Contents lists available at ScienceDirect

Meat Science



journal homepage: www.elsevier.com/locate/meatsci

Avocado, sunflower and olive oils as replacers of pork back-fat in burger patties: Effect on lipid composition, oxidative stability and quality traits

J.G. Rodríguez-Carpena^a, D. Morcuende^b, M. Estévez^{b,*}

^a Food Science, Faculty of Veterinary, Autonomous University of Nayarit, 63190, Tepic, Nayarit, Mexico

^b Animal Production and Food Science, University of Extremadura, 10003, Cáceres, Spain

ARTICLE INFO

Article history: Received 25 October 2010 Received in revised form 2 June 2011 Accepted 3 June 2011

Keywords: Vegetable oils Fat-replacers Oxidative stability Color Texture

ABSTRACT

The present study investigates the effects of avocado, sunflower and olive oils used as back-fat replacers, on the fatty acid composition, oxidative stability, volatiles profile and color and texture properties of cooked pork patties. The vegetable oils modified the fatty acid profiles of the patties by lowering the percentages of SFA (from 36.96% to ~25.30%) and reducing the atherogenic index (from 0.41 to ~0.24). Vegetable oils had higher amounts of antioxidant compounds such as tocopherols (10.8–53.9 mg/100 g) than back-fat (5.9 mg/100 g). Consistently, patties manufactured with the oils had significantly lower amounts of lipid and protein oxidation products than control patties. Avocado oil contributed with specific aroma-active terpenes to patties and had a significant impact on particular color and texture parameters. The results from this study highlight the technological applications of the vegetable oils as food ingredients in the design of healthier meat commodities.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Meat should be an essential component of a healthy and wellbalanced diet owing to its properties as a source of high-quality protein, high-available iron, essential fatty acids and B-group vitamins (Biesalski, 2005). However, recent studies have established a likely relationship between meat consumption and an increased risk of suffering serious health disorders such as colorectal cancer and coronary-heart diseases (CHD) (Ferguson, 2010). As a result of the scientific evidence, health authorities insist on recommending a substantial reduction of cooked meat products consumption while certain processed meat should be directly avoided (World Cancer Research Fund/American Institute for Cancer Research, 2007, 2009). Amongst other factors, animal fat and particularly, saturated fatty acids (SFA), have been recognized as influential factors in the pathogenesis of heart failure and cancer associated to meat consumption (Kratz, 2005; Lin, Zhang, Cook, Lee, & Buring, 2004). Furthermore, the controversy about the possible link between meat consumption and the so-called "affluence" diseases has a great impact on consumers who perceive animal fat and meat as generally unhealthy (reviewed by Webb & O'Neill, 2008). Consumer's increasing demand and concern towards food and nutrition gets into conflict with current trends towards processed and commodity meals (Grunert, 2006). Certain convenience meat products, such as burger patties, are still consumed world-wide despite of the bad image in relation to their impact on consumer's health.

In recent years, great efforts have been exerted in order to improve the nutritional quality of processed meat products and regain consumer's trust in meat. For instance, the replacement of animal fat with vegetable oils in meat products has been found to be an efficient and successful strategy to enhance the nutritional value of muscle foods by decreasing SFA levels and adding natural antioxidants as tocopherols. Olive oil, which has been demonstrated to display protective effects against several cancer types (Escrich, Moral, Grau, Costa, & Solanas, 2007), has been commonly used as animal fat replacer in meat products with positive effects in terms of nutritional value and oxidative stability. The beneficial effect of the partial replacement of animal fat with olive oil, has been investigated in numerous meat products such as frankfurters (Choi et al., 2010; López-López, Cofrades, & Jiménez-Colmenero, 2009), liver pâté (Martín, Ruiz, Kivikari, & Puolanne, 2008) and dry-cured sausages (Muguerza, Gimeno, Ansorena, Bloukas, & Astiasarán, 2001). Amongst other effects, olive oil provides meat products with high levels of oleic acid and monounsaturated fatty acids (MUFA), natural antioxidants such as tocopherols and reduces cholesterol levels without affecting considerably the sensory characteristics of the products (Kayaardi & Gök, 2003; Martín et al., 2008; Muguerza, Fista, Ansorena, Astiasarán, & Bloukas, 2002). The effect of some other vegetable oils such as the sunflower oil has been scarcely studied in meat products. The considerably high amount of polyunsaturated fatty acids (PUFA) in sunflower oil could increase the oxidative instability of the meat product and hence, seriously affect its sensory quality. Yilmaz, Simsek, and Isikli (2002) reported no adverse sensory effects on frankfurters formulated with 15% sunflower oil while Pennisi-Forell, Ranalli, Zaritzky, Andrés, and Califano (2010) added natural antioxidants to



^{*} Corresponding author. Tel.: + 34 927257122; fax: + 34 927257110. *E-mail address:* mariovet@unex.es (M. Estévez).

^{0309-1740/\$ –} see front matter 0 2011 Elsevier Ltd. All rights reserved. doi:10.1016/j.meatsci.2011.06.007

beef burger patties in order to prevent the oxidative instability linked to the addition of sunflower oil. The avocado oil contains considerably high levels of oleic acid (~60%) and natural antioxidants such as tocopherols and phenolic compounds (Ashton et al., 2006; Ozdemir & Topuz, 2004; Wang, Bostic, & Gu, 2010). Whereas the usage of avocado oil as back-fat replacer in meat products might likely lead to positive results in terms of nutritional value and oxidative stability, the impact of formulating meat products with avocado oil has not been studied before.

The present work was devoted to study the impact of using avocado, sunflower and olive oils as back-fat replacers in cooked burger patties in terms of fatty acid composition and the oxidative stability of lipids and proteins. The influence of the vegetable oils on other quality traits of the cooked patties such as their color, texture and volatiles profile was also investigated.

2. Material and methods

2.1. Chemicals

All chemicals and reagents used for the present work were purchased from Panreac (Panreac Química, S. A., Barcelona, Spain), Merck (Merk, Darmstadt, Germany) and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

2.2. Material

Commercial olive and sunflower oils were purchased from a local supermarket in Cáceres (Spain). The avocado oil was purchased from a marketplace in Mexico D.F. Vegetable oils were stored under refrigeration (+4 °C) prior to analysis and the manufacture of burger patties. The meat (porcine longissimus dorsi muscle) and pork backfat belonged to industrial genotypes slaughtered in a local slaughterhouse in Cáceres (Spain). The day after slaughter, the meat was freed from visible fat while the back-fat was cleaned and freed from the skin. Raw materials were immediately chopped into pieces (2 cm³), frozen (-18 °C, 24 h) and used as such for the manufacture of burger patties. The fatty acid composition, tocopherols, total phenolic compounds and total chlorophyll contents of the vegetable oils and the pork back-fat is shown in Table 1.

2.3. Manufacture of burger patties

Four types of porcine burger patties were prepared depending on the addition of different vegetable oils as replacers of pork back-fatavocado (A), sunflower (S) and olive (O)-including a control group manufactured with pork back-fat and no added oil. In the basic formulation, the ingredients per kilogram of patty were as follows: 700 g meat (porcine longissimus dorsi muscle), 180 g distilled water, 100 g pork back-fat and 20 g sodium chloride. In the formulation of patties manufactured with vegetable oils, 50% of the pork back-fat was replaced with each of the oils (50 g oil/kg of burger patty). All ingredients were minced in cutter until a homogeneous emulsiontype raw batter was obtained. Six burger patties per treatment were prepared in two batches, three patties per batch, using similar manufacturing processes each time. Burger patties were formed using a conventional burger-maker (~100 g/patty), to give average dimensions of 10 cm diameter and 1 cm thickness. Preliminary cooking trials were performed to establish the cooking conditions required to achieve a meat core temperature of 73 °C. Patties were placed on trays and cooked at 170 °C for 18 min in a forced-air oven. The cooking loss of burger patties was calculated as follows: Cooking $loss = [(W_b - W_a)/W_b] \times 100$ where W_b and W_a are the weights of the burger patties before and after cooking, respectively. After cooking, samples were allowed to cool down at room temperature and then transferred to a refrigerator overnight. The following day

Table 1

Tocopherols, total phenolics and total chlorophyll content and fatty acid profile^a of the porcine back-fat and the vegetable oils employed for the manufacture of cooked burger patties.

	Back-fat	Avocado oil	Sunflower oil	Olive oil
γ-tocopherol ^b	0.51	1.78	2.03	0.88
α -tocopherol ^b	5.43	9.04	51.90	17.07
Total phenolics ^c	6.73	12.75	7.13	167.81
Total chlorophyll ^d	0.00	65.49	0.00	13.76
C14:0	1.29	0.06	0.06	0.00
C16:0	24.92	12.87	6.48	9.62
C16:1 (n-7)	2.93	3.86	0.11	0.66
C17:0	0.40	0.03	0.04	0.06
C17:1 (n-7)	0.42	0.07	0.03	0.10
C18:0	10.03	1.45	3.62	2.95
C18:1 (n-9)	41.08	57.44	24.70	76.83
C18:1 (n-7)	3.31	3.43	0.99	2.61
C18:2 (n-6)	12.79	18.70	61.99	5.39
C18:3 (n-3)	0.92	0.92	0.28	0.60
C20:0	0.15	0.31	0.42	0.56
C20:1 (n-9)	0.78	0.31	0.24	0.38
C20:2 (n-6)	0.47	0.10	0.14	0.03
C21:0	0.11	0.04	0.00	0.00
C20:4 (n-6)	0.27	0.09	0.00	0.00
C22:0	0.04	0.16	0.67	0.15
C22:1 (n-9)	0.02	0.04	0.08	0.00
C24:0	0.05	0.11	0.14	0.04

^a Data expressed as percentage.

^b Data expressed as mg tocopherol/100 g fat/oil.

^c Data expressed as mg caffeic acid equivalent (CAE)/kg fat/oil.

^d Data expressed as mg chlorophyll/kg fat/oil.

samples were analyzed for instrumental color and texture and then frozen $(-80 \degree C)$ until the remaining chemical analysis were performed (less than four weeks).

2.4. Chemical analysis

2.4.1. Proximate composition of burger patties

Moisture and total protein contents were determined using official methods (A.O.A.C., 2000). The method of Folch, Lees, and Sloane-Stanley (1957) was used for determining fat content in burger patties.

2.4.2. Tocopherol quantification in vegetable oils and pork back-fat

For the determination of α - and γ -tocopherol, the vegetable oils and the liquefied back-fat were dissolved in isopropanol (1:10, v/v)prior to analysis by a Shimadzu "Prominence" HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a quaternary solvent delivery system (LC-20AD), DGU-20AS on-line degasser, SIL-20A auto-sampler, RF-10A XL fluorescence detector, and CBM-20A system controller. Separation was made on a Reversed-phase C18 column (150 mm length × 4.6 mm i.d., 5 µm particle diameter) manufactured by Phenomenex (USA) with the mobile phase being methanol:water (97:3 v/v) at a flow rate of 1.5 mL min⁻¹, and peaks were registered at 285 and 335 nm as excitation and emission wavelength, respectively. The mobile phases were filtered by a Millipore vacuum filtration system equipped with a 0.45 µm pore size filter. The samples (2 µL each) were injected by the aid of the auto-sampler. The system control and data acquisition were performed by Shimadzu "LC solution" software (Shimadzu Corporation, Kyoto, Japan). For guantification purposes, standard curves were prepared using standards α and γ -tocopherol supplied by Sigma-Aldrich (Steinheim, Germany).

2.4.3. Total phenol content (TPC)

TPC in fat materials was determined following to the Folin-Ciocalteu method as described by Capannesi, Palchetti, Mascini, and Parenti (2000). TPC was estimated from a standard curve of caffeic acid, and results were expressed as milligrams of caffeic acid equivalents (CAE) per kg of fat.

2.4.4. Total chlorophyll content

The total chlorophyll content was measured in vegetable oils and the liquefied back-fat by standard analytical method of A.O.C.S. (1997). This method is used to determine mg/kg of chlorophyllrelated pigments (predominantly pheophytin-a) in oils from spectrophotometric absorption measurements at 630 and 710 nm.

2.4.5. Cholesterol quantification in cooked burger patties

The method proposed by Guardiola, Codony, Rafecas, and Boatella (1994) was followed, with some modifications. About 100 mg of fat from burger patties was weighed, and then 10 mL of 1 M KOH was added for cold saponification. Then, the mixture was kept at room temperature for 20 h to complete saponification. The blend was then transferred to a separating funnel, and 10 mL of diethyl ether and 10 mL of distilled water were added. After the separation of phases, the organic phase containing the unsaponifiable fraction was transferred to a second separating funnel, and the aqueous phase was again re-extracted twice with the same solvents. The two organic phases were combined in the second funnel and washed with 5 mL water. The washed organic extract was filtered through anhydrous sodium sulfate, and then, the solvent was evaporated to dryness using rotaevaporator and under nitrogen stream. Afterwards, the unsaponifiable extract was redissolved in 1 mL pyridine. The internal standard (2.5 mg) (5 α -cholestane) was redissolved in 5 mL of pyridine. The cholesterol from the samples and the internal standard were silanized by mixing 25 µL of the unsaponifiable extract, 25 µL of the standard solution and 50 µL of bis(trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (BFSTA) (supplied by Sigma, St. Louis, MO). The mixture was kept at room temperature for 30 min to complete the silanization reaction. Cholesterol was identified using 5-cholesten-3_β-ol as cholesterol standard and determined by GC using a Hewlett-Packard HP-5890-II chromatograph, equipped with a flame ionization detector and a fused silica capillary column (12 m \times 0.2 mm i.d.) with a film thickness of 0.33 μ m, stationary phase of methyl silicone. Helium was used as carrier gas at a flow rate of 1.2 mL/min. The oven temperature program was from 210 to 264 °C at 5 °C/min, from 264 to 290 °C at 7 °C/min, and 2 min at 290 °C. The injector and detector temperatures were 280 and 290 °C, respectively. The split ratio was 1:25. The inlet pressure was 14 psi, and the sample volume injected was 2 µL.

2.4.6. Fatty acid profile of vegetable oils, pork back-fat and burger patties

Fatty acid methyl esters (FAMEs) were prepared by acidic esterification in the presence of sulphuric acid following the method described by Sandler and Karo (1992). FAMEs were analyzed by gas chromatography using a Hewlett-Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethyleneglycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA) (60 m \times 0.32 mm i.d. \times 0.25 μ m film thickness). Gas chromatograph oven program temperature was as follows: initial temperature of 190 °C, 2 °C/min to 235 °C; 15 min at this temperature and thereafter 6 °C/min to 250 °C, and then kept for an additional 20 min. Injector and detector temperatures were 250 °C. Carrier gas was helium at a flow rate of 0.8 mL/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO). Tridecanoic acid was used as internal standard. Results were expressed as grams per 100 g of detected FAMEs.

2.4.7. Determination of TBA-RS numbers

Thiobarbituric acid-reactive substances (TBA-RS) were assessed using the method described by Ganhão, Estévez, and Morcuende (2011) with some modifications. Briefly, 5 g of burger patty were dispensed in cone plastic tubes and homogenized with 15 mL perchloric acid (3.86%) and 0.5 mL BHT (4.2% in ethanol). While homogenisation, the plastic tubes were immersed in an ice bath to minimize the development of oxidative reactions during extraction of TBARS. The slurry was filtered and centrifuged (3000 rpm for 4 min) and 2 mL aliquots were mixed with 2 mL thiobarbituric acid (0.02 M) in test tubes. The test tubes were placed in a boiling water bath (100 °C) for 45 min together with the tubes from the standard curve. After cooling, the absorbance was measured at 532 nm. The standard curve was prepared using a 1,1,3,3-tetraethoxypropane (TEP) solution (0.2268 g) in 3.86% perchloric acid.

2.4.8. Determination of total protein carbonyls

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatisation with dinitrophenylhydrazine (DNPH) according to the method described by Estévez, Ventanas, and Cava (2005) with slight modifications. Burger patties (1 g) were minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an ultraturrax homogenizer for 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 mL) and subsequent centrifugation for 5 min at 5000 rpm. One pellet was treated with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 mL) and washed twice with 1 mL ethanol: ethyl acetate (1:1, v/v) to remove excess of DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 5000 rpm to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 nM-1 cm-1 at 370 nm for protein hydrazones.

2.4.9. Analysis of volatile compounds

Volatile compounds were analyzed from the headspace of cooked burger patties by using the solid-phase micro extraction (SPME) and gas chromatography/mass spectrometry (GC/MS) following the method described by Estévez, Ventanas, Ramírez, and Cava (2004) with minor modifications as follows: The SPME fiber, coated with divinylbenzene-carboxenpoly (dimethylxilosane) (DVB/CAR/PDMS) 50/30 µm, was preconditioned prior to analysis at 220 °C during 45 min. One gram of minced sample was placed in a 4 mL SPME vial and sealed with a silicone septum. The sample was allowed to equilibrate during 30 min while immersed in water at 37 °C. During the extraction, the SPME fiber was inserted through the septum and exposed to the headspace of the vial. After extraction, the SPME fiber was immediately transferred to the injector of the chromatograph (HP5890GC series II gas chromatograph) which was in splitless mode at 220 °C. Volatiles were separated using a 5% phenyl-95% dimethyl polysiloxane column (Restek, USA) (30 m \times 0.25 mm id., 1.05 μ m film thickness). The GC/MS conditions were as follows: the carrier gas was helium at 18.5 psi, resulting in a flow of 1.6 mL min⁻¹ at 40 °C. The SPME fiber was desorbed and maintained in the injection port at 220 °C during the whole chromatography run. The temperature program was isothermal for 10 min at 40 °C and then raised at the rate of 7 °C min⁻¹ to 250 °C and held for 5 min. Transfer line to the mass spectrometer was maintained at 270 °C. The mass spectrometer (Agilent model 5973) operated in the electron impact mode with an electron energy of 70 eV and a multiplier voltage of 1650 V, collecting data at a rate of 1 scan s⁻¹ over a range of m/z 40 to 300. Volatiles compounds were whether tentatively identified by comparing their mass spectra with those from the Wiley library or positively identified by comparing its mass spectra and retention time with those displayed by the standard compounds. Results from the volatiles analysis were provided in arbitrary area units (AAU).

2.4.10. Color measurements

Surface color measurements of cooked burger patties were performed using a Minolta Chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ) which consisted of a measuring head (CR-300), with an 8 mm diameter measuring area and a data processor (DP-301). Before each measuring session the chromameter was calibrated on the CIE color space system using a white tile. The L* value indicates lightness (L*=0 darkness, L*=100 lightness); a* value indicates redness (+60=red, -60=green) and b* value indicates yellowness (+60=yellow, -60=blue). Chroma and Hue angle values were obtained by using the following equations: $C = (a^{*2} + b^{*2})^{0.5}$; H^o = arctg b*/a*. Color measurements were made on the surface of each patty in triplicate at three randomly selected locations. Color measurements were made at room temperature (\approx 22 °C) with illuminant D65 and a 0° angle observer.

2.4.11. Texture measurements

Texture profile analysis (TPA) of cooked burger patties was performed at room temperature with a Texture Analyser TA-XT2i (Stable Micro Systems, Surrey, UK). Three cylinder samples (2.5 cm diameter) were taken from the middle of each patty and subjected to a two-cycle compression test. The samples were compressed to 40% of their original height with a cylindrical probe of 5 cm diameter and a cross-head speed of 5 mm/s. Texture profile parameters were determined following descriptions by Bourne (1978).

2.4.12. Statistical analysis

Six burger patties per treatment were prepared in two batches, making twenty four patties all together (4 treatments × 2 batches × 3 patties). Analyses of variance (ANOVA) and Tukey tests by SPSS for Windows (v. 15.0) were carried out to study the effect of the vegetable oil replacement in burger patties. Differences were considered significant at $p \le 0.05$. Relationships among physicochemical and instrumental parameters were calculated using Pearson's correlation coefficients. A Principal Component Analysis (PCA) was also performed.

3. Results and discussion

3.1. Proximate and fatty acid composition of cooked burger patties

No statistical differences were found amongst treatments for the proximate composition as all types of burger patties had similar amounts of moisture (61.84-63.39 g/100 g), fat (13.85-14.80 g/100 g) and protein (20.98-22.09 g/100 g) (Table 2). Whereas the replacement of back-fat with vegetable oils was expected to increase the amount of total lipids in the final product, the slight differences observed between treatments were not significant (p>0.05). Moreover, the cooking losses ranged from 20.69% to

Table 2

Chemical composition of cooked burger patties manufactured using avocado (A), sunflower (S) and olive (O) oils as replacers of pork back-fat.

.1...(

	C	A	3	0	p-value
Moisture ^a Fat ^a Protein ^a Cholesterol ^b	$\begin{array}{c} 61.84 \pm 1.93 \\ 13.85 \pm 0.38 \\ 22.09 \pm 0.84 \\ 79.60 \pm 11.07 \end{array}$	$\begin{array}{c} 63.11 \pm 0.96 \\ 14.80 \pm 1.10 \\ 22.05 \pm 0.67 \\ 93.27 \pm 11.25 \end{array}$		$\begin{array}{c} 62.38 \pm 0.65 \\ 14.02 \pm 0.40 \\ 20.98 \pm 1.41 \\ 89.46 \pm 10.78 \end{array}$	0.202 0.101 0.137 0.341

Results are expressed as means \pm standard deviation.

^a Expressed as g/100 g burger patty.

^b Expressed as mg cholesterol/100 g burger patty.

^c Statistical significance.

22.20% and no statistical differences were found between treatments. The chemical composition of the burger patties from the present study is within the common scope for this type of meat product (Sánchez-Zapata et al., 2010).

According to the results from the present study, replacing 50% of back-fat with vegetable oils in emulsified pork burger patties containing around 14% of fat, did not affect the amount of cholesterol in the final product. In accordance with the present results, Muguerza, Ansorena, and Astiasarán (2003) reported that replacing up to 25% of pork back-fat with vegetable oils had no impact on the cholesterol levels of fermented sausages containing around 33% fat. On the other hand, other authors found a significant decrease of cholesterol in meat products as a result of a partial substitution of animal fat with vegetable oils such as olive and sunflower oils (Choi et al., 2010; Kayaardi & Gök, 2003; Muguerza et al., 2001). Compared to the burger patties from the present study, the meat products analyzed in those papers had considerably higher fat contents and the replacement of animal fat was combined with a reduction of the total amount of animal fat, which could have more reasonably caused the significant decrease of the cholesterol levels. Although the implication of dietary cholesterol on CHD is still a disputed issue (Kratz, 2005), consumer's rejection of animal fats owing its association with cholesterol is considerably widespread in developed countries. The treated burger patties from the present study do not provide an advantage in terms of cholesterol reduction as compared to conventional pork patties.

Table 3 shows the fatty acid composition of cooked burger patties manufactured with avocado, sunflower and olive oils as replacers of pork back-fat. Four fatty acids namely palmitic, stearic, oleic and linoleic acids comprised around 90% of total fatty acids analyzed in cooked burger patties. As expected, the treatment with vegetable oils had a significant effect on the fatty acid composition of these products. The addition of the vegetable oils to burger patties significantly reduced, in average, 5 percentage points (p.p.) of palmitic, 6 p.p. of stearic and 12 p.p. of total SFA, compared to the control counterparts. Whereas all vegetable oils exerted this effect on SFA, the fatty acid profiles amongst treated patties were considerably different as they reflected, to a great extent, the specific fatty acid composition of each of the oils employed for their manufacture (Table 1). The reduction of SFA in the A-burger patties led to a concomitant and significant increase of the percentages of both MUFA and PUFA (9 and 3 p.p., respectively). In S-burger patties, a significant and noticeably intense increase of PUFA (22 p.p.) caused the simultaneous and significant decrease of the proportion of both SFA and MUFA (11 p.p.). A significant increase of MUFA (15 p.p.) in O-burger patties was compensated with significant decreases of the percentages of both SFA and PUFA (3 p.p.). Amongst treated burger patties, O-burger patties had the highest percentage of oleic acid and MUFA (62.18%) followed by the A-patties (55.87%) and the S-patties (36.47%). The latter had the largest proportion of linoleic acid and PUFA (38.30%), followed by the A-patties (18.78%) and the O-patties (12.53%). Olive oil is known to enrich meat products such as frankfurters (López-López et al., 2009) liver pâtés (Martín et al., 2008) and fermented sausages (Muguerza et al., 2001) with oleic acid. The fatty acid profile of the present O-burger patties is almost identical to that recently reported by Choi et al. (2010) in frankfurters containing around 25% fat and manufactured with 10% olive oil. Whereas the usage of sunflower oil in meat products is not so popular, the study carried out by Yilmaz et al. (2002) on frankfurters treated with such oil led to similar results to those found in the present study. Avocado oil, which is known to be rich in oleic acid (Ozdemir & Topuz, 2004), was employed in the present study for the first time to replace back-fat in burger patties. This oil increased the levels of oleic acid in burger patties to a lesser extent than olive oil and unlike the latter, increased as well PUFA percentages.

The fatty acid composition of muscle foods has a great impact on the nutritional value, oxidative stability and sensory properties of

Table 3

Fatty acid profile of cooked burger patties manufactured using avocado (A), sunflower (S) and olive (O) oils as replacers of pork back-fat.

5 1	0 1	8 (), ()		1	
	С	А	S	0	p-value
	$0.04^a \pm 0.00$	$0.02^{b} \pm 0.01$	$0.02^{b} \pm 0.01$	$0.02^{\rm b} \pm 0.00$	0.001
C14:0	$0.98^{a} \pm 0.01$	$0.52^{b} \pm 0.02$	$0.53 \ ^{\rm b} \pm 0.04$	$0.51^{b} \pm 0.03$	< 0.001
C15:0	$0.06^{a} \pm 0.01$	$0.04^{\rm b} \pm 0.01$	$0.04 \ ^{\rm b} \pm 0.00$	$0.04^{\rm b} \pm 0.00$	< 0.001
C16:0	$22.13^{a} \pm 0.47$	$16.78^{b} \pm 0.37$	$14.81^{\circ} \pm 1.34$	$15.59^{bc} \pm 0.32$	< 0.001
C16:1 (n-7)	$2.14^{b} \pm 0.26$	$2.78^{a} \pm 0.18$	$1.18^{c} \pm 0.16$	$1.40^{\circ} \pm 0.15$	< 0.001
C17:0	$0.36^{a} \pm 0.04$	$0.19^{b} \pm 0.02$	$0.21 \ ^{b} \pm 0.02$	$0.23^{b} \pm 0.01$	< 0.001
C17:1 (n-7)	$0.32^{a} \pm 0.04$	$0.20^{\rm b} \pm 0.02$	$0.18 \ ^{\rm b} \pm 0.02$	$0.22^{b} \pm 0.02$	< 0.001
C18:0	$12.28^{a} \pm 0.69$	$6.73^{\circ} \pm 0.43$	$8.42 \ ^{\mathrm{b}} \pm 1.69$	$7.63^{bc} \pm 0.48$	< 0.001
C18:1 (n-9)	$39.38^{\circ} \pm 0.36$	$47.63^{\rm b} \pm 0.63$	$31.56^{d} \pm 0.37$	$56.14^{a} \pm 1.90$	< 0.001
C18:1 (n-7)	$3.63^{ab} \pm 0.38$	$4.06^{a} \pm 0.25$	$2.43^{\circ} \pm 0.22$	$3.19^{b} \pm 0.21$	< 0.001
C18:2 (n-6)	$12.00^{\circ} \pm 0.37$	$15.20^{b} \pm 0.26$	$34.79^{a} \pm 1.89$	$9.08^{d} \pm 0.45$	< 0.001
C18:3 (n-3)	$0.89^{a} \pm 0.05$	$0.91^{a} \pm 0.11$	$0.65^{b} \pm 0.06$	$0.82^{ab} \pm 0.13$	< 0.001
C20:0	0.58 ± 0.05	0.59 ± 0.13	0.67 ± 0.11	0.75 ± 0.11	0.096
C20:1 (n-9)	$1.46^{a} \pm 0.11$	$1.16^{b} \pm 0.18$	$1.04^{\rm b} \pm 0.15$	$1.15^{b} \pm 0.23$	0.001
C20:2 (n-6)	$0.83^{a} \pm 0.08$	$0.55^{b} \pm 0.07$	$0.58^{\rm b} \pm 0.06$	$0.56^{b} \pm 0.14$	< 0.001
C20:3 (n-6)	0.46 ± 0.07	0.45 ± 0.20	0.43 ± 0.10	0.40 ± 0.15	0.780
C21:0	0.43 ± 0.07	0.37 ± 0.07	0.40 ± 0.07	0.41 ± 0.21	0.415
C20:4 (n-6)	0.89 ± 0.08	0.71 ± 0.13	0.67 ± 0.03	0.73 ± 0.13	0.061
C20:3 (n-3)	0.48 ± 0.07	0.43 ± 0.13	0.45 ± 0.08	0.43 ± 0.17	0.892
C20:5 (n-3)	$0.43^{b} \pm 0.15$	$0.51^{b} \pm 0.11$	$0.72^{a} \pm 0.14$	$0.48^{b} \pm 0.30$	0.001
C22:1 (n-9)	0.09 ± 0.06	0.04 ± 0.03	0.07 ± 0.03	0.08 ± 0.14	0.234
C24:0	$0.10^{ab}\pm0.01$	$0.11^{a} \pm 0.02$	$0.12^{a} \pm 0.01$	$0.08^{b} \pm 0.01$	0.002
C22:6 (n-3)	0.04 ± 0.02	0.01 ± 0.02	0.01 ± 0.02	0.04 ± 0.02	0.091
ΣSFA ^A	$36.96^{a} \pm 0.91$	$25.36^{b} \pm 0.53$	$25.23^{b} \pm 2.91$	$25.28^{b} \pm 0.74$	< 0.001
ΣMUFA ^B	$47.02^{\circ} \pm 0.66$	$55.87^{b} \pm 0.32$	$36.47^{d} \pm 0.75$	$62.18^{a} \pm 1.60$	< 0.001
ΣPUFA ^C	$16.02^{\circ} \pm 0.50$	$18.78^{b} \pm 0.46$	$38.30^{a} \pm 2.22$	$12.53^{d} \pm 1.05$	< 0.001
ΣlcPUFA ^D	3.13 ± 0.26	2.67 ± 0.59	2.86 ± 0.32	2.64 ± 0.86	0.076
$\Sigma n-3$	1.85 ± 0.15	1.87 ± 0.32	1.83 ± 0.21	1.77 ± 0.59	0.243
$\Sigma n-6$	$14.17^{c} \pm 0.40$	$16.91^{\rm b} \pm 0.17$	$36.47^{a} \pm 2.04$	$10.76^{\rm d} \pm 0.53$	< 0.001
n6/n3	$7.71^{bc} \pm 0.56$	$9.30^{\rm b} \pm 1.70$	$20.07^{a} \pm 1.56$	$6.51^{\circ} \pm 1.62$	< 0.001
AIE	$0.41^{a} \pm 0.01$	$0.25^{\rm b} \pm 0.01$	$0.23^{b} \pm 0.03$	$0.24^{\rm b} \pm 0.01$	< 0.001

Results are expressed as means \pm standard deviation. Values with a different letter (^{a-d}) within a row are significantly different (p<0.05).

^A Saturated fatty acids (SFA).

^B Monounsaturated fatty acids (MUFA).

^C Polyunsaturated fatty acids (PUFA).

^D Long chain polyunsaturated fatty acids (lcPUFA).

^E Atherogenic Index (C12:0 + $4 \times C14:0 + C16:0$)/(Σ MUFA + Σ PUFA).

muscle foods (Wood et al., 2008). Hence, the large differences found in the present study amongst treatments would certainly influence relevant quality traits of burger patties as described below. Regarding nutritional aspects, SFA are known to increase low density lipoproteins (LDL) and hence, blood cholesterol levels whereas unsaturated fatty acids exhibit the opposite effect. Amongst unsaturated fatty acids, MUFA display more beneficial effects because, unlike PUFA, do not decrease high-density lipoproteins (HDL) which protects against CHD (Mattson & Grundy, 1985). The nutritional ratios between hypercholesterolemic SFA (C12, C14, C16) and the unsaturated hypocholesterolemic ones (C18:1 n-9; C18:2 n-6) were similar amongst patties treated with vegetable oils (~0.33) and significantly smaller (p < 0.05) than that displayed by the control patty (0.59). Similarly, the atherogenic index (AI) of the control patties was significantly larger than those calculated for A-, S-, and O-burger patties. Large importance has been given to long chain PUFA in meat products because of the role played by the ratio n-6/n-3 in the development of CHD (Okuyama & Ikemoto, 1999). The ratios n-6/n-3 were significantly higher in S-patties followed by A-patties as a result of the higher content of C18:2 (n-6) in these samples. However, no differences were found between samples for long-chain PUFA which are those being directly implicated in the regulation of the inflammatory mechanisms involved in the pathogenesis of CHD (Alshatwi & Alrefai, 2007). In a well-balanced diet, these fatty acids may be supplied by fish and sea-food products. The potential benefits derived from the replacement of animal fat with vegetable oils, as far as the fatty acid composition is concerned, may be derived from the influence of major fatty acids. In this sense, including 5% of avocado, olive and sunflower oils in the recipe of porcine burger patties reduces the atherogenic potential of these cooked meat products. Amongst vegetable oils, the avocado and the olive oil would display the most favorable nutritional influence as a result of the significant enrichment of the anticholesterolemic oleic acid. In fact, both vegetable oils and/or derived by-products of such oils have been highlighted to enhance the health status and protect against CHD (Salazar, Hafifi, Pastelin, Ramírez-Ortega, & Sánchez-Mendoza, 2005).

3.2. Oxidative stability of cooked burger patties

Lipid and protein oxidation products were quantified in cooked burger patties (Table 4). The amount of malonaldehyde (MDA) and other lipid-derived carbonyls were assessed by using the TBA method. The amount of TBA-RS was significantly higher in control patties than in the treated counterparts. Amongst patties manufactured with vegetable oils, A-burger patties displayed significantly smaller TBA-RS numbers than S- and O-burger patties. The effect of using vegetable oils as ingredients in meat products has been profusely studied in terms of the fatty acid composition whereas the impact on lipid

Table 4

TBARS and protein hydrazones of cooked burger patties manufactured using avocado (A), sunflower (S) and olive (O) oils as replacers of pork back-fat.

	С	А	S	0	p-value ^A
TBARS ^B		$0.39^c\pm0.07$			
Protein hydrazones ^C	$1.87^{a} \pm 0.33$	$1.70^{ab} \pm 0.35$	$1.78^{a} \pm 0.40$	$1.46^{b} \pm 0.36$	0.005

Results are expressed as means \pm standard deviation. Values with a different letter (^{a-c}) within a row are significantly different (p<0.05).

^A Statistical significance.

^B Expressed as mg MDA/kg patty.

^C Expressed as nM carbonyls/mg protein.

oxidation is scarcely reported. Additionally, the results from previous works on this issue reveal conflicting effects of vegetable oils. In agreement with the present results, some authors have reported significantly lower amounts of lipid oxidation products in muscle foods produced with olive and high-oleic sunflower oil than in the control counterparts (Ansorena & Astiasarán, 2004; Muguerza et al., 2001). In contrast, results from other studies support that using olive and other vegetable for the manufacture of muscle foods enhances the oxidative instability of the final product (Choi et al., 2010; Kayaardi & Gök, 2003). The vegetable oils employed in the present study as replacers of animal back-fat enhanced the oxidative stability of cooked burger patties. Burger patties are highly susceptible to oxidative reactions as mincing enhances the exposure to oxygen and high temperatures during cooking promotes the degradation of PUFA and the formation of reactive oxygen species (ROS). The protective effect of olive oil against lipid oxidation in O-patties could have been caused by a cooperative effect of the reduction of PUFA and the incorporation of antioxidant compounds such as tocopherols and phenolic compounds. Considerable high amounts of both antioxidant compounds were found to be present in the olive oil employed in the present study (Table 1). The massive levels of α -tocopherol in sunflower oil may be main responsible for the results obtained for Spatties, despite of the high proportion of PUFA in these samples. Compared to the other vegetable oils, the highest oxidative stability provided by the avocado oil was unexpected as A-patties contained significantly higher amounts of PUFA than control and O-patties and the tocopherol content of this oil was considerably lower than in the other vegetable oils. However, the avocado oil had, in comparison with the other vegetable oils, considerable high amount of chlorophylls. This oil is known to be a good source of polyphenols, carotenoids and a large variety of chlorophylls with proven antioxidant activity (Wang et al., 2010). In fact, the intense antioxidant activity of avocado pigments has been highlighted to be closely linked to the health-promoting effects of avocado fruit and oil (Ashton et al., 2006). It is plausible to consider that these phenolic compounds contributed to enhance the oxidative stability of A-burger patties. The accumulation of MDA and other TBA-RS in muscle foods causes a straight loss of quality as most of these compounds contribute to the deterioration of color and flavor of meat products (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). TBA-RS numbers above about 0.5 are critical since they indicate a level of lipid oxidation products which produce a rancid odor and taste which can be detected by consumers (Wood et al., 2008). In addition, some TBA-RS, such as MDA, have been reported to be compounds with mutagenic and toxic potential (Del Río, Stewart, & Pellegrini, 2005). Hence, the vegetable oils from the present study could contribute to improve the overall quality of burger patties through the inhibition of TBA-RS formation.

Cooked burger patties were also analyzed for the accumulation of protein oxidation products (Table 4). Amongst treatments, only Oburger patties contained significantly smaller amounts of protein carbonyls than the control samples. In meat systems, the formation of carbonyl compounds is a well-establish consequence of the metalcatalyzed oxidation of certain amino acids such as lysine, arginine and proline (Estévez, in press). The occurrence of protein oxidation in muscle foods is thought to take place along with the oxidation of lipids. In fact, numerous meat researchers have reported a timely interaction between lipid and protein oxidation (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008a; Ventanas, Ventanas, Tovar, García, & Estévez, 2007) which is supported by the fact that ROS formed during early stages of PUFA oxidation can attach susceptible amino acid residues to trigger their oxidative degradation (Lund, Heinonen, Baron, & Estévez, 2011). Like this, the extent of protein oxidation in meat products has been reported to be enhanced with increasing amounts of lipids (Estévez et al., 2005). However, the impact of the modification of the characteristics of the lipid fraction on the occurrence and extent of protein oxidation in meat products has not been studied before. In agreement with the TBA-RS results, the partial replacement of back-fat with olive oil reduced the intensity of protein oxidation in burger patties. A significant correlation was found between both lipid and protein oxidation measurements (r=0.40; p<0.05). Besides the protective effect of olive oil against protein oxidation through the inhibition of lipid oxidation, certain components of olive oil such as α -tocopherol could specifically have protected proteins against ROS as this compound has been proved to be a reliable inhibitor of myofibrillar protein oxidation (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008b). The lack of correspondence between the TBA-RS and DNPH results for the other two treatments (A and S) may be due to a lack of sensitivity of the DNPH method. Numerous authors have reported a significant effect of particular antioxidant treatments on lipid oxidation and no effect at all when the DNPH method was employed for assessing protein oxidation in the same samples (Lund, Hvii, & Skibsted, 2007; Smet et al., 2008). Ganhão, Morcuende, and Estévez (2010a) found that, compared to the DNPH method, the detection of specific protein carbonyls using LC-MS increased the sensitivity of the analysis and enabled revealing significant differences between particular antioxidant treatments. Should more advanced methodology had employed for assessing protein oxidation, significant antioxidant effects might have been found for the addition of avocado and sunflower oils on burger patties.

The impact of the oxidative degradation of muscle proteins on meat quality and consumer's health requires further research. It is known however, that protein oxidation involves the loss of essential amino acids and may lead to the deterioration of specific quality traits in burger patties such as color and texture (Ganhão, Morcuende, & Estévez, 2010b). The usage of olive oil on burger patties would contribute to improve the quality of cooked burger patties through the effective inhibition of protein oxidation reactions.

3.3. Volatiles profile of cooked burger patties

The analysis of volatile compounds in meat and meat products provides objective and valuable information regarding their aroma characteristics as certain volatile components of meat are aromaactive compounds and hence, contribute particular aroma notes (Mottram, 1998). Other volatile compounds are reliable indicators of enzymatic, microbial and/or biochemical alteration occurred during storage or manipulation of meat products (Estévez, Morcuende, Ventanas, & Cava, 2003; Mottram, 1998). Amongst the total volatile compounds identified in the HS of cooked burger patties, three straight-chain saturated aldehydes-hexanal, octanal and nonanaland a number of volatile terpenes were selected and analyzed (Table 5). The aforementioned volatile aldehydes are derived from the oxidative degradation of unsaturated fatty acids and particularly hexanal, has generally been employed as indicator of lipid oxidation and rancidity (Shahidi & Pegg, 1994). In agreement with the TBA-RS numbers, the A-burger patties had significantly lower amounts of hexanal than control patties. On the other hand, the counts of octanal and nonanal were significantly higher in O-patties than in the samples from the other treatments. The pathways for the formation of oxidation-induced volatiles from particular fatty acids are fairly specific and therefore, the large differences between types of burger patties in terms of fatty acid composition could have affected their volatiles profile, as a result, their aroma profile. The aromatic notes of linoleic and PUFA-derived volatiles such as hexanal have been described as intense 'grass-like' and related to rancidity in cooked meat and other food systems (Shahidi & Pegg, 1994) while oleic acidderived volatiles such as octanal and nonanal are associated to pleasant notes, described as 'floral' and 'sweet' (Specht & Baltes, 1994). The ratio between linoleic acid- and oleic acid-derived volatiles has been previously employed as an indicator of the balance between unpleasant and pleasant aromatic notes linked to lipid oxidation (Estévez et al., 2003; Estévez et al., 2005). Consistently with the fatty

_		_
Ta	hle	5

Selected volatile components of cooked burger patties manufactured using avocado (A), sunflower (S) and olive (O) oils as replacers of pork back-fat.

	С	А	S	0	p-value ^A
Aldehydes					
Hexanal	$139.22^{a} \pm 3.32$	$103.80^{b} \pm 2.16$	$145.48^{a} \pm 6.78$	$136.04^{ab} \pm 1.87$	0.017
Octanal	$0.80^{b} \pm 0.28$	$1.07^{b} \pm 0.15$	$0.65^{b} \pm 0.24$	$1.72^{a} \pm 0.24$	0.002
Nonanal	$0.49^{b} \pm 0.01$	$0.36^{\circ} \pm 0.04$	$0.34^{c} \pm 0.18$	$0.77^{a} \pm 0.17$	0.012
Terpenes					
α-thujene	ND ^b	$0.29^{a} \pm 0.10$	ND ^b	ND ^b	0.000
α-pinene	ND ^c	$0.43^{a} \pm 0.14$	$0.24^{ab} \pm 0.03$	$0.19^{\rm b} \pm 0.05$	0.033
δ-3-carene	ND ^b	$0.28^{a} \pm 0.05$	ND ^b	ND ^b	0.000
α -terpinene	ND ^b	$0.09^{a} \pm 0.02$	ND ^b	ND ^b	0.000
limonene	ND ^c	$0.52^{a} \pm 0.07$	ND ^c	$0.10^{\rm b} \pm 0.02$	0.001
β-terpinene	ND ^b	$0.24^{a} \pm 0.03$	ND ^b	ND ^b	0.000
γ-terpinene	ND ^c	$0.38^{a} \pm 0.05$	ND ^c	$0.12^{b} \pm 0.01$	0.001

Results are expressed as means \pm standard deviation. Values with a different letter (a^{-c}) within a row are significantly different (p<0.05).

^A Statistical significance.

^B ND: non detected.

acid profiles of the cooked patties, this ratio resulted significantly different among treatments and followed the increasing order: O (54) < A (72) < C (107) < S (145); suggesting a more pleasant aromatic profile in samples with small ratio values, namely O- and A-patties. In the present study, oleic acid was found to have positive and significant (p<0.001) correlations with octanal (r=0.88) and nonanal (r=0.61). The high content of oleic acid and its oxidation-derived aldehydes have been ascribed to enhanced quality traits in dry-cured products and cooked meats (Estévez et al., 2003; 2005; Ventanas et al., 2007).

Volatile terpenes are natural constituents of the essential oils of plant materials. The vegetable oils are responsible for the occurrence of several volatile terpenes in the HS of treated patties which were not detected in C-patties. These terpenes, such as α -pinene, limonene and γ -terpinene, are amongst the common volatile components of vegetable oils (Guzmán-Gerónimo, López, & Dorantes-Alvarez, 2008). The S-patties had only one monoterpene hydrocarbon (α pinene) while O-patties had three of them (α -pinene, limonene and γ -terpinene). Burger patties with added avocado oil had significantly higher amounts of the previously described terpenes and considerable quantities of other compounds not detected in the S- and Ocounterparts. Several of the volatile terpenes detected are recognized odorants and are commonly used in the food industry as flavor and fragrance ingredients (Ibáñez, López-Sebastián, Ramos, Tabera, & Reglero, 1998). For instance, α -pinene, which has been related to 'spices, sweet, pine needles' odors, is an aroma-active component of avocado oil (Guzmán-Gerónimo et al., 2008) and has been highlighted as relevant contributor to the aroma of spiced cooked sausages (Chevance & Farmer, 1999). The usage of avocado oil as a back-fat

replacer could have a positive impact on the aroma of burger patties through the contribution of fruity and spicy odors. However, in the absence of a sensory assessment of the patties, the contribution of these compounds to the overall aroma of the final product remains unknown, and therefore, the attitude of consumers towards patties with such odor notes would be a future work of interest.

3.4. Instrumental color and texture of cooked burger patties

Table 6 shows the color and texture characteristics of the cooked burger patties as measured by instrumental means. The color parameters displayed by C-patties were comparable with those reported in a previous study (Ganhão et al., 2010b). The typical redbrownish color of cooked burger patties is mainly determined by the presence of denatured-globin hemochromes formed as a result of high temperatures, the formation of colored Maillard products upon heating, the physicochemical state of proteins and other meat components (Lawrie, 1998). The partial replacement of pork backfat with vegetable oils led to a significant modification of the color measured on the surface of the cooked patties. Patties treated with vegetable oils showed significantly higher L*-values. A-patties also had significantly lower a*-values than C-patties and A- and Opatties displayed significantly higher b*- and chroma values compared to the control counterparts. Similar results have been found in previous studies devoted to assess the effect of the replacement of pork back-fat with olive and other vegetable oils on the instrumental color of fermented and cooked sausages (López-López et al., 2009; Muguerza et al., 2002). Amongst vegetable oils, the avocado had the greatest impact on the color of burger patties as A-patties were

Table 6

Instrumental color and texture of cooked burger patties manufactured using avocado (A), sunflower (S) and olive (O) oils as replacers of pork back-fat.

	С	А	S	0	p-value ^A
L*	$74.43^{c} \pm 1.30$	$75.63^{\rm b} \pm 1.09$	$77.09^{a} \pm 1.45$	$76.17^{a} \pm 0.72$	< 0.001
a*	$2.63^{a} \pm 0.44$	$1.58^{\rm b} \pm 0.52$	$2.82^{a} \pm 0.34$	$2.38^{a} \pm 0.55$	< 0.001
b*	$13.45^{\circ} \pm 0.93$	$16.37^{a} \pm 0.40$	$13.36^{\circ} \pm 1.39$	$14.82^{b} \pm 0.45$	< 0.001
Chroma	$13.71^{\circ} \pm 0.85$	$16.45^{a} \pm 0.38$	$13.66^{\circ} \pm 1.30$	$15.02^{b} \pm 0.39$	< 0.001
Hue	$78.85^{bc} \pm 2.45$	$84.53^{a} \pm 1.86$	$77.90^{\circ} \pm 2.52$	$80.86^{b} \pm 2.29$	< 0.001
Hardness ^B	$41.64^{a} \pm 7.26$	$36.07^{\rm b} \pm 6.69$	$39.08^{ab} \pm 5.80$	$42.59^{a} \pm 5.47$	0.014
Adhesiveness ^C	-0.005 ± 0.007	-0.002 ± 0.005	-0.004 ± 0.009	-0.003 ± 0.006	0.491
Springiness ^D	0.92 ± 0.04	0.91 ± 0.02	0.93 ± 0.01	0.92 ± 0.01	0.296
Cohesiveness ^D	0.63 ± 0.01	0.63 ± 0.01	0.63 ± 0.01	0.63 ± 0.01	0.959
Gumminess ^D	$26.01^{a} \pm 4.24$	$22.55^{b} \pm 3.89$	$24.49^{ab} \pm 3.49$	$26.69^{a} \pm 3.16$	0.007
Chewiness ^D	$23.90^{a} \pm 3.43$	$20.56^{b} \pm 3.46$	$22.73^{ab} \pm 3.18$	$24.56^{a} \pm 2.74$	0.002
Resilience ^D	$0.37^{\rm b} \pm 0.02$	$0.38^{\rm ab} \pm 0.02$	$0.38^{a} \pm 0.02$	$0.37^{ab} \pm 0.01$	0.030

Results are expressed as means \pm standard deviation. Values with a different letter (^{a-c}) within a row are significantly different (p<0.05).

^A Statistical significance.

^B N.

 C N×s.

^D Dimensionless.

lighter, less red and more yellow than conventional pork patties. In addition, the avocado oil increased the color saturation of the samples and increased the hue angle value. As aforementioned, a large variety of polyphenols and chlorophylls are natural components of the avocado oil and contribute to the typical green color of the avocado pulp and the derived products (Ashton et al., 2006). The modification of the desirable red-brownish color of cooked patties by adding certain grayish and greenish tones might not be appreciated by consumers although this extent may be clarified by further sensory analyses.

Amongst vegetable oils, the avocado oil was, again, the most influential in the texture parameters displayed by cooked burger patties. Compared to C-patties, patties with added avocado oil presented significantly lower values for hardness, gumminess and chewiness (p<0.05). The composition and characteristics of the fat are highly influential on the textural properties of meat products (Lawrie, 1998). In the present study, the substitution of SFA with unsaturated fats as a result of the replacement of back-fat with avocado oil could explain the observed soft consistency of the A-patties. However, other vegetable oils with higher unsaturation indexes, such as the sunflower oil, did not show a similar effect. On the other hand, Ganhão et al. (2010b) recently proposed that the oxidation of myofibrillar proteins would cause an increase of hardness in burger patties through the formation of protein cross-links. In the present study, such effect was not observed as the olive oil

had the greatest effect against protein oxidation whereas the impact on the texture properties of burger patties was negligible. Whereas the reason for the significant effect of the avocado oil on the texture properties of burger patties remains unknown, it is reasonable to consider that the soft texture of A-patties could be interpreted by consumers as a drawback in terms of sensory quality.

3.5. Principal component analysis

A PCA was performed using data obtained from the chemical and instrumental analyses of cooked burger patties in order to determine the relationship amongst chemical and instrumental parameters and to discriminate between samples from different treatments. Fig. 1A shows the similarity map of the measured parameters defined by the two first Principal Components (PC#1 and PC#2 respectively) that accounted for the 50% of the total variability. The relative position of the variables in the similarity map suggests close relationships between certain parameters supporting the results obtained from the Pearson correlations. As expected, SFA were positioned on the positive axis of PC#1, far from the origin, close to the AI and opposite to oleic acid. The latter was located on the left upper guadrant, also far from the origin and close to the volatile compounds formed as a result of its oxidation, namely, octanal and nonanal. The hexanal was situated on the positive axis of PC#1 and closely related to other oxidation parameters such as the TBA-RS and the carbonyls derived

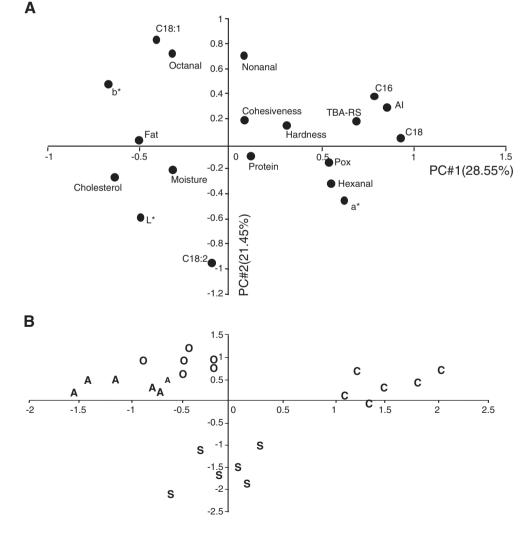


Fig. 1. Cooked burger patties: projection of the variables (1A) and the samples from the four treatments (1B) onto the space defined by the principal components (PC#1/PC#2).

from protein oxidation (Pox) which is in agreement with the Pearson correlation coefficients. Some other variables such as the texture parameters and the major chemical components of patties, such as protein, were located close to the origin. The projection of the samples onto the space of PC (Fig. 1B) showed that C-patties were situated on the positive axis of PC#1, in the plane area corresponding to high values of SFA, the AI, and the lipid and protein oxidation indexes. According to the PCA, A- and O-patties are mainly defined by high levels of oleic acid and volatile compounds which are related to favorable nutritional and sensory features, as aforementioned. On the contrary, S-patties are mainly defined by high levels of linoleic acid. According to the results obtained in the PCA, the variables computed in the model clearly discriminated between patties from different recipes and the replacement of pork back-fat with vegetable oil noticeably leads to products with different composition and quality. The relative proximity between samples from O- and A-patties suggest that using avocado oil as a back-fat replacer in burger patties imitates the composition and quality traits of the products manufactured with olive oil.

4. Conclusions

The manufacture of cooked burger patties with vegetable oils as replacers of pork-back-fat lead to products with enhanced nutritional properties as a result of a more favorable fatty acid profile and higher oxidative stability. In addition, the volatile components of burger patties with added avocado and the olive oils indicate a more pleasant aroma profile in these products. Whereas the avocado oil may impart positive spicy and fruity flavor notes to burger patties, its impact on other quality traits such as color and texture could be considered as a noticeable drawback. The results from the present study highlight remarkable technological applications of these vegetable oils as food ingredients in the design of healthier meat commodities. The accomplishment of further sensory analyses should confirm the extent of the impact of back-fat replacement on particular sensory features and on consumer's acceptance.

Acknowledgements

Mario Estévez thanks the Spanish Ministry of Science and Innovation for the contract through the "Ramón y Cajal (RYC-2009-03901)" program and the support through the project "*Protein oxidation in frozen meat and dry-cured products: mechanisms, consequences and development of antioxidant strategies*; AGL2010-15134". The European Community (Research Executive Agency) is acknowledged for the Marie Curie Reintegration Fellowship (PERG05-GA-2009-248959; Pox-MEAT). The excellent technical assistance of Dr. Antonio Silva from the Innovation Service in Animal Products (S.I.P.A.) from the University of Extremadura is also appreciated.

References

- A.O.A.C. (2000). Official methods of analysis (17th ed.). . Gaithersburgh, Maryland: Association of Official Analytical Chemists.
- A.O.C.S. (1997). Official method Cc 13d-55. Official methods and recommended practices of the AOCS (fifth ed.). Champaign: AOCS Press.
- Alshatwi, A. A., & Alrefai, N. A. (2007). A comparison of serum omega-3 fatty acid concentration between patients with coronary heart disease and healthy subjects. *Pakistan Journal of Nutrition*, 6, 72–74.
- Ansorena, D., & Astiasarán, I. (2004). Effect of storage and packaging on fatty acid composition and oxidation in dry fermented sausages made with added olive oil and antioxidants. *Meat Science*, 67, 237–244.
- Ashton, O. B. O., Wong, M., McGhie, T. K., Vather, R., Wang, Y., Requejo, J. C., et al. (2006). Pigments in avocado tissue and oil. *Journal of Agricultural and Food Chemistry*, 54, 10151–10158.
- Biesalski, H. K. (2005). Meat as component of a healthy diet are there any risks or benefits if meat is avoided in the diet? *Meat Science*, 70, 509–524.
- Bourne, M. C. (1978). Texture profile analysis. Food Technology, 33, 62-66.
- Capannesi, C., Palchetti, I., Mascini, M., & Parenti, A. (2000). Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chemistry*, 71, 553–562.

- Chevance, F. F. V., & Farmer, L. J. (1999). Identification of major volatile odor compounds in frankfurters. *Journal of Agricultural and Food Chemistry*, 47, 5151–5160.
- Choi, Y. S., Choi, J. H., Han, D. J., Kim, H. Y., Lee, M. A., Jeong, J. Y., et al. (2010). Effects of replacing pork back fat with vegetable oils and rice bran fiber on the quality of reduced-fat frankfurters. *Meat Science*, 84, 557–563.
- Del Río, D., Stewart, A. J., & Pellegrini, N. (2005). Una revisión de estudios recientes en malondialdehyde como la molécula tóxica y marcador biológico de la tensión oxidative. Nutrition, Metabolism, and Cardiovascular Diseases, 15, 316–328.
- Escrich, E., Moral, R., Grau, L., Costa, I., & Solanas, M. (2007). Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer. *Molecular Nutrition & Food Research*, 51, 1279–1292.
- Estévez, M. Protein carbonyls in meat systems: A review. *Meat Science, in press,* doi: 10.1016/j.meatsci.2011.04.025.
- Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., & Heinonen, M. (2008a). Fluorescence spectroscopy as a novel approach for the assessment of myofibrillar protein oxidation in oil-in-water emulsions. *Meat Science*, 80(4), 1290–1296.
- Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., & Heinonen, M. (2008b). Oxidation of skeletal muscle myofibrillar proteins in oil-in-water emulsions: Interaction with lipids and effect of selected phenolic compounds. *Journal of Agricultural and Food Chemistry*, 56, 10933–10940.
- Estévez, M., Morcuende, D., Ventanas, S., & Cava, R. (2003). Analysis of volatiles in meat from Iberian pigs and lean pigs alter refrigeration and cooking by using SPME-GC-MS. Journal of Agricultural and Food Chemistry, 51, 3429–3435.
- Estévez, M., Ventanas, S., & Cava, R. (2005). Protein oxidation in frankfurters with increasing levels of added rosemary essential oil: effect on colour and texture deterioration. *Journal of Food Science*, 70, 427–432.
- Estévez, M., Ventanas, S., Ramírez, R., & Cava, R. (2004). Analysis of volatiles in porcine liver pâtés with added sage on rosemary essential oils by using SPME-GS-MS. Journal of Agricultural and Food Chemistry, 52, 5168–5174.
- Ferguson, L. R. (2010). Meat and cancer. Meat Science, 84, 308-313.
- Folch, L., Lees, M., & Sloane-Stanley, C. H. (1957). A simple method for the isolation and purification of total lipid from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Ganhão, R., Morcuende, D., & Estévez, M. (2010a). Tryptophan depletion and formation of α -aminoadipic and γ -glutamic semialdehydes in porcine burger patties with added phenolic-rich fruit extracts. *Journal of Agricultural and Food Chemistry*, 58, 3541–3548.
- Ganhão, R., Morcuende, D., & Estévez, M. (2010b). Protein oxidation in emulsified cooked burger patties with added fruit extracts: influence on colour and texture deterioration during chill storage. *Meat Science*, 85, 402–409.
- Ganhão, R., Estévez, M., & Morcuende, D. (2011). Suitability of the TBA method for assessing lipid oxidation in meat system with added phenolic-rich material. *Food Chemistry*, 126, 772–778.
- Grunert, K. G. (2006). Future trends and consumer lifestyles with regard to meat consumption. *Meat Science*, 74, 149–160.
- Guardiola, F., Codony, R., Rafecas, M., & Boatella, J. (1994). Selective gas chromatographic determination of cholesterol in eggs. *Journal American Oil Chemist's Society*, 71, 867–871.
- Guzmán-Gerónimo, R. I., López, M. G., & Dorantes-Alvarez, L. (2008). Microwave processing of avocado: Volatile flavor profiling and olfactometry. *Innovate Food Science and Emerging Technologies.*, 9, 501–506.
- Ibáñez, E., López-Sebastián, S., Ramos, E. U., Tabera, J., & Reglero, G. (1998). Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chemistry*, 63, 281–286.
- Kayaardi, S., & Gök, V. (2003). Effect of replacing beef fat with olive oil on quality characteristics of Turkish soudjouk (sucuk). *Meat Science*, 66, 249–257.
- Kratz, M. (2005). Dietary cholesterol, atherosclerosis and coronary heart disease. Handbook of Experimental Pharmacology, 170, 195–213.
- Lawrie, R. A. (1998). The eating quality of meat. In R.A. Lawrie (Ed.), Meat science (6th ed.). Cambridge: Woodhead Publishing.
- Lin, J., Zhang, S. M., Cook, N. C. R., Lee, I. M., & Buring, J. E. (2004). Dietary fat and fatty acids and risk of colorectal cancer in women. *American Journal of Epidemiology*, 160, 1011–1022.
- López-López, I., Cofrades, S., & Jiménez-Colmenero, F. (2009). Low-fat frankfurters enriched with n-3 PUFA and edible seaweed: effects of olive oil and chilled storage on physicochemical, sensory and microbial characteristics. *Meat Science*, 83, 148–154.
- Lund, M. N., Heinonen, M., Baron, C. P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research*, 55, 83–95.
- Lund, M. N., Hvii, M. S., & Skibsted, L. H. (2007). The combined effect of antioxidants and modified atmosphere packaging on protein and a lipid oxidation in beef patties during chill storage. *Meat Science*, 76, 226–233.
- Martín, D., Ruiz, J., Kivikari, R., & Puolanne, E. (2008). Partial replacement of pork fat by conjugated linoleic acid and/or olive oil in liver pâtés: Effect on physicochemical characteristics and oxidative stability. *Meat Science*, 80, 496–504.
- Mattson, F. H., & Grundy, S. M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research*, 26, 194–202.
- Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49, 73–86.
- Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. Food Chemistry, 62, 415–424.
- Muguerza, E., Ansorena, D., & Astiasarán, I. (2003). Improvement of nutricional properties of Chorizo de Pamplona by replacement of pork backfat with soy oil. *Meat Science*, 65, 1361–1367.

- Muguerza, E., Fista, G., Ansorena, D., Astiasarán, I., & Bloukas, J. G. (2002). Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, 61, 397–404.
- 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.
- Okuyama, H., & Ikemoto, A. (1999). Needs to modify the fatty acids composition of meat for human health. Proceedings 45nd International Congress of Meat Science and Technology, Vol. II. (pp. 638–640) Yokohama. Japan.
- Ozdemir, F., & Topuz, A. (2004). Changes in dry matter, oil content and fatty acids composition of avocado during harvesting time and post.harvesting ripening period. *Food Chemistry*, 86, 79–83.
- Pennisi-Forell, S. C., Ranalli, N., Zaritzky, N. E., Andrés, S. C., & Califano, A. N. (2010). Effect of type emulsifiers and antioxidants on oxidative stability, colour and fatty acid profile of low-fat beef burgers enriched with unsaturated fatty acids and phytosterols. *Meat Science*, 86, 364–370.
- Salazar, M. J., Hafifi, M. E., Pastelin, G., Ramírez-Ortega, M. C., & Sánchez-Mendoza, M. A. (2005). Effect of an avocado oil-rich diet over an angiotensin II-induced blood pressure response. *Journal of Ethnopharmacology*, 98, 335–338.
- Sánchez-Zapata, E., Muñoz, C. M., Fuentes, E., Fernández-López, J., Sendra, E., Sayas, E., et al. (2010). Effect of tiger nut fibre on quality characteristics of pork burger. *Meat Science*, 85, 70–76.
- Sandler, S., & Karo, W. (1992). Sourcebook of advanced organic laboratory preparations. San Diego: Academic Press.
- Shahidi, F., & Pegg, R. B. (1994). Hexanal as an indicator of meat flavour deterioration. Journal of Food Lipids, 1, 177–186.

- Smet, K., Raes, K., Huyghebaert, G., Haak, L., Arnouts, S., & de Smet, S. (2008). Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. *Poultry Science*, 87, 1682–1688.
- Specht, K., & Baltes, W. (1994). Identification of volatile flavor compounds with high aroma values from shallow-fried beef. *Journal of Agricultural and Food Chemistry*, 42, 2246–2253.
- Ventanas, S., Ventanas, J., Tovar, J., García, C., & Estévez, M. (2007). Extensive feeding versus oleic acid and tocopherol enriched mixed diets for the production of Iberian dry-cured hams: Effect on chemical composition, oxidative status and sensory traits. *Meat Science*, 77, 246–256.
- Wang, W., Bostic, T. R., & Gu, L. (2010). Antioxidant capacities, procyanidins and pigments in avocado of different strains and cultivars. *Food Chemistry*, 122, 1193–1198.
- Webb, E. C., & O'Neill, H. A. (2008). The animal fat paradox and meat quality. *Meat Science*, 80, 28–36.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343–358.
- World Cancer Research Fund/American Institute for Cancer Research (2007). Food, nutrition and the prevention of cancer: A global perspective. Washington, DC: A.I.C.R. http://www.dietandcancerreport.org/
- World Cancer Research Fund/American Institute for Cancer Research (2009). *Policy and action for cancer prevention*. Food, nutrition, and physical activity: A global perspective Washington, DC.
- Yilmaz, I., Simsek, O., & Isikli, M. (2002). Fatty acid composition and quality characteristics of low-fat cooked sausages made with beef and chicken meat, tomato juice and sunflower oil. *Meat Science*, 62, 253–258.