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ANTIOXIDANTS AND OXIDATIVE PROCESSES OF LIPIDS AND CHOLESTEROL IN A DRY FERMENTED MEAT PRODUCT (SALAME MILANO)

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Background

Oxidative phenomena, and lipid autoxidation in particular, play important roles (positive and negative) in quality features of meat and meat products. Rancidity and flavour are just the two better known facets of the relationship. Oxidation of lipid moieties, and among them cholesterol, appears to be important also for human health since cholesterol oxides and lipid free radicals are involved in the pathogenesis of various chronic metabolic diseases (Kubow, 1990, Guardiola et al., 1996). Dry fermented sausages make up a significant part of meat products in many parts of the world and their production technology entails phases (mincing, mixing with salt, maturing) which could promote or facilitate oxidation. Moreover, such products make use, almost without exceptions, of nitrates and nitrites, a class of additives which has been under criticism in particular for the alleged link with nitrosamine production. Nitrates and nitrites are involved in oxidation-reduction reactions in meat products as clearly exemplified by the chemical pathways known to bring to the formation of nitrosomyoglobin in cured products (Freybler et al., 1993; Skibsted, 1992). Sodium nitrite, per se a strong oxidant, in cured meats has antioxidant activity through the production of nitric oxide (Kanner et al., 1984; Skibsted, 1992) and a number of investigations have reported its positive effects in controlling rancidity and off flavours development in cured meat products (Freybler et al., 1993; Igene et al., 1979a; Morrissey and Tichivangana, 1985). The effort for safer and more tasty meat products needs to strike a balance between negative and positive effects on lipid stability or degradation in fermented meat products processing.

Objectives

The research, of which some preliminary results are here presented, was intended to clarify the antioxidant role of nitrates and nitrites, used in combination with or without ascorbic acid, in relation with lipid oxidative processes in a dry fermented meat product. Lipid oxidation would be looked at in a comprehensive way covering the rancidity (TBARS), the flavour (aldehydes) and the toxic (cholesterol oxides) aspects. Only part of the results dealing with TBARS and cholesterol oxides are reported.

Methods

A set of batches of salame Milano was produced according to standard technology with 72% pork shoulder, 28% jowl, 2.5% NaCl and 2.8% skimmed milk. The starter cultures were the same for all batches. The batches differed for types and amount of additives in the following way: (*A*) sodium nitrite (80ppm), potassium nitrate (120ppm), ascorbic acid (0.03%), sucrose (0.5%), dextrose (0.3%), black pepper (0.07%), white pepper (0.03%) and garlic (0.01%); (*B*) the same as (*A*) but without spices (i.e., pepper and garlic); (*C*) without spices and nitrate; (*D*) without spices, nitrate and nitrite; (*E*) without spices, nitrate, nitrite and ascorbic acid; (*F*) only with 150ppm nitrite; (*G*) only with 250ppm nitrate. The number of batches was 5 for the normal formulation (*A*) and 2 for each of the other formulations. Total processing time was 40 days. Residual nitrates, nitrites (Slack, 1987), pH (homogenisation with distilled water, 10/1 water/sample), water activity (Aqualab CX/2) and TBARS (Novelli et al., 1998) were determined on the mince just before stuffing and on the matured products. Total cholesterol, cholesterol oxides (Zanardi et al., 1998) and fatty acids oxidation products (aldehydes) were measured in finished products.

Results

The data on pH values (Table 1) of the minces and the matured salami are typical of the so called low-acid mediterranean fermented meat products. Initial pH is above 6.0 due to the presence of the shoulder muscles. Final pH values are on average higher than 5.5 as in this case the amount of sugar added was limited and the acidification process was not encouraged by the type of technology adopted (starter cultures and temperature of the fermentation and drying rooms). The lowest pH values (values not reported) have been found to be in the range of 5.25-5.35 at days 10-15 of processing. Water activity values in matured products were around 0.89-0.90 as is normal for this type of products. The determination of residual nitrites and nitrates has presented some analytical difficulties, especially for nitrates. Residual nitrites in finished products have been found to be lower than 10ppm whereas, in some cases, residual nitrates have been found to be higher than expected.

The measurement of thiobarbituric reactive substances (TBARS) (Table 2) has given values lower than, or near to, 0.5 mgMDA/kg fresh matter in the normal formulation (*A*) and in the formulations in which either nitrate or nitrite were present. Values were significantly higher in the batches with only ascorbic acid (*D*) and, even higher, in batches without any antioxidant molecule (*E*). A value of 0.5mg MDA/kg has been suggested as a threshold for the appearance of rancidity off flavour in fresh pork (Lanari et al., 1995) while a value of 1.0 has been linked with the perception of warmed over flavour in cooked meat (Igene et al.,, 1979b). The absence of spices did not seem to have significant negative effects on stability of lipids towards oxidation as measured by the TBARS value. Cholesterol oxidation has confirmed TBARS data with the difference that batches (*D*) did not differ from all the others whereas batches (*E*) showed a remarkable increase in total cholesterol oxides and, therefore, in the percentage of oxidised cholesterol. The latter, in all batches except (*E*), was in line with the results of previous investigations (Zanardi et al., 1998, Zanardi et al., 1999) in which values for cooked chops and for samples of salame Milano and Parma ham were always lower than or around 0.1%. From the

results obtained, therefore, it appears that ascorbic acid alone, without nitrite or nitrate, can sufficiently control cholesterol oxidation but is not so efficient in stopping secondary oxidation expressed by TBARS values.

Pertinent Literature

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TABLE 1. Average values of pH, Aw, $NaNO_2$ (ppm) and KNO_3 (ppm) of fresh mince and matured salame Milano (mean of duplicates±S.D.) (N.D.: Not Detected)

FORMULATION	MINCE				MATURED SALAME			
	pH	Aw	$NaNO_2$	KNO_3	pH	Aw	$NaNO_2$	KNO_3
A	6.32±0.04	0.949	3.8±0.5	46.9±14.8	5.55±0.06	0.887	0.7±0.1	3.8±1.0
B	6.20±0.00	0.944	46.2±0.3	44.4±1.8	5.46±0.06	0.889	6.1±0.4	75.6±1.0
C	6.11±0.01	0.943	52.6±2.6	6.4±7.4	5.46±0.11	0.896	6.8±0.5	N.D.
D	6.31±0.04	0.954	N.D.	N.D.	5.85±0.10	0.901	N.D.	N.D.
E	6.18±0.00	0.938	N.D.	N.D.	5.44±0.02	0.880	N.D.	N.D.
F	6.20±0.04	0.952	13.1±0.8	13.4±1.3	5.84±0.06	0.901	6.0±0.1	29.1±7.2
G	6.49±0.12	0.964	N.D.	68.1±5.6	5.85±0.03	0.903	8.0±0.7	63.6±0.3

TABLE 2. TBARS (mg MDA/kg fresh tissue), cholesterol (mg/100g) and cholestereol oxides (μg/g) content (mean of duplicates±S.D.) (N.D.: Not Detected) (* only 1 sample)

FORMULATION	MINCE	MATURED SALAME						
	TBARS	TBARS	Cholest.	7β-OHchol.	5,6α-epox.	7-ketochol.	Total COPs	% ox-chol
A	0.20±0.03	0.33±0.12	92.1±1.4	0.11±0.06	0.13±0.05	0.28±0.19	0.52±0.25	0.06±0.03
B	0.16±0.01	0.31±0.02	84.3±0.3	0.11±0.01	0.23±0.03	0.11±0.03	0.45±0.03	0.05±0.00
C	0.46±0.04	0.41±0.04	87.6±2.2	0.16±0.01	0.42±0.08	0.11±0.01	0.69±0.09	0.08±0.01
D	0.21±0.01	0.82±0.03	95.3±2.9	0.16±0.05	N.D.	0.18±0.05	0.33±0.03	0.03±0.00
E	1.23±0.13	1.64±0.11	88.8±3.2	0.97±0.15	0.33±0.08	0.73±0.02	2.02±0.16	0.23±0.02
F	0.18±0.02	0.56±0.02	93.0±1.9	0.22±0.10	N.D.	0.11±0.02	0.33±0.13	0.03±0.01
G	0.23±0.01	0.51±0.01	96.6±3.2	0.21±0.04	N.D.	0.054*	0.23±0.04	0.02±0.00