EFFECT OF CURING AGENTS AND SPICES ON LIPOLYSIS AND OXIDATION IN FERMENTED SAUSAGES

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Background

Fermented meat products make up a distinct class of foods both for their technology and for their quality features. Such products have been the object of intensive studies to clarify the role of raw materials, bacterial cultures and additives used for their manufacture. Lipolysis and lipid oxidation, and their mutual relationship, are among the subjects which have raised the biggest interest in recent years for the importance of such phenomena in regards with safety and flavour production. Mincing disrupts cellular structure and, coupled with NaCl, fosters oxidation. Nitrite, nitrate and ascorbic acid have well known antioxidant effects and spices are credited with similar aptitudes, too. Nitrite and nitrate affect also microbial growth and the two factors (oxidation and microbial growth) have profound influences on lipid changes which, in turn, play major roles in the production of compounds responsible for the flavour of matured sausages (Aguirrezabal et al., 2000; Sanz et al., 2000).

Objectives

A research has been conducted on a typical Mediterranean fermented meat product with the aim of clarifying the effect of nitrite, nitrate, ascorbic acid and spices on the oxidation of fatty acids and cholesterol during processing and of matured sausages sliced and exposed to fluorescent light. To complete the picture, the production of free fatty acids due to processing was controlled to verify possible relationships between the use of the above mentioned additives and lipolysis.

Methods

A set of batches of Milano-type sausage was produced as described by Chizzolini et al. (1999). The batches differed for type and amount of additives according the following plan in which formulation A stood for standard production:

Formulation	$NaNO_2$ (ppm)	KNO_3 (ppm)	Ascorbic acid (%)	Spices (%)
A	80	120	0.03	0.11
B	150	-	-	-
C	-	-	0.03	-
D	-	-	-	0.11

Proximate composition, NaCl, pH, non protein nitrogen, residual nitrites and nitrates, total fatty acid composition (Zanardi et al., 2000), free fatty acid composition (Garcia Regueiro et al., 1994), TBARS (Novelli et al., 1998), total cholesterol and cholesterol oxides (Zanardi et al., 1998) were determined on fresh minces and sausages at the end of processing, which lasted 40 days. The sausages of formulations A, B and C were also sliced, packed under vacuum and exposed, 12 hours a day, to fluorescent light in a display cabinet for 60 days. The packs were sensory evaluated for colour stability three times a week by a panel of 8 members (Ghiretti et al., 1997) and submitted to determination of TBARS and cholesterol oxides after 15, 30, 45 and 60 days of light exposure.

Results and discussion

Proximate composition, NaCl content and non protein nitrogen of fresh minces and matured sausages have shown a remarkable degree of standardisation among the four formulations (data not shown). The initial pH of formulation A was 6.28, decreased to 5.32 in 10 days and rose 5.53 at the end of maturation. Formulations B and C had a minimum pH value of 5.62 after 10 days whereas minimum pH was 5.49 at the same time in formulation D. Final pH values at the end of maturation were 6.21, 6.01 and 5.92 respectively for formulations B, C and D. Nitrite and nitrate contents were 62 and 91ppm in fresh mince of formulation A and decreased respectively to 6 and 26ppm in matured sausages. Formulation B was found to have a nitrite content of 115 and 10ppm in fresh mince and matured sausages; nitrate was 20ppm in fresh mince and disappeared in matured products.

The measurement of total and free fatty acids (Table 1), inside the variation that can be considered normal in these products, has shown that the four formulations did not differ appreciably. Indeed, although free fatty acids contents in the mince of formulations B and C were found to be significantly different from those of formulations A and D, such a result can be attributed partly to inherent raw material variability and partly to analytical limitations. The different batches were produced the same day with raw materials of the same origin. Free fatty acids in the minces varied from a maximum of 1.9% of total fatty acids in formulation D to a minimum of 1.2% in formulation B whereas in matured sausages they ranged from 4.8% of formulation D to 5.3% of formulation A. The presence or absence of additives in the four formulations, therefore, did not appear to have significant effects on lipid hydrolysis. Similar results have been reported by Aguirrezabal et al.(2000) who observed that, in Spanish dry sausages, the increase of free fatty acids during ripening was not dependent on the presence/absence of spices (garlic and paprika) and/or curing agents (nitrite, nitrate and ascorbic acid). Toldra' and Flores (1998) also reported that curing agents did not affect lipolysis in dry cured ham, although the same authors observed that the presence of ascorbic acid produced a slight inhibition on enzyme activities.

The results of TBARS values and percentage of oxidised cholesterol in mince and matured sausages (Table 2) have revealed some differences among the formulations. Formulations A and B, in which nitrite and nitrate or nitrite alone were used, showed the lowest oxidation levels both in the minces and in matured sausages. Nitrite alone, with no ascorbic acid like in formulation B, seemed to be able to keep fatty acids oxidation at the same level as standard production, but cholesterol oxidation of matured sausages increased to the levels of the formulations without nitrite. Formulation C, in which ascorbic acid was used without nitrite and nitrate, had higher oxidation values and even higher levels were observed in formulation D, in which only pepper and garlic were employed. Antioxidant properties of garlic and paprika in dry fermented sausages have been reported by different authors (Palić et al., 1993; Aguirrezabal et al., 2000) but were probably due to the higher amounts used. Garlic, white and black pepper were employed at a total dose of 0.11% in formulation D, sufficient for flavour purposes but not for controlling fatty acid oxidation. The antioxidant effect of

nitrite observed right from the beginning of processing (see the values of the minces) is probably due to a chelating action of nitrite towards non-haem iron released during chopping and mincing of raw materials (Morrissey and Tichivangana, 1985). The increased of cholesterol oxides is a possible hint that oxidation of such a compound follows different pathways compared with fatty acids. Ascorbic acid was able to scavenge in part free radicals or reactive molecules released or produced at the beginning of processing. The comparison between the results of the minces with those of matured sausages shows that oxidation increased from fresh to finished products in all formulations except in formulation D. It would appear from such a result that most of oxidative processes take place at the beginning of processing of fermented sausages, probably as a consequence of the disrupting action of mincing and mixing with salt and additives.

Exposure to fluorescent light (Table 3) produced a sharp increase in TBARS values of formulation A from 0.087 to 0.217mgMDA/kg fresh tissue in 15 days, followed by a small increment at the end of the trial. Something similar happened with formulation B: TBARS values increased significantly during the first 15-30 days and remained more or less stable afterwards. In formulation B, though, final TBARS values (0.667mgMDA/kg fresh tissue) were much higher than those of formulation A. Lipid oxidation of formulation C grew steadily from the beginning to the end of exposure going from 0.143 to 1.085 mgMDA/kg fresh tissue. Cholesterol oxidation increased in all formulations during light exposure (Table 3): formulation B, with nitrite, had values higher but not too far from standard production (formulation A) whereas the batch with only ascorbic acid was nearly 50% higher. Sensory evaluation of brown colour appearance has shown a significant lower colour oxidative stability of formulation C compared with those containing nitrite (data not shown).

In conclusion sodium nitrite alone seems to be able to control oxidation of fatty acids and, to a lower extent, also cholesterol oxidation. Ascorbic acid, without nitrite, appears to moderate cholesterol oxidation during sausage maturation but it is not so efficient with regards to fatty acids oxidation. Spices are not able to curb lipid oxidation, at least at the concentrations used in standard formulation. Exposure to light of sliced and vacuum packed sausages has confirmed, as a whole, the ability of nitrite in controlling oxidation but the absence of ascorbic acid, probably, makes this formulation less efficient than the standard one (formulation A). In the same conditions ascorbic acid on its own had limited antioxidant effects both on fatty acids and on cholesterol oxidation.

Pertinent literature

Aguirrezabal et al. (2000) *Meat Science*, **54**, 77-81. - Chizzolini et al. (1999) *Proc.45th ICoMST*, Yokohama (Japan), 662-663. - Garcia Regueiro et al. (1994) *Journal of Chromatography A*, **667**, 225-233. - Ghiretti et al. (1997) *Meat Science*, **47**, 167-176. - Morrissey et al. (1985) *Meat Science*, **14**, 175-190. - Novelli et al. (1998) *Meat Science*, **48**, 29-40. - Palić et al. (1993) *Fleischwirtsch*, **73**, 670-672. - Sanz et al. (2000) *International Journal of Food Microbiology*, **42**, 213-217. - Toldra' et al. (1998) *Critical Reviews in Food Science*, **38**, 331-352. - Zanardi et al. (1998) *Meat Science*, **49**, 309-320. - Zanardi et al. (2000) *Meat Science*, **55**, 169-175.

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Table 1. Total and free fatty acid composition (mg/g fat)

		FORMULATIONS			
		A	B	C	D
Mince	Total fatty acids	699±49^{a}	556±38^{a}	617±66^{a}	651±38^{a}
	Free fatty acids	10.09±0.14^{ab} (1.4%)	6.71±0.62^{b} (1.2%)	7.73±0.33^{b} (1.3%)	12.57±1.73^{a} (1.9%)
Matured sausage	Total fatty acids	701±21^{a}	718±18^{a}	689±32^{a}	709±2^{a}
	Free fatty acids	37.24±1.00^{a} (5.3%)	35.05±0.02^{a} (4.9%)	34.69±3.90^{a} (5.0%)	33.74±4.20^{a} (4.8%)

(Different superscripts, within a raw, stand for significant differences, P≤0.05)

Table 2. TBARS (mgMDA/kg dry tissue) and % of oxidised cholesterol

		FORMULATIONS			
		A	B	C	D
Mince	TBARS	0.073±0.010^{c}	0.087±0.013^{c}	0.174±0.017^{b}	0.347±0.013^{a}
	%oxid. cholest.	0.10±0.01^{c}	0.15±0.02^{b}	0.10±0.02^{c}	0.26±0.01^{a}
Matured sausage	TBARS	0.158±0.013^{c}	0.155±0.009^{c}	0.250±0.010^{b}	0.371±0.014^{a}
	%oxid. cholest.	0.08±0.01^{b}	0.17±0.01^{a}	0.14±0.04^{ab}	0.20±0.03^{a}

(Different superscripts, within a raw, stand for significant differences, P≤0.05)

Table 3. TBARS (mgMDA/kg fresh tissue) and % of oxidised cholesterol during exposure to fluorescent light.

Formulations		EXPOSURE TIME				
		End maturation	15 days	30 days	45 days	60 days
A	TBARS	0.087±0.001^{b}	0.217±0.003^{b}	0.256±0.001^{c}	0.203±0.010^{b}	0.247±0.006^{c}
B		0.092±0.007^{b}	0.337±0.047^{b}	0.606±0.089^{b}	0.694±0.135^{a}	0.667±0.061^{b}
C		0.143±0.006^{a}	0.504±0.094^{a}	0.795±0.052^{a}	0.951±0.182^{a}	1.085±0.194^{a}
A	%oxid. cholest.	0.08±0.01^{b}	0.12±0.06^{a}	0.12±0.01^{b}	0.13±0.01^{b}	0.23±0.01^{a}
B		0.17±0.01^{a}	0.17±0.02^{a}	0.23±0.04^{a}	0.18±0.03^{b}	0.27±0.05^{a}
C		0.14±0.04^{ab}	0.13±0.04^{a}	0.29±0.08^{a}	0.27±0.03^{a}	0.34±0.05^{a}

(Different superscripts, within a column, stand for significant differences, P≤0.05)