Friulian Journal of Science **11.** 2009, 19-28

On the presence of cholesterol oxides (COPs) in ham

LORENA PIZZALE, ORNELLA BOSCHELLE, LANFRANCO CONTE*

Abstract. Development of flavour in food, and in meat food is a very complex process, that involves a number of chemical reactions on lipid and proteins. Lipid oxidation does not involve fatty acids only, but sterols as well, in meat foods this happens on cholesterol, mainly.
Cholesterol oxides (COPs) are extensively studied because of their cytotoxic activity and they are a risk factor for consumers health.
In this paper, COPs were measured in *longissimus dorsi*, subcutaneous, intramuscular fat and lean meat volatile compounds of the same samples were also analysed.
Results highlighted the presence of COPs, mainly 7-keto cholesterol: average concentrations were 0,6 mg/kg in muscle and 0,9 mg/kg in fat. Data are in good agreement with those already published for other kinds of hams.
The volatile fraction too was analysed on the same samples, and results demonstated that aldehydes and ketones, were present as in Parma ham, but unlike Spanish ham.

Key-words. Raw ham, lipid oxidation, volatile compouds, GC-MS analysis.

Introduction. Development of aroma in meat foodstuff products is a complicated process involving mechanisms not yet explained because of the high number of reactions involved.

Generally, typical aroma compounds arise from chemical reactions, such as Maillard, Strecker degradation and others.

The lipid fraction can undergo oxidative rancidity that on one hand is useful for the development of typical aromas, but on the other, has as side

* Department of Food Science, University of Udine, Italy.
E-mail: ornella.boschelle@uniud.it

and sometimes main effects, the formation and accumulation of potentially toxic products. Carbonylic compounds responsible for flavour (but also off-flavours), originate from fatty acid hydroperoxide, however also compounds other than fatty acids can meet the same fate and accumulate then oxidized products, among these particularly studied are products of cholesterol oxidation (COPs).

The first evidence of the toxiticy of COPs was in 1976, thanks to Tayor and Lee's work. These authors fed rabbits with purified and oxidized cholesterol and showed that it caused derious damage to arteries, angiotoxicity and arteriosclerosis. The products "incriminated" were triol and 25-hydroxy-cholesterol.

Other COPs, important toxicologicaly were identified and are: 5a6a-epoxy 5b6b-epoxy 7a hydroxyl and 7b hydroxyl 7-keto and 3,5-dien7-one cholesterol.Actually more tha 80 different COPs have been reported in literature. In figure 1 formulas of some of these compounds are reproduced.

Materials and methods. On the basis of research work in the literature, we evaluated raw ham oxidation as follow:

– At slaughter, in the group of pigs from which the haunch is collected, samples of muscle *longissimus dorsi* and subcutanaeus and intermuscolar fat and of lean meat were obtained for analysis of oxidative status (cholesterol oxides, volatile substances profile).
– Analysis of the volatile fraction of the product using Head Space Solid Phase Micro Extraction (HS-SPME) analysis by gas chromatography coupled with mass spectrometry.
– Analysis of oxidation compounds of cholesterol by extraction of the lipid fraction, separation of the unsaponifiable fraction, purification, derivatization and gas-chromatographic analysis coupled with mass spectrometry as the scheme reported in Figure 2.

A preliminary investigation was carried out on samples obtained from the market, with the aim of verifying the presence of COPs particularly in sliced ham preserved in the light.

We applied coordinated chromatographic techniques such as solid phase extraction (SPE), capillary gas-chromatography (CGC) coupled with mass spectrometer (GCMS). The research developed measured the concentration of the following compounds: 7-hydroxy-cholesterol, 7-keto-cholesterol, 3,5,6 triol-cholesterol, 5-6epoxy-cholesterol.

For each compound we achieved identification by GC-MS, recovery, and analytical repeatability, were evaluated and procedures were optimized within this frame.

Because of poor homogeneity of the sample, a number of problems affected repeatability of measurements, this problem was solved by carrying out replicated measurements which results were then averaged. Sampling was carried out on lean and fat Analysis of the volatile fraction was carried out by HS-SPME, according to the scheme reported in Figure 3.

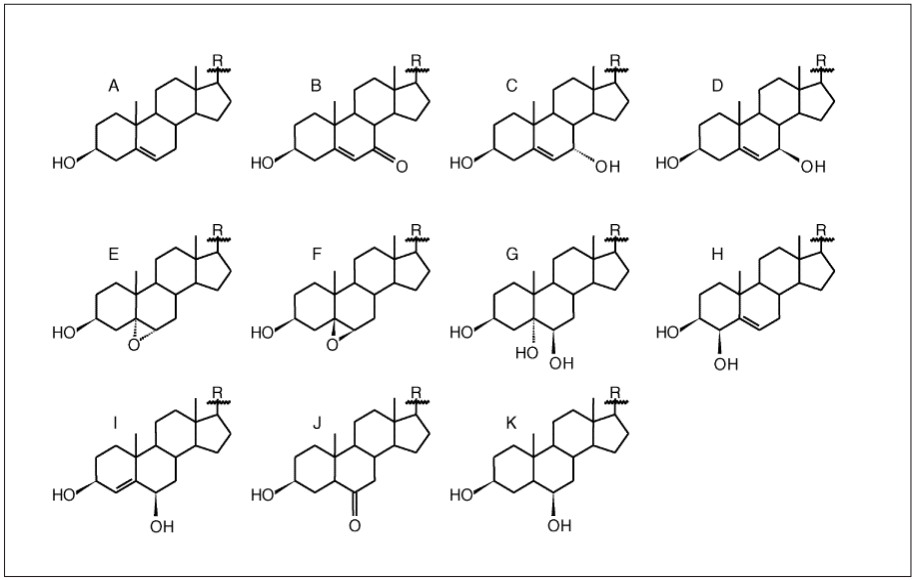

Figure 1. Structural formula of some COPs: A cholesterol, B 7 keto, C e D 7 hydroxy, E e F 5,6 epoxy, G 3,5,6 triol, H 4 hydroxy, J 6 keto, K 6 hydroxycolestane.

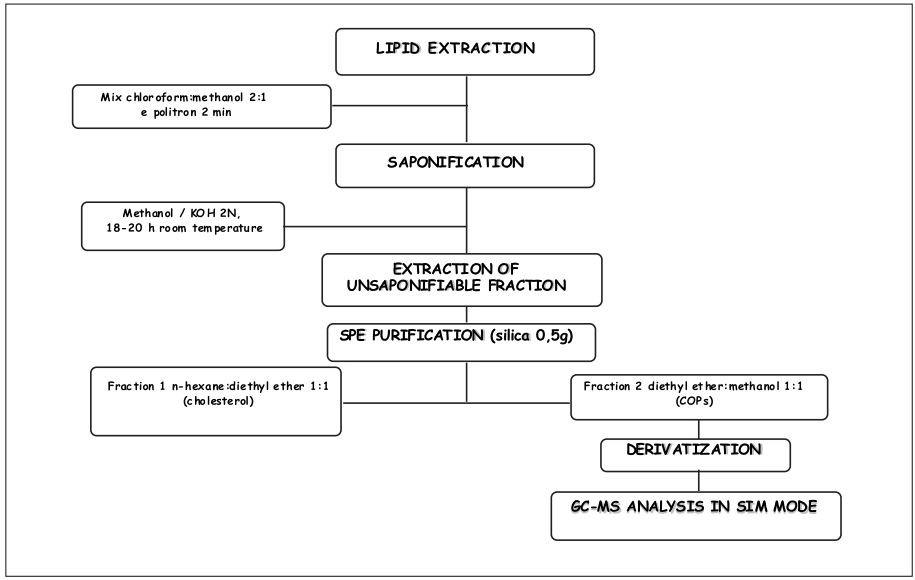

Figure 2. Scheme of analytical protocol used for determination of cholesterol oxides.

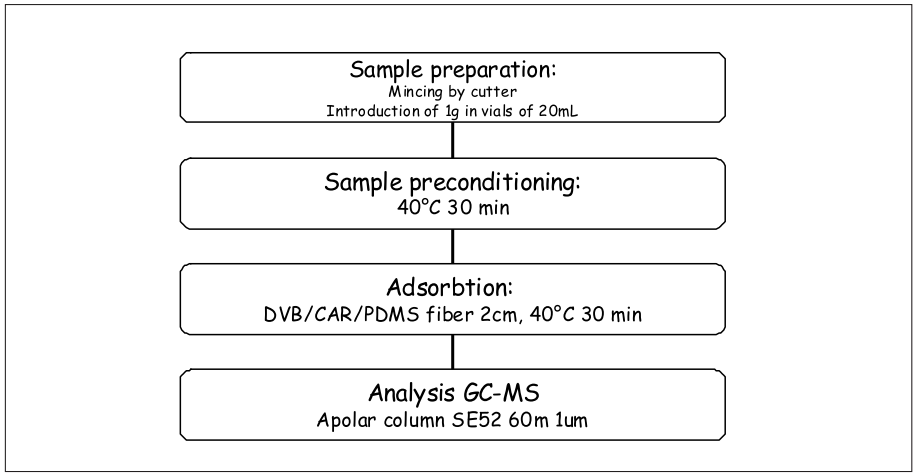

Figure 3. Analytical protocol used for volatile fraction analysis.

Results: COPs. In Tables 1-5 are reported histograms for COPs (microgram/kg of sample) both lean and fatty portions.

On the whole, as was somewhat expected, the data obtained show COPs concentration highter, in particular 7-ketocholesterol, in the fatty

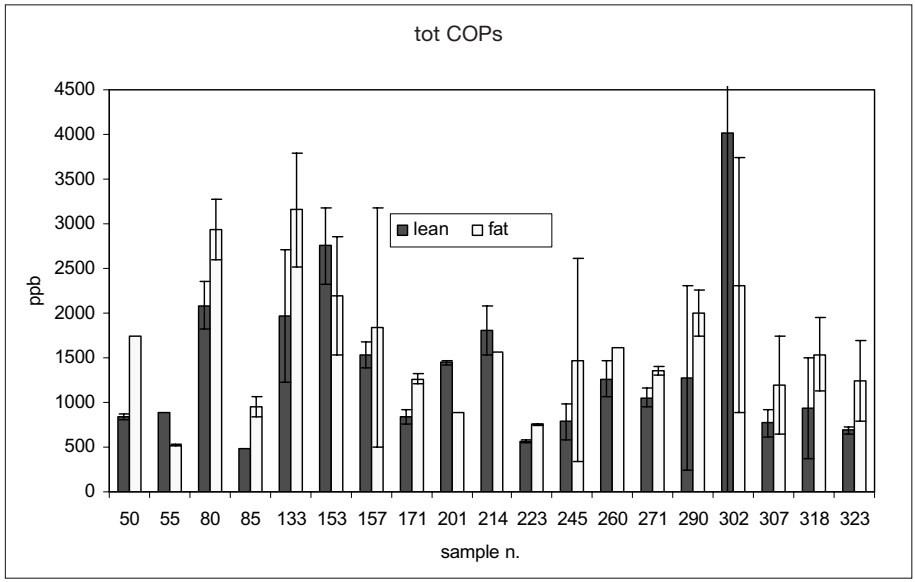

Table 1.

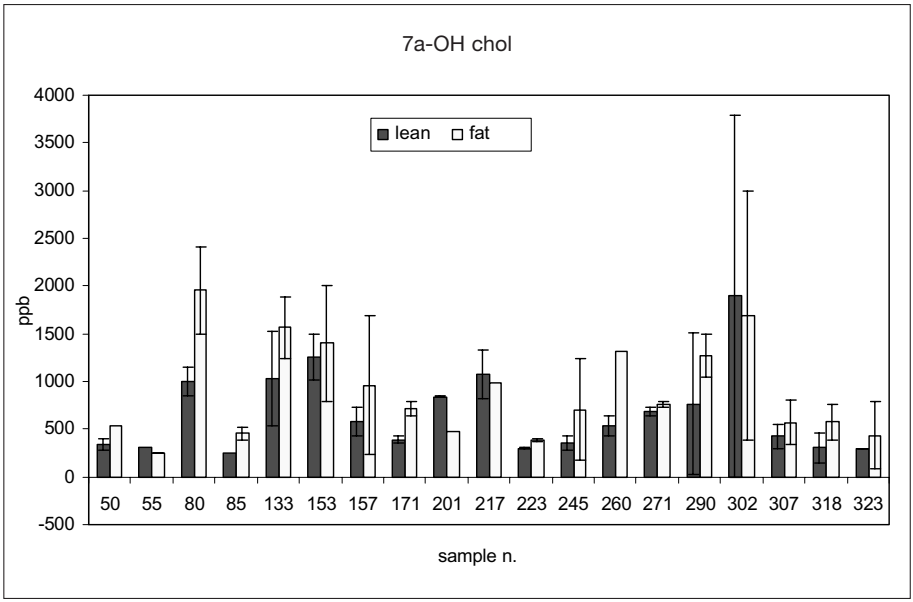

Table 2.

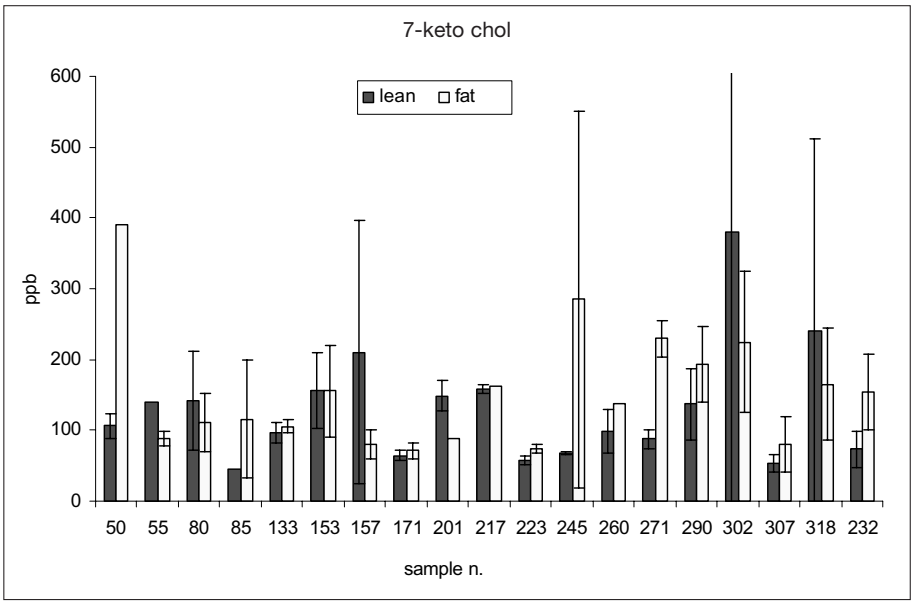

Table 3.

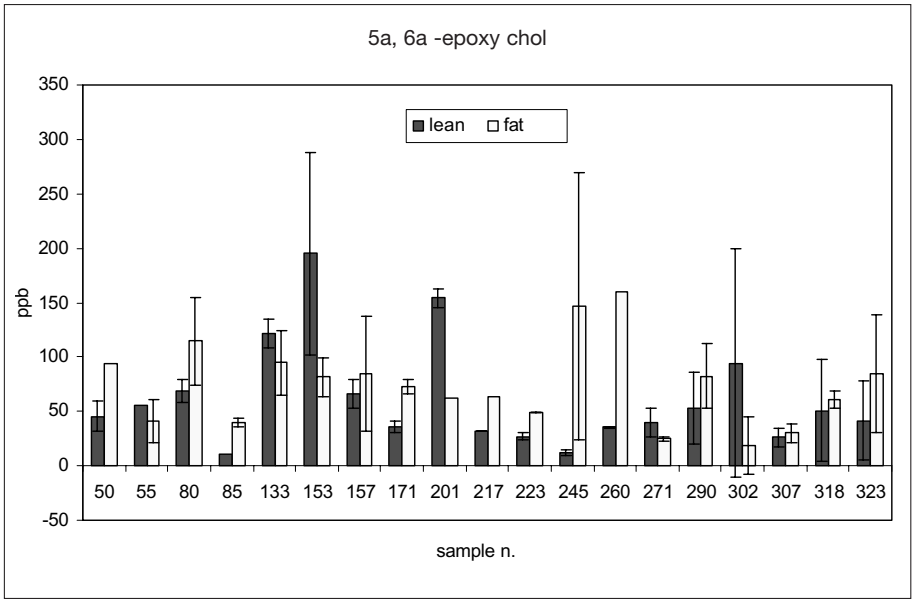

Table 4.

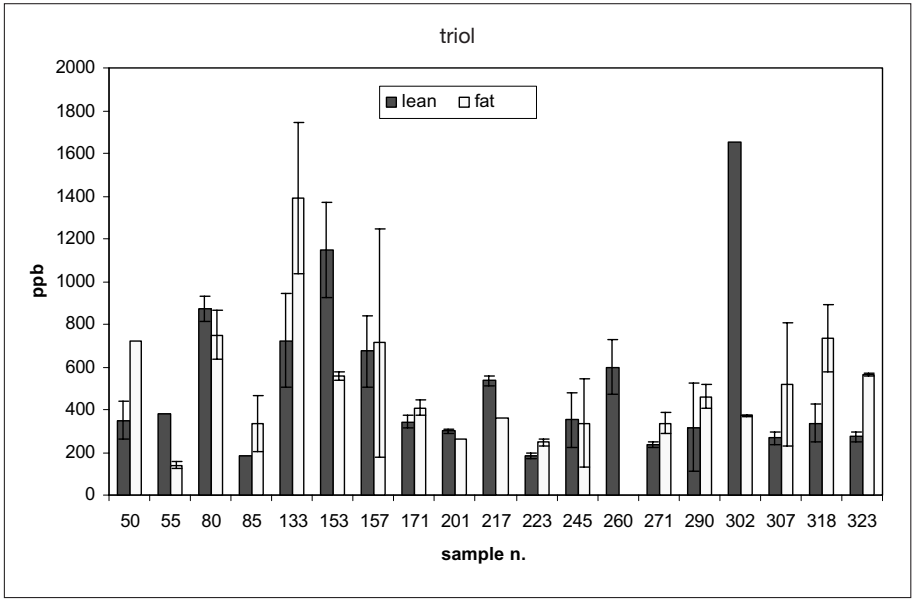

Table 5.

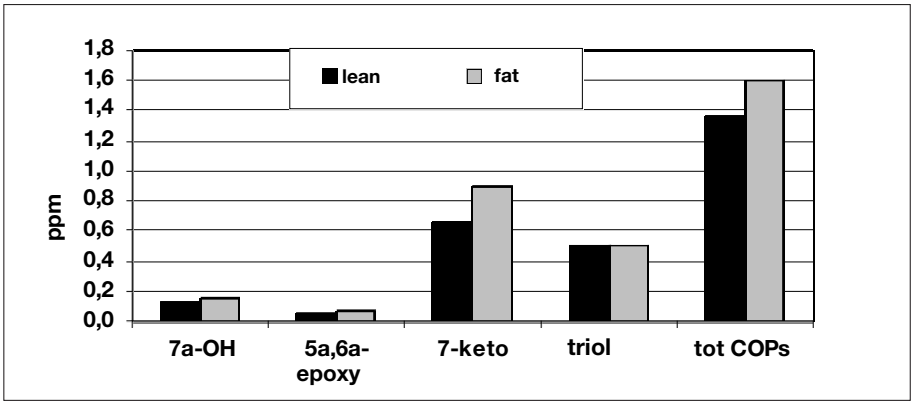

Figure 4. Average content of COPs analyzed.

fraction of tissue, in comparison to the lean fraction.

Concentration of COPs are reported in the literature from 1 to 5mg/kg in raw ham and from 0,5 to 2 mg/kg in pork meat. Samples studied in this work, show a value of 7-keto-cholesterol ranging from 0,6 to 0,9 mg/kg and total COPs value from 1,4 to 1,2 mg/kg as shown in figure 4.

Future research will verify if correlations exist between COP level found in this phase and peculiar characteristics of the seasoned product.

Results: Volatile fraction. In this first phase we optimized the analytical method, on the basis of an experimental protocol reported in figure 3. We tested different conditions particularly as regards sampling in relation to mincing or not of the sample, the relation weight of the sample vs volume of the vial, time of the sample balancing in the vial, time of exposure of the fiber in the head space. The method so optimized will be applied in the second part of the research on seasoned products. In this first phase we used the method on samples of ham obtained from the marked in order to verify the situation in the product purchased by consumers. In Figures 5 and 6 are represented the plots of a freshly sliced ham and one with a higher level of oxidation.

The composition points out an increase in carbonyl compounds (aldehydes) bound with oxidation of fatty acids. Besides the evidence of the oxidative level, the aromatic compound profile has been associated with typology of the product. A preliminary investigation of data obtained seems to show for San Daniele a substantial preponderance of aldehydes and ketones as was already revealed in Parma ham and unlike Spanish ham.

Conclusions. The SPME technique coupled with mass spectrometry highlight volatile compounds present

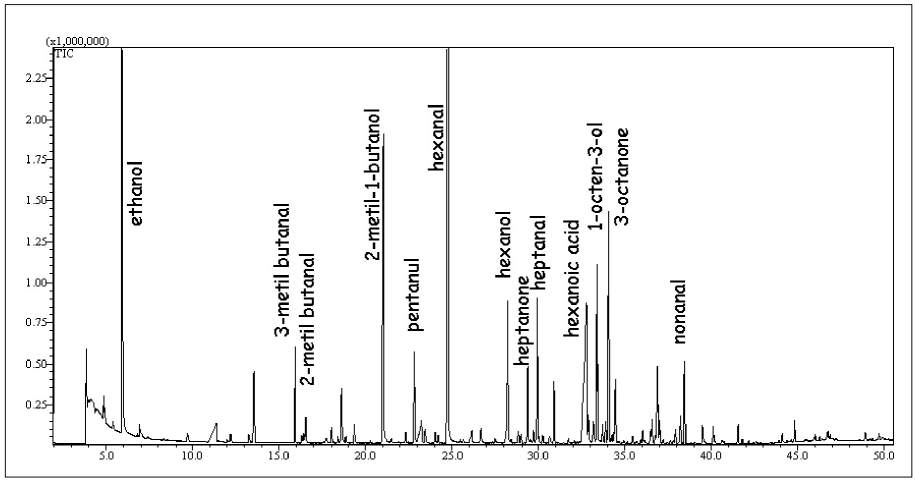

Figure 5. GLC-MS trace of the head space of a raw fresh ham.

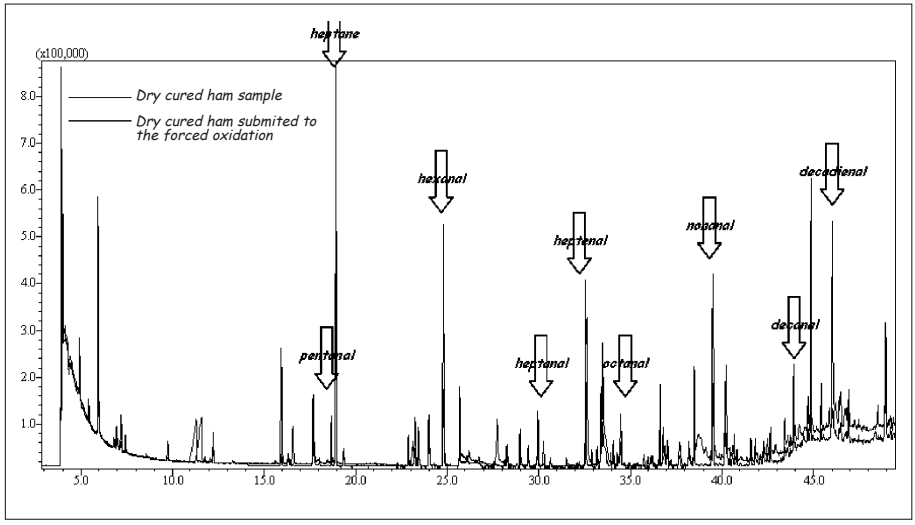

Figure 6. GLC-MS trace of the head space of a raw ham following oxidation.

in San Daniele ham individualizing aldehydes responsible unpleasant odours that indicate decay of product. Measuring oxidation products of cholesterol in raw materials showed low values, a finding that enter the range of those published in the literature for products of the same type.

Bibliografie/ References

Adamsen E.C., Moller K.S.J., Laursen K., Olsen K., Skibsted H.L. (2005). Zn-porphyrin formation in cured meat products: Effect of added salt and nitrite. *Meat Science*, 72: 672-679.

Carrapiso A.I., Bonilla F., Garcìa C. (2003). Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. *Meat Science*, 65: 623-629.

Andrès A.I., Cava R., Ventanas J., Thovar V., Ruiz J. (2004). Sensory characteristics of Iberian ham: influence of salt content and processing conditions. *Meat Science* 68: 45-51.

Antequera T., Caro A., Rodrìguez P.G., Pèrez T. (2007). Monitoring the ripening process of Iberian ham by computer vision on magnetic resonance imaging. *Meat Science*, 76: 561-567.

Baggio S.R., Rauen Miguel A.M., Bragagnolo N. (2005). Simultaneous determination of cholesterol oxides, cholesterol and fatty acids in processed turkey meat products. *Food chemistry*, 89: 475-484.

Careri M., Mangia A., Musci M., Parolari G. (1998). Development of Gas Chromatographic Method for Determination of Cholesterol Oxides in Meat Products. *Chromatographia*, 47, 1/2.

Chizzolini R., Novelli E., Zanardi E. (1998). Oxidation in Traditional Mediterranean Meat Products. *Meat Science*, 49: S87-S99.

Coutron-Gambotti C., Gandemer G., Rousset S., Maestrini O., Casabianca F. (1999). Reducing salt content of dry-cured ham: effect on lipid composition and sensory attributes. *Food Chemisry*, 64: 13-19.

Countron-Gambotti C., Gandemer G. (1999). Lypolysis and oxidation in subcutaneous adipose tissue during dry-cured ham processing. *Food Chemistry*, 64: 95-101.

Ferrari G., Lambertini P., Manzini D., Marchetti A., Sighinolfi S., Della Casa G. (2007). Evaluation of the oxidation state and metal concentration in the adipose tissue of Parma ham. *Meat Science*, 75: 347-352.

Flores M., Gianelli M.P., Pèrez-Juan M., Toldrà F. (2007). Headspace concentration of selected dry-cured aroma compounds in model systems as affected by curing agents. *Food Chemistry*, 101: 1588-1593.

Gilles G. (2002). Lipids muscles and adipose tissues, changes during processing and sensory properties of meat products. *Meat Science*, 62: 309-321.

Garcia-Esteban M., Ansorena D., Astiasaran I., Ruiz J. (2004). Study of the effect of different fiber coatings and extraction conditions on dry cured ham volatile compounds extracted by solid-phase microextraction (SPME). *Talanta*, 64: 458-466.

Garcia Regueiro J.A., Maraschiello C. (1997). Procedure for determination of eight cholesterol oxides in poultry meat using on-column and solvent venting capillary gas chromatography. *Journal of Chromatography*, 764: 279-293.

Imai H., Werthessen N.T., Taylor C.B., Lee K.T. (1976). Angiotoxicity and arteriosclerosis due to contaminants of USP – grade cholesterol. *Archives of Pathology and Laboratory Medicine*, 100: 565-575.

Luna G., Aparicio R., Garcìa-Gonzàlez D.L. (2006). A tentative characterizazion of white dry-cured hams from Teruel (Spain) by SPME-GC. *Food Chemistry*, 97: 621-630.

Jerkovìc I, Mastelic' J., Tartaglia S. (2007). A study of volatile flavour substances in Dalmatian traditional smoked ham: Impact of dry curing and frying. *Food Chemistry*, 104: 1030-1039.

Muriel E., Antequera T., Petròn M.J., Andrès A.I., Ruiz J. (2004). Volatile compounds in Iberian dry-cured loin. *Meat Science*, 60: 391-400.

Muriel E., Andres A.I., Petròn M.J., Antequera T., Ruiz J. (2007). Lipolytic and oxidative changes in Iberian dry-cured loin. *Meat Science*, 75: 325-333.

Novelli E., Zanardi E., Ghiretti G.P., Campanini G., Dazzi G., Madarena G., Chizzolini R. (1998). Lipid and Cholesterol Oxidation in Frozen Stored Pork, Salame Milano and Mortadella. *Meat Science*, 48, 1/2: 29-40.

Pastorelli G., Magni S., Rossi R.,.Pagliarini E., Baldini P., Dirinck P., Van Opstaele F., Corino C. (2003). Influence of dietary fat, on fatty acid composition and sensory properties of dry-cured Parma ham. *Meat Science*, 65: 571-580.

Sabio E., Vidal-Aragon M.C., Bernalte M.J., Gata J.L. (1998). Volatile compounds present in six types of dry-cured ham from south European countries. *Food Chemistry*, 61, 4: 493-503.

Schivazappa C., Degni M., Nanni Costa L., Russo V., Buttazzoni L., Virgili R. (2002). Analysis of raw meat to predict proteolysis in Parma ham. *Meat Science*, 60: 77-83.

Ventanas S., Estevez M., Andrès A.I., Ruiz J. (2008). Analysis of volatile compounds of Iberian dry-cured loins with different intramuscolar fat contents using SPME-DED. *Meat Science*, 79: 172-180.

Vestergaard C.S., Parolari G. (1999). Lipid and cholesterol oxidation products in dry-cured ham. *Meat Science*, 52: 397-401.