

## Sterols, Sterol Oxides and CLA in Typical Meat Products from Pigs Fed with Different Diets

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**Abstract:** The effect on sterol stability and CLA content in meats from pigs fed with diets having different contents of oleic and linoleic acid was studied, considering typical Italian products seasoned with or without nitrates and ascorbates. Results showed that the increase of oleic acid in the diet leads to seasoned products with higher contents of oleic acid and lower content of linoleic acid and CLA. A different partition of sterols and sterol oxides was observed in the fat and in the muscle of all the products, with slightly higher amounts of cholesterol and sterol oxides in the muscle. Meat products seasoned in presence of ascorbates showed slightly lower amounts of sterol oxides in respect to those added with nitrates. The statistical treatment of the data showed that fatty acids distribution allow to discriminate between meats from different diets.

**Keywords:** sterol oxides; CLA; oleic acid; pigs; diet

### INTRODUCTION

Several studies have demonstrated the possibility of influencing the composition of the lipids of meat (DAZA *et al.* 2005; OLIVARES *et al.* 2009) and other foods such eggs (AYERZA & COATES 2001), by feeding animals with diets richer of polyunsaturated fatty acids, mainly to improve the nutritional value, increasing the food content of n-6- and n-3-fatty acids and also of conjugated linoleic acid (CLA). Moreover, it is known that cholesterol oxidation can be influenced by diets and by meat processing (ZANARDI *et al.* 2000; PETRON *et al.* 2003; PIESZKA 2007). So, the aim of this work was to determine the effect of diets rich in oleic or in linoleic acid on the fatty acids, in particular CLA, of pork meats, and to evaluate the influence on cholesterol oxidation. Effects were checked on a series of typical seasoned products of the Parma and Piacenza regions (Italy): Pancetta, Coppa and Culatello, obtained from different anatomic parts of the pork and consequently with different lipid contents. A series of meat products seasoned in presence of nitrates and/or ascorbates were also analysed, in order to control the effect of these additives on the cholesterol oxidation.

### MATERIALS AND METHODS

**Samples.** A total of 64 seasoned pork meat samples were analysed, 24 of Pancetta, 24 of Coppa and 16 of Culatello. Meat samples were from half-cast pigs (Duroc × Large White) fed with four different diets (D1, D2, D3, D4), having different contents of linoleic and oleic acid obtained by adding maize varieties with increasing linoleic acid content: low (D1), medium (D2), high (D3), and a variety of sunflower oil rich of oleic acid (D4). Pancetta and Coppa samples were seasoned for 3 months, after adding sodium nitrate (150 ppm, series A), or sodium ascorbate (1000 ppm, series B1 and B2). Samples of Culatello were seasoned for 10 months, part with 150 ppm of nitrates (A1) and part without nitrates (A0).

**Sample preparation.** Meat samples (100 g) was homogenised, and fat extracted according to Folch method. For Coppa and Culatello, analyses were done both on muscle and fat fractions. Analysis of fatty acids and sterols. 0.2 g of fat were dissolved in 5 ml hexane and transesterified with 2 ml of 5% KOH in methanol. The hexane phase was recovered, added with 1 ml stigmasterol (50 ppm) as internal standard for sterols, and fractionated

on a silica gel cartridge (2 ml), eluting first with hexane to recover fatty acid methyl esters (fraction 1) and subsequently with ethyl acetate for sterols and oxysterols (fraction 2). Fraction 1 was concentrated and analysed by GC-MS on an Agilent instrument (GC-6890, MSD-5973A), scan mode ( $m/z$  50–500) and split (1:20), with a DB5 capillary column (J&W, 30 m  $\times$  0.25 mm), temperature increasing from 120°C to 280°C. Fractions 2 were evaporated to dryness, recovered with 0.6 ml hexamethyldisilazane (HMDS) and 0.3 ml trimethylchlorosilane (TMCS), heated 5 min at 60°C and analysed by GC-MS, under the following conditions: DB5 column (30 m  $\times$  0.25 mm), temperature program from 240 to 290°C, splitless (0.5 min), and SIM mode with  $m/z$ : 368, 458, 382, 472, 456, 484, 396, 486.

**Analysis of CLA.** Fraction 1 was fractionated with hexane/ether (8/2, v/v) on a preparative silica gel plate (Merck 20  $\times$  20 cm) treated with a 10% silver nitrate solution in acetonitrile. The fractions of monounsaturated methyl esters containing CLA and of linoleates containing CLnA, detected with

Rhodamine B, were recovered with ethyl acetate by the scraped silica, added with an internal standard (C25-methyl ester) and analysed by GC-MS split (1/20), both scan ( $m/z$  41–400) and SIM ( $m/z$ : 55, 67, 79, 87, 292, 294). A Supelco SLB column (30 m  $\times$  0.25 mm) was used with temperature from 120°C to 270°C.

## RESULTS AND DISCUSSION

**Fatty acids.** The series of 24 more important peaks, from capric acid (C10) to the C22 series, recorded as area percentages for all the 64 samples, showed major contents of oleic acid for samples marked D1 (39.7%) and D4 (41.6%) than for D2 (39.5%) and D3 (38.0%), richer in linoleic acid. The fatty acids compositions, elaborated with multivariate statistical methods (discriminant analysis), allowed to distinguish between the four different diets as reported in Figure 1 for Pancetta samples. Analogous results were obtained for Coppa and Culatello. The most important variables contribut-

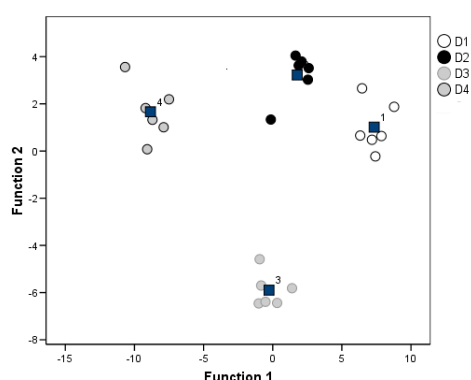


Figure 1. Discriminant analysis on fatty acids percentage composition for Pancetta samples

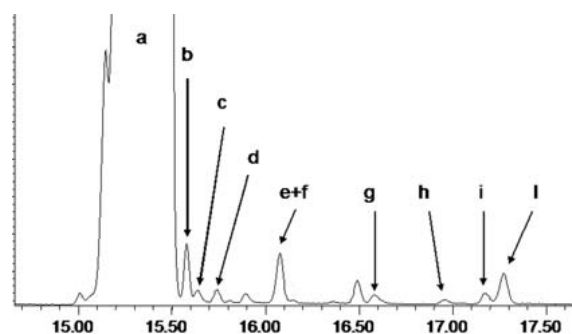


Figure 2. Enlargement of the GC-MS profile of monounsaturated fatty acids of Culatello, containing also CLA (a) 18:1 ( $\Delta$ 9); (b) 18:1 ( $\Delta$ 11); (c) 18:1; (d) 18:1; (e) CLA C/T; (f) T/C; (g) C/C; (h) T/T; (i) 19:1 ( $\Delta$ 9); (l) 19:1 ( $\Delta$ 11)

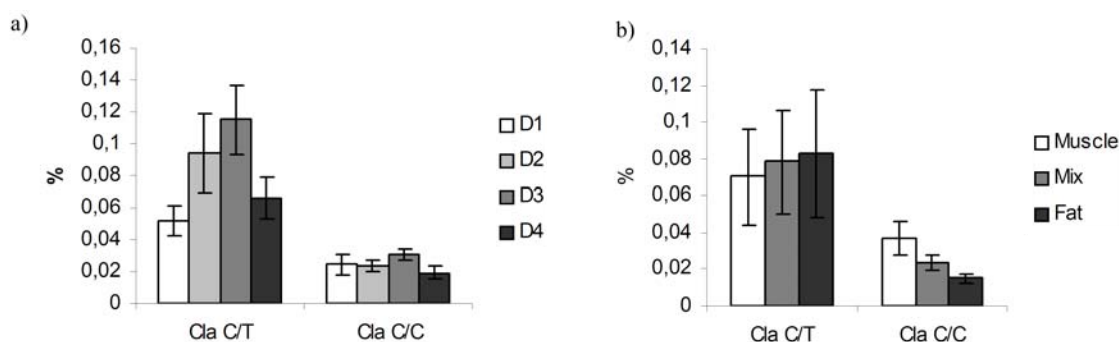


Figure 3. Variation of CLA isomers due to: (a) the different diets and (b) the meat tissue

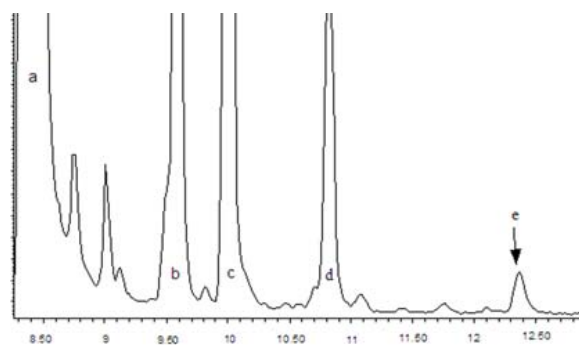


Figure 4. GC-MS profile of sterols and sterol oxides in a meat sample: (a) cholesterol; (b) campesterol; (c) stigmasterol (i.s.); (d)  $\beta$ -sitosterol; (e) 7-ketocholesterol

ing to discrimination were, as expected, oleic and linoleic acids, but also pentadecanoic, margaroleic and palmitic acids.

**CLA.** The fractionation of methyl ester mixture, executed on Culatello, allowed to recover a band richer on CLA, eluting together with monounsaturated methyl esters (Figure 2).

Results show an interesting total content of CLA (0.10% of meat lipids), unexpected for non ruminants. The total content of CLA is slightly influenced by the diet: increasing contents of linoleic acid from D1 to D3 lead to enrichments in CLA (Figure 3a). The prevalent isomer in the fat fraction was the 9c-11t-CLA; the other isomers (mainly 9c-11c) were more evident in the muscle fraction (Figure 3b), reaching the 30% of the total content of CLA.

The oleic fraction contained also an unknown conjugated eicosadienoic acid and the fraction richer in linoleic acid showed small amounts of two conjugated linolenic acids (CLnA).

**Sterols and sterol oxides.** the GC-MS procedure allowed to well determine cholesterol,  $\beta$ -sitosterol, campesterol and the oxide 7-ketocholesterol (Figure 4).

Diets seem not influence the total content of cholesterol in the samples, even if lower amounts were detected in Culatello samples from pigs fed with diets richer in linoleic acid. Moreover, Coppa and Culatello muscles were, on average, slightly richer of cholesterol than the fatty fraction. The main secondary sterols were  $\beta$ -sitosterol and campesterol; the first is mainly accumulates in the fat, while campesterol, surprisingly, in the muscle, as cholesterol (Figure 5).

The levels of 7-ketocholesterol (ppm) were slightly higher in the more seasoned Culatello than in

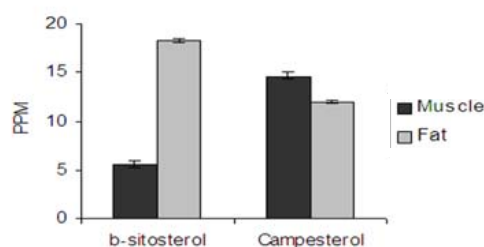


Figure 5. Distribution of phytosterols in fat and muscle of Culatello products

Table 1. 7-Ketocholesterol content (ppm) in meat samples with different additives addition (A0, no addition; A1, nitrates addition; B1 and B2 ascorbate addition). Values are reported as a mean of all the samples analysed

	A0	A1	B1	B2
Coppa	–	0.5 ± 0.2	0.3 ± 0.2	0.3 ± 0.1
Pancetta	–	0.6 ± 0.5	0.5 ± 0.3	0.7 ± 0.3
Culatello	1.8 ± 0.6	1.3 ± 0.6	–	–

Coppa and Pancetta, and were partially reduced, at least in coppa samples, by seasoning in presence of ascorbates (B1 and B2 samples, Table 1).

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