Effect of type of emulsifiers and antioxidants on oxidative stability, colour and fatty acid profile of low-fat beef burgers enriched with unsaturated fatty acids and phytosterols

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Running title: **Low-fat burgers enriched with unsaturated fatty acids and phytosterols**

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Abstract

Low-fat beef burgers were formulated using fresh lean meat, 9.9% oleic sunflower oil and 0.1% deodorized fish oil to obtain a product enriched in unsaturated fatty acids. The effect of two emulsifiers (whey proteins or egg white) and natural antioxidants (tocopherols and/or oregano-rosemary), as well as the influence of frozen storage on the oxidative stability, colour, and fatty acid (FA) profile was determined on the cooked products. Whey proteins protected better against oxidation than egg white, and tocopherols demonstrated an adequate antioxidant effect in formulations with egg white. For all the formulations the unsaturated/saturated FA ratio was higher than 5.8, showing a good lipid balance in the products. The consumption of 100 g of the cooked product would provide 6% of the recommended daily intake of phytosterols suggested to decrease cholesterol and the risk of heart disease. Formulated low-fat burgers with pre-emulsified oils and phytosterols could be considered to be potentially functional foodstuffs.

Key words: low-fat beef burgers, oxidative stability, colour, whey protein concentrate, dry egg white, fatty acids profile

1. Introduction

The importance of the link between nutrition and health is a hot topic. Like other food-related sectors, the meat industry is undergoing major transformations, driven among other things by changes in consumer demands (Jiménez-Colmenero, 2007).

There exist varying possibilities to improve the different categories of processed meats. For ground products additives with a “healthy perception” can be added, such as phytosterols or “special” oils. Of importence to the market are fat and salt (sodium) reduction (Vandendriessche, 2008). The manufacture of low-fat products generally follows two basic approaches: the use of leaner raw materials (which raises the cost) and/or the reduction of fat and calorific contents by adding water and other ingredients that contribute few or no calories. These approaches can be supplemented by the use of technological procedures that help offset undesirable side effects produced as a result of changes to the composition and nature of the product (Muchenje, Dzama, Chimonyo, Strydom, Hugo, & Raats, 2009).

Not only the total amount of fat consumed but also the type of fat has health implications. Beef is one of the widely consumed protein sources in the world. Modern consumers are increasingly concerned about the production of safe meat with no undesirable effects on their health. For meat products the energy (fat) level, the sodium level, and fat quality in terms of fatty acid composition are the main priorities.

Beef fat is a significant source of saturated fatty acids in the human diet because red meat has a relatively high proportion of saturated fatty acids in its lipids (Muchenje et al., 2009). Thus palmitic acid, the predominate saturated fatty acid present in red meat, has a cholesterol-elevating effect (Wolmarans, 2009).

The most often cited criteria for saturated fat recommendations are that saturated fats increase LDL-cholesterol, but also increase HDL-cholesterol and decrease triglyceride levels (Mensink, Zock, Kester, & Katan, 2003). However limits on saturated fat intake should be considered in the context of the replacement nutrient, as replacement with carbohydrates may have little benefit (Smit, Mozaffarian, & Willet, 2009).

Regarding the effect of *n-6* and *n-3* fatty acid consumption, both types of fatty acids have anti-inflammatory properties that are protective against atherogenic changes in vascular endothelial cells (De Caterina, Liao, & Libby, 2000). Linoleic acid is an essential n-6 fatty acid that favourably affects the blood lipid profile, and is associated with a lower risk of coronary heart disease and reduced risk of type 2 diabetes (Smit et al., 2009). Long chain polyunsaturated *n-3* fatty acids have been implicated as critical nutrients for human health, and fortification of foods with these fatty acids is an emerging area of commercial and academic interest (Lee, Decker, Faustman, & Mancini, 2005; Jiménez-Colmenero, 2007). There is considerable evidence from epidemiological, clinical and biochemical studies that eicosapentaenoic acid (EPA) and docosahexaenoic acis (DHA) consumption have physiological benefits on blood pressure, heart rate, triglycerides, and a reduced risk of fatal coronary heart disease and sudden cardiac death (Smit, Mozaffarian,& Willett, 2009).

A variety of non-meat fats of plant (olive, high oleic sunflower, linseed, soybean) and marine origin have been added to meat products as partial substitutes for meat fats (mainly pork and beef) (Park, Rhee, Keeton, & Rhee, 1989; Park, Rhee, & Ziprin, 1990; Paneras, & Bloukas, 1994; Pappa, Bloukas, & Arvanitoyannis, 2000; Yilmaz, Şimşek, & Işikh, 2002; Lee, Faustman, Djordjevic, Faraji, & Decker, 2006a; Jimenez-Colmenero, 2007; Pelser, Linssen, Legger, & Houben, 2007) since they are

rich sources of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and are cholesterol-free.

Oil in water (o/w) emulsions may be easier to disperse into water-based foods (such as muscle foods), than bulk oil that could physically separate from the aqueous phase during storage. Oil pre-emulsion technology with a non-meat protein improves the stability of the system, since the oils can be stabilized or immobilized in a protein matrix. This reduces the chances of bulk oil physically separating from the structure of the product so that it remains stable throughout processing, storage and consumption.

Whey proteins can be used to produce a low-viscosity O/W emulsion at oil concentration ranging from 5% to 30%, with excellent physical stability that is not adversely affected by thermal processing (Djordjevic, McClements, & Decker, 2004).

Siegel, Church and Schmidt (1979) demonstrated that egg white is a good binder for meat pieces. Due to its crude protein concentration and stronger gel strength, dried egg white may be more useful in restructuring muscle products than raw egg white (Lu & Chen, 1999).

Prevention of lipid oxidation during processing and storage of meat is essential to maintain quality and safety. Recently, interest in using natural antioxidants to prevent meat lipid oxidation has increased so as to avoid the possible harmful effects of synthetic substances. Many researchers have evaluated the antioxidant properties of extracts from different herbs and spices. Rosemary (*Rosmarinus officianalis*) is a popular *Labitae* herb with a verified potent antioxidant activity (Estévez & Cava, 2006, Estévez, Ramírez, Ventanas, & Cava, 2007) which has been used traditionally to improve the sensory characteristics and extend the shelf-life of foods. Essential oils are regarded as ‘‘natural’’ alternatives to chemical preservatives and their use in foods

meets the demand of consumers for minimally processed products. Oregano is a characteristic spice of Mediterranean cuisine, obtained by drying leaves and flowers of *Origanum vulgare subsp. hirtum* plants, well known for its antioxidative and antimicrobial properties (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, & Guerrero Legarreta, 2009). However, the practical application of several essential oils in foods is limited due to the strong flavor they impart to foods and also due to their interaction with some food ingredients (Chouliara et al., 2007).

Another alternative is the use of mixed tocopherol isomers, by-products of the vegetable oil industry, which may be more effective in preventing lipid oxidation in muscle foods than α-tocopherol as some of the tocopherol isomers have superior antioxidant activity than α-tocopherol. Tocopherols are usually used at levels up to 500 mg/kg in food products because above this concentration they may act as pro-oxidants (Channon & Trout, 2002).

Phytosterols/stanols are used as a novel food ingredient with plasma cholesterol lowering activity. There is substantial evidence that plant sterols and stanols lower total and LDL-cholesterol, by partly inhibiting cholesterol absorption, and that their effect is additional to that achieved by other strategies (e.g. a low-fat diet and/or the use of cholesterol-lowering drugs such as statins). A wide variety of phytosterol structures exist but the phytosterols found most frequently in nature are β-sitosterol, campesterol and stigmasterol. Many pre-market controlled clinical trials have demonstrated that an intake of 2 g/day of phytosterol/-stanols reduces serum LDL cholesterol by approximately 10% (Ostlund, 2007). In addition, the US Food and Drug Administration has accepted that phytosterols/-stanols are Generally Recognized as Safe (GRAS) (de

Jong, Ros, Ocké, & Verhagen, 2008) and the Scientific Comittee on Foods of the Comission of European Comunities came to the conclusion that the addition of phytosterols is safe, provided that the daily consumption does not exceed 3 g (Scientific Committee on Food, 2002).

The aim of the present work was to study the effect of two emulsifiers (whey protein concentrate or dry egg white) and natural antioxidants (tocopherols and/or oleoresins) on the oxidative stability, colour, and fatty acid profile of low-fat meat burgers enriched in unsaturated fatty acids and phytosterols, kept frozen, and subsequently cooked.

2. Materials and methods

2.1 Materials

Low-fat burgers were prepared using fresh lean beef meat (*adductor femoris* and *semimembranosus muscles*) obtained from local processors (pH: 5.48±0.01). As fat sources, high oleic acid sunflower oil (85% *n-9*, Ecoop, Cooperativa Obrera, Bahía Blanca, Argentina) and deodorized refined fish oil (26% *n-3*, Omega Sur S.A., Mar del Plata, Argentina) were used. Mixed phytosterols (Advasterol 90% with 16-24% campesterol, 19-32% stigmasterol and 32-50% β-sitosterol, AOM SA, Buenos Aires, Argentina) were included.

As emulsifiers, whey protein concentrate (W, 80%, Arla Foods Ingredients S.A., Martínez, Argentina) or dry egg white (E, 80%, Tecnovo SA, Entre Ríos, Argentina) were used. Mixed tocopherols (TO, Mixed tocopherols 70% with d-γ-/d-β-tocopherol 43.81%, d-δ-tocopherol 19.31% and d-α-tocopherol 7.40%, AOM SA, Buenos Aires,

Argentina), a mixture of rosemary oleoresin (5%) and distilled fraction of oregano (5%) adsorbed in an inert matrix of calcium carbon (OR, Río Arnedo SA, Buenos Aires, Argentina) were added alone or in combination as antioxidants. Analytical grade sodium chloride (NaCl) and tripolyphosphate (TPP), cold distilled water (4 ºC), food-grade commercial corn starch (Parafarm, Saporiti SACIFIA, Buenos Aires, Argentina) were used. TPP and corn starch were added to improve binding and water-holding properties. Water was incorporated to replace part of the beef fat.

2.2 Burger manufacture

Beef without visible fat and connective tissue was ground with a commercial food processor (Universo, Rowenta, Germany) equipped with a 14 cm blade for 5 minutes at the highest speed. NaCl and TPP were added to the ground meat and processing continued for 2 min.

Emulsifiers, either whey protein or egg white, were solubilised in cold distilled water, homogenized 30 sec. and rested for 10 min for a better hydration. Then, oils, tocopherols, phytosterols and OR were added and emulsified with a hand-held food processor (Braun, Buenos Aires, Argentina) for 1.5 min to form a coarse emulsion. The emulsion was added to the meat, mixing all ingredients for 4 min. Finally the starch was added, mixing for one additional minute. The batter obtained was stored for 1 h at 4 °C. The ingredients are listed in Table 1 where W indicates those formulations containing 3% whey protein concentrate while E corresponds to 3% added dry egg white. Numbers 1 to 4 designate the type of antioxidant considered: 1 = none, control, 2 = 0.1% TO + 0.05% OR, 3 = 0.1% TO, and 4 = 0.05% OR.

Burgers (40±1g) were formed using a hamburger mould (5 cm diameter and 1.2

cm in high), wrapped separately in polyethylene cling film and sealed in lots of eight in Zip-Lock pouches (C. S. Johnson & Sons de Argentina S.A.I.C., Buenos Aires). They were frozen and stored at –20 °C for up to 6 months.

At different times (0, 2, 4, and 6 months) burgers were removed from the freezer and immediately cooked (without previously defrosting) in a commercial, double-sided electric household grill (3882, Oster, China) preheated for 30 min to reach the maximum temperature (210 °C). The burgers were cooked until a final internal temperature of 73 ºC for 15 s was reached, according to the recommendations of the FDA-CFSAN (2003). Temperature was monitored by a type-T (copper-constantan) thermocouple inserted in the centre of a burger, and connected to an acquisition system (TESTO175, TESTO AG, Germany). The samples were then cooled immediately at room temperature over absorbent paper and further processed to determine lipid oxidation, colour, FA profiles, and phytosterols content. The procedure was replicated twice.

2.3 Proximate analysis and energy content

Moisture, ash, and protein contents of the cooked products were determined according to AOAC (1980) methods 24.003, 24.009 and 24.027, respectively, in triplicates. Fat content was determined on samples previously dried with anhydrous sodium sulphate (SO_4Na_2) by the Soxhlet method, using petroleum ether (Bp: 35-60 ºC) as extraction solvent. Carbohydrates were calculated by difference.

Total calories (kcal) were calculated using the Atwater values corresponding to lipids (9 kcal/g), proteins (4.02 kcal/g) and carbohydrates (3.87 kcal/g) (Cáceres, García, & Selgas, 2006).

2.4 Colour

Colour was measured at room temperature on the internal surface of recently cut cooked burgers (five replicates), using a Chroma Meter CR-400 colorimeter (Minolta Co., Osaka, Japan) and CIE-LAB parameters (L*, a*, and b*) were determined. Within the approximate uniform colour space CIELAB, two colour coordinates, redness, a*, and yellowness, b*, and lightness, L*, are defined. Coordinate a* takes positive values for reddish colours and negative values for the greenish colours, whereas b* takes positive values for yellowish colours and negative values for the bluish colours. L* is an approximate measurement of luminosity, which is the property according to which each colour can be considered as equivalent to a member of the grey scale, between black and white, within the range 0–100.

2.5 Lipid oxidation

Lipid oxidation was measured by the thiobarbituric acid reactive substances (TBARS) test. TBARS values were determined in duplicate on cooked burgers according to Andrés, Zaritzky and Califano (2009) to evaluate the extent of oxidative rancidity development. Results are expressed as mg malonaldehyde (MDA)/kg product.

2.6 Fatty acid profile

Total lipids of the cooked burgers were extracted (in duplicate) using chloroform-methanol (2:1, v/v) according to Folch, Lees and Sloane Stanley (1957), and then methylated with 10% boron-trifluoride methanol complex in methanolic solution (Morrison & Smith, 1964).

GC analysis of fatty acid methyl esters (FAME) was performed on a Hewlett Packard 6890 gas chromatograph (Hewlett Packard, USA) equipped with a flame ionization detector (FID) and a split/splitless injector (Chrompack, Middleburg, The Netherlands). Analysis of FAME was achieved with a fused silica capillary column (Chrompack CP-SIL 88) of length 50 m, i.d. 0.25 mm and film thickness 0.1 μm. Operating conditions were: temperature program of 185 °C for 3 min, rising to 230 °C at 3 °C/min, keeping constant at 230 ºC for 25 minutes. Other GLC conditions were: injector temperature, 250 °C; detector temperature, 250 °C; carrier gas, nitrogen at a flow rate of 1 ml/min (Arici, Tasan, Gecgel, & Ozsoy, 2002).

FAME were identified by comparing their retention times with those of commercial, standard FAME (NuChek Prep, Inc., MN, USA). Peak areas were determined and calculated as normalized area percentages of fatty acids (Pereira, Tarley, Matsushita, & de Souza, 2000; Ayo, Carballo, Serrano, Olmedilla-Alonso, Ruiz-Capillas, & Jiménez-Colmenero, 2007).

2.7 Phytosterol content

Phytosterols content was determined at AOM S.A.'s laboratory over the lipid phase extracted of the cooked burgers by Folch´s procedure (Folch et al., 1957) using gas chromatography. An Agilent 6890N capillary gas chromatograph equipped with an automatic split/splitless injection (1 μl), flame-ionization detector and HP-5 MS column (30 m x 0.25 mm I.D., 0.25 μm film thickness; Agilent Technologies, CA, USA) was used. Operating conditions were: injection temperature, 260 °C; detector temperature, 320 °C, oven temperature 140 °C to 300 °C, at 10 °C/min, holding at 300 ºC for 14 minutes, helium as carrier gas (57.1 ml/min) based on AOCS recommended practice Ce 7-87 (AOCS, 1998). Identification and quantitation was based on external standards

using campesterol, stigmasterol and β-sitosterol from Sigma-Aldrich Inc, St. Louis, USA

2.8 Experimental design and statistical analysis

A full factorial design (2 x 4 x 4) was used, and the analysed factors were: type of emulsifier (2 levels: W or E), antioxidant (4 levels: none, TO, OR, or TO+OR), storage time at -20 ºC (4 levels: 0, 2, 4, and 6 months), and their interactions.

Analysis of variance was applied to evaluate the influence of the variables. For simultaneous pairwise comparisons, Tukey's test was chosen, calculating in each case the Honestly Significant Difference (HSD). Differences in means and F-tests were considered significant when $P < 0.05$. The symbol ± stands for standard error of the mean (SEM) values.

3. Results and Discussion

3.1 Proximate analysis and caloric content

Proximate analysis of all the cooked formulations showed no significant differences ($P > 0.05$) between them. Mean values of the main components were: protein 23.61±0.48%, lipids 13.84±0.15%, ash 2.21±0.09%, and moisture 58.80±0.25%. Due to the formulation, more than 87% of the protein content of the products was from meat, and the rest from milk or egg, all proteins of high nutritional value. The low salt in the products (1% NaCl) produced low ash levels.

The energy content of the burgers was 225.4 kcal/100 g, of which total lipids represented 55.2% (124.6 kcal/100g), and 105.9 kcal/100g came from oils.

3.2 Colour

During cooking of meats, a hemichrome pigment (denatured globin and oxidized heme iron) is formed that is tan in color (Foegeding, Lanier, & Hultin, 1996).

Samples at zero storage time (0 months) had higher L* values than those of commercial "light" hamburgers (52.4±0.26) with less than one months frozen storage, which could be attributed to a milky appearance imparted by the oil emulsion (Table 2). Similar results were obtained by Lee et al. (2006 a and b), who developed meat products (patties, sausages, restructured hams), fortified with *n-3* fatty acids incorporated as a pre-emulsion of algal oil stabilized with whey protein isolate.

For L*, all the factors considered (emulsifier, antioxidant and storage time) and their interactions were significant. Formulations containing egg albumin were lighter than products that contained whey proteins (58.9±0.66 and 56.8±0.77, respectively, $P < 0.05$). Regardless of storage time, the control group containing whey proteins had the lowest lightness (53.7±0.75), and the highest L* value (60.7±1.65) corresponded to the tocopherols + OR formulation with egg white ($P < 0.05$). There were no significant differences between the formulations containing these antioxidants (tocopherols or OR) added individually ($P > 0.05$). As the third level interaction was highly significant ($P < 0.01$) and considering that this term explained the highest percentage of total variance (23%), the changes produced by storage time depended on the emulsifier and antioxidant combination (Table 2). Comparing for each formulation, the initial and final mean L* values (at 0 and 6 months storage), non significant differences were found, even though several authors found that L* increased during refrigerated storage of lamb (Linares, Bórnez, & Vergara., 2006, 2008; Linares, Berruga, Bórnez, & Vergara, 2007; Sante´-Lhoutellier, Engel, & Gatellier, 2008; Soldatou, Nerantzaki, Kontominas, &

Savvaidis, 2009), in contrast, Rojas and Brewer (2008) reported an increase in L* of vacuum-packaged pork hamburgers after 2 months of frozen storage, remaining constant thereafter.

Products containing whey proteins as emulsifier were redder (9.03±0.74) than egg white formulations (8.30±0.57, $P < 0.05$). At the beginning of storage individual a* values of the cooked hamburgers ranged between 4.31 and 12.39; formulations with individual antioxidants (TO or OR, W3, W4, E3, and E4) showed no significant differences during storage at -20 ºC. Control and TO + OR samples (W1, W2, E1, and E2) showed an increase in redness up to the fourth month of frozen storage, decreasing thereafter (Table 2). The decrease in a* values has frequently been associated with with meat discolouration (Jeremiah, 2001).

There were no significant differences in yellowness (b*values, $P > 0.05$) attributable to the different factors and values ranged between 11 and 17 during frozen storage.

3.3 Lipid oxidation

Oxidation processes are slowed during frozen storage but not completely prevented. In fact, some lipid soluble radicals may even be more stable at lower temperatures and thereby propagate oxidation (Kanner, 1994)

Georgantelis, Blekas, Katikou, Ambrosiadis, and Fletouris (2007) reported that rancid flavour is detected in meat products with TBARS values higher than 0.6 mg MDA/kg. Campo, Nute, Hughers, Enser, Wood, and Richardson (2006) reported that a TBARS value of around 2 mg MDA/kg could be considered the limiting threshold for the acceptability of oxidized beef.

The effects of different antioxidant treatments on lipid oxidation during storage at -20 ºC and subsequent cooking are presented in Table 3. In the evaluation of the lipid oxidation of the products all the factors (type of emulsifier, antioxidant, and storage time) and their interactions were highly significant ($P < 0.01$). When the type of emulsifier was compared, whey proteins protected better from the oxidation than egg white, and agrees with the antioxidant properties of whey proteins described by other authors (Hu, McClements, & Decker, 2003).

The results showed that all cooked burgers containing whey proteins as emulsifier (even the control W1) were protected against lipid oxidation, maintaining TBARS levels less than 0.6 mg MDA/kg after 6 months of frozen storage and average values were not significantly different ($P > 0.05$).

Tocopherols were effective in the burgers with egg white but brought no additional effect to the whey protein burgers. TBARS content in formulations with dry egg white without tocopherols (control E1 and E4 with OR antioxidant) increased during storage and exceeded the level of lipid oxidation which produces a rancid odor and taste. On the other hand those samples containing tocopherols (E2 and E3) maintained low TBARS levels until the end of frozen storage ($P > 0.05$).

It can be concluded that when whey proteins were used as emulsifier it was not necessary to incorporate any antioxidant, while in the products containing egg white proteins it was necessaary to add tocopherols to control lipid oxidation. This can be explained through the combination of two different mechanisms: the tocopherols may act as free radical scavengers while the TPP present in the formulations may act as chelator. Lee, Decker, Faustman and Mancini (2005) found antioxidant combinations (sodium citrate, sodium erythorbate and rosemary) had beneficial effects in *n-3* fortified

patties.

3.4 Phytosterol contents

Particular and total phytosterol levels were determined in the lipid phases and expressed on dry weight basis of the burgers. The concentrations of individual and total phytosterols were not affected either by formulation or storage time at -20 ºC ($P > 0.05$). The total phytosterols content of the cooked burgers was 0.314±0.020% db, while campesterol, stigmasterol and β-sitosterol levels were 0.071±0.005, 0.103±0.006 and 0.138±0.011% db, respectively. The results were in accordance with the proportions of each type of phytosterol in the mix (22.8% campesterol, 25.3% stigmasterol and 40.6% β-sitosterol).

Total phytosterol content of 100 g of cooked burgers represented 6% of the daily recommended intake necessary to decrease cholesterol contents and reduce the risk of heart disease (de Jong et al., 2008).

3.5 Fatty acid profile

Fatty acid profiles of the different burgers initially and after six months of frozen storage are presented in Table 4. No differences were found for elaidic, linoleic and araquidonic acid contents between formulations or storage times ($P > 0.05$).

Saturated fatty acid content (SFA) of the products were between 9.4 and 14.6% FA, palmitic acid being the major component of the SFA, followed by stearic acid. Monounsaturated fatty acids contents (MUFA) were in the range 78.8-85.0% FA, with oleic acid comprising over 97% of MUFA. The difference between unsaturated and MUFA contents corresponded to linoleic and linolenic acids (4.4-6.7% FA).

Unsaturated/SFA ratio for all the formulations was higher than 5.8, showing a good lipid balance.

The PUFA content ((18:2 *n-6* + 18:3 *n-3* + 20:4 *n-6*) of the burgers was 5.78±0.42% and was not affected by the time of frozen storage or formulation ($P > 0.05$). Linoleic acid (18:2 *n-6*) accounts more than 95% of this PUFA content.

When SFA and MUFA contents of the hamburgers formulated with vegetable and marine oils are compared to those quoted by USDA (2009) for a commercial product (pan-broiled cooked patty, 90% lean meat, 10% fat, NDB No: 23564) with similar beef fat content (49.8% SFA, 41.8% MUFA and 4% PUFA) it can be seen that SFA were reduced by at least 60% and the MUFA content nearly doubled (Table 4).

4. Conclusions

Low-fat beef burgers enriched with unsaturated fatty acids and phytosterols were produced using only natural antioxidant. Whey proteins protected the burgers against lipid oxidation, maintaining TBARS levels of all formulations below 0.6 mg MDA/kg after 6 months of frozen storage. Formulations containing egg albumin were lighter in colour than the whey protein products, and were more susceptible to lipid oxidation. In products containing egg white proteins tocopherols demonstrated a measurable antioxidant effect.

The fatty acids profile of these low-fat meat burgers had a high monounsaturated FA content with oleic acid comprising more than 97% of the MUFA. Total phytosterol content of 100 g of cooked burgers represented 6% of the daily recommended intake, necessary to decrease cholesterol and the risk of heart disease; thislevel was not affected by any of the factors analysed. Thus low-fat beef burgers formulated with pre-

emulsified vegetable and fish oils (10%), tocopherols, and phytosterols could be considered potentially valuable functional meat products.

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Table 1: Formulation (%) of the different products*

COMPONENTS	W1	W2	W3	W4	E1	E2	E3	E4
Beef	74.65	74.50	74.55	74.60	74.65	74.50	74.55	74.60
Distilled water	10	10	10	10	10	10	10	10
High oleic sunflower oil	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9
Fish oil	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Whey protein concentrate (W)	3	3	3	3	-	-	-	-
Dry egg white (E)	-	-	-	-	3	3	3	3
Tocopherols (TO)	-	0.1	0.1	-	-	0.1	0.1	-
Oregano-rosemary antioxidants (OR)	-	0.05	-	0.05	-	0.05	-	0.05

* All the samples also contained 1% NaCl, 0.2% TPP, 0.15% mixed phytosterols, and 1% corn starch.

Table 2: Changes in lightness (L*) and redness (a*) of low-fat cooked beef burgers formulated with whey protein concentrate (W) or dry egg white (E), as a function of the antioxidant added and frozen storage time. Δ HSD L*: 4.3.

	Month 0	Month 2	Month 4	Month 6
Lightness (L*)				
W1	53.2^{aD}	56.3^{aAB}	52.8^{aD}	52.6^{aD}
W2	59.6^{aABC}	56.7^{abAB}	52.7^{bD}	60.3^{aAB}
W3	57.2^{abCD}	56.7^{bAB}	61.3^{aA}	60.3^{abAB}
W4	56.6^{abCD}	53.5^{bB}	59.9^{aAB}	59.6^{aB}
E1	61.7^{abAB}	58.3^{bA}	53.2^{cD}	62.9^{aAB}
E2	63.8^{aA}	58.8^{bA}	56.3^{bBCD}	64.0^{aA}
E3	56.1^{aCD}	58.0^{aA}	58.1^{aAC}	56.4^{aCD}
E4	58.0^{aB}	59.2^{aA}	58.1^{aAC}	59.0^{aBC}
Redness (a*)				
W1	7.6^{cABC}	10.1^{bA}	15.4^{aA}	13.4^{aA}
W2	5.7^{cCD}	9.6^{bAB}	12.5^{aB}	7.2^{cB}
W3	9.2^{aA}	7.3^{aC}	7.8^{aC}	7.5^{aB}
W4	8.9^{aAB}	7.0^{aBC}	7.7^{aC}	7.6^{aB}
E1	5.1^{cD}	8.2^{bA}	12.7^{aB}	6.9^{bcB}
E2	6.2^{cCD}	9.5^{bA}	14.2^{aAB}	8.5^{bcB}
E3	6.6^{aBCD}	7.6^{aBC}	7.6^{aC}	7.9^{aB}
E4	7.1^{aABD}	8.3^{aAC}	7.3^{aC}	8.4^{aB}

Δ HSD a*: 2.3.

$^{a-b}$ Means with the same superscript within same row do not differ significantly (P>0.05)
$^{A-B}$ Means with the same superscript within same column do not differ significantly (P>0.05)

Table 3: Lipid oxidation of low-fat cooked beef burgers expressed as TBARS (mg MDA/kg of cooked product) for samples formulated with whey protein concentrate (W) or dry egg white (E), as a function of the antioxidant added and frozen storage time. Δ HSD: 0.323.

	Month 0	**Month 2**	**Month 4**	**Month 6**
W1	0.31^{aA}	0.24^{aB}	0.34^{aB}	0.48^{aABC}
W2	0.35^{aA}	0.37^{aB}	0.52^{aB}	0.43^{aBC}
W3	0.24^{aA}	0.33^{aB}	0.42^{aB}	0.50^{aABC}
W4	0.46^{aA}	0.51^{aAB}	0.43^{aB}	0.47^{aBC}
E1	0.40^{cA}	0.46^{bcB}	1.12^{aA}	0.77^{bA}
E2	0.16^{aA}	0.27^{aB}	0.29^{aB}	0.26^{aC}
E3	0.21^{aA}	0.38^{aB}	0.46^{aB}	0.40^{aBC}
E4	0.22^{cA}	0.82^{abA}	0.98^{aA}	0.65^{bAB}

$^{a-b}$ Means with the same superscript within same row do not differ significantly (P>0.05)
$^{A-B}$ Means with the same superscript within same column do not differ significantly (P>0.05)

Table 4: Effect of frozen storage time on fatty acid profile (% of total fatty acids) of the cooked formulations (n=2).

	W1		W2		W3		W4		E1		E2		E3		E4		SEM
Storage (months)	**0**	**6**	**0**	**6**	**0**	**6**	**0**	**6**	**0**	**6**	**0**	**6**	**0**	**6**	**0**	**6**	
Miristic 14:0	0.61^{cd}	0.58^{cd}	0.98^{ab}	0.55^{cd}	0.55^{cd}	0.59^{cd}	0.58^{cd}	0.56^{cd}	0.59^{cd}	0.63^{bc}	0.38^{d}	0.30^{d}	0.77^{abc}	1.12^{a}	0.6^{bcd}	1.09^{a}	0.05
Palmitic 16:0	7.16^{bcd}	6.89^{cd}	8.13^{b}	7.21^{bcd}	7.32^{bcd}	6.63^{cd}	7.84^{bc}	5.88^{d}	7.25^{bcd}	8.11^{b}	6.78^{cd}	8.9^{ab}	8.53^{ab}	9.45^{a}	6.37^{cd}	9.23^{a}	0.20
Palmitoleic 16:1 *n-7*	0.76^{d}	0.70^{d}	0.74^{d}	0.70^{d}	0.81^{cd}	0.86^{cd}	0.60^{d}	0.66^{d}	1.29^{bcd}	1.51^{bcd}	0.55^{d}	0.83^{cd}	1.57^{abc}	1.77^{ab}	1.25^{bcd}	1.96^{a}	0.09
Stearic 18:0	4.89^{ab}	4.52^{ab}	5.45^{a}	5.21^{ab}	4.39^{ab}	3.73^{b}	5.24^{ab}	2.91^{b}	4.30^{ab}	3.76^{b}	4.25^{ab}	3.24^{b}	3.64^{b}	3.80^{b}	3.55^{b}	3.71^{b}	0.29
Oleic 18:1 *n-9* c	79.76^{bc}	82.09^{cd}	77.43^{d}	81.28^{ab}	80.9^{ab}	81.28^{ab}	78.95^{bcd}	84.19^{a}	81.54^{cd}	78.76^{abc}	82.81^{ab}	80.81^{b}	78.52^{bcd}	78.13^{cd}	83.27^{a}	78.19^{cd}	0.34
Total 18:1 *trans*	N.D.	N.D.	0.64^{a}	N.D.	0.01^{a}	0.28^{a}	0.07^{a}	0.1^{a}	N.D.	N.D.	0.14^{a}	N.D.	0.24^{a}	0.59^{a}	0.48^{a}	N.D.	0.15
Linoleic 18:2 *n-6*	5.61^{a}	5.37^{a}	5.52^{a}	5.10^{a}	5.95^{a}	5.47^{a}	6.69^{a}	6.21^{a}	5.31^{a}	5.72^{a}	4.72^{a}	4.8^{a}	5.9^{a}	5.45^{a}	4.42^{a}	6.02^{a}	0.42
Linolenic 18:3 *n-3*	0.33^{b}	0.25^{b}	1.17^{a}	0.21^{b}	0.15^{b}	N.D.	0.09^{b}	N.D.	0.19^{b}	N.D.	N.D.	0.26^{b}	N.D.	N.D.	N.D.	N.D.	0.06
Arachidonic 20:4 *n-6*	N.D.	N.D.	N.D.	N.D.	N.D.	0.63^{a}	N.D.	0.21^{a}	N.D.	N.D.	0.46^{a}	N.D.	0.2^{a}	N.D.	N.D.	N.D.	0.15
SFA	12.66^{abcd}	11.99^{abcde}	14.56^{a}	12.97^{abc}	12.26^{abcd}	10.95^{cde}	13.66^{ab}	9.35^{e}	12.14^{abcd}	12.5^{abcd}	11.41^{bcde}	12.44^{abcd}	12.94^{abcd}	14.37^{a}	10.52^{de}	14.03^{ab}	0.42
MUFA	80.52^{def}	82.79^{bc}	78.81^{g}	81.98^{bcd}	81.72^{cde}	82.42^{bc}	79.62^{fg}	84.95^{a}	82.83^{bc}	80.27^{efg}	83.5^{ab}	81.64^{cde}	80.33^{efg}	80.49^{def}	85.00^{a}	80.15^{efg}	0.24
Unsat	86.46^{bcd}	88.41^{abcd}	85.5^{d}	87.29^{bcd}	87.82^{bcd}	88.52^{abcd}	86.40^{bcd}	91.37^{a}	88.33^{abcd}	85.99^{d}	88.68^{abc}	86.70^{bcd}	86.43^{bcd}	85.94^{d}	89.42^{ab}	86.17^{cd}	0.48
PUFA	5.94^{a}	5.62^{a}	6.69^{a}	5.31^{a}	6.1^{a}	6.1^{a}	6.78^{a}	6.42^{a}	5.5^{a}	5.72^{a}	5.18^{a}	5.06^{a}	6.1^{a}	5.45^{a}	4.42^{a}	6.02^{a}	0.42
Unsat/ SFA	6.83^{cdef}	7.37^{bcde}	5.87^{f}	6.73^{def}	7.16^{cdef}	8.08^{bc}	6.33^{ef}	9.77^{a}	7.28^{bcde}	6.88^{cdef}	7.77^{bcd}	6.97^{cdef}	6.68^{def}	5.98^{f}	8.50^{ab}	6.14^{ef}	0.20

Different superscripts within the same file indicate that average values differ significantly ($P < 0.05$).

N.D.: not detected

SFA: saturated fatty acids (14:0 + 16:0 + 18:0); MUFA: monounsaturated fatty acids (16:1 n-7 + 18:1 *n-9 c* + 18:1 *n-9 t*), PUFA: polyunsaturated fatty acids (18:2 *n-6* + 18:3 *n-3* + 20:4 *n-6*)