

**ENHANCEMENT OF THE NUTRITIONAL STATUS AND QUALITY OF
FRESH PORK SAUSAGES FOLLOWING THE ADDITION OF LINSEED OIL,
FISH OIL AND NATURAL ANTIOXIDANTS**

I. Valencia^a, M.N. O'Grady^b, D. Ansorena^a, I. Astiasarán^a and J.P. Kerry^{b*}

^aDepartment of Nutrition, Food Science, Physiology and Toxicology, Faculty of
Pharmacy, University of Navarra, Irunlarrea s/n, 31008-Pamplona, Spain.

^bDepartment of Food and Nutritional Sciences, University College Cork, National
University of Ireland, Western Road, Cork, Ireland.

*Corresponding author: Tel.: +353-21-4903798; Fax: +353-21-4270001.

E-mail address: joe.kerry@ucc.ie

ABSTRACT

Fresh pork sausages were manufactured where 15% of the pork back fat was substituted with linseed oil (LO) or fish oil (FO). Green tea catechins (GTC) and green coffee antioxidant (GCA) were added to both LO (LGTC 200 and LGCA 200) and FO (FGTC 200 and FGCA 200) substituted sausages at a level of 200 mg/kg. Raw and cooked pork sausages were either over-wrapped with oxygen permeable film (aerobic storage) or stored in modified atmosphere packages (MAP) containing 80% O₂ : 20% CO₂ or 70% N₂ : 30% CO₂, respectively for 7 days at 4°C. Effects on fatty acid profiles, lipid oxidation, colour and sensorial properties were investigated. α -Linolenic acid increased from 1.34% (control) to 8.91% (LO) and up to 11.2% (LGTC 200 and LGCA 200). Addition of fish oil increased levels of EPA from 0.05% (control) to 2.83% (FO), 3.02% (FGTC 200) and 2.87% (FGCA 200) and DHA levels increased from 0.04% (control) to a maximum of 1.93% (FGTC 200). Lipid oxidation was low in linseed oil containing sausages. GTC (200 mg/kg) significantly ($P < 0.05$) reduced lipid oxidation in fish oil containing sausages after 7 days of storage. Colour parameters were unaffected by the packaging atmosphere. L* lightness values were lower ($P < 0.05$) in LGTC 200 and a* redness values lower ($P < 0.05$) in LGTC 200 and FGTC 200 after 7 days of storage. Sensory scores were unaffected by linseed oil addition. Flavour and overall acceptability scores in fish oil containing sausages were improved by GTC addition. Results obtained demonstrate potential for the production of nutritionally enhanced fresh pork sausages.

Keywords: Linseed oil, Fish oil, green tea catechins, green coffee antioxidant, lipid oxidation.

1. INTRODUCTION

Meat and meat products are considered to be vital components of a healthy diet. Negative concerns regarding meat consumption and its impact on human health have prompted research into development of novel functional meat products (Arihara, 2006). The terms functional food and nutraceutical are used interchangeably and usually defined as any substance that may be considered a food or part of a food which provides medical or health benefits including the prevention and treatment of disease (DeFelice, 1992). Dietary recommendations for humans, favouring the consumption of less saturated fat, have led to an increased interest in meats containing more unsaturated fatty acids. Scientific evidence suggests that certain dietary fats, for example n-3 polyunsaturated fatty acids (PUFA's), may prevent and modulate diseases such as coronary heart disease, cancer, hypertension and arthritis (Connor, 2000).

Manipulation of the fatty acid profiles of meat and meat products may be achieved by, feeding animals diets rich in polyunsaturated fatty acids (PUFA's), for example fish oil, linseed oil and soya oil (Monahan, Buckley, Morrissey, Lynch and Gray, 1992; Raes, De Smet & Demeyer, 2004) or alternatively, by processing specific lipid ingredients into meat products (Fernández-Ginés, Fernández-López, Sayas-Barberá & Pérez-Alvarez, 2005). Oils such as linseed oil, fish oil olive oil, and soya oil alter product fatty acid profiles producing healthier meat products, for example, dry fermented sausages (Ansorena, & Astiasarán, 2004; Muguerza, Ansorena & Astiasarán, 2003 , 2004; Muguerza, Gimeno, Ansorena, Bloukas & Astiasarán, 2001; Severini, De Pilli & Baiano, 2003; Valencia, Ansorena, & Astiasarán, 2006).

Lipid oxidation is a major quality deteriorative process in muscle foods resulting in a variety of breakdown products which produce off-odours and flavours. Susceptibility to lipid oxidation increases with increased levels of PUFA's. Bryhni,

Kjos, Ofstad and Hunt (2002) reported that supplementation of porcine diets with polyunsaturated fat and fish oil resulted in increased lipid oxidation in whole muscle and sausages. Therefore while oils rich in n-3 polyunsaturated fatty acids may contribute to the production of healthier meat, negative effects on meat palatability may be observed. Lipid oxidation in muscle foods may be controlled using synthetic or natural antioxidants. Concerns regarding the safety and toxicity of synthetic antioxidants have prompted research into natural antioxidants derived from plant sources. In recent times, the functional properties of plant extracts have been investigated due to their potent antioxidant and nutraceutical activity.

Tea catechins are a major group of polyphenolic flavonoids found in green tea. The principle catechins found in green tea (*Camellia sinensis*) are (-) epicatechin (-EC), (-)-epigallocatechin (-EGC), (-)-epicatechin gallate (-ECG), and (-)-epigallocatechin gallate (-EGCG). The antioxidant activity of tea catechins has been demonstrated in a variety of test systems (Huang & Frankel, 1997) and in beef, pork and poultry meats (McCarthy, Kerry, Kerry, Lynch & Buckley, 2001; Mitsumoto, O'Grady, Kerry & Buckley, 2005; Nissen, Byrne, Bertelsen & Skibsted 2004; Tang, Kerry, Sheehan, Buckley & Morrissey, 2001). Reported human health benefits of tea catechins include anti-carcinogenic anti-inflammatory and cardioprotective activity (Higdon & Frei, 2003; Sato & Miyata, 2000). Green coffee antioxidant (GCA[®]) is a commercially available natural chlorogenic acid (> 50%) extract derived from raw coffee beans. Green coffee beans are a rich dietary source of chlorogenic acid, an ester of caffeic acid with quinic acid (Clifford, 1999). In addition to antioxidant activity (Charurin, Ames & del Castillo, 2002; Daglia, Racchi, Papetti, Lanni, Govoni & Gazzani, 2004), chlorogenic acid may modulate the onset of diabetes in humans (McCarty, 2005) thereby demonstrating human health promoting properties. Also epidemiological and

experimental studies have shown positive effects of regular coffee consumption on various aspects of human health such as psychoactive responses, neurological and metabolic disorders, gonad and liver function (Dórea & da Costa, 2005). The antioxidant activity of coffee extract in cooked pork patties has been reported previously (Nissen et al., 2004).

Fresh pork sausages are popular meat products in Ireland and traditionally presented for retail sale either, in trays over-wrapped with oxygen permeable film or sealed into plastic pouches. Martínez, Djenane, Cilla, Beltrán and Roncalés (2006b) packaged fresh pork sausages in modified atmosphere packages (MAP) containing increased oxygen levels and reported a limited positive effect of oxygen level of sausage colour and increased levels of lipid oxidation. In a further study, plant extracts (rosemary, borage, green and pu-erh tea) effectively reduced lipid oxidation and extended the shelf-life of fresh pork sausages stored in MAP (Martínez, Cilla, Beltrán and Roncalés, 2006a). The influence of health promoting green tea catechins (GTC) and green coffee antioxidant (GCA) on the quality of nutritionally enhanced fresh pork sausages stored aerobically (over-wrapped) and in MAP merits investigation.

The objective of this study was to manipulate the fatty acid profile of fresh pork sausages with oils high in n-3 PUFA's namely linseed oil (LO) (rich in α -linolenic acid (C18:3(n-3))) and fish oil (FO) (rich in eicosapentaenoic acid (EPA) (C22:5(n-3)) and docosahexaenoic acid (DHA) (C22:6(n-3))). The effects of LO, FO, GTC and GCA on quality parameters such as lipid oxidation, colour and sensorial properties was investigated.

2. MATERIALS AND METHODS

2.1 Materials and reagents

All chemicals used were AnalaR grade and obtained Sigma Chemical Co. Ltd., Poole, Dorset UK and Merck KGaA, Darmstadt, Germany. Green tea catechins (GTC) (81.43%) were supplied by New Kinglong Natural Products Co. Ltd, Hunan, China and stated by the manufacturer to contain (-C) 4.82%, (-EC) 11.51%, (-ECG) 16.22%, (-EGC) 9.64%, (-EGCG) 37.62% and (-GCG) 1.62%. Green coffee antioxidant (GCA[®]) was obtained from Applied Food Sciences, LLC, Austin, Texas. GCA, a natural chlorogenic acid extract derived from raw coffee beans, contained >65% total polyphenols and was standardized to contain >50% chlorogenic acid.

Linseed oil (Oxyguard fresh cold pressed) was obtained from Biona, United Kingdom and fish oil (omega-3 18/12) from LYSI, Reykjavik, Iceland. Rusk (wheat, flour, salt, E503) and sausage seasoning (salt, wheatflour, stabilisers E450 and E451, spice and spice extract, preservative E221, dextrose, flavour enhancer E621, antioxidant E301, flavourings and colour E128) were supplied by National Food Ingredients (Limerick, Ireland). Collagen casing was obtained from Devro casings, Moodiesburn, Glasgow, Scotland. Lean pork meat (shoulder) and pork back fat were supplied by Dairygold Ltd., Mitchelstown, Co Cork, Ireland). Pork meat and back fat were minced through a 10 mm plate (Talsa Mincer, Talsabell S. A., Valencia, Spain), vacuum packaged and stored (approximately 1 week) at -20°C until required for sausage manufacture.

2.2 Sausage formulation and manufacture

A standard fresh pork sausage (~ 5 kg batch) mixture was formulated to contain 1.63 kg pork shoulder, 1.63 kg pork back fat, 1.00 kg water, 0.63 kg rusk and 0.13 kg

seasoning (Control). Batches were manufactured where 15% of the pork back fat was substituted with linseed oil (LO) and fish oil (FO) in an emulsion. Linseed oil (245 g) and fish oil (245 g) were emulsified with 245 g water (50°C) and 49 g soya protein by mixing vigorously for 2 min. The natural antioxidants, GTC and GCA were also incorporated into both the LO (LGTC 200 and LGCA 200) and FO (FGTC 200 and FGCA 200) substituted sausages at a level of 200 mg/kg. GTC and GCA were dissolved in the water (1.00 kg) component of the sausage mixture.

Fresh pork sausages were manufactured by mixing and chopping pork meat, back fat, water (0.5 kg) and seasoning for 35 sec in a bowl chopper (Seydleman bowl chopper, Burgstallstrabe, Germany). The remainder of the water (0.5 kg), rusk and the emulsion were added and further chopped for 20 sec. The sausage batter was subsequently stuffed into 21 mm diameter collagen casings and chilled at 4°C for 1 hour prior to further processing and packaging.

2.3 Sausage packaging

Sausages were cooked in a conventional oven by grilling for approx. 15 min at 200°C. Raw and cooked pork sausages were placed in low oxygen permeable ($< 1\text{ cm}^3/\text{m}^2/24\text{h}/\text{atm}$) polystyrene/ethylvinylalcohol (EVOH) / polyethylene (PE) trays and flushed with 80% O₂ : 20% CO₂ and 70% N₂ : 30% CO₂, respectively (modified atmosphere packs (MAP), using a vacuum sealing unit (VS 100, Gustav Müller & Co. KG, Bad Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH and Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable ($3\text{ cm}^3/\text{m}^2/24\text{h}/\text{atm}$) laminated barrier film with a polyolefin heat sealable layer. For aerobic storage, sausages were placed in PE trays which were over-wrapped

with oxygen permeable film. All samples were stored for up to 7 days under fluorescent lighting conditions (approximately 660 lx) at 4°C.

2.4 Moisture and fat content

The moisture and fat content of fresh pork sausages was determined using the SMART Trac rapid moisture/fat analyser (CEM Corporation, NC, USA). For comparative purposes, a range of commercially available pork sausages (n = 5), currently available on the Irish market, were also analysed for moisture and fat content. The instrument was operated according to the manufacturer's instruction and results were expressed as percentage moisture and fat.

2.5 Fatty acid analysis

Lipid was extracted from cooked pork sausage samples following the method of Folch, Lees and Stanley (1957). The fatty acid composition of linseed oil and fish oil was also determined. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters dissolved in hexane (AOAC, 2002). Fatty acids profiles were measured by gas chromatography (Perkin-Elmer Clarus 500 gas chromatograph (PE, Shelton, CT, USA) fitted with a capillary column SPTM-2560 (100 m x 0.25 mm x 0.2 µm) and a flame ionization detector). The temperatures of the injection port and detector were 250°C and 260°C, respectively. The oven temperature was programmed to 175°C for 10 min and increased to 200°C at a rate of 10°C/min, increased to 220°C at a rate of 4°C/min and held for 15min. The carrier gas was hydrogen, and the pressure was 20.5psi. The split ratio was 120:1. Pure standards were used for identification of the fatty acid method esters and quantitative determination of fatty acids was achieved

using heptadecanoic acid methyl ester as an internal standard. Results were expressed as a percentage of the total fatty acids.

2.6 Measurement of lipid oxidation

Lipid oxidation was determined in raw and cooked pork sausages following the method of Vyncke (1975) with modifications (Juncher, Vestergaard, Søltøft-Jensen, Weber, Bertelsen and Skibsted, 2000). Sample absorbances were measured spectrophotometrically (Cary 300 Bio, UV-Vis spectrophotometer, Varian Instruments, CA, USA) at 532 nm and 600 nm (turbidity). Results were expressed as mg malondialdehyde (MDA)/kg sausage sample using 1,1,3,3-tetraethoxypropane (TEP) as a standard. Lipid oxidation measurements were made on days 0, 3 and 7 in raw and cooked sausage samples.

2.7 Colour determination

Surface colour measurements in raw sausages were determined using a CR-300 Chroma Meter (Minolta Co., Osaka, Japan) which consisted of a measuring head (CR-300), with an 8 mm diameter measuring area, and a data processor (DP-301). The chroma meter was calibrated on the CIE colour space system using a white tile (Dc: L=97.79, a=-0.11, b=2.69). The 'L*' value represents lightness and 'a*' and 'b*' values represent redness and yellowness, respectively. Colour measurements were made on days 0, 3 and 7 in raw sausage samples.

2.8 Sensory evaluation

A trained sensory panel (n = 12) from the Department of Food and Nutritional Sciences, University College Cork evaluated cooked pork sausages stored aerobically

and in MAP on days 0 and 7. Samples were sliced, placed on paper plates and served to panelists. Panelists were asked to evaluate sample colour, texture, flavour and overall acceptability on a 5-point hedonic scale ranging from very poor (1) to very good (5). Results are expressed as mean sensory scores.

2.9 Statistical analysis

Four sausage samples were analysed from each treatment and all analyses were performed in duplicate. Data was analysed by one-way ANOVA and differences among treatment means were determined by Tukey's b posteriori test. A student-t test was used to determine differences between packaging systems (aerobic and MAP). The analysis was carried out using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA) software package.

3. RESULTS AND DISCUSSION

3.1 Moisture and fat content of fresh pork sausages

Similar fat and moisture contents for linseed oil and fish oil substituted sausages were observed (Table 1). The fat and moisture contents of five commercially available brands of fresh pork sausages ranged from $29.03 \pm 4.69\%$ – $38.92 \pm 1.50\%$ and 44.69 ± 0.16 – $48.68 \pm 0.40\%$, respectively. Therefore, the sausages manufactured in the present study had slightly lower fat and similar moisture contents compared to the range of commercially available Irish pork sausages analysed.

3.2 Fatty acid profiles of cooked pork sausages

The addition of linseed oil altered the fatty acid profiles of cooked pork sausages (Table 2). Significant decreases ($P < 0.05$) in the total levels of saturated (Σ SFA) and monounsaturated (Σ MUFA) fatty acids were observed in cooked pork sausages containing linseed oil (LO) and antioxidants (LGTC 200 and LGCA 200), relative to controls. The level of α -linolenic acid (n-3) increased from approximately 1.34% to 8.91% in control and LO sausages, respectively. Higher levels (~11.2%) of α -linolenic acid (n-3) were present in LGTC 200 and LGCA 200 sausages indicating that addition of natural antioxidants such as GTC and GCA protected α -linolenic acid from deterioration during cooking. Similar findings were reported by Ansorena and Astiasarán (2004) where synthetic antioxidants (BHA and BHT, 100 mg/kg) significantly protected linoleic and α -linolenic acids from deterioration in linseed oil substituted dry fermented sausages. From a nutritional perspective, LO, LGTC and LGCA pork sausages contained 2.32, 2.75 and 2.72% α -linolenic acid, respectively. Simopoulos, Leaf and Salem, (1999) reported a recommended adequate intake of 2.22 g

α -linolenic acid/day based on a 2000 kcal diet. Therefore in the present study, 100 g of fresh pork sausages would supply a sufficient amount of dietary α -linolenic acid.

A host of health agencies and organisations worldwide have issued recommendations on the intake of n-3 PUFA's (Garg, Wood, Singh and Moughan, 2006). A ratio of 5:1 was recommended for dietary n-6/n-3 fatty acids (Nordic working group on Diet and Nutrition, 1996). Also, it was previously reported that the ratio of n-6/n-3 in the diet should not exceed 4:1 in order to optimize the bioavailability, metabolism and incorporation of fatty acids into membrane phospholipids (Garg, Wierzbicki, Sebkova, Thomson, & Clandinin, (1988); Garg, Thomson & Clandinin, 1990; Volker & Garg, 1996). In the present study, the addition of linseed oil to fresh pork sausages decreased the n-6/n-3 ratio from 8.25:1 (control) to 1.64:1 (LO) and 1.3:1 (LGTC 200 and LGCA 200). Similar findings were reported by Pelsler, Linssen, Legger and Houben, (2007) where the n-6/n-3 ratio decreased from 11.20 to 1.05 in Dutch style fermented sausages substituted with 20% flaxseed oil.

The addition of fish oil to fresh pork sausages (Table 3) increased the long chain n-3 PUFA's EPA and DHA, characteristic of this oil type. Levels of EPA increased from approximately 0.05% in control sausages to 2.83%, 3.02% and 2.87% in FO, FGTC 200 and FGCA 200 sausages, respectively. Also DHA levels increased from 0.04% up to a maximum of 1.93% in FGTC 200 sausages. Addition of GTC and GCA did not result in higher levels of EPA and DHA in fish oil substituted sausages after cooking. By contrast, Bhale, Xu, Prinyawiwatkul, King and Godber (2007) reported that addition of oregano and rosemary extracts to menhaden oil protected EPA and DHA from oxidation during heating and storage. From a cardiovascular health perspective, a minimum combined intake of EPA and DHA of 500 mg/day is recommended (ISSFAL, 2004). In the current study FO, FGTC 200 and FGCA 200

contained combined levels of approximately 1.10%, 1.16% and 1.12% EPA+DHA, respectively. Therefore consumption of two sausages (~ 30 g each) daily containing added fish oil would provide an adequate recommended daily intake of EPA and DHA. In a previous study, Valencia et al. (2006) manufactured dry fermented sausages, where pork back fat was substituted with 25% fish oil, containing 1.10% EPA+DHA.

3.3 Lipid stability of raw and cooked pork sausages containing added linseed oil, fish oil, GTC and GCA

In general, lipid oxidation did not increase in raw pork sausages after 3 days of aerobic storage or in MAP (Table 4). After 7 days of storage, compared to days 0 and 3, levels of lipid oxidation significantly increased in raw pork sausages irrespective of treatment (LO, LGTC 200 or LGAC 200) or packaging atmosphere (aerobic or MAP). In cooked pork sausages, levels of lipid oxidation were higher than in raw sausages due to the lipid pro-oxidant nature of the cooking process (Table 4). Significantly lower levels of lipid oxidation were observed in cooked pork sausages after 3 and up to 7 days of storage in MAP, compared to aerobic storage, presumably due to the lack of oxygen within the MAP (70% N₂ : 30% CO₂).

Overall, levels of lipid oxidation in raw and cooked linseed oil containing sausages were low ranging from 0.25 – 0.57 mg MDA/kg muscle and 0.50 – 0.98 mg MDA/kg muscle, respectively. Gray and Pearson (1987) suggested a threshold value of 1 mg MDA/kg muscle for organoleptic detection of rancidity which is in excess of lipid oxidation values observed in the present study. Martínez et al. (2006b) reported increased levels of lipid oxidation and off-odour development in raw fresh pork sausages stored in 80% O₂ : 20% CO₂. In contrast with the present study, Martinez et al. (2006b) did not include sausage seasoning in their product formulation. The

commercial sausage seasoning used in the current experiments contains antioxidant compounds (see section 2.1) which may have contributed to the low levels of lipid oxidation detected.

In raw pork sausages containing fish oil (FO) significantly ($P < 0.05$) higher levels of lipid oxidation, compared to controls, were observed after 7 days of storage either aerobically or in MAP (Table 5). The inclusion of GTC (200 mg/kg) in fish oil containing sausages (FGTC 200) significantly reduced the extent of lipid oxidation after 7 days of storage. Stabilisation of fish oil against oxidation with GTC was previously reported by O'Sullivan, Mayr, Shaw, Murphy & Kerry (2005). Also, Jo, Son, Son and Byun (2003) reported that green tea extract reduced lipid oxidation in raw and cooked pork patties. GCA (200 mg/kg) did not exert antioxidant activity in raw fish oil containing sausages (FGCA 200) on any of the storage days. The antioxidant activity of chlorogenic acid, the main antioxidant component of GCA, has been previously reported in coffee model systems (Charurin et al., 2002) but to date, specific reports of GCA or chlorogenic acid activity in meat systems have not been cited.

In cooked pork sausages containing fish oil, lipid oxidation values were higher than in raw pork sausages on days 0 and 3 of storage. By contrast with raw pork sausages, lipid oxidation did not significantly increase between days 3 and 7 in cooked sausages (FO and FGCA) and a plateau effect on lipid oxidation values was observed (Table 5). The reason for this is unclear. As reported for cooked linseed oil containing sausages (Table 4), lipid oxidation was lower in fish oil containing sausages stored in MAP (70% N₂ : 30% CO₂) than in those stored under aerobic conditions.

3.4 Colour stability of raw pork sausages containing added linseed oil, fish oil, GTC and GCA

On days 0 and 3 of storage, significantly higher L* lightness values ($P < 0.05$), compared to controls, were observed due to the addition of an emulsion containing linseed oil (LO, LGTC 200, LGCA 200) (Table 6) or fish oil (FO, FGTC 200, FGCA 200) (Table 7). Similarly, Lee, Faustman, Djordjevic, Faraji and Decker, (2006) reported that addition of an emulsion containing algal oil and whey protein isolate to fresh pork sausages increased the L* values. This was attributed to the milky appearance of the emulsion. L* values for LGTC 200 sausages were significantly lower than LO and LGCA 200 sausages after 7 days of storage. This trend was not observed in fish oil containing sausages (Table 7). After 7 days of storage, a* redness values were significantly lower in LGTC 200 and FGTC 200 compared to LO, LGCA 200 and FO, FGCA 200, respectively. Polyphenolic flavonoids, present in GTC, react or bind with proteins (Arts et al., 2002; Kroll & Rawel, 2001) for example, oxymyoglobin which is responsible for the red colour of fresh meat. Individual catechin isomers exhibit varying degrees of pro-oxidant activity on oxymyoglobin resulting in oxidation to metmyoglobin (O'Grady, Maher, Buckley, Troy and Kerry, 2005). Metmyoglobin formation results in lower a* redness values. A decrease in a* values over time in fresh pork sausages stored in high oxygen packs was also reported by Martínez et al. (2006b). By contrast McCarthy et al. (2001) reported that GTC (2500 mg/kg) did not reduce a* values of raw pork patties, relative to controls, over a 9 day storage period. The b* yellowness values were higher than controls in fresh pork sausages containing linseed oil on days 0 and 3 of storage. Lower b* values were detected in LGTC 200 and FGTC 200 after 7 days of storage. In general, the packaging atmosphere (aerobic and MAP) did not affect the colour parameters tested in linseed oil and fish oil containing sausages.

3.5 Sensory evaluation of cooked pork sausages containing added linseed oil, fish oil, GTC or GCA

Sensory scores for parameters such as colour, texture, flavour and overall acceptability were not significantly affected by the addition of linseed oil, GTC or GCA (Table 8). In addition, after 7 days of storage, sensory scores were not significantly lower than day 0 values in cooked pork sausages stored aerobically or in MAP. Chlorogenic acids are responsible for coffee bitterness following roasting of green coffee beans (Campa, Doubeau, Dussert, Hamon and Noirot, 2005). GCA which contains chlorogenic acid did not affect the flavour of cooked pork sausages in the present study. In sausages containing fish oil, textural properties were not affected over the 7 day storage period. The addition of fish oil resulted in lower sensory scores for flavour and overall acceptability most probably due to higher levels of lipid oxidation. By contrast, Bryhni et al. (2002) reported that while dietary fish oil increased lipid oxidation in pork loin, sensory evaluation scores were not affected. The addition of GTC increased flavour and overall acceptability scores for fish oil containing sausages (FGTC 200).

4. CONCLUSION

Scientific evidence suggests that increased intake of n-3 PUFA's can have a positive influence of human health. Nutritionally enhanced fresh pork sausages were manufactured containing increased levels of n-3 PUFA's namely α -linolenic acid from linseed oil and eicosapentaenoic acid (EPA) and docosahexaenoic acid from fish oil. Susceptibility to lipid oxidation was controlled by the addition of natural antioxidants such as GTC and GCA, both of which are reported to exert health promoting properties. Lower levels of lipid oxidation were observed in linseed oil containing sausages

compared to fish oil containing sausages. GTC resulted in lower surface lightness and redness in linseed oil containing sausages and displayed potent antioxidant activity in fish oil containing sausages. Sensorial properties of cooked pork sausages were not affected by linseed oil addition. Fish oil addition affected the flavour and acceptability of fresh pork sausages and this effect was overcome by the addition of GTC. In conclusion, a dual approach to the manufacture of healthier functional meat products may be adopted i.e. via manipulation of the fatty acid profiles and addition of natural antioxidant compounds with health promoting properties.

ACKNOWLEDGEMENTS

We thank Fundación-Empresa of the University of Navarra, Gobierno de Navarra (Departamento de Educación) and Ministerio de Educación y Tecnología (Programa Ramón y Cajal, 2002) for their financial support of this research work. We are grateful for the provision of facilities and to staff at the Department of Food and Nutritional Sciences in University College Cork, Ireland where this research work was carried out.

REFERENCES

- AOAC. (2002). Preparation of methyl ester. 969.33. In *Official methods of analysis* (17th ed.) Gaithersburg, Maryland: Association of Official Analytical Chemist.
- Ansorena, D., & Astiasarán, I. (2004). The use of linseed oil improves nutritional quality of the lipid fraction of dry fermented sausages. *Food Chemistry*, 87, 69-74.
- Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science*, 74, 219-229.
- Arts, M. J. T. J., Haenen, G. R. M. M., Wilms, L. C., Beetstra, S. A. J. N., Heijnen, C. G. M., Voss, H. P., & Bast, A. (2002). Interactions between flavonoids and proteins: Effect on the total antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 50, 1184-1187.
- Bhale, S. D., Xu, Z., Prinyawiwatkul, W., King, J. M., & Godber, J. S. (2007). Oregano and rosemary extracts inhibit oxidation of long-chain n-3 fatty acids in menhaden oil. *Journal of Food Science* (in press).
- Bryhni, E.A., Kjos, N.P., Ofstad, R., & Hunt, M. (2002). Polyunsaturated fat and fish oil in diets for growing-finishing pigs: effects on fatty acid composition and meat, fat, and sausage quality. *Meat Science*, 62, 1-8.
- Campa, C., Doubeau, S., Dussert, S., Hamon, S., & Noiro, M. (2005). Qualitative relationship between caffeine and chlorogenic acid contents among wild *coffea* species. *Food Chemistry*, 93, 135-139.
- Charurin, P., Ames, J. M., & Del Castillo, M. D. (2002). Antioxidant activity of coffee model systems. *Journal of Agricultural and Food Chemistry*, 50, 3751-3756.

- Clifford, M.N. (1999). Chlorogenic acids and other cinnamates – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, 79, 362-372.
- Connor, W. E. (2000). Importance of n-3 fatty acids in health and disease. *American Journal of Clinical Nutrition*, 71, 171S-175S.
- Daglia, M, Racchi, M., Papetti, A., Lanni, C., Govoni, S., & Gazzani, G. (2004). In vitro and ex vivo antihydroxyl radical activity of green and roasted coffee. *Journal of Agricultural and Food Chemistry*, 52, 1700-1704.
- DeFelice, S.L. (1992). The nutraceutical initiative: a recommendation for U.S. economic and regulatory reforms. *Genetic Engineering News*, 12, 13-15.
- Dórea, J.G. & da Costa, T.H.M. (2005). Is coffee a functional food? *British Journal of Nutrition*, 93, 773-782.
- Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, & Pérez-Alvarez, J. A. (2005). Meat products as functional foods: a review. *Journal of Food Science*, 70 (2), 37-43.
- Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biology and Chemistry*, 226, 497-509.
- Garg, M.L., Thomson, A.B.R., & Clandinin, T. (1990). Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. *Journal of Lipid Research*, 31, 271-277.
- Garg, M.L., Wierzbicki, A., Sebokova, E., Thomson, A.B.R., & Clandinin, M.T. (1988). Differential effects of dietary linoleic and alpha-linolenic acids on lipid metabolism in rat tissues. *Lipids*, 23, 847-852.

- Garg, M. L., Wood, L. G., Singh, H., Moughan, P. J. (2006). Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. *Journal of Food Science*, 71, 66-71.
- Gray, J.I., & Pearson, A.M. (1987). Rancidity and warmed-over flavour. In A.M. Pearson, & T. R. Dutson, *Advances in meat research* (Vol. 3) (pp. 221-269). New York: Van Nostrand.
- Higdon, J.V. & Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition*, 43, 89-143.
- Huang, S.W. & Frankel, E.N. (1997). Antioxidant activity of tea catechins in different lipid systems. *Journal of Agricultural and Food Chemistry*, 45, 3033-3038.
- International society for the study of fatty acids and lipids (ISSFAL) (2004). Report of the sub-committee on recommendations for intake of polyunsaturated fatty acids in healthy adults, 1-22.
- Juncher, D., Vestergaard, C. S, Søltoft-Jensen, J., Weber, C. J., Bertelsen, G., & Skibsted, L. H. (2000). Effects of chemical hurdles on microbiological and oxidative stability of a cooked cured emulsion type meat product. *Meat Science*, 55(4), 483-491.
- Jo, C., Son, J.H., Son, C.B., & Byun, M.W. (2003). Functional properties of raw and cooked pork patties with added irradiated, freeze-dried green tea leaf extract powder during storage at 4°C. *Meat Science*, 64, 13-17.
- Kroll, J., & Rawel, H.M. (2001). Reaction of plant phenols with myoglobin: Influence of chemical structure of the phenolic compounds. *Journal of Food Science*, 66, 48-58.

- Lee, S., Faustman, C., Djordjevic, D., Faraji, H., & Decker, E. A. (2006). Effect of antioxidants on stabilization of meat products fortified with n-3 fatty acids. *Meat Science*, *72*, 18-24.
- Martínez, L., Cilla, I., Beltrán, J. A., & Roncalés, P. (2006a). Antioxidant effect of rosemary, borage, green tea, pu-erh tea and ascorbic acid on fresh pork sausages packaged in a modified atmosphere: influence of the presence of sodium chloride. *Journal Science Food and Agriculture*, *86*, 1298-1307.
- Martínez, L., Djenane, D., Cilla, I., Beltrán, J. A., & Roncalés, P. (2006b). Effect of varying oxygen concentrations on the shelf-life of fresh pork sausages packaged in modified atmosphere. *Food Chemistry*, *94*, 219-225
- McCarty, M.F. (2005). A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. *Medical Hypotheses*, *64*, 848-853.
- McCarthy, T. L., Kerry, J. P., Kerry, J. F., Lynch, P. B., & Buckley, D. J. (2001). Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Science*, *57*, 45-52.
- Mitsumoto, M., O'Grady, M. N., Kerry, J. P., & Buckley, J. (2005). Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. *Meat Science*, *69*, 773-779.
- Monahan, F.J., Buckley, D.J., Morrissey, P.A., Lynch, P.B., & Gray, J.I. (1992). Influence of dietary fat and α -tocopherol supplementation on lipid oxidation in pork. *Meat Science*, *31*, 229-241.

- Muguerza, E., Ansorena, D., & Astiasarán, I. (2004). Functional dry fermented sausages manufactured with high levels of n-3 fatty acids: nutritional benefits and evaluation of oxidation. *Journal of the Science of Food and Agriculture*, 84, 1061-1068.
- Muguerza, E., Ansorena, D., & Astiasarán, I. (2003). Improvement of nutritional properties of Chorizo de Pamplona by partial replacement of pork backfat with soy oil. *Meat Science*, 65, 1361-1367.
- Muguerza, E., Gimeno, O., Ansorena, D., Bloukas, J. G., & Astiasarán, I. (2001). Effect of replacing pork backfat with pre-emulsified olive oil on lipid fraction and sensory quality of Chorizo de Pamplona-a traditional Spanish fermented sausage. *Meat Science*, 59, 251-258.
- Nissen, L.R., Byrne, D.V., Bertelsen, G., & Skibsted, L.H. (2004). The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. *Meat Science*, 68, 485-495.
- Nordic working group on diet and nutrition (1996). Nordic nutrition recommendations. *Scandinavian Journal of Nutrition*, 40, 161-165.
- O'Grady M.N., Maher, M., Buckley, D.J., Troy, D. and Kerry, J.P. (2005). Studies of the effects of catechin isomers on oxymyoglobin oxidation and lipid oxidation in muscle model systems. In *Proceedings 51st International Congress of Meat Science and Technology*, (pp.660-665), 7-12 August Baltimore, Maryland, USA.
- O'Sullivan, A., Mayr, A., Shaw, N.B., Murphy, S.C., & Kerry, J.P. (2005). Use of natural antioxidants to stabilise fish oil systems. *Journal of Aquatic Food Product Technology*, 14, 75-94.
- Pelser, W. M., Linssen, J. P. H., Legger, A., & Houben, J. H. (2007). Lipid oxidation in n-3 fatty acid enriched Dutch style fermented sausages. *Meat Science*, 75, 1-11.

- Raes, K., De Smet, S., & Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. *Animal Feed Science and Technology*, *113*, 199-221.
- Sato, T. & Miyata, G. (2000). The nutraceutical benefit, part 1: green tea. *Nutrition*, *16*, 315-317.
- Simopoulos, A. P., Leaf, A., & Salem, N. (1999). Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Journal of the American College of Nutrition*, *18*, 487-489.
- Severini, C., De Pilli, T., & Baiano, A. (2003). Partial substitution of pork backfat with extra-virgin olive oil in 'salami' products: effects on chemical, physical and sensorial quality. *Meat Science*, *64*, 323-331
- Tang, S., Kerry, J. P., Sheehan, D., Buckley, D. J., & Morrissey, P.A. (2001). Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. *Food Research International*, *34*, 651-657.
- Valencia, I., Ansorena, D., & Astiasarán, I. (2006). Nutritional and sensory properties of dry fermented sausages enriched with n - 3 PUFAs. *Meat Science*, *72*, 727-733.
- Volker, D & Garg, M.L. (1996). Dietary n-3 fatty acid supplementation in rheumatoid arthritis – mechanisms, clinical outcomes, controversies and future directions. *Journal of Clinical Biochemistry and Nutrition*, *20*, 83-97.
- Vyncke, W. (1975). Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (*Scomber scombrus* L.). *Fette Seifen Anstrichmittel*, *77*, 239-240.

Table 1. Fat and moisture content of fresh pork sausages.

Treatment ¹			Treatment ²		
	Fat, %	Moisture, %		Fat, %	Moisture, %
Control	23.68 ± 1.16	51.67 ± 0.41	Control	21.23 ± 1.40	53.74 ± 0.84
LO	26.02 ± 0.45	49.92 ± 1.09	FO	23.64 ± 1.12	50.26 ± 1.12
LGTC 200	24.65 ± 0.95	51.21 ± 0.83	FGTC 200	23.46 ± 0.89	50.68 ± 0.89
LGCA 200	24.31 ± 0.69	51.72 ± 1.00	FGCA 200	23.76 ± 0.77	50.85 ± 0.76

¹Linseed oil, 15% substitution of pork back fat. ²Fish oil, 15% substitution of pork back fat. Mean ± standard deviation.

Table 2. Fatty acid composition (%) in cooked pork sausages containing linseed oil* green tea catechin (GTC) and green coffee antioxidant (GCA).

	Treatment ¹			
	Control	LO	LGTC 200	LGCA 200
Lauric C12:0	0.09 ± 0.00 ^c	0.08 ± 0.00 ^b	0.07 ± 0.00 ^a	0.08 ± 0.00 ^b
Myristic C14:0	1.34 ± 0.01 ^d	1.13 ± 0.00 ^c	1.05 ± 0.05 ^a	1.09 ± 0.01 ^b
Palmitic C16:0	23.74 ± 0.04 ^c	21.23 ± 0.15 ^b	20.27 ± 0.92 ^a	20.44 ± 0.14 ^a
Stearic C18:0	13.48 ± 0.06 ^c	12.32 ± 0.20 ^b	11.90 ± 0.59 ^a	11.89 ± 0.04 ^a
Arachidic C20:0	0.05 ± 0.00 ^a	0.06 ± 0.00 ^{ab}	0.07 ± 0.01 ^b	0.06 ± 0.00 ^{ab}
ΣSFA	38.71 ± 0.08^c	34.82 ± 0.36^b	33.38 ± 1.56^a	33.56 ± 0.19^a
Palmitoleic C16:1	2.04 ± 0.09 ^c	1.72 ± 0.03 ^b	1.62 ± 0.07 ^a	1.60 ± 0.00 ^a
Oleic C18:1 (n-9)	37.61 ± 0.05 ^b	34.88 ± 0.04 ^a	34.54 ± 1.91 ^a	34.21 ± 0.02 ^a
Vaccenic C18:1 (n-7)	2.78 ± 0.00 ^d	2.46 ± 0.00 ^c	2.35 ± 0.10 ^a	2.37 ± 0.00 ^b
Eicosenoic C20:1 (n-9)	0.83 ± 0.22 ^b	0.62 ± 0.00 ^a	0.59 ± 0.00 ^a	0.59 ± 0.00 ^a
Erucic C22:1	0	0	0	0
ΣMUFA	43.26 ± 0.16^c	39.69 ± 0.06^b	39.11 ± 0.08^{ab}	38.78 ± 0.02^a
Linoleic C18:2 (n-6)	14.97 ± 0.04 ^b	15.05 ± 0.12 ^b	14.70 ± 0.63 ^a	14.94 ± 0.11 ^b
α-Linolenic C18:3 (n-3)	1.34 ± 0.02 ^a	8.91 ± 0.21 ^b	11.16 ± 0.47 ^c	11.18 ± 0.12 ^c
γ-Linolenic C18:3 (n-6)	0.04 ± 0.00 ^b	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a	0.03 ± 0.00 ^a
Arachidonic C20:4 (n-6)	0.33 ± 0.01 ^b	0.27 ± 0.01 ^a	0.27 ± 0.01 ^a	0.27 ± 0.01 ^a
Eicosadienoic C20:2 (n-3)	0	0	0	0
Eicosapentaenoic C22:5(n-3)	0.04 ± 0.00 ^a	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b
Eicosatrienoic C20:3 (n-3)	0.16 ± 0.00 ^b	0.14 ± 0.00 ^a	0.14 ± 0.01 ^a	0.14 ± 0.00 ^a
Docosapentaenoic 22:5 (n-6)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.08 ± 0.00 ^b	0.00 ± 0.00 ^a
Docosapentaenoic 22:5 (n-3)	0.25 ± 0.00 ^c	0.22 ± 0.00 ^b	0.21 ± 0.01 ^a	0.21 ± 0.00 ^a
Docosahexaenoic C22:6 (n-3)	0.06 ± 0.00 ^c	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b	0.04 ± 0.00 ^a
Σn-3	1.86 ± 0.01 ^a	9.37 ± 0.21 ^b	11.60 ± 0.48 ^c	11.62 ± 0.13 ^c
Σn-6	15.34 ± 0.05 ^b	15.35 ± 0.13 ^b	15.07 ± 0.64 ^a	15.24 ± 0.12 ^{ab}
ΣPUFA	17.19 ± 0.06^a	24.72 ± 0.34^b	26.68 ± 1.13^c	26.87 ± 0.24^c
t-Palmitoleic C16:1t	0.37 ± 0.04 ^a	0.29 ± 0.00 ^a	0.46 ± 0.34 ^a	0.28 ± 0.00 ^a
t-Linoleic C18:2t	0	0	0	0
Elaidic C18:1t	0.45 ± 0.06 ^b	0.41 ± 0.05 ^b	0.30 ± 0.05 ^a	0.44 ± 0.06 ^b
Brassicidic C20:1t	0.01 ± 0.00 ^a	0.07 ± 0.00 ^b	0.07 ± 0.00 ^b	0.07 ± 0.00 ^b
ΣTRANS	0.84 ± 0.03^a	0.77 ± 0.05^a	0.83 ± 0.30^a	0.79 ± 0.06^a
PUFA/SFA	0.44 ± 0.00 ^a	0.71 ± 0.02 ^b	0.80 ± 0.00 ^c	0.80 ± 0.01 ^c
MUFA+PUFA/SFA	1.56 ± 0.00 ^a	1.85 ± 0.03 ^b	1.97 ± 0.04 ^c	1.96 ± 0.02 ^c
n-6/n-3	8.27 ± 0.04 ^c	1.64 ± 0.02 ^b	1.30 ± 0.00 ^a	1.31 ± 0.00 ^a

* Fatty acid composition (%) of linseed oil: lauric (0.02), myristic (0.02), palmitic (4.78), t-palmitoleic (0.02), palmitoleic (0.02), stearic (3.87), oleic (19.80), vaccenic (0.40), linoleic (15.01), γ-linolenic (0.20), α-linolenic (55.62) and docosahexaenoic n-3 (0.11).

¹Linseed oil, 15 % substitution of pork back fat (LO), LO + 200 mg GTC/kg sausage (LGTC 200) or 200 mg GCA/kg sausage (LGCA 200). ^{abc}Mean values (± standard deviation) in the same row bearing different superscripts are significantly different, P < 0.05.

Table 3. Fatty acid composition (%) in cooked pork sausages containing fish oil* green tea catechin (GTC) and green coffee antioxidant (GCA).

	Treatment ¹			
	Control	FO	FGTC 200	FGCA 200
Lauric C12:0	0.07 ± 0.00 ^a	0.09 ± 0.00 ^b	0.08 ± 0.00 ^b	0.09 ± 0.00 ^b
Myristic C14:0	1.28 ± 0.00 ^a	2.26 ± 0.01 ^b	2.32 ± 0.01 ^c	2.31 ± 0.02 ^c
Palmitic C16:0	24.02 ± 0.02 ^c	23.41 ± 0.02 ^a	23.59 ± 0.12 ^b	23.99 ± 0.06 ^c
Stearic C18:0	14.21 ± 0.08 ^d	12.20 ± 0.14 ^a	12.64 ± 0.22 ^b	13.09 ± 0.01 ^c
Arachidic C20:0	0.05 ± 0.00 ^a	0.10 ± 0.00 ^c	0.09 ± 0.00 ^c	0.07 ± 0.00 ^b
ΣSFA	39.64 ± 0.09^b	38.07 ± 0.11^a	38.73 ± 0.33^a	39.56 ± 0.07^b
Palmitoleic C16:1	1.91 ± 0.00 ^a	3.05 ± 0.03 ^b	3.12 ± 0.08 ^c	3.05 ± 0.02 ^b
Oleic C18:1 (n-9)	37.96 ± 0.09 ^c	33.76 ± 0.12 ^a	34.14 ± 0.13 ^b	33.91 ± 0.11 ^a
Vaccenic C18:1 (n-7)	2.75 ± 0.00 ^a	2.87 ± 0.01 ^b	2.92 ± 0.01 ^c	2.87 ± 0.01 ^b
Eicosenoic C20:1 (n-9)	0.71 ± 0.00 ^a	0.90 ± 0.00 ^b	0.91 ± 0.00 ^c	0.90 ± 0.00 ^b
Erucic C22:1	0.00 ± 0.00 ^a	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b	0.05 ± 0.00 ^b
ΣMUFA	43.33 ± 0.09^c	40.63 ± 0.17^a	41.16 ± 0.18^b	40.79 ± 0.13^a
Linoleic C18:2 (n-6)	14.44 ± 0.04 ^c	13.48 ± 0.08 ^b	11.86 ± 0.07 ^a	11.93 ± 0.03 ^a
α-Linolenic C18:3 (n-3)	1.17 ± 0.00 ^c	1.28 ± 0.00 ^d	1.15 ± 0.00 ^b	1.13 ± 0.00 ^a
γ-Linolenic C18:3 (n-6)	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.07 ± 0.00 ^b	0.07 ± 0.00 ^b
Arachidonic C20:4 (n-6)	0.33 ± 0.02 ^a	0.43 ± 0.03 ^b	0.41 ± 0.00 ^b	0.42 ± 0.04 ^b
Eicosadienoic C20:2 (n-3)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eicosapentaenoic C22:5 (n-3)	0.05 ± 0.00 ^a	2.83 ± 0.00 ^b	3.02 ± 0.08 ^c	2.87 ± 0.04 ^b
Eicosatrienoic C20:3 (n-3)	0.16 ± 0.00 ^b	0.11 ± 0.00 ^a	0.17 ± 0.01 ^b	0.16 ± 0.00 ^b
Docosapentaenoic 22:5 (n-6)	0	0	0	0
Docosapentaenoic 22:5 (n-3)	0.24 ± 0.00 ^a	0.44 ± 0.07 ^b	0.44 ± 0.09 ^b	0.50 ± 0.00 ^b
Docosahexaenoic C22:6(n-3)	0.04 ± 0.00 ^a	1.83 ± 0.07 ^b	1.93 ± 0.06 ^b	1.83 ± 0.06 ^b
Σn-3	1.66 ± 0.00 ^a	6.49 ± 0.06 ^b	6.71 ± 0.06 ^c	6.50 ± 0.12 ^b
Σn-6	14.79 ± 0.02 ^c	13.93 ± 0.07 ^b	12.44 ± 0.07 ^a	12.42 ± 0.02 ^a
ΣPUFA	16.45 ± 0.02^a	20.42 ± 0.07^d	19.15 ± 0.13^c	18.92 ± 0.18^b
t-Palmitoleic C16:1t	0.35 ± 0.01 ^b	0.36 ± 0.02 ^b	0.36 ± 0.00 ^b	0.33 ± 0.00 ^a
t-Linoleic C18:2t	0.06 ± 0.00 ^c	0.04 ± 0.00 ^b	0.04 ± 0.00 ^b	0.00 ± 0.00 ^a
Elaidic C18:1t	0.16 ± 0.02 ^a	0.46 ± 0.04 ^c	0.54 ± 0.01 ^d	0.41 ± 0.03 ^c
Brassicidic C20:1t	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ΣTRANS	0.58 ± 0.03^a	0.87 ± 0.03^c	0.96 ± 0.02^d	0.74 ± 0.03^b
PUFA/SFA	0.42 ± 0.00 ^a	0.54 ± 0.00 ^d	0.49 ± 0.01 ^c	0.48 ± 0.00 ^b
MUFA+PUFA/SFA	1.51 ± 0.01 ^a	1.60 ± 0.01 ^c	1.56 ± 0.02 ^b	1.51 ± 0.00 ^a
n-6/n-3	8.92 ± 0.02 ^d	2.15 ± 0.05 ^c	1.85 ± 0.01 ^a	1.91 ± 0.03 ^b

* Fatty acid composition (%) of fish oil: lauric (0.16), myristic (7.04), palmitic (17.33), t-palmitoleic (0.03), palmitoleic (7.96), stearic (3.50), oleic (8.69), vaccenic (3.11), linoleic (1.26), γ-linolenic (0.22), α-linolenic (1.16), arachidonic (1.14), eicosapentaenoic n-3 (16.92) and docosahexaenoic n-3 (13.44).

¹Fish oil, 15 % substitution of pork back fat (FO), FO + 200 mg GTC/kg sausage (FGTC 200) or 200 mg GCA/kg sausage (FGCA 200). ^{abcd}Mean values (± standard deviation) in the same row bearing different superscripts are significantly different, P < 0.05.

Table 4. Effect of linseed oil, green tea catechin (GTC) and green coffee antioxidant (GCA) on lipid oxidation (TBARS) in raw and cooked pork sausages stored aerobically and in modified atmosphere packs (MAP).

Treatment ¹	Packaging Atmosphere	Storage time at 4°C, d					
		Raw ²			Cooked ³		
		0	3	7	0	3	7
Control	Aerobic	0.36 ± 0.01 ^{aA}	0.32 ± 0.02 ^{abA}	0.49 ± 0.05 ^{aB}	0.52 ± 0.01 ^{aA}	0.77 ± 0.03 ^{aB}	0.76 ± 0.02 ^{aB}
	MAP	0.36 ± 0.01 ^{aA}	0.36 ± 0.02 ^{bA}	0.49 ± 0.04 ^{abB}	0.52 ± 0.01 ^{aA}	0.49 ± 0.03 ^{aA}	0.48 ± 0.04 ^{aA}
LO	Aerobic	0.36 ± 0.01 ^{aA}	0.32 ± 0.05 ^{abA}	0.57 ± 0.03 ^{bB}	0.60 ± 0.02 ^{cA}	0.96 ± 0.05 ^{bB}	0.98 ± 0.03 ^{bB}
	MAP	0.36 ± 0.01 ^{abB}	0.29 ± 0.03 ^{abA}	0.50 ± 0.01 ^{abC}	0.60 ± 0.02 ^{cA}	0.56 ± 0.02 ^{cA}	0.48 ± 0.01 ^{bA}
LGTC 200	Aerobic	0.33 ± 0.04 ^{aA}	0.28 ± 0.01 ^{aA}	0.49 ± 0.03 ^{aB}	0.54 ± 0.01 ^{abA}	0.71 ± 0.02 ^{aA}	0.76 ± 0.03 ^{aB}
	MAP	0.33 ± 0.04 ^{abB}	0.25 ± 0.04 ^{aA}	0.46 ± 0.02 ^{aC}	0.54 ± 0.01 ^{abB}	0.50 ± 0.02 ^{abA}	0.50 ± 0.02 ^{abA}
LGCA 200	Aerobic	0.39 ± 0.05 ^{aA}	0.37 ± 0.03 ^{bA}	0.48 ± 0.03 ^{aB}	0.59 ± 0.04 ^{bcA}	0.90 ± 0.03 ^{bB}	0.97 ± 0.03 ^{bB}
	MAP	0.39 ± 0.05 ^{abB}	0.28 ± 0.07 ^{abA}	0.53 ± 0.02 ^{bc}	0.59 ± 0.04 ^{bcB}	0.54 ± 0.02 ^{cAB}	0.50 ± 0.02 ^{abA}

¹Linseed oil, 15 % substitution of pork back fat (LO), LO + 200 mg GTC/kg sausage (LGTC 200) or 200 mg GCA/kg sausage (LGCA 200). ²Stored in 80% O₂ : 20% CO₂. ³Stored in 70% N₂ : 30% CO₂. ^{abc}Within each packaging system (aerobic or MAP) mean values in the same column bearing different superscripts are significantly different, P < 0.05. ^{ABC}Within each treatment (raw or cooked) mean values in the same row bearing different superscripts are significantly different, P < 0.05. A student t-test was used to determine differences between aerobic and MAP, ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001. TBARS, mg malondialdehyde/kg muscle, mean ± standard deviation.

Table 5. Effect of fish oil, green tea catechin (GTC) and green coffee antioxidant (GCA) on lipid oxidation (TBARS) in raw and cooked pork sausages stored aerobically and in modified atmosphere packs (MAP).

Treatment ¹	Packaging Atmosphere	Storage time at 4°C, d					
		Raw ²			Cooked ³		
		0	3	7	0	3	7
Control	Aerobic	0.54 ± 0.04 ^{aA}	0.73 ± 0.09 ^{aB}	1.16 ± 0.08 ^{aC}	0.69 ± 0.06 ^{aA}	0.88 ± 0.03 ^{aB}	0.96 ± 0.06 ^{aB}
	MAP	0.54 ± 0.04 ^{aA}	0.63 ± 0.04 ^{aA}	1.11 ± 0.16 ^{aB}	0.69 ± 0.06 ^{aA}	1.08 ± 0.08 ^{aB}	1.13 ± 0.07 ^{aB}
FO	Aerobic	0.91 ± 0.06 ^{cA}	0.93 ± 0.06 ^{bA}	5.27 ± 0.23 ^{bB}	2.94 ± 0.13 ^{dA}	2.90 ± 0.18 ^{dA}	2.52 ± 0.12 ^{cA}
	MAP	0.91 ± 0.06 ^{cA}	1.09 ± 0.12 ^{bA}	5.27 ± 0.17 ^{bB}	2.94 ± 0.13 ^{dB}	1.68 ± 0.09 ^{cA}	1.78 ± 0.19 ^{cA}
FGTC 200	Aerobic	0.73 ± 0.04 ^{bA}	0.78 ± 0.00 ^{aA}	0.94 ± 0.07 ^{aB}	2.04 ± 0.19 ^{bA}	2.00 ± 0.10 ^{bA}	2.05 ± 0.11 ^{bA}
	MAP	0.73 ± 0.04 ^{cA}	0.78 ± 0.08 ^{aA}	0.86 ± 0.08 ^{aA}	2.04 ± 0.19 ^{bA}	1.97 ± 0.11 ^{dA}	1.94 ± 0.09 ^{cA}
FGCA 200	Aerobic	0.93 ± 0.06 ^{cA}	1.13 ± 0.09 ^{cA}	5.22 ± 0.38 ^{bB}	2.43 ± 0.24 ^{cA}	2.56 ± 0.06 ^{cA}	2.74 ± 0.3 ^{cA}
	MAP	0.93 ± 0.06 ^{cA}	1.52 ± 0.19 ^{cB}	5.18 ± 0.40 ^{bC}	2.43 ± 0.24 ^{cB}	1.42 ± 0.09 ^{bA}	1.49 ± 0.13 ^{bA}

¹Fish oil, 15 % substitution of pork back fat (FO), FO + 200 mg GTC/kg sausage (FGTC 200) or 200 mg GCA/kg sausage (FGCA 200). ²Stored in 80% O₂ : 20% CO₂. ³Stored in 70% N₂ : 30% CO₂. ^{abcd}Within each packaging system (aerobic or MAP) mean values in the same column bearing different superscripts are significantly different, P < 0.05. ^{ABC}Within each treatment (raw or cooked) mean values in the same row bearing different superscripts are significantly different, P < 0.05. A student t-test was used to determine differences between aerobic and MAP, ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001. TBARS, mg malondialdehyde/kg muscle, mean ± standard deviation.

Table 6. Effect of linseed oil, green tea catechin (GTC) and green coffee antioxidant (GCA) on the surface lightness (L* value), redness (a* value) and yellowness (b* value) of raw pork sausages stored aerobically and in modified atmosphere packs (MAP).

Treatment ¹	Packaging Atmosphere	Storage time at 4°C, d								
		L* lightness value			a* redness value			b* yellowness value		
		0	3	7	0	3	7	0	3	7
Control	Aerobic	71.83 ± 0.94 ^{aA}	71.55 ± 0.83 ^{aA}	72.60 ± 2.14 ^{aA}	13.90 ± 0.32 ^{cA}	13.97 ± 0.59 ^{cA}	14.73 ± 0.91 ^{cA}	12.49 ± 0.70 ^{aB}	11.60 ± 0.68 ^{aAB}	11.04 ± 0.69 ^{aA}
	MAP ²	71.83 ± 0.94 ^{aA}	71.02 ± 0.59 ^{aA}	70.74 ± 1.12 ^{aA}	13.90 ± 0.32 ^{cA}	13.63 ± 0.60 ^{bA}	14.99 ± 0.55 ^{cB}	12.49 ± 0.70 ^{aB}	11.10 ± 0.56 ^{aA}	10.75 ± 0.22 ^{aA}
LO	Aerobic	76.84 ± 0.79 ^{cA}	76.28 ± 0.20 ^{cA}	76.81 ± 0.41 ^{bA}	12.03 ± 0.46 ^{aA}	11.89 ± 0.40 ^{bA}	12.93 ± 0.75 ^{bA}	14.11 ± 0.69 ^{bB}	13.97 ± 0.33 ^{bB}	12.96 ± 0.44 ^{bA}
	MAP	76.84 ± 0.79 ^{cA}	76.52 ± 0.71 ^{dA}	75.75 ± 0.81 ^{bA}	12.03 ± 0.46 ^{aA}	12.23 ± 0.45 ^{aA}	13.04 ± 0.36 ^{bB}	14.11 ± 0.69 ^{bB}	13.65 ± 0.40 ^{bcAB}	12.86 ± 0.30 ^{bA}
LGTC 200	Aerobic	73.56 ± 1.02 ^{bA}	74.02 ± 0.42 ^{bA}	72.76 ± 0.44 ^{aA}	13.07 ± 0.46 ^{bcB}	10.81 ± 0.30 ^{aA}	10.94 ± 0.55 ^{aA}	14.45 ± 0.52 ^{bc}	12.33 ± 0.48 ^{aB}	10.81 ± 0.37 ^{aA}
	MAP	73.56 ± 1.02 ^{bB}	73.29 ± 0.56 ^{bB}	71.59 ± 0.99 ^{aA}	13.07 ± 0.46 ^{bcC}	11.78 ± 0.35 ^{aB}	10.76 ± 0.12 ^{aA}	14.45 ± 0.52 ^{bc}	13.12 ± 0.16 ^{bB}	10.62 ± 0.33 ^{aA}
LGCA 200	Aerobic	75.92 ± 0.90 ^{cA}	75.59 ± 0.35 ^{cA}	76.19 ± 0.16 ^{bA}	12.45 ± 0.70 ^{abA}	11.85 ± 0.39 ^{bA}	12.73 ± 0.34 ^{bA}	14.40 ± 0.52 ^{bB}	13.82 ± 0.40 ^{bAB}	13.26 ± 0.44 ^{bA}
	MAP	75.92 ± 0.90 ^{cB}	74.48 ± 0.72 ^{cA}	75.70 ± 0.40 ^{bAB}	12.45 ± 0.70 ^{abA}	12.06 ± 0.14 ^{aA}	12.41 ± 0.23 ^{bA}	14.40 ± 0.52 ^{bB}	14.27 ± 0.14 ^{cB}	13.03 ± 0.68 ^{bA}

¹Linseed oil, 15 % substitution of pork back fat (LO), LO + 200 mg GTC/kg sausage (LGTC 200) or 200 mg GCA/kg sausage (LGCA 200). ²Stored in 80% O₂ : 20% CO₂. ^{abcd}Within each packaging system (aerobic or MAP) mean values in the same column bearing different superscripts are significantly different, P < 0.05. ^{AB}Within each colour value (L*, a* or b*) mean values in the same row bearing different superscripts are significantly different, P < 0.05. A student t-test was used to determine differences between aerobic and MAP, ns = not significant, * = P < 0.05, ** = P < 0.01. L*, a* and b* values, mean ± standard deviation.

Table 7. Effect of fish oil, green tea catechin (GTC) and green coffee antioxidant (GCA) on the surface lightness (L*value), redness (a* value) and yellowness (b* value) of raw pork sausages stored aerobically and in modified atmosphere packs (MAP).

Treatment ¹	Packaging Atmosphere	Storage time at 4°C, d								
		L* lightness value			a* redness value			b* yellowness value		
		0	3	7	0	3	7	0	3	7
Control	Aerobic	71.03 ± 1.22 ^{aAB}	69.26 ± 0.50 ^{aA}	71.47 ± 1.15 ^{aB}	14.65 ± 0.50 ^{cB}	14.15 ± 0.71 ^{cB}	12.91 ± 0.26 ^{dA}	11.82 ± 0.62 ^{aA}	11.01 ± 0.28 ^{bA}	11.06 ± 0.24 ^{bA}
	MAP ²	71.03 ± 1.22 ^{aB}	69.09 ± 0.46 ^{aA}	69.76 ± 0.67 ^{aAB}	14.65 ± 0.50 ^{cB}	14.08 ± 0.28 ^{cAB}	13.84 ± 0.22 ^{dA}	11.82 ± 0.62 ^{aB}	10.62 ± 0.19 ^{bA}	11.42 ± 0.52 ^{bAB}
FO	Aerobic	78.17 ± 0.62 ^{bbB}	75.83 ± 0.74 ^{cA}	76.22 ± 1.21 ^{bA}	12.92 ± 0.07 ^{bbB}	12.64 ± 0.40 ^{bbB}	11.26 ± 0.48 ^{bA}	11.30 ± 0.87 ^{aA}	11.53 ± 0.52 ^{bA}	11.62 ± 0.45 ^{cA}
	MAP	78.17 ± 0.62 ^{bbB}	76.20 ± 1.17 ^{cA}	76.23 ± 0.89 ^{cA}	12.92 ± 0.07 ^{bbB}	12.59 ± 0.28 ^{bbB}	11.36 ± 0.23 ^{bA}	11.30 ± 0.87 ^{aA}	11.10 ± 0.79 ^{bA}	12.14 ± 0.25 ^{cA}
FGTC 200	Aerobic	78.11 ± 0.20 ^{bcC}	73.44 ± 0.85 ^{bA}	75.06 ± 0.16 ^{bbB}	11.87 ± 0.37 ^{aC}	10.88 ± 0.52 ^{aB}	9.45 ± 0.09 ^{aA}	10.95 ± 0.32 ^{aC}	9.61 ± 0.45 ^{aB}	8.89 ± 0.17 ^{aA}
	MAP	78.11 ± 0.20 ^{bbB}	72.88 ± 1.29 ^{bA}	73.03 ± 0.70 ^{bA}	11.87 ± 0.37 ^{aB}	11.09 ± 0.74 ^{aB}	9.78 ± 0.23 ^{aA}	10.95 ± 0.32 ^{aC}	9.26 ± 0.42 ^{aB}	8.58 ± 0.27 ^{aA}
FGCA 200	Aerobic	77.78 ± 0.52 ^{bbB}	75.17 ± 0.14 ^{cA}	74.81 ± 0.24 ^{bA}	13.02 ± 0.28 ^{bbB}	12.93 ± 0.56 ^{bbB}	12.02 ± 0.25 ^{cA}	11.06 ± 0.49 ^{aA}	11.02 ± 0.37 ^{bA}	10.83 ± 0.21 ^{bA}
	MAP	77.78 ± 0.52 ^{bbB}	73.99 ± 0.77 ^{bA}	74.10 ± 0.86 ^{bA}	13.02 ± 0.28 ^{bbAB}	13.29 ± 0.69 ^{abbB}	12.26 ± 0.34 ^{cA}	11.06 ± 0.49 ^{aA}	11.42 ± 0.46 ^{bA}	11.66 ± 0.28 ^{bcA}

¹Fish oil, 15 % substitution of pork back fat (FO), FO + 200 mg GTC/kg sausage (FGTC 200) or 200 mg GCA/kg sausage (FGCA 200). ²Stored in 80% O₂ : 20% CO₂. ^{abc}Within each packaging system (aerobic or MAP) mean values in the same column bearing different superscripts are significantly different, P < 0.05. ^{AB}Within each colour value (L*, a* or b*) mean values in the same row bearing different superscripts are significantly different, P < 0.05. A student t-test was used to determine differences between aerobic and MAP, ns = not significant, * = P < 0.05. L*, a* and b* values, mean ± standard deviation.

Table 8. Effect of linseed oil, fish oil, green tea catechin (GTC) and green coffee antioxidant (GCA) on the sensory properties of cooked pork sausages stored aerobically and in modified atmosphere packs (MAP).

Sensory parameter	Day	Packaging Atmosphere	Treatment							
			Linseed oil ¹				Fish oil ²			
			Control	LO	LGTC 200	LGCA 200	Control	FO	FGTC 200	FGCA200
Colour	0	Aerobic	3.62 ^{aA}	3.43 ^{aA}	3.28 ^{aA}	3.29 ^{aA}	3.10 ^{aA}	3.00 ^{aA}	3.40 ^{aA}	3.30 ^{aA}
	7		3.33 ^{aA}	3.66 ^{aA}	3.33 ^{aA}	3.33 ^{aA}	3.37 ^{aA}	2.75 ^{aA}	2.50 ^{aA}	2.75 ^{aA}
	7		MAP	3.14 ^{aA}	2.86 ^{aA}	2.85 ^{aA}	2.71 ^{aA}	2.87 ^{aA}	2.37 ^{aA}	3.12 ^{aA}
Texture	0	Aerobic	3.62 ^{aA}	3.71 ^{aA}	4.00 ^{aA}	3.57 ^{aA}	3.30 ^{aA}	3.40 ^{bA}	3.20 ^{aA}	3.10 ^{aA}
	7		3.66 ^{aA}	4.16 ^{aA}	3.66 ^{aA}	4.00 ^{aA}	3.50 ^{aA}	3.00 ^{abA}	3.40 ^{aA}	2.87 ^{aA}
	7		MAP	3.71 ^{aA}	3.28 ^{aA}	3.28 ^{aA}	3.57 ^{aA}	3.12 ^{aA}	2.37 ^{aA}	3.50 ^{aA}
Flavour	0	Aerobic	3.12 ^{aA}	3.42 ^{abA}	4.00 ^{abA}	3.43 ^{aA}	3.50 ^{aA}	3.10 ^{aB}	3.00 ^{aA}	3.10 ^{aB}
	7		3.00 ^{aA}	4.00 ^{bB}	4.16 ^{bB}	3.83 ^{bA}	3.12 ^{aA}	2.25 ^{abA}	3.00 ^{aA}	2.12 ^{abA}
	7		MAP	3.28 ^{aA}	3.83 ^{aA}	3.28 ^{aA}	2.71 ^{aA}	3.00 ^{bA}	1.62 ^{aA}	3.25 ^{bA}
Overall Acceptability	0	Aerobic	3.50 ^{aA}	3.43 ^{aA}	3.14 ^{aA}	3.57 ^{aA}	3.20 ^{aA}	3.20 ^{aB}	2.90 ^{aA}	3.10 ^{aA}
	7		3.00 ^{aA}	3.83 ^{aA}	3.83 ^{aA}	3.50 ^{aA}	3.37 ^{bA}	2.50 ^{abA}	2.87 ^{aA}	2.25 ^{aA}
	7		MAP	3.28 ^{aA}	3.00 ^{aA}	3.1 ^{aA}	2.85 ^{aA}	3.00 ^{bA}	1.62 ^{aA}	3.25 ^{bA}

¹Linseed oil, 15 % substitution of pork back fat (LO), LO + 200 mg GTC/kg sausage (LGTC 200) or 200 mg GCA/kg sausage (LGCA 200). ²Fish oil, 15 % substitution of pork back fat (FO), FO + 200 mg GTC/kg sausage (FGTC 200) or 200 mg GCA/kg sausage (FGCA 200). ^{ab}Within each sensory parameter mean values (\pm standard deviation) in the same column bearing different superscripts are significantly different, $P < 0.05$. ^{AB}Within each treatment (linseed oil or fish oil) mean values (\pm standard deviation) in the same row bearing different superscripts are significantly different, $P < 0.05$.