Research Article

Meat-based functional foods for dietary equilibrium omega-6/omega-3

Guillermo Reglero[1], Paloma Frial[2], Alejandro Cifuentes[3], Mónica R. García-Risco[1], Laura Jaime[1], Francisco R. Marin[1], Vicente Palanca[2], Alejandro Ruiz-Rodríguez[1], Susana Santoyo[1], Francisco J. Señoráns[1], Cristina Soler-Rivas[1], Carlos Torres[1] and Elena Ibañez[3]

[1] Sección Departamental Ciencias de la Alimentación, Universidad Autónoma de Madrid, Ciudad Universitaria de Cantoblanco, Madrid, Spain
[2] Grupo Frial, Tres Cantos, Madrid, Spain
[3] Instituto de Fermentaciones Industriales, CSIC, Madrid, Spain

Nutritionists encourage improving the diet by combining meat products with fish or other sea-related foods, in order to equilibrate the omega-6/omega-3 ratio. Strong scientific evidence supports the beneficial health effects of a balanced omega-6/omega-3 PUFA (poly unsaturated fatty acids) diets. In the present work, the scientific bases of new functional meat products with both a balanced omega-6/omega-3 ratio and a synergic combination of antioxidants are discussed. The aim is to contribute to the dietary equilibrium omega-6/omega-3 and to increase the antioxidant intake. Conventional meat products supplemented with a specific fatty acids and antioxidants combination led to functional foods with healthier nutritional parameters.

Keywords: Antioxidants / Functional meat products / Omega-6/omega-3 ratio

Received: September 18, 2007; revised: January 17, 2008; accepted: January 17, 2008

1 Introduction

The scientific evidence on the relationship between food and health has promoted the rapid development of a new food market in the last years: the market of functional foods. Consumer's increasing interest for maintaining or improving their health by eating specific food products supplemented with bioactive ingredients has led to the development of many new functional foods. Most of these new formulations are dairy products, vegetable based products, specific fats, *etc*., but so far only few of them are based on meat products.

Nutritionists encouraged improving the nutritional value of meat products intakes by combining them, for instance, with fish or other sea-related foods (a rich source of omega-3 fatty acids). Strong scientific evidence supports the beneficial health effects of a balanced omega-6/omega-3 PUFA (poly unsaturated fatty acids) diets [1].

PUFA omega-3 and omega-6 are functionally and metabolically different and have important opposing physiological functions [2, 3]. While both are precursors of metabolic products such as prostaglandins, thromboxanes, leukotrienes, hydroxyl fatty acids, and lipoxins, all of them are formed in larger quantities from omega-6 fatty acids being biologically active in very small quantities. Moreover, the nature and the balance between the different prostaglandins formed depends on the PUFA precursor. Evidence has demonstrated that the contribution of such compounds to thrombus and atheromas formation, allergic and inflammatory disorders and cells proliferation is higher in omega-6 PUFA [4]. Moreover, excessive amounts of omega-6 PUFA and a very high omega-6/omega-3 ratio disturbs cellular redox balance (shifting to more oxidizing conditions) altering the expression of key regulatory proteins and thus promoting the pathogenesis of many diseases, including cardiovascular disease, cancer, inflammatory, and autoimmune diseases [5 – 8]. A balance between the omega-6 and omega-3 fatty acids is a better physiological state in terms of gene expression, eicosanoid metabolism and cytokine production [9].

Conventional meat products have an omega-6/omega-3 ratio higher than 15 while the healthy effects have been

Correspondence: Professor Guillermo Reglero, Sección Departamental Ciencias de la Alimentación, Universidad Autónoma de Madrid, Ciudad Universitaria de Cantoblanco, E-28049 Madrid, Spain
E-mail: guillermo.reglero@uam.es
Fax: +34-91-4978255

Abbreviations: AA, antioxidant activity; **TBA,** 2-thiobarbituric acid; **TEAC,** trolox equivalent antioxidant capacity; **TBARS,** thiobarbituric acid reactive substances

associated to ratios lower than four [4]. Scientific evidence, based on controlled intervention trials in humans, suggested dietary omega-6/omega-3 fatty acids recommendations depending on specific physiological situations (adults with different pathologies, infant, newborns, *etc.*) [4]. Therefore, the best strategy to improve the omega-6/omega-3 ratio in meat products is the addition of an appropriate amount of omega-3 PUFA, being the most convenient the long chain EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) [4, 10, 11] fatty acids.

The higher omega-3 PUFA intake might promote increasing of oxidative stress in human body [12]. To minimize this effect and to protect the added PUFA from autooxidation, the PUFAs should be combined with antioxidants [13].

The most interesting food grade antioxidants are those obtained from natural sources, in particular from plants such as *Rosmarinus officinalis*. Rosemary extracts obtained with supercritical fluid technologies showed high antioxidant properties [14] even at low concentrations [15 – 17].

The use of supercritical fluids technologies to extract key compounds such as antioxidants have many advantages compared to traditional extraction processes, such as the operation at mild and nonoxidative conditions that allows obtaining bioactive extracts without toxic residues [18]. Other advantages, compared to traditional extraction techniques, are the use of solvents generally recognized as safe (GRAS) and the higher efficiency of the extraction process (in terms of increasing yields and lower extraction times).

The supercritical rosemary extracts are heat-resistant, easily dissolved in different type of foods [19] and do not change the sensory properties (color, taste, or flavour) of the food matrix in which they are added as supplement. Rosemary antioxidant extracts mainly contain phenolic diterpenes such as carnosic acid [20, 21] which act as primary antioxidants showing a high synergistic effect with other antioxidants such as α-tocopherol [22]. Moreover, it has been demonstrated that these products have an activity similar to superoxide dismutase (SOD) and positive interactions with glutation reductase and NADPH-quinone reductase enzymes, regenerating them and increasing their effects as radical scavengers. The protective effects of phytochemicals against cancer agents in lungs, kidney, and stomach have been linked to their association with the mentioned enzymes [23].

The goal of the present work was to design new functional meat products based on a healthy combination of omega-3 PUFA and supercritical rosemary extracts with antioxidant activity (AA). The effect of processing (including different technological steps), storage, and culinary treatment was studied on the designed products. Different parameters were used to control the quality and to evaluate the biological activity of the functional meat products. They were based on exhaustive chemical and functional characterization using chromatographic techniques and *in vitro* assays. Designed products were chemical- and nutritionally compared to other commercial products considered "healthy" food such as salmon and ACE fruit beverages (vitamin A, C, and E supplemented juices).

2 Materials and methods

2.1 Samples, processing, and storage conditions

Sheets (100 g) of vacuum-packed cold-smoked salmon and fresh salmon slices (100 g) utilized in this study were both from Norway and produced by aquaculture. They were purchased at local retailers. Six fruit drinks enriched with antioxidants vitamins (A + C + D or E) were also purchased at local supermarkets.

Pork and Turkey meats for Frankfurt-sausages, cooked ham, and cooked Turkey breast were obtained from Grupo Frial (Tres Cantos, Spain). The functional meat products were manufactured at Grupo Frial facilities following their quality control standards mixing the meats with the patented formula® P200402755.2004. The amount of supercritical rosemary extract, deodorized salmon oil and vitamin E used was the necessary to achieve a level of, respectively, 0.02% w/w, 0.6% w/w, and 0.001% w/w. The functional ingredients were incorporated into the meat products through a brine. The meat pieces were prepared by first eliminating as much fat as possible. Then, the brine containing the bioactive ingredients was incorporated at a rate of 16.6% in a drum massage specially designed for this purpose that contained the meat products. Contact times were selected according to the norm for the different meats (ham and pork tenderloin 18 h, and Turkey breast 4 h). Then, the meat was introduced into the mould and was cooked in ovens to get special cooking as smooth as possible while minimizing the temperature difference between the meat and the ambient; the final temperature of the products was 66°C and cooking lasted 10 h. After, the products were cooled for 12 h below 5°C and vacuum-packed in plastic bags Cryovac® with high barrier effect. Vacuum preserved samples were then stored at 4°C inside industrial climatic controlled chambers.

Traditional meat products were prepared as mentioned above, without addition of the functional ingredients, and immediately submitted to vacuum shielding and refrigeration.

Afterwards, samples were supplied to UAM laboratories for further analysis. All samples were transported under refrigerated conditions and immediately analyzed. Samples were collected and analyzed at 0, 60, and 90 days.

2.2 Functional ingredients

Commercial supercritical (Flavex Naturextrakte, Germany) and conventional rosemary extracts were obtained at Grupo Frial's usual provider and were compared to extracts

obtained at UAM supercritical facilities (pilot plant scale) using the conditions previously described [20]. Food grade commercial vitamin E (BTSA, Madrid) and food grade commercial deodorized salmon oil (Grupo Frial's supplier) were also purchased and used together with rosemary extracts as functional ingredients.

2.3 Reagents

2-Thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane (TEP), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and potassium persulfate were purchased from Sigma/Aldrich (St. Louis, USA). TCA (trichloroacetic acid) and analytical grade organic solvents were obtained from Panreac Quimica SA (Barcelona, Spain) and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) from Fluka Chemie AG (Buchs, Switzerland).

2.4 Samples preparation for analysis

A wide internal slice of cooked ham and cooked Turkey breast (from both functional and traditional) was cut from the whole piece. Five portions of 60 g were obtained from the internal slice. Each portion was minced using a grinder and individually analyzed as sample (quintuplicate).

Two portions of 60 g pork Frankfurt-sausage and salmon products were minced using a grinder and individually analyzed (duplicate).

2.5 Culinary treatment

To reproduce a culinary homemade process, fresh salmon slices (100 g) and functional pork sausages (75 g) were grilled in a frying pan as follows. Firstly, the frying pan was brought up to 180°C. Salmon slices and Frankfurt-sausages were grilled during 4 min, by changing the side in contact with the frying pan each minute. Immediately after, grilled salmon slices and pork Frankfurt-sausages were ground in a grinder (Ecron microchop Mec 1126, Madrid, Spain) and submitted to the chemical and functional analysis below described.

Similarly, smoked and fresh salmon slices and functional pork sausages (not heat treated) were also ground and used as control samples.

2.6 Lipid oxidation measurement: Thiobarbituric acid reactive substances (TBARS) value

Lipid oxidation in meat products was measured as TBARS using the TBA method of Pfalzgraf *et al*. (1995) [24]. This method is based on the malondialdehyde (MDA) reaction with TBA to obtain a pink pigment, which results form the condensation of two molecules of TBA with one molecule of MDA [25]. The substances that react with TBA are called TBA-reactive substances. TBARS value expressed as mg MDA/kg meat product was calculated in duplicate from a standard curve of TEP.

2.7 AA by trolox equivalent antioxidant capacity (TEAC) assay

To carry out the TEAC assay, meat or salmon products (20 ± 0.10 g) were homogenized with 20 mL of pure acetone using an Ultra Turrax® T25 from IKA Werke, Jane & Kunkel (Stanfen, Germany) for 45 s at 15 000 rpm. The homogenate was centrifuged using a Beckman GS-6R (Germany) centrifuge (20 min at 3500 rpm) and the supernatant filtrated through a Whatman No. 1 paper filter. A second extraction was performed and supernatants were pooled together and stored in dark at –18°C. Acetone was removed using a vacuum rotary evaporator (Heidolph Instrument Laborota 4000, Germany) at 35°C. Dry extracts were dissolved on acetone before analysis. TEAC values assessment of fruit juices was performed taking the liquid directly as sample.

The TEAC assay was performed essentially as described by Re *et al*. [26] with minor modifications. $ABTS^{\bullet+}$ radical cation was generated by incubating during 16 h at room temperature in the dark 7 mM ABTS in the presence of 2.45 mM potassium persulfate (final concentration). The $ABTS^{\bullet+}$ radical solution was diluted with ethanol for meat and salmon products or phosphate buffer saline (PBS), pH 7.4, for enriched fruit drinks to an absorbance of 0.70 (± 0.02) at 734 nm. The reaction was initiated by the addition of 10 μL sample extracts to 0.990 mL of diluted $ABTS^{\bullet+}$. The reactive mixture was allowed to stand at room temperature for 10 min (until the reaction reached a steady state) and the absorbance was immediately recorded at 734 nm. Trolox was used as reference standard and results were expressed as TEAC values. These values were obtained from at least four different concentrations of each sample giving a linear response.

2.8 AA by β-carotene bleaching assay

Meat samples (20 g) were homogenized with 20 mL of ethanol using an Ultra turrax for 1 min (15 000 rpm). The blended sample was centrifuged at 3000 rpm and filtrated. The filtrate was evaporated to dryness using a rotary vacuum evaporator and the residue dissolved in ethanol to a concentration of 30 mg/mL.

The extracts were tested for their abilities to inhibit the autooxidation of linoleic acid and β-carotene as previously described [27]. For each sample, 0.2 mL extract were added to 0.2 mL β-carotene (1 mg/mL in chloroform), 20 mg linoleic acid, and 200 mg Tween-20. This mixture was firstly homogenized, then concentrated in a vacuum rotary evaporator, dried under a nitrogen stream and diluted with distilled water (50 mL). The mixture was vigorously shaken to

form a liposome solution. The samples were then submitted to thermal autooxidation at 50°C for 3 h. Afterwards, their absorbance was measured at 470 nm. Samples were measured in duplicate. The AA was expressed as inhibition percentage relative to the control (sample with no meat extract) using the following equation:

$$AA\ (\%) = [(R_{control} - R_{sample})/R_{control}] \times 100$$

where $R_{control} = \ln[Abs(t_0)/Abs(t_{180})]/180$

2.9 Lipid extraction

Meat samples (2 g) plus 5 mL H_2O Milli-Q were extracted twice with 25 mL hexane using an Ultra Turrax at 12 000 rpm during 1 min. After extraction, samples were centrifuged at 3800 rpm for 5 min. The organic phases were transferred to another vial and centrifuged again with Na_2SO_4 anhydrous (under the former conditions) to remove any trace of water. The supernatant was collected and concentrated on a rotary vacuum evaporator until dryness.

2.10 Analysis of fatty acids profile in meat samples by GC

To prepare methyl esters of free and esterified fatty acids, samples were mixed with chloroform/ethanol 2:1 v/v and methylated by addition of 1 mL of 0.1 M methanolic NaOH. This mixture was allowed to stand for 30 min at 60°C. Then, 200 μL water was added. The resulting mixture was extracted with two 1 mL portions of *n*-hexane. The final extract was then dried with sodium sulfate.

The method of GC utilized has been published elsewhere [28]. Derivatized sample (1 μL) was injected into a Perkin-Elmer autosystem XL (Wellesley, MA, USA) gas chromatograph with a 30 m BTR-Carbowax column (0.25 mm id). Injector and detector temperatures were set at 220 and 230°C, respectively. The temperature program was as follows: starting at 100°C and then heating to 180°C at 20°C/min; followed by heating from 180 to 220°C at 15°C/min. The final temperature (220°C) was held for 30 min. Identification of the ethyl esters of the various fatty acids was based on a menhaden oil fish standard (#4-7085) obtained from Supelco (Bellefonte, PA).

2.11 Statistical analysis

Means comparison has been performed using GraphPad Prism v. 4 for Windows (www.graphpad.com) run in a PC.

3 Results and discussion

A proper design of a functional food requires developing a precise strategy to guarantee the product efficiency, while reducing the risks of side effects. Therefore, the main parameter considered in the present study was the benefit/risk ratio, raising the benefits to a maximum and decreasing the risks to a minimum, at the same time.

To increase the benefits, the following statements have to be considered:

(i) Search for extensive physiological benefits. For instance, antioxidants [29] and PUFAs [1] ingested through diet can influence gene expression.

(ii) To guarantee bioavailability. *In vitro* tests can be used to select the functional ingredients previous to animal assays and clinical trials.

(iii) Test integrity maintenance. Analytical techniques and biological assays can be used to ensure the chemical and biological integrity of the functional ingredients after processing operations, during preservation time and cooking.

To reduce risks, the layout is the following:

(i) To use commonly consumed food products as natural ingredients. It is important to use ingredients occurring naturally in foods [30], obtained using mild transformation techniques [31] or preparations with well defined food activities, extracted from natural sources [32].

(ii) To add the minimum effective dosage of functional ingredients. Some products, commonly used in the design of functional foods, such as tocopherols, can have negative effects at high dose [33].

(iii) To perform an exhaustive characterization of the product. It is important to ensure that negative chemical changes have been avoided or detect the presence of residues or contaminants [34]. Considering that most of the functional ingredients are extracts, the possibility of concentrating toxic compounds along with the main product should be eliminated.

(iv) To carry out toxicity studies at higher dosage than those used in the formulation to guarantee the absence of negative side effects [35].

3.1 Design of functional meat foods

Functionalizing meat products has a great interest, considering their occurrence in the diet [36, 37]. Meat and meat products have a great nutritional value with high quality proteins, around 40% of essential amino acids, vitamins, and minerals. Their high fat content [38] is the only deleterious aspect raising strong controversy since it has been frequently related with a higher incidence of chronic diseases [39, 40].

Following the general strategy previously mentioned, several steps were taken into consideration in the present work before designing a functional meat product. Basically, the first attempt was to modify the fat profile, replacing the fat excess by fish oil (rich in EPA and DHA) at the minimum dosage. The precise dose was enough to fulfill the requirements imposed by the meat fat composition, adding

Table 1. Nutritional information for the conventional and functional meat products used in the present study

Values per 100 g	Pork Frankfurt-sausage	Functional pork Frankfurt-sausage	Cooked ham	Functional cooked ham	Cooked Turkey breast	Functional cooked Turkey breast
Energy (kcal)	373	274	110	110	106	107
Proteins (g)	13	16.5	19.0	19.0	19.0	19.0
Carbohydrates (g)	0.3	1.2	0.5	0.5	1.0	1.0
Fat (g)	31.7	22.5	2.8	2.8	1.3	2.0
Saturated (g)	13.2	9.1	1.0	1.0	0.5	0.7
Monounsaturated (g)	15.1	11.1	1.6	1.4	0.6	0.8
Polyunsaturated (g)	3.4[a)]	2.3[b)]	0.2[a)]	0.4[b)]	0.2[a)]	0.5[b)]
Na (g)	0.8	0.8	0.8	0.8	0.5	0.5
Ratio *n*-6/*n*-3	>20	2.8	20	1.0	>20	0.5

a) *n*-6.
b) *n*-6 + *n*-3.

fish oil until the omega-6/omega-3 ratio was lower than 4. As mentioned above, it is mandatory to combine the omega-3 PUFAs with antioxidants. In the present study, supercritical rosemary extract was added to the designed functional meats as a technological and physiological antioxidant.

Supercritical rosemary extract was selected considering its high AA, as compared to other rosemary extracts obtained using traditional extraction methods (that involved extraction with organic solvents). As previously reported [20, 21], supercritical extracts showed a high carnosic acid content, ranging from 20 to 80% w/w depending on the supercritical conditions employed. The extracts tested in this study showed antioxidant activities (AA50%, measured using the β-carotene bleaching test) between 1.8 and 16.4 μg/mL for supercritical rosemary extracts (depending on the extraction conditions) and around 180 μg/mL for traditional rosemary extracts. Considering that the AA expressed as AA 50% accounts for the extract concentration needed to reduce 50% the oxidation, the smallest the amount, the highest the antioxidant capacity of the extract. Thus, results indicated a 100-fold higher AA when using supercritical extracts compared to conventional extracts typically added to traditional foods.

Vitamin E was also included in the formulation of the functional meat products because of its synergistic antioxidant effect with the phenolic diterpenes of rosemary extract [22].

Concluding, the result was a combination of omega-3 fatty acids and synergic antioxidants (patented formula) [41].

3.2 Functionality evaluation

Several parameters were evaluated to verify the nutritional and functional value of the developed meat products. First of all, the nutritional values and omega-6/omega-3 ratios of the conventional and functional meat products were compared (Table 1). As expected, results indicated that the highest nutritional difference between traditional and functional products was their fat content and PUFAs composition. Thus, one of the objectives of the new formulation was fulfilled since, for all the functional products studied, the omega-6/omega-3 ratio was lower than 3 and one order of magnitude lower than the conventional meat products.

Functional pork Frankfurt-sausages showed around 30% lower fat content (9.2 g less) than the traditional ones with a different fat profile. In this particular example, to obtain a functional sausage less pork fat, containing basically omega-6, was utilized (following the nutritional recommendations of International Organizations), and substituted by fish oil (containing basically EPA and DHA) in a 3.5% weight. This fat reduction led also to a lower energy intake (*per* 100 g of sausages) while the rest of parameters did not significantly change.

Cooked ham showed a slightly different behavior since part of its fat was mechanically removed before the fish oil addition (0.6% w/w) in order to avoid an excessive increase in the total fat concentration. Also in this case, the polyunsaturated omega-6 levels, typical from a traditional cooked ham, were changed to omega-6 plus omega-3 through the new formulation. When cooked Turkey breasts were compared, the functional version of the product showed a slightly higher fat content due to the fish oil addition (0.6% weight), since it was not possible to remove the fat from the conventional product before processing with the new formula.

Moreover, the lipidic profiles (in terms of saturated, monounsaturated, omega-6 and omega-3 content) of the functional meat products were closer to salmon products (in different presentations: fresh, smoked and grilled) than to traditional meat products, especially in terms of omega-6 and omega-3 fatty acids (Fig. 1). The omega-6/omega-3 ratio in analyzed salmon products ranged from 0.5 to 0.6, in functional meat products from 0.5 to 2.8, while traditional meat products showed ratios higher than 20. The closest profile to the marine products corresponded to cooked Turkey breast which indeed showed the lower fat content.

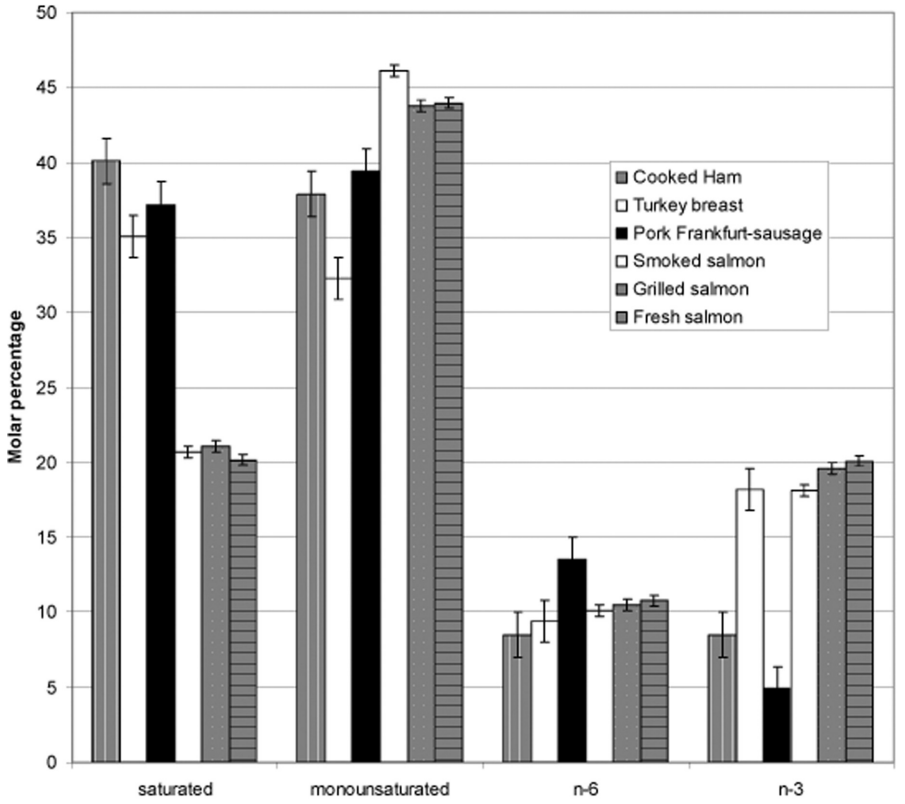

Figure 1. Lipidic profile of different functional meat products compared to salmon products (means and error bars shown).

Thus, its functional formula was the most effective treatment using the minimal fish oil dosage of all the functional products. Worth to mention is the fact that in all the studied products, the omega-6/omega-3 ratio was lower than 3, demonstrating the possibility of modifying the fatty acids profile in a meat product by correcting or adjusting a specific formulation to produce functional foods.

As previously mentioned, besides salmon oil, the developed functional meat products contained a combination of supercritical rosemary extract and vitamin E as antioxidants. Their AA was evaluated using several methods: the β-carotene bleaching test, TEAC assay, and TBARS value test.

The β-carotene bleaching test was used as reference of unsaturated fatty acids peroxidation [27] (Table 2). The antioxidant capacity (AA%) of the functional meat products was statistically significantly higher than the traditional products being the highest the functional pork Frankfurt-sausage. In this samples, the supercritical rosemary extract + vitamin E were added in higher concentrations (0.08% + 0.004%) to overcome the problems associated to the higher fish oil percentage added (necessary to fulfill the requirements: n-6/n-3 < 4). Similar AA was found in cooked ham and Turkey breast indicating that the activity was mainly due to the amount of antioxidants added to the formula.

The TEAC assay has been described as the most suitable to assess the AA of biological samples, food components, and food extracts [42]. The AA in the functional meat products was compared with some functional beverages specifically designed to provide a potent antioxidant effect (Fig. 2). The designed functional meat products provided an AA statistically lower than the analyzed juices but close to, at least, one of them. Nevertheless, a 50% less AA than those commercial functional beverages enriched with antioxidants vitamins (A + C + D or D) was still a very high antioxidant capacity.

To compare the efficiency of the added antioxidants in the formulation in terms of preserving the fat from autooxidation, the TBARS value was investigated and used as oxidation index (Table 3). As expected, a higher oxidation

Table 2. Mean and SD of AA (%) of the meat products considered in the present study using the β-carotene bleaching method

Sample	Mean ± SD
Cooked ham	34.71a ± 2.98
Functional cooked ham	52.80b ± 3.13
Cooked Turkey breast	34.26a ± 2.42
Functional cooked Turkey breast	50.75b ± 3.56
Pork Frankfurt-sausage	43.61a ± 2.61
Functional pork Frankfurt-sausage	68.75b ± 1.87

a – b) Means within a same type of samples, with a different letter are significantly different ($p < 0.05$) (from unpaired t-test, using GraphPad Prism program, www.graphpad.com).

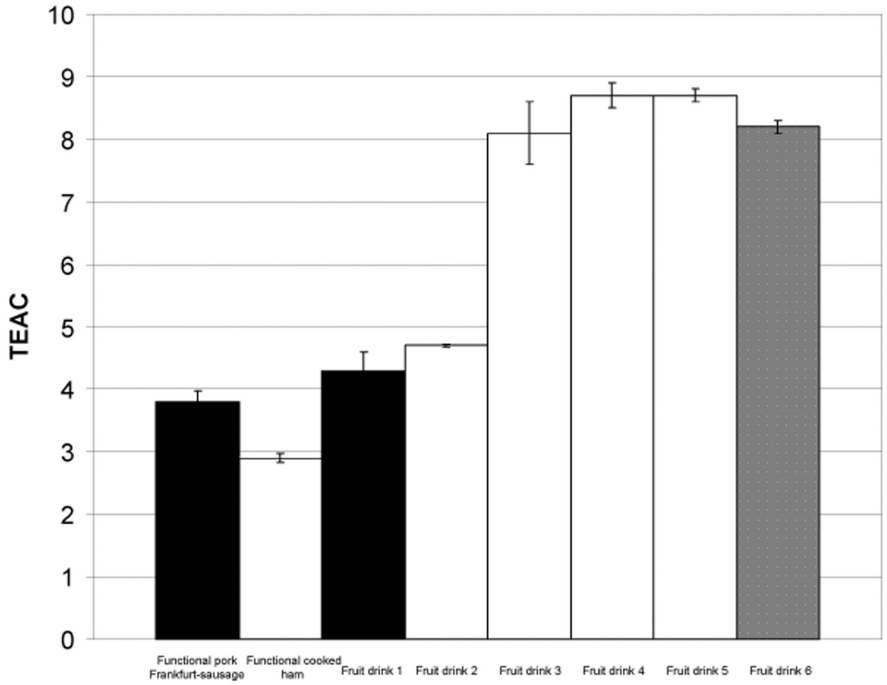

Figure 2. Antioxidant capacity (expressed as TEAC, trolox equivalent antioxidant capacity, in μmol/g product) of the developed functional meat products (with PUFA + natural antioxidants) and functional fruit drinks vitamins enriched (means and error bars shown).

Table 3. TBARS values of meat and salmon products (mg MDA/kg product)

Samples		Mean ± SD
Pork Frankfurt-sausage	Control	0.206a ± 0.004
	Functional	0.209a ± 0.019
	Functional grilled	0.395b ± 0.010
Cooked ham	Control	0.100a ± 0.007
	Functional	0.102a ± 0.011
Cooked Turkey breast	Control	0.087a ± 0.002
	Functional	0.101a ± 0.012
Salmon	Control (fresh)	0.965a ± 0.013
	Grilled	1.296b ± 0.037
	Smoked	1.071a ± 0.089

a – b) Means within a same type of samples, with a different letter are significantly different ($p < 0.05$) (from Newman-Keuls test for multiple comparison, and unpaired *t*-test, using GraphPad Prism program, www.graphpad.com).

index was observed in salmon samples, containing a higher omega-3 fatty acids concentration, that in all the functional meat products. When traditional (control) and functional meat products were compared, no significant differences were observed.

3.3 Effect of processing, storage, and culinary treatments

Once the combination of functional ingredients was added to the meat products, control analysis were performed to guarantee the integrity of the formulation during technological processing operations, preservation under refrigeration and cooking. Obtained data (not shown) indicated that no significant differences were found in all the analyzed parameters (lipidic profile and AA evaluated using the described methods). After 90 days and culinary treatment, the increase of oxidation index and the reduction of AA were lower than 10% indicating that the products, just before consumption, maintained their high AA.

When comparing different samples before and after the culinary treatment, oxidation largely increased during grilling of fish samples (Table 3). No significant differences were observed between fresh and smoked salmon. Concerning the meat products, even after cooking (grill) operations, the oxidation index of the pork Frankfurt-sausage increased less than salmon. Functional pork sausages after the treatment showed a statistically significant increase in the oxidation index but these effect could be due to the water evaporation (weight lost) occurring during the heat treatment. Nevertheless, oxidation levels were acceptable and under the normal oxidation occurring in normal food confirming the efficiency of the ingredient combination used in the present work.

4 Concluding remarks

In conclusion, the designed functional meat products, with a omega-6/omega-3 ratio lower than 4 and a combination of natural antioxidants, showed a lipidic profile closer to fresh salmon than other meat products and an AA similar to fruit "ACE" functional beverages. These properties were maintained during 90 days in refrigeration and were not degraded by culinary treatments.

This work was supported by Grupo Frial and Centro para el Desarrollo Tecnológico e Industrial (CDTI) from the Spanish Ministry of Industry. Authors want to thank Dr. P. J. Martin-Alvarez for his advice in the statistical treatment of the data.

The authors have declared no conflict of interest.

5 References

[1] Jump, D. B., Fatty acid regulation of gene transcription, *Crit. Rev. Clin. Lab. Sci.* 2004, *41*, 41 – 78.

[2] Dyerberg, J., Bang, H. O., Haemostatic function and platelet polyunsaturated fatty acids in Eskimos, *Lancet* 1979, *2*, 33 – 435.

[3] Hirai, A., Hamazaki, T., Terano, T., Nishikawa, T., *et al*., Eicosapentaenoic acid and platelet function in Japanese, *Lancet* 1982, *2*, 1132 [Letter].

[4] Simopoulos, A. P., The importance of the ratio omega-6/omega-3 essential fatty acids, *Biomed. Phamacother.* 2002, *56*, 365 – 379.

[5] Simopoulos, A. P., Omega 6/Omega-3 essential fatty acid ratio and chronic diseases, *Food Rev. Int.* 2004, *20*, 77 – 90.

[6] Kromann, N., Green, A., Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950 – 1974, *Acta Med. Scand.* 1980, *208*, 401 – 406.

[7] Burr, M. L., Fehily, A. M., Gilbert, J. F., Rogers, S., *et al*., Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART), *Lancet* 1989, *2*, 757 – 761.

[8] Von Schacky, C., Prophylaxis of atherosclerosis with marine omega-3 fatty acids: a comprehensive strategy, *Ann. Intern. Med.* 1988, *107*, 890 – 899.

[9] Simopoulos, A. P., The role of fatty acids in gene expression: Health implications, *Ann. Nutr. Metab.* 1996, *40*, 303 – 311.

[10] Brown, A. J., Roberts, D. C. K., Fish and fish oil intake: effect on haematological variables related to cardiovascular disease, *Thromb. Res.* 1991, *64*, 169 – 178.

[11] GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial, *Lancet* 1999, *354*, 447 – 455.

[12] Pinchuk, I., Lichtenberg, D., The mechanism of action of antioxidants against lipoprotein peroxidation, evaluation based on kinetic experiments, *Prog. Lipid Res.* 2002, *41*, 279 – 285.

[13] Medina, I., González, M. J., Pazos, M., Medaglia, D. D., *et al*., Activity of plant extracts for preserving functional food containing n-3-PUFA, *Eur. Food Res. Technol*. 2003, *217*, 301 – 307.

[14] Cavero, S., Jaime, L., Martín-Álvarez, P. J., Señoráns, F. J., *et al*., *In vitro* antioxidant analysis of supercritical fluid extracts from rosemary (*Rosmarinus officinalis* l.), *Eur. Food Res. Technol.* 2005, *221*, 478 – 486.

[15] Nguyen, U., Evans, D. D., Frakman, G., in: Rizvi, S. S. H. (Ed.), *Supercritical Fluid Processing of Foods and Biomaterials*, Chapman & Hall, London 1994, pp. 103.

[16] Mendes, R. L., Coelho, J. P., Fernandes, H. L., Marrucho, I. J., *et al*., Applications of supercritical CO2 Extraction to microalgae and plants, *J. Chem. Technol. Biotechnol.* 1995, *62*, 53 – 59.

[17] Quirin, K. W., SFE of natural antioxidants from rosemary and sage, *Innov. Food Technol.* 2003, *18*, 186 – 191.

[18] Lopez-Sebastián, S., Ramos, E., Ibáñez, E., Bueno, J. M., *et al*., Dearomatization of antioxidant rosemary extracts by treatment with supercritical carbon dioxide, *J. Agric. Food Chem.* 1998, *46*, 13 – 19.

[19] Gerard, D., Quirin, K. W., Schwarz, E., CO2 extracts from rosemary and sage, *Food Mark. Technol.* 1995, *9*, 46 – 55.

[20] Señorans, F. J., Ibañez, E., Cavero, S., Tabera, J., Reglero, G., LC/MS analysis of supercritical fluid extracts of rosemary plants, *J. Chromatogr. A* 2000, *870*, 491 – 499.

[21] Ramírez, P., Ibáñez, E., Señoráns, F. J., Reglero, G., Separation of rosemary antioxidant compounds by SFC with coated packed capillary columns, *J. Chromatogr. A* 2004, *1057*, 241 – 245.

[22] Hopia, A. I., Shu, W. H., Schwartz, K., German, J. B., Frankel, E. N., Effect of different lipid systems on antioxidant activity of rosemary constituents carnosol and carnosic acid with and without alpha-tocopherol, *J. Agric. Food Chem.* 1996, *44*, 2030 – 2036.

[23] Offord, E. A., Macé, K., Avanti, O., Pfeifer, A. M. A., Mechanisms involved in the chemoprotective effects of rosemary extract studied in human liver and bronchial cells, *Cancer Lett.* 1997, *114*, 275 – 281.

[24] Pfalzgraf, A., Frigg, M., Steinhart, H., α-Tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage, *J. Agric. Food Chem.* 1995, *43*, 1339 – 1342.

[25] Sinnhuber, R. O., Yu, I. C., Yu, T. C., Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity, *Food Res.* 1958, *23*, 624 – 634.

[26] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., *et al*., Antioxidant activity applying and improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 1999, *26*, 1231 – 1237.

[27] Velioglu, Y. S., Mazza, G., Gao, L., Oomah, B. D., Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, *J. Agric. Food Chem.* 1998, *46*, 4113 – 4117.

[28] Mendiola, J. A., Torres, C. F., Toré, A., Martín-Álvarez, P. J., *et al*., Use of supercritical CO2 to obtain extracts with antimicrobial activity from *Chaetoceros muelleri* microalga. A correlation with their lipidic content, *Eur. Food Res. Technol*. 2007, *224*, 505 – 510.

[29] Demmig-Adams, B., Adams, W. W., Antioxidants in photosynthesis and human, *Nutr. Sci.* 2002, *298*, 2149 – 2153.

[30] Ibáñez, E., Kubátová, A., Señoráns, F. J., Cavero, S., *et al*., Subcritical water extraction of antioxidant compounds from rosemary plants, *J. Agric. Food Chem.* 2003, *51*, 375 – 382.

[31] Torres, C. F., Lin, B., Moeljadi, M., Hill, C. G., Lipase-catalyzed synthesis of designer acylglycerols rich in residues of eicosapentaenoic, docosahexaenoic, conjugated linoleic, and/or stearic acids, *Eur. J. Lipid Sci. Technol.* 2003, *105*, 614 – 623.

[32] Jaime, L., Mendiola, J. A., Herrero, M., Soler-Rivas, C., *et al*., Separation and characterization of antioxidants from *Spirulina platensis* microalga combining pressurized liquid extraction, TLC, and HPLC-DAD, *J. Sep. Sci.* 2005, *28*, 2111 – 2119.

[33] Meagher, E. A., Barry, O. P., Lawson, J. A., Rokach, J., Fitz-Gerald, G. A., Effects of vitamin E on lipid peroxidation in healthy persons, *JAMA* 2001, *285*, 1178 – 1182.

[34] Mendiola, J. A., Marín, F. R., Hernández, S. F., Arredondo, B. O., *et al*., Characterization via liquid chromatography coupled to diode array detector and tandem mass spectrometry of supercritical fluid antioxidant extracts of *Spirulina platensis* microalga, *J. Sep. Sci.* 2005, *28*, 1031 – 1038.

[35] Anadon, A., Martinez-Larranaga, R. M., Factors increasing the toxicity of anticoccidial ionophores in poultry, *Rev. Med. Vet.* 1990, *141*, 17 – 24.

[36] Fernandez, J. M., Fernandez, J., Sayas, E., Perez, J. A., Meat products as functional foods: A review, *J. Food Sci.* 2005, *70*, R37 – R43.

[37] Sanchez-Muniz, F. J., Jimenez-Colmenero, F., Olmedila, B. (Eds.), *Derivados Cárnicos Funcionales: Estrategias y Perspectivas*, Fundación Española de la Nutrición, Madrid 2005.

[38] Ahrens, E. H., Blankenhorn, D. H., Tastas, T. T., Effect on human serum lipids of substituting plant for animal fat in the diet, *Proc. Soc. Exp. Biol. Med.* 1954, *86*, 872 – 878.

[39] Higgs, J. D., The changing nature of red meat: 20 years of improving nutritional quality, *Trends Food Sci. Technol.* 2000, *11*, 85 – 95.

[40] Key, T. J., Allen, N. E., Spencer, E. A., Travis, R. C., The effect of diet on risk of cancer, *Lancet* 2002, *14*, 861 – 868.

[41] Reglero, G., Frial, P., Señoráns, F. J., Ibáñez, E., *et al*., Inventors; Oficina Española de Patentes y Marcas., assignee. Mezcla oleosa de ingredientes bioactivos naturales para la preparación de un producto alimenticio enriquecido. Spanish Patent P200402755. 2004 November 16.

[42] Van den Berg, R., Haenen, G. R. M. M., Van den Berg, H., Bast, A., Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures, *Food Chem.* 1999, *66*, 511 – 517.

