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Effect of different cooking methods on lipid oxidation and formation of free cholesterol oxidation products (COPs) in *Latissimus dorsi* muscle of Iberian pigs

J.M. Broncano *, M.J. Petrón, V. Parra, M.L. Timón

Department of Animal Production and Food Science, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Ctra. de Cáceres s/n, 06071 Badajoz, Spain

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ABSTRACT

The aim of this work was to study the influence of different cooking methods (grilled (GR), fried (FP), microwave (MW) and roasted (RO)) on lipid oxidation and formation of free cholesterol oxidation products (COPs) of meat from Iberian pigs that have been fed on an intensive system. Moisture and total lipid content, TBARs, hexanal and COPs were measured in *Latissimus dorsi* muscle samples. Cooking did not produce changes in total lipid content in meat but induced significantly higher lipid oxidation (TBARs and hexanal values) ($p < 0.001$) and cholesterol oxidation (COPs) ($p < 0.01$). When the different cooking methods were studied, the grilled method was the least affected by lipid oxidation (TBARs and hexanal) compared to the others. There were no significant differences among different cooking methods on COPs values. The most abundant cholesterol oxides were both 7α-hydroxycholesterol and 7β-hydroxycholesterol in all groups studied.

1. Introduction

Usually, meat has to be cooked before being consumed. Cooking causes several positive effects on meat such as taste and flavour enhancement, microorganism destruction, shelf life increase (Bognar, 1998) and improved digestibility (Rodriguez-Estrada, Penazzi, Caboni, Bertacco, & Lercker, 1997), but also produces some negative effects like aromatic polycyclic hydrocarbons (APHs) generation and nutritional losses (Rodriguez-Estrada et al., 1997). In this respect, lipid oxidation in meat occurring during cooking is the most important source for taste and odour compound formation but also is the main reason for the deterioration of this product giving undesirable odours, rancidity, texture modification, nutritional losses or toxic compound production. It has been accepted that lipid oxidation products are related to atherosclerosis (Esterbauer, Wäg, & Puhl, 1993), Alzheimer's disease (Markesbery & Lovell, 1998), cancer (Boyd & McGuire, 1991), inflammation or aging processes (Packer & Walton, 1977).

Development of oxidation reactions in meat depends on the method, temperature and time of cooking (Gandemer, Girard, & Desnoyers, 1983; Gandemer, Viau, Maho, Metro, & Laroche, 1985). It is known that qualitative changes in food during cooking are larger when temperature increases (Hoac, Daun, Trafikowska, Zackrisson, & Åkesson, 2006; Mielche, 1995) and there are also differences related to cooking times at specific temperatures. Moreover, high temperatures and long times produce oxidative changes in food which could be really negative for quality (Kesava Rao, Kowale, Babu, & Bisht, 1996). Several studies (Hernández, Navarro, & Toldrá, 1999; Rodriguez-Estrada et al., 1997) have shown that roasting, which uses high temperatures during a long time, produces an increase of lipid oxidation compared to other methods. However, Rodriguez-Estrada et al. (1997) observed that microwave treatment (shorter time and lower temperature) caused high oxidation also. Therefore, microwave cooking promotes oxidation reactions. Several authors explained this fact by showing a decrease of polyunsaturated fatty acid (PUFA) from phospholipids in different foods after microwave cooking, relating this result, to a likely increase of oxidation products originated from these fatty acids (Hernández et al., 1999; Yoshida, Hirakawa, Tomiyama, Nagamizu, & Mizushina, 2005).

On the other hand, in frying, heat is transferred to the meat by oils and fats and oxidation changes are produced in both, meat and the oil or fat, when the hot oil contacts with food surface. Moreover, oils can modify fatty acid composition and antioxidant content in meat which could therefore affect the oxidation in final products (Cuesta & Sánchez-Muniz, 2001; Ramírez, Morcuende, Estévez, & Cava, 2004; Saghir, Wagner, & Elmadfa, 2005; Sánchez-Muniz, Viejo, & Medina, 1992).

The thiobarbituric acid (TBA) test is a common method to measure lipid oxidation in meat (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004; Juntachote, Berghofer, Siebenhandl, & Bauer, 2007) which determines malondialdehyde (MDA) content. Hexanal has been also used to follow lipid oxidation and volatile compound

* Corresponding author. Tel.: +34 924286200; fax: +34 924286201.
E-mail address: jbroant@unex.es (J.M. Broncano).

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formation in meat during cooking (Brunton, Cronin, Monahan, & Durcan, 2000). Lipid oxidation in meat during cooking also affects cholesterol. COPs formation is related to temperature and time of heat treatment (Morgan & Armstrong, 1992; Paniangvait, King, Jones, & German, 1995). COPs determination in cooked meat is very important since many studies have demonstrated that these compounds are more dangerous for arterial cells than cholesterol and they are directly related to atherosclerosis, coronary diseases and mutagenic activity (Garcia-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002; Schroepfer, 2000).

Many different methods have been used to determine COPs in food, but the most used are the ones that include a cold saponification and a solid-phase extraction (SPE) to purify COPs, which improve the limits of determination, using usually GC or HPLC (García Regueiro & Maraschiello, 1997; Guardiola, Codony, Rafecas, & Boatella, 1994; Petrón, García Regueiro, Martín, Muriel, & Antequera, 2003; Ulberth & Rössler, 1998). However, some authors prefer a simple fractionation (SPE) to purify COPs in meat products (Ahn, Nam, Du, & Jo, 2001; Lercker & Rodriguez-Estrada, 2000; Maraschiello, Esteve, & García Regueiro, 1998), since total COPs detection is improved (García Regueiro & Maraschiello, 1997).

The implication of lipid oxidation products in different pathologies makes necessary the study of oxidation reactions in meat during cooking. The aim of our work was to study Iberian pork oxidation according to different cooking methods and determine their effect on COPs content. No studies have been found dealing with the effect of cooking method on oxidation in Iberian pork, a special meat having different fatty acid composition compared to other pig meat.

2. Material and methods

2.1. Samples

This study was carried out using meat from Iberian pigs ($n = 9$) fed on an intensive system. Animals were slaughtered and *Latissimus dorsi* muscle was selected for the study. Each muscle ($n = 9$) was cut into slices and randomly divided into five groups. A total of 45 samples were obtained (9 muscles $\times$ 5 groups). One group was used as raw control (RA), and the rest was subjected to the following common cooking methods: grilled (GR) at 190 °C during 2 min on each surface; fried (FP) using 8 ml refined olive oil, at 170 °C during 2 min on each surface; microwave (MW) at 450 W for 90 s (80 °C) and roasted (RO) at 150 °C during 20 min. Thickness of steaks was 8 mm.

After cooking, the steaks were minced, vacuum-packed and stored at −80 °C until needed for analysis.

2.2. Reagents and chemicals

The solvents used were of the following origin and quality: HPCLC, Synthesis grade, extra pure, PRS and reagent grade, and they were purchased from Panreac Química S.A. (Barcelona, Spain) and Scharlau Chemie S.L. (Barcelona, Spain).

The gas used in GC–MS (helium) and evaporation of solvents (nitrogen) was from the following origin: Abelló Linde S.A. (Barcelona, Spain).

The malondialdehyde and hexanal standards used in quantification and determination themselves were supplied by Merk Schuchardt (Hohenbrunn, Germany) and Sigma–Aldrich Inc. (St. Louis, MO, US), respectively. The standards of cholesterol oxides (7α-hydroxycholesterol, 7β-hydroxycholesterol, 20α-hydroxycholesterol, cholestanetriol, 25-hydroxycholesterol, 19-hydroxycholesterol and 7-ketocholesterol) were supplied by Steraloids Inc. (Newport R.I., USA).

2.3. Methods

2.3.1. Chemical analysis and thiobarbituric acid-reactive substances (TBARs)

Moisture content of meat was determined following the ISO recommended method (ISO-1442, 1997). Total lipids of muscle were extracted according to the method described by Folch, Lees, and Stanley (1957). TBARs were measured by following the extraction method described by Sørensen and Jørgensen (1996). Samples were dispersed in trichloroacetic acid, centrifuged and filtered. The extract was mixed with thiobarbituric acid and heated to 100 °C for 40 min. The samples were immediately cooled on ice and the absorbance was measured at 532 nm and 600 nm on a spectrophotometer (model HEλIOS UNICAM). The concentration of malondialdehyde (MDA) was calculated from a standard curve with solutions of MDA (Merk, Schuchardt) in triplicate, extracted and analyzed under exactly the same conditions as the samples. MDA contents were expressed as mg MDA/kg muscle.

2.3.2. Hexanal content

Hexanal was quantified by headspace–solid-phase microextraction (SPME) and GC/MS (gas chromatograph AGILENT 6890, coupled to a mass selective detector AGILENT 5973 Network) following the method described by Andrés et al. (2004). Minced samples, 0.5 g, and distilled water, 1.5 ml, were thoroughly mixed in a vial, which was then closed with a teflon/silicone septum. An SPME fibre (75 μm carboxene–polydimethylsiloxane coating) was inserted through the septum and exposed to the headspace of the vial. Extraction was carried out at 40 °C for 30 min with stirring in a water bath. After extraction, the SPME fibre was immediately transferred to the injector of the chromatograph which was in splitless mode at 250 °C. The separation of hexanal was performed on a 5% phenyl-methyl silicone (HP-5)-bonded phase fused silica capillary column (Hewlett–Packard) (length: 30 m; i.d.: 0.25 mm) with a film thickness of 0.25 mm), operating with a helium flow at 1.5 ml/min. Oven program was 35 °C initially for 5 min, 4 °C/min to 150 °C, and held at 20 °C/min to 250 °C. Transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained using a mass selective detector in electronic impact mode at 70 eV, a multiplier voltage of 1259 V and data collection at 1 scan/s over the *m/z* range 30–500. Hexanal was tentatively identified by comparing mass spectra and retention time with that of a standard (Sigma, St. Louis, US) injected under the same conditions and with the spectra library (Wiley & NIST/EPA/NIH). Hexanal content was calculated using a standard curve with solutions of hexanal in triplicate, extracted and analyzed under exactly the same conditions as the samples. Hexanal content was expressed as μg hexanal/g muscle.

2.3.3. Determination of the free cholesterol oxide composition

Six COPs were identified according to the method of Petrón et al. (2003), by the procedure described below. A column fractionation without previous cold saponification was performed to determine free COPs. The COPs coming from esterified cholesterol were not quantified.

Each sample (50 mg of total lipids) and 5 ml of hexane:diethyl ether (95:5, v/v) were added to a silica-SPE cartridge and washed successively with three mixtures of hexane:diethyl ether in the following quantities and proportions: 10 ml of hexane:diethyl ether (95:5, v/v), 25 ml of hexane:diethyl ether (90:10, v/v) and 15 ml of hexane:diethyl ether (80:20, v/v). This procedure allowed recovery of both apolar lipids and the cholesterol fraction. After that, COPs were extracted with 10 ml of acetone. Then, acetone was removed under vacuum at 25 °C on a rotary evaporator. To reduce contaminants (traces of cholesterol and/or partial glycerides) to a further degree, the COP-containing residue of the silica-SPE