or functional dairy products.

The aim of the present work was to provide new information on the effect of addition of functional lipids on the oxidative stability of dairy products. Towards this end, the oxidation state in a variety of commercial functional dairy products, along with

conventional dairy products, was evaluated at initial and expiry dates through quantitation of non-volatile oxidation compounds by combined chromatographic techniques. Also, fatty acid composition, tocopherol contents and peroxide values were determined.

2. Materials and Methods

2.1. Samples and reagents

In this benchmarking study, 12 functional dairy products and 10 conventional dairy products from the most representative national and international manufacturers were acquired in local supermarkets. A brief description of each product and total fat content, as stated in the labels, along with the abbreviations used in this study, is listed in Table 1. Also, the shelf-life period, according to the expiry date, was included for all products. Functional dairy products included 6 products enriched with ω -3 PUFAs (FP ω 3), 4 products with added CLA (FPCLA), and 2 products enriched with stanol esters (FPSE). As to conventional dairy products, 2 pasteurized whole milks (PM), 2 Ultra-High Temperature-treated whole milks (UHTM), 2 fermented milks (FM), 2 evaporated whole milks (EM) and 2 powdered whole milks (POM) were selected.

Six containers of the same lot were acquired for each commercial product, 3 of them were analyzed in triplicate when were acquired and the other 3 containers were stored in their original containers (sealed) until the expiry date stated by the manufacturer (duration of storage is included in Table 1) and then analyzed. Products were stored at their respective recommended temperatures, i.e., refrigeration temperature (2-4°C) for PM-A, PM-B, FM-A, FM-B, FP ω 3-E, FPCLA-C, FPCLA-D, FPSE-A and FPSE-B; and room temperature (20-23°C) for the rest of the samples.

All reagents used for lipid extraction and separation of polar compounds by SPE were purchased from Panreac (Barcelona, Spain). An anhydrous milk fat (reference material BCR-164; EU Commission, purchased from Fedelco Inc., Madrid, Spain) was used for quantitative determinations of total fatty acid methyl esters. Methyl esters of ω -3 fatty acids and CLA were identified by GC-FID using standards purchased from Nu-Check Prep. Inc. (Elysian, MN, USA). The chemicals and solvents used for other methods were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Lipid Extraction

Lipids were extracted according to the International Standard Method ISO 14156-IDF for milk and milk products (ISO-IDF, 2002), except for POM samples where two lipid fractions were extracted separately, i.e., the free, non-encapsulated and the encapsulated fat fractions. The extraction of the free fat was carried out according to Sankarikutty et al. (Sankarikutty, Sreekumar, Narayanan & Mathew, 1988). Thus, 200 mL of light petroleum ether (60-80°C) was added to 4 g of powder. Stirring was applied at room temperature for 15 min. After filtration through a filter paper, the solvent was evaporated in a rotatory evaporator and the extracted oil was dried to constant weight using a stream of nitrogen. The extraction of encapsulated fat was carried out in the powder sample devoid of free fat and dried to constant weight, following the International Standard Method ISO 14156-IDF (ISO-IDF, 2002). Extracted lipids for all dairy samples were calculated as weight percentage (grams of lipids extracted from 100 grams of product).

2.3. Peroxide Value

Peroxide value (PV) was determined in extracted lipids by the iodometric assay according to IUPAC Standard Method 2.501 (IUPAC, 1992).

2.4. Fatty acid composition

Extracted lipids were converted into fatty acid methyl esters (FAMES) by base-catalysed transmethylation using 2 N KOH in methanol, as described by International Standard Method ISO 15884-IDF (ISO-IDF, 2002). FAMES were analyzed on a Perkin-Elmer chromatograph (model Autosystem, Beaconsfield, UK) with a flame-ionization detector. FAMES were separated using a fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 µm film thickness, CP-Sil 88, Chrompack, Middelburg, the Netherlands). The column was held at 100°C for 1 min after injection, then the temperature was increased at 7°C/min to 170°C, held for 55 min, then increased at 10°C/min to 230°C, and held there for 33 min. Helium was the carrier gas with a column inlet pressure set at 214 kPa (30 Psig) and a split ratio of 1:20. The injection volume was 0.5 µL.

2.5. Non-volatile oxidation compounds

Quantitative analysis of total non-volatile oxidation compounds in extracted lipids was carried out according to the method of Márquez-Ruiz et al. (Márquez-Ruiz, Jorge, Martín-Polvillo, & Dobarganes, 1996; Márquez-Ruiz & Dobarganes, 2006). Oxidation compounds were separated from the non-oxidized triacylglycerols by solid-phase extraction (SPE). Briefly, 50 mg of extracted lipids were dissolved in 2 mL of hexane containing 1 mg of monostearin added as internal standard. The sample solution was poured into a 1-g silica Sep-Pak cartridge (Waters, Darmstadt, Germany) and the non-oxidized triacylglycerols were eluted with 15 mL of hexane/diethyl ether (90:10, v/v). After that, the polar fraction was eluted with 25 mL of diethyl ether. The solvent of the polar fraction was evaporated in a rotary evaporator and redissolved in 1 mL of diethyl ether. Then, the polar fraction was analyzed by HPSEC in a liquid chromatograph equipped with a Rheodyne injector with a 20 µL sample loop, a Waters 510 pump (Waters, Milford, MA, USA), and a Waters refractive index detector. The separation was performed on two PLgel columns (Agilent

Technologies, Palo Alto, CA, USA) packed with 5 μ m particles of 100 and 500 Å pore size, respectively, and placed into an oven set at 35 °C. HPLC-grade tetrahydrofuran was the mobile phase with a flow of 1 mL/min. Under these conditions, resolved peaks of triacylglycerol polymers (TGP), triacylglycerol dimers (TGD), oxidized triacylglycerol monomers (oxTGM), diacylglycerols (DG), monostearin (internal standard), and finally a peak corresponding to free fatty acids of various chain lengths and the polar unsaponifiable matter, were obtained. Total non-volatile oxidation compounds were calculated as the sum of TGP, TGD and oxTGM. The methodology has been described in detail, including precision and repeatability data, in a previous publication (Márquez-Ruiz, Jorge, Martín-Polvillo, & Dobarganes, 1996).

2.6. Tocopherols

Determination of tocopherols in extracted lipids was carried out by HPLC with fluorescence detection according to IUPAC Standard Method 2.411 (IUPAC, 1992).

2.7. Statistical Analysis

Data were analyzed using the ANOVA procedure of the SPSS package (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL). Tukey's test was applied to determine significance and *p* values less than 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Fatty acid composition and lipid content of initial dairy products

Tables 2 and 3 show the composition of the fatty acid methyl esters (% of FAMES) and lipid content of all the dairy products analyzed. The lipid contents of PM, UHTM and FM samples were within the levels expected, between 3.6 and 4.2 % (grams of lipids in 100 grams of product), and were also, as expected, higher in the evaporated and dried milks in consistency with the dehydration degree applied. Very similar fatty acid compositions were found for the conventional milk products, matching with most common values for bovine milk fat (Rodríguez-Alcalá, Alonso & Fontecha, 2014). The distinct fatty acid compositions found in the functional products derived from the contribution of different sources of oils or lipids added. All the FP ω 3 products had been enriched with fish oils, but in different amounts, as reflected by the ω -3 contents, ranging from 0.6 to 4.3%. The high oleic acid content in FP ω 3-A, FP ω 3-B, FP ω 3-D and FP ω 3-E accounted for the addition of olive oil and/or high-oleic sunflower oil, as stated in the labels (Table 1). In contrast, the major fatty acid in FP ω 3-F was linoleic acid, highly abundant in sunflower oil and other seed oils commonly used in the preparation of functional products. As to FP ω 3-C, the significant proportions of palmitic acid, short-chain and medium-chain fatty acids found indicate that the skimmed milk used contained some remaining milk fat. The same was observed in some functional dairy products enriched with CLA oil, especially in FPCLA-A, as already reported (Rodríguez-Alcalá & Fontecha, 2007). In this line of functional products, similar concentration of *cis*-9, *trans*-11 CLA and *cis*-10, *trans*-12 CLA were found, in sum ranging from 52.2 to 75.8%. The functional dairy products with plant sterols were abundant in monounsaturated fatty acids coming from the vegetable oils used as plant stanol vehicles (García-Llatas et al., 2013).

3.2. Oxidation parameters in conventional dairy products

Table 4 shows values of PV, quantitative data of non-volatile, triacylglycerol oxidation compounds (oxTGM, TGD and TGP) and tocopherol contents in initial conventional dairy products. All the conventional dairy products showed PV below 1 meq O₂/kg oil and oxidation compounds as determined by chromatographic techniques were not detectable, except for the POM samples. In POM-A and POM-B, 9.6 and 9.9% of the total lipids was non-encapsulated or free, respectively, and results showed that the free lipid fractions were significantly more oxidized than the encapsulated fractions. Thus, 16.1 and 10.4 mg/g total oxidation compounds were respectively found in the free lipid fractions, in contrast to values of 1.5 and 2.3 mg/g observed in the encapsulated lipid fractions of POM-A and POM-B, respectively. Clearly, the free lipid fraction was more susceptible to oxidation than the fraction embedded in the matrix, as already reported in powder milks (Vignolles, Jeantet, López & Schuck, 2007) and powdered infant formulas (García-Martínez et al., 2010).

Following the shelf-life period, the samples were examined at their corresponding expiry dates. Since none of the conventional dairy products showed significant changes in any of the oxidation parameters measured, data has not been included for the sake of brevity. Even in the POM samples, which have been reported to oxidize significantly under certain storage temperature and water activity (Stapeldefeldt, Nielsen & Skibsted, 1997), no significant changes were found at the expiry dates in the present study, probably because of the protection provided by the vacuum-sealed bags used for packaging.

3.3. Oxidation parameters in functional dairy products

As compared with the negligible level of oxidation found in the conventional products (Table 4), the oxidation state in the initial time was apparently high in the functional products (Table 5). However, the results found were in concordance with those reported for the refined vegetable oils used in their formulation. Thus, PV was always lower than the limit allowed for refined vegetable oils, 10 meq O₂/kg oil, and a small amount of TGD remained as marker of the oil refining process (Ruiz-Méndez, Márquez-Ruiz & Dobarganes, 1997). The tocopherol contents were generally very high in the functional products as compared to levels found in conventional products (Table 4), as expected from the amounts of vitamin E or different tocopherol types commonly added to bioactive lipid-functional products. Surprising results were found in products enriched with CLA oil, showing unusual occurrence of TGP, between 1.9 and 2.5% expressed on total lipids extracted, while oxTGM and TGD levels were low. Generally, the presence of TGP is indicative of advanced oxidation stages and is concomitant with high levels of oxTGM which form earlier in the autooxidation process through the hydroperoxide pathway (Márquez-Ruiz & Dobarganes, 2006). Thus, the typical oxidation pattern consists in the initial formation of oxTGM, mainly comprised of hydroperoxides during early oxidation, then the accumulation of sufficient amounts of oxTGM leads to formation of TGD and likewise TGP do not build up until a notable increase of TGD takes place. However, oxidation of conjugated linoleic acid seems to proceed through different mechanisms (Brimberg & Kamal-Eldin, 2003; Yurawecz, Delmonte, Vogel & Kramer, 2003; Luna, De la Fuente, Salvador & Márquez-Ruiz, 2007; García-Martínez, Márquez-Ruiz, Fontecha & Gordon, 2009).

Results of oxidation parameters in the functional products at expiry times are also shown in Table 5. Among the products analyzed, it is important to stand out that neither of the FPS samples showed oxidative changes at expiry times as compared to initial samples, in agreement with results obtained in previous works (Soupas, Huikko, Lampi & Piironen, 2006; García-Llatas et al., 2013).

299 With respect to the FP ω 3 samples, FP ω 3-A, FP ω 3-B and FP ω 3-D at expiry times
300 showed significantly increased level of oxidation as compared to initial samples, in
301 contrast with FP ω 3-C, FP ω 3-E and FP ω 3-F. Specifically, a significant increase of
302 hydroperoxides was observed in FP ω 3-A, FP ω 3-B and FP ω 3-D, as denoted by the
303 values obtained for PV and oxTGM, along with significant losses of tocopherols (21-35%),
304 especially α -tocopherol. Nor the fatty acid composition, nor the tocopherol contents, nor
305 the initial oxidation levels accounted for the differences found among FP ω 3 samples. In
306 samples FP ω 3-C and FP ω 3-E, fish oil was employed as microencapsulated fish oil
307 (information provided by the companies) rather than emulsified directly in the skimmed
308 milk, which could have contributed to increasing protection towards oxidation. In fact,
309 microencapsulation enables supplementation of functional foods with higher amounts of
310 fish oil by virtue of solubility enhancement, masking of unpleasant fishy taste and odor
311 and protection from pro-oxidant agents (Beindorff, Zuidam, Zuidam, & Nedovic, 2010).

312
313 Among the products enriched with CLA, FPCLA-A and FPCLA-D at expiry times
314 showed significantly higher total oxidation compounds as compared to initial samples. In
315 contrast with results obtained for FP ω -3 samples, values of PV and oxTGM remained low
316 in FDPCLA-A and FDPCLA-D. Therefore, in such samples the increase of total oxidation
317 compounds during storage was exclusively attributable to significant formation of TGP.
318 Tocopherol decreases were also significant (about 14%) but lower than in the case of
319 FP ω -3 samples and only due to δ -tocopherol losses.

320
321 Figure 1 shows exclusion chromatograms of the polar fractions of samples FP ω 3-A
322 and FPCLA-A at the expiry dates, illustrating their different distribution of oxidation
323 compounds. For similar total oxidation levels (Figure 1), oxTGM were major compounds
324 in FP ω 3-A, while TGP were predominant in FPCLA-A. The early retention time at which
325 TGP started to elute, implying unusually high molecular weight or size, indicates that

formation of very complex molecules was favoured even under the mild shelf-life conditions recommended by producers, room temperature in the case of FPCLA-A. This large degree of polymerization has already been observed in a previous work in which conjugated methyl linoleate was used as a model compound (Luna, De la Fuente, Salvador & Márquez-Ruiz, 2007).

It is difficult to ascertain the main sources of lipid oxidation in commercial functional dairy products containing ω -3 PUFAs or CLA, which include a complex mixture of major ingredients, oils, mineral salts and vitamins. Most of these products are oil-in-water emulsions highly susceptible to oxidation where the oil-water interface greatly affects the distribution of the compounds that impact oxidation. For example, the right selection of antioxidants is of the utmost importance to ensure that they are located at the major site of the lipid oxidation reactions. It is also clear that the initial quality of the oils used as well as the protection against pro-oxidants agents in the emulsion systems are determinant parameters for the oxidative stability of dairy products (Jacobsen, 2010). A large number of studies have been carried out by Jacobsen and co-workers to evaluate the effects of ω -3 PUFAs addition to commercial milk as substrate. The authors have consistently found significant but low increases of PV in milk emulsions containing fish oil (Let, Jacobsen, Frankel & Meyer, 2003; Let, Jacobsen, & Meyer, 2004, 2005).

Regarding dairy products containing CLA, most published studies have shown that processing parameters such as heating or fermentation had no significant effect on the CLA levels (Bisig, Eberhard, Collomb & Rehberger, 2007). However, loss of CLA based on gas-chromatography analysis of FAME derivatives is not sensitive enough since a significant decrease of polyunsaturated fatty acids is not detected until well within the advanced oxidation stage (Angulo, Romera, Ramírez & Gil, 1998; García-Martínez, Holgado, Velasco & Márquez-Ruiz, 2012). Even so, significant losses of CLA isomers have been found after HTST pasteurization (up to 10%) and further losses upon

refrigerated storage (up to 6%) (Campbell, Drake & Larick, 2003). Moreover, losses of CLA as high as 22% have recently been reported in an enriched milk after UHT treatment, while only slight increases of hydroperoxides were detected (Martínez-Monteagudo, Leal-Dávila, Curtis & Saldaña, 2015). Since hydroperoxides are only minor products in the oxidation of CLA (Luna, De la Fuente, Salvador & Márquez-Ruiz, 2007), it is clear that the determination of PV provided misleading results.

4. Conclusions

The method proposed to evaluate major, non-volatile oxidized triacylglycerols in dairy products has provided complete and quantitative information on the oxidation state, including individual quantitation of primary and final oxidation products. Application of this method to commercial dairy products at initial and expiry dates under shelf-life conditions has provided complete data on oxidative changes. In contrast with the high stability against oxidation of conventional dairy products, half of the analyzed products enriched with ω -3 fatty acids or CLA showed significantly increased total oxidation compounds. For the former, changes were attributable to hydroperoxide formation, clearly denoted by increases of peroxide values and oxidized triacylglycerol monomers. However, in the case of CLA products, formation of polymers is prevalent and would have gone undetected if a global measurement of the oxidation state had not been carried out. This is of utmost importance since PV, which only enables to detect hydroperoxide formation, is at present the only determination included in quality specifications of CLA-rich ingredients used in commercialized dairy products. Polymer determination has therefore proven to be a highly valuable technique and could be an alternative routine method to evaluate oxidation of CLA-rich products.

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539 **Legends for Figure**

540 **Figure 1.-** High-performance size-exclusion chromatograms of polar fractions of lipids
541 extracted from a functional dairy product with omega-3 fatty acids (FP ω 3-A) and a
542 functional dairy product with conjugated linoleic acid (FPCLA-A) at the expiry dates.
543 Abbreviations: TGP, triacylglycerol polymers; TGD, triacylglycerol dimers; oxTGM,
544 oxidized triacylglycerol monomers; DG, diacylglycerols and IS, internal standard
545 (monostearin).

Table 1.- Brief description of the conventional and functional dairy products analyzed

Commercial product	Description (as in label)	Total fat (g/100mL) (as in label)	Shelf-life (days)	Brand	Abbreviation
Pasteurized milk	Whole milk	3.7	10	A	PM-A
	Whole milk	4.4	12	B	PM-B
Ultra-High-Temperature milk	Whole milk	4.2	90	A	UHTM-A
	Whole milk	3.8	94	B	UHTM-B
Fermented milk	Whole milk	4.0	23	A	FM-A
	Whole milk	3.8	19	B	FM-B
Evaporated milk	Whole milk	19.0	240	A	EM-A
	Whole milk	18.2	216	B	EM-B
Powder milk	Whole milk	30.0	498	A	POM-A
	Whole milk	34.5	463	B	POM-B
Functional dairy products with ω -3 polyunsaturated fatty acids	Skimmed milk with vegetable oils and fish oil	2.8	65	A	FP ω 3-A
	Skimmed milk with high-oleic sunflower, olive and fish oils	2.9	54	B	FP ω 3-B
	Skimmed milk with high-oleic sunflower, olive and fish oils	3.0	62	C	FP ω 3-C
	Skimmed milk with virgin olive oil and marine oils	3.3	75	D	FP ω 3-D
	Fermented skimmed milk with high-oleic sunflower, olive and fish oils	2.0	18	E	FP ω 3-E
	Skimmed milk with sunflower, corn and fish oils	1.6	71	F	FP ω 3-F
Functional dairy products with conjugated linoleic acid	Skimmed milk with oil rich in conjugated linoleic acid	1.0	79	A	FPCLA-A
	Skimmed milk with oil rich in conjugated linoleic acid	1.5	87	B	FPCLA-B
	Fermented skimmed milk with oil rich in conjugated linoleic acid	2.0	18	C	FPCLA-C
	Fermented skimmed milk with oil rich in conjugated linoleic acid	1.5	19	D	FPCLA-D
Functional dairy products with plant sterols	Fermented skimmed milk with stanol esters	5.0	20	A	FPSE-A
	Fermented skimmed milk with stanol esters	4.6	22	B	FPSE-B

Table 2.- Fatty acid composition and total lipids extracted from initial conventional dairy products.

FAME (wt % on oil)	PM-A	PM-B	UHTM-A	UHTM-B	FM-A	FM-B	EM-A	EM-B	POM-A	POM-B
C4:0	3.3 ± 0.32	3.4 ± 0.20	3.2 ± 0.17	3.1 ± 0.05	3.2 ± 0.27	3.1 ± 0.58	3.5 ± 0.26	3.0 ± 0.20	3.8 ± 0.18	3.6 ± 0.26
C6:0	2.6 ± 0.21	2.4 ± 0.03	2.5 ± 0.13	2.4 ± 0.22	2.2 ± 0.11	2.1 ± 0.09	2.4 ± 0.16	2.3 ± 0.17	2.4 ± 0.12	2.3 ± 0.12
C8:0	1.1 ± 0.08	1.0 ± 0.05	1.1 ± 0.06	1.0 ± 0.01	1.1 ± 0.04	1.2 ± 0.06	1.1 ± 0.07	1.2 ± 0.06	1.3 ± 0.07	1.3 ± 0.06
C10:0	2.6 ± 0.12	2.6 ± 0.04	2.5 ± 0.06	2.3 ± 0.22	2.5 ± 0.10	2.6 ± 0.05	2.9 ± 0.14	2.7 ± 0.08	2.9 ± 0.05	2.8 ± 0.11
C10:1	0.3 ± 0.02	0.2 ± 0.00	0.3 ± 0.01	0.3 ± 0.00	0.3 ± 0.01	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.02	0.3 ± 0.01	0.2 ± 0.01
C12:0	3.1 ± 0.07	3.1 ± 0.16	3.2 ± 0.05	2.9 ± 0.13	3.1 ± 0.07	2.9 ± 0.14	3.8 ± 0.06	2.8 ± 0.10	3.8 ± 0.09	3.5 ± 0.15
C13:0	0.2 ± 0.02	0.3 ± 0.02	0.2 ± 0.01	0.2 ± 0.04	0.3 ± 0.04	0.2 ± 0.01	0.3 ± 0.05	0.2 ± 0.04	0.3 ± 0.06	0.2 ± 0.03
C14:0	11.2 ± 0.33	10.9 ± 0.12	10.4 ± 0.65	11.3 ± 0.24	10.0 ± 0.49	10.0 ± 0.56	11.6 ± 0.46	11.8 ± 0.55	10.4 ± 0.70	10.4 ± 0.94
C15:0	1.0 ± 0.05	0.9 ± 0.03	1.1 ± 0.02	1.0 ± 0.05	0.9 ± 0.05	0.9 ± 0.02	0.9 ± 0.02	1.0 ± 0.08	0.9 ± 0.02	0.8 ± 0.06
C16:0	32.7 ± 0.87	34.7 ± 1.08	31.8 ± 1.10	34.3 ± 1.22	32.7 ± 1.29	31.6 ± 1.21	35.3 ± 1.38	33.5 ± 1.37	27.3 ± 1.30	29.0 ± 1.47
C16:1	2.0 ± 0.16	1.1 ± 0.02	0.2 ± 0.01	1.1 ± 0.03	1.2 ± 0.02	1.4 ± 0.02	2.0 ± 0.00	1.6 ± 0.02	1.5 ± 0.02	1.5 ± 0.01
C18:0	11.8 ± 0.22	12.3 ± 0.12	12.4 ± 0.60	11.9 ± 0.79	11.9 ± 0.50	11.9 ± 0.47	10.8 ± 0.72	12.0 ± 0.84	12.9 ± 0.75	12.5 ± 0.60
C17:0	2.0 ± 0.16	2.1 ± 0.07	1.9 ± 0.06	2.0 ± 0.03	2.0 ± 0.00	2.0 ± 0.02	2.1 ± 0.05	2.0 ± 0.08	1.9 ± 0.11	2.1 ± 0.05
C17:1	0.1 ± 0.02	0.1 ± 0.00	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.02	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	ND	ND
C18:1	23.6 ± 0.77	22.2 ± 0.34	22.7 ± 0.32	23.0 ± 1.25	23.0 ± 0.84	24.3 ± 1.21	21.1 ± 0.58	22.7 ± 0.67	25.9 ± 1.27	25.4 ± 0.90
C18:2 9c,12c	1.7 ± 0.16	1.1 ± 0.03	2.3 ± 0.01	1.7 ± 0.12	1.9 ± 0.02	1.9 ± 0.00	1.1 ± 0.02	1.0 ± 0.00	2.2 ± 0.01	2.2 ± 0.02
C18:2 9c,11t	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.02	0.3 ± 0.03	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	ND	1.0 ± 0.00	0.7 ± 0.00
C18:2 10t,12c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:3 n-3	ND	0.2 ± 0.02	0.1 ± 0.03	0.2 ± 0.00	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.01
C20:0	0.1 ± 0.02	ND	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.00	0.1 ± 0.00	ND	ND	0.1 ± 0.01	0.1 ± 0.02
C20:5 n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:6 n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ΣSFA	71.7	73.7	70.4	72.5	69.0	67.6	74.7	72.5	68.0	68.6
ΣMUFA	26.0	23.6	23.4	24.5	24.7	26.2	23.5	24.7	27.7	27.1
ΣPUFA	1.8	1.4	2.5	2.2	2.2	2.2	1.3	1.1	3.3	3.0
Extracted Lipids (wt % on product)	3.6 ± 0.32	4.2 ± 0.25	4.1 ± 0.28	3.7 ± 0.29	3.9 ± 0.20	3.7 ± 0.35	18.3 ± 2.51	17.1 ± 1.90	28.3 ± 2.08	34.8 ± 2.51

Data are expressed as Mean ± SD, *n* = 3.

Abbreviations: FAME, fatty acid methyl esters; PM, pasteurized whole milk; UHTM, ultra-high temperature-treated whole milk; FM, fermented milk; EM, evaporated whole milk; POM, powdered whole milk; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

Table 3. - Fatty acid composition and total lipids extracted from initial functional dairy products.

FAME (wt% on oil)	FPw3-A	FPw3-B	FPw3-C	FPw3-D	FPw3-E	FPw3-F	FPCLA-A	FPCLA-B	FPCLA-C	FPCLA-D	FPSE-A	FPSE-B
C4:0	0.2 ± 0.01	0.1 ± 0.01	0.7 ± 0.04	ND	0.1 ± 0.01	0.1 ± 0.01	0.6 ± 0.04	0.3 ± 0.02	ND	ND	0.1 ± 0.01	ND
C6:0	0.1 ± 0.01	0.1 ± 0.01	0.4 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.8 ± 0.01	0.3 ± 0.01	ND	ND	0.2 ± 0.01	ND
C8:0	0.1 ± 0.00	0.1 ± 0.01	0.8 ± 0.03	0.1 ± 0.01	0.1 ± 0.00	0.1 ± 0.02	0.5 ± 0.03	ND	ND	ND	0.4 ± 0.02	ND
C10:0	0.1 ± 0.01	0.1 ± 0.01	1.6 ± 0.10	0.9 ± 0.08	0.1 ± 0.01	0.1 ± 0.01	1.0 ± 0.10	0.6 ± 0.03	ND	ND	0.3 ± 0.01	ND
C10:1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C12:0	0.2 ± 0.01	0.3 ± 0.01	2.3 ± 0.20	1.0 ± 0.07	0.3 ± 0.02	0.4 ± 0.03	1.2 ± 0.07	0.7 ± 0.03	0.1 ± 0.00	0.1 ± 0.01	0.5 ± 0.01	ND
C13:0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C14:0	0.5 ± 0.03	1.2 ± 0.10	9.5 ± 0.88	5.3 ± 0.42	1.2 ± 0.08	0.5 ± 0.02	3.7 ± 0.21	1.9 ± 0.10	ND	ND	1.4 ± 0.05	ND
C15:0	ND	ND	0.1 ± 0.01	ND	ND	ND	0.1 ± 0.01	0.1 ± 0.00	ND	ND	ND	ND
C16:0	9.4 ± 1.10	10.1 ± 0.71	31.9 ± 1.02	26.7 ± 0.91	9.0 ± 0.32	6.7 ± 0.20	11.7 ± 0.90	7.8 ± 0.41	1.6 ± 0.11	6.0 ± 0.22	10.9 ± 0.75	6.3 ± 0.39
C16:1	0.1 ± 0.01	ND	2.3 ± 0.11	2.0 ± 0.10	0.1 ± 0.00	0.1 ± 0.03	0.5 ± 0.03	0.5 ± 0.00	ND	ND	1.1 ± 0.04	1.0 ± 0.05
C18:0	8.7 ± 0.72	6.0 ± 0.33	12.1 ± 0.59	9.0 ± 0.35	7.4 ± 0.51	3.3 ± 0.28	5.5 ± 0.27	3.5 ± 0.31	2.5 ± 0.22	3.8 ± 0.18	5.1 ± 0.21	5.4 ± 0.14
C17:0	0.1 ± 0.03	0.1 ± 0.02	0.2 ± 0.00	ND	ND	ND	0.2 ± 0.01	ND	ND	ND	0.1 ± 0.00	ND
C17:1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:1	65.0 ± 1.50	63.7 ± 1.65	28.3 ± 0.73	44.6 ± 1.05	68.8 ± 2.10	24.2 ± 1.10	17.5 ± 1.10	15.6 ± 0.75	14.7 ± 0.87	16.2 ± 1.02	57.9 ± 1.34	68.1 ± 1.47
C18:2 9c,12c	12.1 ± 0.55	10.4 ± 0.41	4.9 ± 0.30	3.7 ± 0.22	7.9 ± 0.44	62.7 ± 2.07	1.3 ± 0.08	1.4 ± 0.09	0.4 ± 0.02	0.8 ± 0.02	16.9 ± 0.76	14.2 ± 0.91
C18:2 9c,11t	ND	ND	ND	ND	ND	ND	26.6 ± 1.00	29.2 ± 1.08	38.0 ± 0.99	34.1 ± 0.77	ND	ND
C18:2 10t,12c	ND	ND	ND	ND	ND	ND	25.6 ± 0.86	30.1 ± 0.96	37.8 ± 0.84	33.1 ± 0.93	ND	ND
C18:3 n-3	0.4 ± 0.02	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.03	0.1 ± 0.02	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	ND	ND	0.4 ± 0.02	2.3 ± 0.10
C20:0	ND	ND	0.1 ± 0.00	0.1 ± 0.01	ND	ND	0.1 ± 0.00	0.1 ± 0.01	ND	ND	ND	ND
C20:5 n-3	1.4 ± 0.01	1.5 ± 0.12	1.4 ± 0.15	1.6 ± 0.15	1.4 ± 0.03	0.4 ± 0.00	ND	ND	ND	ND	ND	ND
C22:6 n-3	2.3 ± 0.01	2.7 ± 0.08	2.0 ± 0.11	2.0 ± 0.27	0.6 ± 0.01	0.2 ± 0.01	ND	ND	ND	ND	ND	ND
ΣSFA	17.4	18.1	59.7	43.2	18.3	11.3	25.4	15.2	4.2	9.9	19.0	11.7
ΣMUFA	65.1	66.7	30.6	46.6	68.9	24.3	18.0	16.1	14.7	16.2	59.0	69.1
ΣPUFA	16.2	14.7	7.5	7.5	10.0	63.5	53.6	60.8	76.2	68.0	17.3	16.5
Σn-3	4.1	4.3	3.6	3.7	2.1	0.8	0.1	0.1	ND	ND	ND	ND
ΣCLA	ND	ND	ND	ND	ND	ND	52.2	59.3	75.8	67.2	ND	ND
Extracted Lipids (wt % on product)	3.0 ± 0.15	2.6 ± 0.23	2.8 ± 0.11	3.1 ± 0.34	1.9 ± 0.18	1.7 ± 0.22	1.0 ± 0.20	1.6 ± 0.15	2.0 ± 0.25	1.5 ± 0.10	5.1 ± 0.37	4.0 ± 0.30

Data are expressed as Mean ± SD, $n = 3$.

Abbreviations: FAME, fatty acid methyl esters; CLA, conjugated linoleic acids; FPw3, functional dairy product with omega-3 fatty acids; FPCLA, functional dairy product with conjugated linoleic acid; FPSE, functional dairy product with stanol esters; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; ND, not detected.

Table 4. - Oxidation parameters in lipids extracted from conventional dairy products at initial time.

	PM-A	PM-B	UHTM-A	UHTM-B	FM-A	FM-B	EM-A	EM-B	POM-A		POM-B	
									Free	Encapsulated	Free	Encapsulated
PV	0.8 ± 0.34	0.7 ± 0.40	1.0 ± 0.36	0.9 ± 0.28	0.6 ± 0.44	0.7 ± 0.38	0.9 ± 0.26	1.0 ± 0.35	6.9 ± 0.57 ^a	1.3 ± 0.20 ^b	7.5 ± 0.61 ^a	1.5 ± 0.29 ^b
TG (mg/g):												
- OxTGM	ND	ND	ND	ND	ND	ND	1.8 ± 0.30	2.1 ± 0.28	16.1 ± 1.36 ^a	1.5 ± 0.20 ^b	10.4 ± 2.17 ^a	2.3 ± 0.25 ^b
- TGD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
- TGP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TOC (mg/kg):												
- α	28.6 ± 4.16	29.2 ± 4.18	28.4 ± 3.06	27.0 ± 4.12	26.1 ± 4.10	21.9 ± 5.14	37.9 ± 5.21	32.6 ± 4.16	20.5 ± 5.09	31.7 ± 6.86	26.1 ± 4.32	27.3 ± 2.99
- β	27.2 ± 4.62	ND	26.4 ± 4.28	ND	9.8 ± 3.33	27.0 ± 5.69	ND	ND	ND	ND	ND	ND
- γ	ND	21.3 ± 3.14	ND	17.6 ± 2.05	ND	ND	ND	ND	32.1 ± 3.22 ^a	44.8 ± 4.70 ^b	18.4 ± 2.56 ^a	26.0 ± 3.01 ^b
- δ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	55.9 ± 6.22	50.4 ± 5.22	54.7 ± 5.26	44.6 ± 4.60	35.9 ± 5.28	48.9 ± 7.67	37.9 ± 5.21	32.6 ± 4.16	52.6 ± 4.31 ^a	76.5 ± 5.03 ^b	44.5 ± 4.67 ^a	53.3 ± 3.28 ^b

Data are expressed as Mean ± SD, $n = 3$. Different superscript letters in POM samples indicate significant differences between free and encapsulated lipid fractions ($p < 0.05$).

Abbreviations: PM, pasteurized whole milk; UHTM, ultra-high temperature-treated whole milk; FM, fermented milk; EM, evaporated whole milk; POM, powdered whole milk; PV, peroxide value; TG, triacylglycerols; OxTGM, oxidized triacylglycerol monomers; TGD, triacylglycerol dimers; TGP, triacylglycerol polymers; TOC, tocopherols; ND, not detected.

Table 5.- Oxidation parameters in lipids extracted from functional dairy products at initial and expiry times.

	FPw3-A	FPw3-B	FPw3-C	FPw3-D	FPw3-E	FPw3-F	FPCLA-A	FPCLA-B	FPCLA-C	FPCLA-D	FPSE-A	FPSE-B
Initial time												
PV (meq O ₂ /kg)	8.2 ± 2.26 ^a	6.7 ± 2.22 ^a	3.9 ± 2.34	8.8 ± 2.41 ^a	9.1 ± 2.61	9.6 ± 2.48	6.6 ± 3.50	7.3 ± 2.62	6.0 ± 2.81	7.9 ± 2.61	4.9 ± 3.49	3.3 ± 2.18
TG (mg/g):												
- OxTGM	9.1 ± 1.12 ^a	6.1 ± 1.14 ^a	5.2 ± 1.12	9.2 ± 1.15 ^a	9.3 ± 1.19	9.0 ± 1.22	8.5 ± 1.13	8.0 ± 1.28	7.0 ± 1.03	5.2 ± 1.23	6.9 ± 1.30	5.0 ± 0.41
- TGD	8.5 ± 1.11	3.2 ± 0.30	1.1 ± 0.10	7.1 ± 1.10	6.9 ± 1.08	6.1 ± 1.17	3.2 ± 0.41	2.8 ± 0.31	3.5 ± 0.20	2.5 ± 0.88	3.4 ± 0.37	ND
- TGP	1.9 ± 0.18	ND	ND	ND	1.7 ± 0.20	ND	24.3 ± 1.35 ^a	21.2 ± 2.07	19.3 ± 2.12	24.9 ± 1.91 ^a	ND	ND
TOC (mg/kg):												
- α	532.0 ± 15.52 ^a	445.6 ± 14.54 ^a	406.1 ± 21.37	335.1 ± 12.86 ^a	524.4 ± 14.62	526.4 ± 15.04	65.1 ± 4.13	93.4 ± 5.44	78.9 ± 5.99	28.1 ± 2.14	12.3 ± 1.55	220.1 ± 10.34
- β	34.5 ± 2.20	29.0 ± 3.03	25.1 ± 3.08	7.7 ± 1.27	10.3 ± 1.04	36.1 ± 4.39	13.1 ± 1.22	22.1 ± 1.30	ND	ND	ND	ND
- γ	147.5 ± 8.81	102.8 ± 5.19	92.0 ± 6.04	26.7 ± 0.91	166.1 ± 6.26	44.7 ± 4.46	312.0 ± 13.74	593.1 ± 15.61	511.6 ± 20.11	324.0 ± 17.22	116.2 ± 7.56	55.3 ± 3.39
- δ	43.8 ± 2.45	40.1 ± 2.66	337.0 ± 18.11	45.7 ± 1.62	84.0 ± 5.49	3.2 ± 0.40	250.3 ± 8.15 ^a	318.6 ± 11.01	268.0 ± 7.44	215.2 ± 12.99 ^a	35.0 ± 1.18	19.9 ± 2.05
Total	757.6 ± 18.15 ^a	617.3 ± 15.96 ^a	860.2 ± 28.82	396.9 ± 13.06 ^a	784.7 ± 16.86	610.4 ± 16.29	640.4 ± 16.55 ^a	1027.4 ± 19.90	855.0 ± 22.26	567.3 ± 21.68 ^a	163.5 ± 7.81	295.3 ± 11.07
Expiry time												
PV (meq O ₂ /kg)	65.9 ± 4.16 ^b	26.0 ± 3.01 ^b	4.1 ± 3.22	44.5 ± 5.01 ^b	10.9 ± 3.87	11.5 ± 4.01	7.2 ± 5.18	8.5 ± 2.09	7.1 ± 2.30	8.3 ± 3.99	3.8 ± 2.98	4.2 ± 2.87
TG (mg/g):												
- OxTGM	41.1 ± 2.88 ^b	19.1 ± 1.55 ^b	6.1 ± 1.30	34.5 ± 1.64 ^b	10.2 ± 2.30	11.2 ± 2.37	8.0 ± 1.17	9.3 ± 1.39	8.3 ± 1.88	10.4 ± 1.88	5.7 ± 1.51	7.3 ± 2.06
- TGD	9.9 ± 1.05	3.2 ± 0.29	1.0 ± 0.36	7.9 ± 0.87	7.9 ± 1.97	7.4 ± 2.26	3.5 ± 0.50	3.7 ± 1.23	3.6 ± 0.65	5.0 ± 1.01	ND	ND
- TGP	2.1 ± 0.14	ND	ND	ND	1.6 ± 0.47	ND	48.9 ± 3.22 ^b	25.9 ± 4.47	21.9 ± 2.94	32.8 ± 2.98 ^b	ND	ND
TOC (mg/kg):												
- α	332.6 ± 11.41 ^b	346.2 ± 13.10 ^b	399.0 ± 18.21	198.7 ± 9.31 ^b	511.7 ± 21.85	490.4 ± 23.26	60.0 ± 2.91	82.5 ± 7.56	75.6 ± 7.01	21.8 ± 2.31	15.9 ± 1.22	199.6 ± 12.58
- β	29.1 ± 2.87	25.1 ± 1.04	20.8 ± 5.01	ND	8.7 ± 2.09	25.0 ± 5.22	10.7 ± 0.53	23.4 ± 2.56	ND	ND	ND	ND
- γ	93.0 ± 7.18	80.9 ± 4.59	91.3 ± 5.93	20.0 ± 1.22	146.3 ± 9.11	39.9 ± 4.87	283.5 ± 10.18	512.7 ± 20.31	498.8 ± 24.87	309.9 ± 18.02	136.1 ± 8.36	47.5 ± 3.70
- δ	40.2 ± 3.04	36.1 ± 2.89	330.2 ± 24.60	38.9 ± 3.03	73.8 ± 8.62	2.4 ± 0.56	201.4 ± 9.31 ^b	294.4 ± 19.89	211.9 ± 10.20	154.2 ± 12.11 ^b	29.7 ± 1.45	21.2 ± 2.44
Total	494.5 ± 14.11 ^b	487.0 ± 14.22 ^b	853.1 ± 33.76	259.6 ± 9.87 ^b	729.1 ± 18.03	584.8 ± 23.60	551.7 ± 14.11 ^b	990.82 ± 33.22	827.0 ± 19.98	486.1 ± 19.47 ^b	170.3 ± 6.62	286.9 ± 12.20

Data are expressed as Mean ± SD, *n* = 3. Different superscript letters indicate significant differences between samples at initial and expiry times (*p* < 0.05).

Abbreviations: FPw3, functional dairy product with omega-3 fatty acids; FPCLA, functional dairy product with conjugated linoleic acid; FPSE, functional dairy product with stanol esters; PV, peroxide value; TG, triacylglycerols; OxTGM, oxidized triacylglycerol monomers; TGD, triacylglycerol dimers; TGP, triacylglycerol polymers; TOC, tocopherols; ND, not detected

Figure 1