ION CHROMATOGRAPHY AS A TOOL TO CHARACTERIZE ENDOGENOUS ANTIOXIDANTS IN MEAT AND MEAT PRODUCTS

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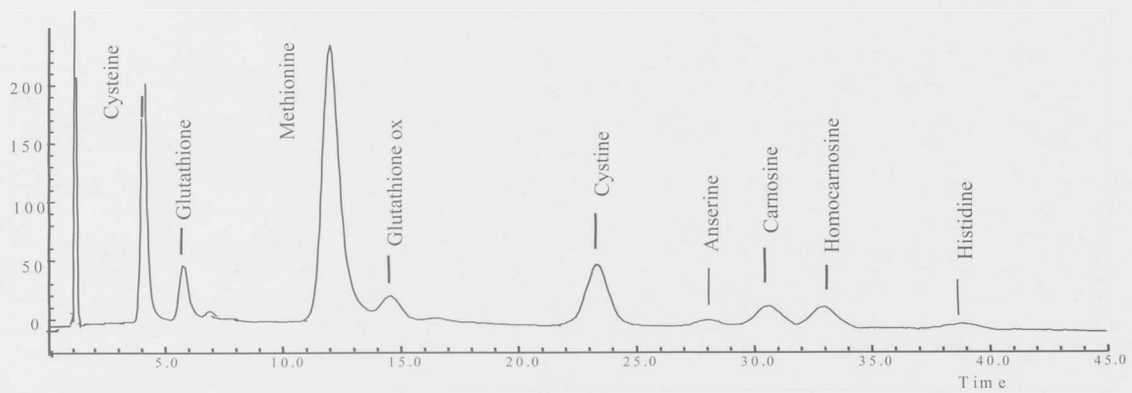
Background:

Oxidation is a leading cause for quality deterioration during processing and storage of muscle foods. Susceptibility of meat to oxidative processes comes from the relatively high content of unsaturated lipids, heme pigments, metal catalysts and many prooxidant agents present in muscle tissue (1). Quality deterioration in oxidized meat products are generally characterized by colour and flavour deterioration, loss of nutritive value and sometimes by production of toxic compound.
To prevent or delay oxidation reactions, several endogenous antioxidant systems are found in muscle tissue. (2-3)

Objectives:
The aim of this study was to characterize fresh meat cuts (*m.Longissimus dorsi* and *m. Masseter*) and Italian dry-cured ham (Parma ham) in terms of endogenous antioxidants (sulphur amino acids, dipeptides containing histidine and glutathione) and to evaluate changes of muscle antioxidants as affected by processing and ageing phases.

Methods:
A chromatographic method for the simultaneous determination of endogenous antioxidants in meat and meat products has been developed. Determination of thiols and peptide antioxidants was performed by cation-exchange chromatography with isocratic elution and integrated pulsed amperometric detection (IPAD). The analytes were extracted from the muscle with acid buffer solution (pH=2.5) without additional derivative step or sample clean-up. (4)
Separation of analytes was achieved in 45 minutes as shown in Fig. 1.

Fig.1: Chromatogram of a standard solution containing amino acids and peptides antioxidants



The analytical method was used to characterize endogenous antioxidants in fresh meat and their changes during maturation. Muscles *masseter* and *longissimus dorsi* (N= 24) were removed from heavy pig carcasses at slaughter-house and from hams (N=37) at various phases of the process (green state, end of salting, end of resting , half maturing, 12-months aged, 15-months aged and 18-months aged). Proximate composition and endogenous antioxidants were determined. (4)

Results and discussion:
Content of endogenous antioxidants are reported in table 1 according to muscles under investigation (white *L. dorsi* and red *Masseter*). Data show that muscle type has a significant effect ($p < 0.05$) on peptides content, with carnosine and homocarnosine being significantly lower ($p < 0.05$) in oxidative m. *Masseter*, while glutathione is higher (Fig.1). These results are in agreement with the reported data (5- 6) and the difference observed in oxidative stability between oxidative and glycolitic muscles (red muscles are oxidized more rapidly than white ones) might be explained by their different peptide content. It is noteworthy that the ratio between glutathione oxide and total glutathione was correlated to sensory colour ($r= - 0.65$), indicating that a major meat quality, such as visual redness, may be affected by the relative content of these antioxidants.

In table 2 the antioxidant content of dry cured hams s are reported according to their age. To compare hams at different processing stages, hence different shrinkage, the concentrations are referred to the meat. ANOVA treatment of data shows that during the process there is no significant depletion of carnosine and homocarnosine, while glutathione seems to be affected mainly during the salting and the resting phases. Glutathione decreases more rapidly than histidine-containing dipeptides and its depletion is complete after prolonged ageing (>15 mounths). The bar graph in fig.2 shows the relationship between glutathione depletion and glutathione oxide increase during the dry-curing process.

Conclusion:

The analytical method proposed in this study can provide useful information about endogenous antioxidants in meat and meat products. In fresh meat, histidine-containing dipeptides and glutathione are closely related to the metabolic muscle type, with the latter prevailing in oxidative red muscle fiber.

It appears that skeletal muscle contains a multicomponent antioxidant system that can effectively cope with oxidative reactions occurring during processing or storage. Therefore, the absence of added antioxidant in Parma ham might explain glutathione loss in the early phases of curing, while carnosine would account for extend protection against oxidation in the latest phases of ageing. Understanding how meat processing can affect natural antioxidants content can help to develop technologies able to protect meat products from the oxidative damage.

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Tab. 1: Endogenous antioxidants in fresh pork meat.

ENDOGENOUS ANTIOXIDANT	Units	M. *Longissimus dorsi*	M. *Masseter*
Cysteine	mg/kg	59 ± 11	63 ± 3
Glutathione GSH	mg/kg	201 ± 55*	265 ± 34*
Methionine	mg/kg	78 ± 27	68 ± 5
Glutathione oxide GSSG	mg/kg	61 ± 44	49 ± 22
Carnosine	mg/kg	6755 ± 630*	3109 ± 940*
Homocarnosine	mg/kg	392 ± 84*	201 ± 33*

(*) denote significant difference along row $p < 0.05$

Tab. 2: Antioxidants in fresh meat and dried hams at several ageing stages. Data in mg/kg muscle.

Ageing phase	Cysteine	Glutathione	Methionine	Glutathione ox	Carnosine	Homocarnosine
fresh	3.4 ± 0.3	8.4 ± 1.5 *[a]	3.5 ± 1 *[a]	< 0.01	221 ± 40	19 ± 3 *[a]
salting phase	3.2 ± 0.3	8.0 ± 1.4 *[a]	3.3 ± 0.8 *[a]	2.3 + 0.1 *[a]	204 ± 29	18 ± 3 *[a]
resting phase	3.2 ± 0.2	1.9 ± 0.6 *[b]	53.5 ± 6.3 [b]*	3.2 + 0.3 *[b]	194 ± 39	13 ± 1 *[b]
half ageing	4.6 ± 1.2	1.4 ± 0.1 *[c]	52.0 ± 9.6 *[b]	3.7 + 0.2 *[b]	214 ± 21	12 ± 2 [b]*
12 months	4.4 ± 1.8	0.6 ± 0.7 *[d]	60.0 ± 8.0 *[b]	5.0 + 1.1 *[c]	220 ± 18	10 ± 2 *[b]
15 months	4.9 ± 1.0	< 0.01	69.4 ± 5.8 *[b]	4.0 + 0.6 *[c]	242 ± 13	11 ± 1 *[b]
18 months	4.5 ± 1.5	< 0.01	61.5 ± 9.1 *[b]	4.6 + 1.4 *[c]	201 ± 9	13 ± 3 *[b]

Different superscripts along columns denote significant difference * = $p < 0.05$

Fig.2:Endogenous antioxidants muscle content.

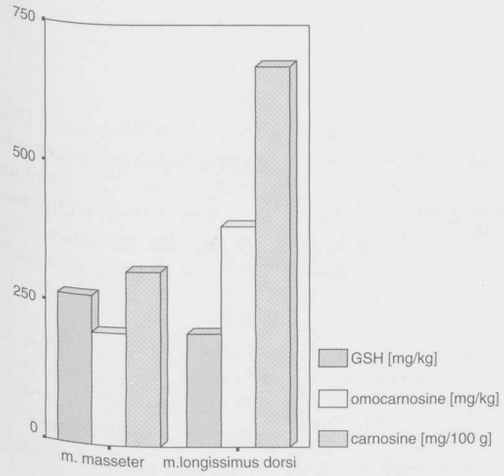

Fig.3: Glutathione and Glutathione oxide changes in dry cured ham during ageing stages.