Oxidative changes in vacuum packed cured and salted meat products

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SUMMARY : Two sliced and vacuum packaged products, a cured pork loin and a Bologna-type sausage were used in the study of oxidative and bacteriological changes during storage. The number of total aerobic bacteria and lactics developed rapidly in Bologna sausage at 5, 10 and 20°C. Numbers of 10^7 /g or more were reached within 5 days. In pork loin, total numbers, *B. thermosphacta* and lactics reached 10^7 /g after app. 4 weeks at 10°C, while Gram negative bacteria were practically absent. At 5°C the bacteriological flora never reached 10^7/g.
The only major change observed during storage was an increase in thiobarbituric acid (TBA) reactive substances, in Bologna sausage increasing app. 5 times during storage; in pork loin levels were higher after 6 weeks incubation. Levels of total carbonyl compounds were relatively constant, and also fluorescent substances showed only minor changes, whether in chloroformic or methanolic extract. Levels of free fatty acids in both products were constant during storage. It is concluded that oxidative changes during storage of these kinds of products are very small.

INTRODUCTION : Sliced, salted and cooked meat products are subject to oxidative changes during storage, when exposed to aerobic conditions. Packaging under proper vacuum may greatly reduce these changes (Lin et al 1980), however, not eliminate them. Addition of nitrite inhibits the oxidation of lipids (Kanner et al.1984), and thus a study was done on two products, one with added nitrite and the other without nitrite addition.

MATERIALS AND METHODS :Two meat products were used, both produced commercially. A cured pork loin was sliced and vacuum packaged (100 g packages) at the manufacturer. This product was thus "naturally" contaminated. A Bologna-type sausage was made without the addition of nitrite. This product was sliced in the laboratory using aseptic procedures and subsequently inoculated with a suspension comprising a "normal" bacteriological flora (100 g packages). This flora was obtained by washing three packages of Bologna sausage with peptone-salt water, and appropriate dilution of this suspension. The packaging film consisted of a laminate of polyethylene-polyamid.
Packages of cured pork loin were stored at 5 and 10°C; and those of Bologna sausage at 5, 10 and 20°C.
Bacteriological examinations were done on duplicate samples homogenized in peptone-salt water using a Stomacher. Total counts were measured on plate count agar and numbers of lactic acid bacteria were counted on Man Rogosa Sharp agar (Oxoid). Additionally, pork loins were tested for *Brochothrix thermosphacta* on streptomycin thallous acetate actidione medium (Merck) and Gram negative bacteria were followed on desoxycholate hydrogensulphide lactose medium (Merck) .
Oxidative changes were followed using material from the same packages as used for microbiological examinations.
Flourescens A 5 g sample was used for direct measurement of fluorescent compounds (modified after Kamarei and Karel 1984, Nolan et al. 1989 and Pikul et al.1984). The sample was homogenized with 25 ml chloroform-methanol (2:1). Centrifugation was done for 5 min at 10.000 rpm and fluorescens was measured on the separated phases. The methanol phase was diluted 1:20. Fluorescence spectra were determined on a Kontron SFM25 fluorometer using quininsulphate (10-8 M in 0.1 % sulfuric acid) as standard. However, the reference values were different for the two products, i.e. they cannot be compared. Emission spectra were made at 632-380 nm using λ_{ex} 360 nm and excitation spectra were made at 410-200 nm using λ_{em} 438. Values reported are for λ_{ex} 360nm and λ_{em}438nm.
Thiobarbituric (TBA) reactive compounds were examined using distillation methodology (Lindeløv 1978 and Rhonda et al. 1988). A 20 g sample was homogenized with 50 ml water (containing 0.1 % propylgallate and 0.1 % EDTA) for 1 min. The mixed sample was transferred to a Kjeldahl distillation tube with a further 25 ml. 2 ml HCl (half concentrated) were added and the sample was destilled using a Tecator Kjeltec System (1002 Distilling Unit). Within 2 minutes 50 ml distillate was collected and after appropriate dilution, 5 ml distillate was mixed with 5 ml 0.02 M thiobarbituric acid either in 90 % glacial acetic acid or water. Blanks were included. The samples were heated in a water bath (100°C) for 45 min, cooled quickly and measured at 532 nm using a Ultrospec II LKB spectrophotometer.
Further, fluorescence of TBA reactive compounds were measured at λ_{ex} 532nm λ_{em} 553nm (Halliwell and Gutteridge1l985). A standard curve was made with 1.1.3.3. tetraethoxypropan.
Total carbonyl compounds were measured as 2,4-dinitrophenylhydrazones. A 5 g sample was mixed with 25 ml cold ethanol. The ethanol was previously distilled with 2,4-dinitrophenylhydrazin to remove any carbonyl compounds present. The homogenized sample was centrifugated at 2000 rpm for 30 min, and 5 ml of the supernatant was mixed with 5 ml 2,4-dinitrophenylhydrazin (DNPH) for 30 min at 60°C. The DNPH solution was made using 0.5 g DNPH in 500 ml 1 N HCl (this solution was previously extracted twice with hexan). Extraction of the sample followed twice with hexan. The hexan phases were combined and, after

appropriate dilution, the spectra of the carbonyl compounds were measured at 320 nm to 380 nm. The carbonyl content was also measured after KOH addition (modified after Henick et al.1954). 0.2 ml hexan extract, 1 ml 4% alcoholic KOH and 3 ml ethanol were mixed and the absorbance was measured at 460 nm, using an Ultrospec II LKB spectrophotometer.

Fatty acids the concentration of free fatty acids were examined. A 10 g sample was homogenized with 20 ml distilled water, pH was adjusted to 2, and the cooled sample was further homogenized with cold 15 ml heptan. After centrifugation (10 min 10.000 rpm) the heptan phase was recoved and 3 ml of this was titrated with app. 3.5 mM alcoholic NaOH using 0.02 % thymol blue as indicator.

Fat content was determined using a Soxtec System HT 1043 Extraction Unit. Water was measured by drying (Anon. 1955) and salt content was also determined (Anon. 1974).

RESULTS and DISCUSSION : The pork loin had a sodium chloride concentration of 3.0 %, and a water content of 69.7 %. The fat content was 9 % (only 1 % in the lean portion of the product).

The salt concentration of the Bologna sausage was 2.3 %, water content was 61.6 % and fat content 18.2 % .

Bacteriological analysis on pork loin showed that total aerobic plate count, numbers of lactic acid bacteria and *B.thermosphacta* reached approx.10^5/g within a storage period of 6-7 weeks at 5°C (not shown). At 10°C, these numbers were reached within 3-4 weeks (Tabel 1).

Generally, numbers of Gram negative bacteria were insignificant, less than 10^2 cfu/g.

During storage, the pH values in the packages, decreased from 6.3 - 6.4 to 6.10-6.30.

Total aerobic plate count and lactic acid bacteria on Bologna sausage exceeded 10^8/g after 12 days at 5°C and within 5 days at 10 and 20°C. During storage pH values decreased to app. 5.6, 5.7 and 5.8 at 20°C, 10°C and 5°C respectively (Table 2).

The concentration of TBA reactive compounds were increasing (app. 5 times) in Bologna sausage at both 5, 10°C and 20°C during storage, Table 3. This happened whether the TBA reagent was made in water solution or acetic acid solution. Generally, differences were small whether the reaction was done with TBA in pure water or acetic acid solutions.

In pork loin, however, higher levels were only observed in the acid solution and only after 6 weeks storage (Table 4). The values shown are for TBA-reactive substances measured by fluorometry, this method is much more sensitive than spectroscopy.

Fluorescens spectra on the chloroformic and methanolic extracts were very similar during storage, however levels of fluorescent compounds in the two products varied during storage. As already mentioned the reference values for the two products were different, so the actual figures cannot be compared.

In Bologna-sausage samples, the fluorescens did not change irrespective of temperature or storage length. Although the actual percent values measured in methanol were higher, neither did they change significantly (Table 5). Thus it was not possible to indicate any association between TBA-reactive compounds and fluorescent compunds as shown by others (Kim and LaBella 1987).

The values for pork loin were higher after 6 weeks storage than initially, but again no definite trend could be observed (Tabel 6).

The concentrations of total carbonyl compounds in the Bologna sausage, showed only minor changes during storage. Values are not in μM/g of aldehydes, but in absorbance values adjusted to 1 gram product (Table 7). These results indicate that oxidation of fatty acids and subsequent degradation to smaller carbonyl compounds does not happen during storage. The presence or absence of nitrite does not appear to influence oxidative changes during storage.

The concentration of free fatty acids in Bologna sausage were app. 3-4 μmol/g (Table 8) and 10 times less on pork loin (Table 9). The levels were relatively constant during storage irrespective of temperature and storage time. The constant level of free fatty acids suggests that any profound lipolytic activity in the microbial flora is absent in these products.

CONCLUSIONS :

Bacteriological examination of two vacuum packaged products showed an increase in total count on storage at all temperatures. The Flora consisted primarely of *B. thermosphacta* and lactics. The concentration of free fatty acids was constant during storage, i.e. lipolytic activity was abscent. Oxidative changes were very small and could only be measured flourometrically as TBA-active compounds. The precense or absence of nitrite apparently only had minor effect

Spoilage of these kinds of products are exclusively a microbiological problem, organoleptic changes due to oxidative changes are insignificant.

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Table 1. Bacteriological flora at 10°C (log cfu/g pork loin)

weeks	PCA	MRS	STAA
0	1.72	< 1.00	< 1.00
1	3.34	2.70	2.27
2	5.27	5.17	2.20
3	6.69	6.72	4.66
4	7.47	7.09	6.94
5	7.26	6.86	6.12
6	7.26	6.52	6.58

Table 2. Total counts and lactics on Bologna sausage (log cfu/g)

days	substrate	temperature, °C		
		5	10	20
0	PCA	4.3		
	MRS	3.83		
5	PCA	7.47	8.44	8.75
	MRS	6.96	8.40	8.98
12	PCA	8.94	9.06	9.10
	MRS	8.88	8.89	9.06
19	PCA	8.74	8.92	9.12
	MRS	8.72	9.09	8.88
26	PCA	9.04	9.11	-
	MRS	8.90	9.07	-

Table 3. TBA reactive substances in Bologna ($\cdot 10^8$ mol /g)

days	5°C	10°C	20°C
0	0.5/0.5		
5	1.3/1.6	1.6/1.8	1.8/2.1
12	1.0/2.1	1.1/2.49	1.2/2.58
19	2.7/2.4	2.4/2.2	2.3/2.2
26	2.7/2.4	2.9/2.4	-/-

Table 4. TBA-reactive substances measured by fluorescens in pork loin. ($\cdot 10^9$ mol/g)

weeks	water solution		acetic acid solution	
	5°C	10°C	5°C	10°C
3	0.6	0.5	0.7	0.6
4	0.8	0.8	0.7	0.7
5	0.6	0.6	0.6	0.5
6	0.7	0.6	1.5	0.9

Table 5. Fluorescent compounds in Bologna, values are % pr g product in chloroform/methanol extract

days	5°C	10°C	20°C
0	9.72/85.8		
5	10.02/88.4	9.23/76.0	8.20/77.2
12	11.57/87.0	8.98/84.0	9.00/84.0
19	9.00/88.1	8.83/91.8	7.30/83.0
26	8.48/84.4	11.10/76.6	-/-

Table 6. Fluorescent compounds in pork loin, % pr. g product in chloroformic extract.

weeks	5°C	10°C
1	39.8	33.8
2	43.1	42
3	45.8	52.4
4	34.8	39.3
5	51.4	48
6	67	65.9

Table 7. Carbonyl compounds abs 440nm/g Bologna

days	5°C	10°C	20°C
0	7.3		
5	5.4	5.9	6.2
12	6.2	8.7	6.3
19	7.1	6.6	6.4
26	4.0	4.6	-

Table 8. Concentration of fatty acids, μmol fatty acid/g Bologna

days	5°C	10°C	20°C
0	4.32		
5	3.90	3.38	3.71
12	4.07	4.19	4.12
19	3.71	3.53	3.77
26	4.83	4.26	-

Table 9. Concentration of fatty acids in pork loin

weeks	μmol/g pork loin	
0	.38	
1	.34	.36
2	.28	.38
3	.69	.50
4	.26	.29
5	.51	.38
6	.50	.45