EFFECT OF NITRATE AND ASCORBATE ON COLOUR AND COLOUR STABILITY OF DRY, FERMENTED SAUSAGE PREPARED USING " BACK SLOPPING ".

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SUMMARY

The effect of various levels of nitrite and nitrate with and without ascorbate, in sausages prepared using " back slopping " as inoculation was investigated in three series of experiments. In all experiments, nitrite was rapidly depleted and nitrate formed. Nitrate was not used, probably because of the lack of (active ?) Micrococci in the starter sausage. Surface colour was found to be darker with increasing levels of nitrite, irrespective of substitution of nitrate for nitrite. Irrespective of the presence of nitrate, colour stability was mainly promoted by residual ascorbate. The latter decreased with increasing nitrite levels.

INTRODUCTION

The stable, red colour of fermented, dry sausage is obtained by treating the sausage mix with salt containing nitrite and/or nitrate. Nitrate was traditionally used and is still preferentially used for dry sausages and hams requiring long ripening times. To be effective it must be reduced to nitrite by micro-organisms. Acid sensitive Micrococcaceae are considered to be essential in this respect as long as pH is above 5.4.The use of nitrite involves an initial oxidation of red, oxygenated myoglobin (MbO_2) to grey metmyoglobin (MMb), with reduction of nitrite to Nitric oxyde (NO). The latter is rapidly oxidized to nitrate. Second, indigenous and added reductants (e.g. ascorbate) reduce MMb to Mb and nitrite to NO, which then combine to form the red nitric oxyde myoglobin (NOMb). Stabilization of the red pigment is obtained through

denaturation of the globin moiety of the molecule (Lücke, 1985) (Demeyer et al, 1986). It is often assumed that the presence of nitrate is required for optimal colour stability (Puolanne, 1977) (Puolanne, 1986). Also residual nitrite (as a source of NO to inhibit NOMb dissociation) and ascorbate (to inhibit MMb and N oxyde formation) are considered essential for colour stability (Ranken, 1981). Belgian legislation allows the use of both sodium and potassium salts of nitrite and nitrate in joint amounts up to 200 mg/Kg (expressed as $NaNO_2$ and KNO_3 respectively) in both prepared meats and meat preparations produced using a " ripening process ".
The experiments reported here were carried out to evaluate the effects of nitrate and ascorbate on colour formation and stability in dry sausages prepared using " back slopping ". The latter procedure involves the use of finished fermented sausage as inoculum (starter) for a new production.

MATERIALS AND METHODS

Preparation of sausages : In a series of 3 experiments (Table 1), various levels of KNO_3 with or without sodium ascorbate (NaAsc) were added with varous levels of $NaNO_2$ to a sausage mix cuttered in the sequence (%w/w): beef (30.6) and pork(30.6)(-5°C); glucose (0.7); pepper(0.09); nutmeg(0.01); ascorbate and starter (1 min.40 sec.; -2 °C), followed by lard(35) (-20°C)(3min.30 sec.; -5°c) and finally NaCl containing nitrite and/or nitrate (2.85)(4min.20 sec.; -7°C). As starter, 1% of a mixture of 2 two day old sausages (1 from each company) containing ca. 10^7/g and 10^5/g of Lactobacilli and Micrococci respectively was used. In one experiment a suspension of Micrococci (Staphylococcus Carnosus, Bacto 61, Müller, 10^7/g) was used as starter.The mixture was cuttered (cutter 201, Rex type HK 20 II) at the laboratory, the resulting mix vacuum packed for removal of air and then stuffed into pimaricin treated Naturin casing (diam.6cm). The sausages (250 à 500 g) were transferred to one of two companies, fermented for 2 days (20-24°C; 92-98% r.h.) and then transferred to drying chambers for further drying up to 21 d.(gradual decrease to 13 or 16°C and 80 or 83% r.h.).

Analyses : Measurement of pH was done directly in the sausages, using pointed electrodes (Ingold LOT 406M6) and a digital pH meter (Knick portamess 651). The redox potential was measured continuously by permanent longitudinal insertion of a combined Ag/AgCl reference electrode + Pt electrode (Consort 42A - Ingold Pt 4 200 K7) into a sausage.

Table 1 Scheme of Experiments

Added		$NaNO_2$ mg/Kg	KNO_3 mg/Kg	NaAsc. mg/Kg	Starter sausage	Starter culture
Expt. 1	s.1	171	-	-	+	-
	s.2	171	-	600	+	-
	s.3	86	125	-	+	-
	s.4	86	125	600	+	-
Expt.2	s.1	-	-	-	+	-
	s.2	-	-	600	+	-
	s.3	296	-	-	+	-
	s.4	296	-	600	+	-
	s.5	-	325	-	+	-
	s.6	-	325	600	+	-
	s.7	150	162	-	+	-
	s.8	150	162	600	+	-
Expt.3	s.1	74	108	-	+	-
	s.2	74	108	600	+	-
	s.3	74	108	600	+	+
	s.4	148	216	-	+	-
	s.5	148	216	600	+	-
	s.6	148	216	600	+	+

A sausage was removed at various times after stuffing, twice ground in a meat grinder and used for analysis of nitrite, nitrate and ascorbate. Nitrite and nitrate were immediately extracted in .01 M NaOH at 80 °C as described by Keliher & Norwitz (1987). After deproteinization with $ZnSO_4.7H_2O$ 10%, pH is adjusted to 10 with .1 M NaOH to prevent nitrite losses during storage. Extracts are stored at 0 °C for nitrite analysis based on the Griess reaction (AOAC, 1984) as adapted by Davis et al (1985). Nitrate was determined after reduction to nitrite (Beernaert et al, 1987) adapting the Griess reaction to an auto-analyzer. Ascorbate was extracted in meta-phosphoric acid (15% w/v) and determined using ascorbate oxidase (Boehringer, Mannheim) as described by Deneke et al (1978).

Using a Hunter reflectance colorimeter, colour was measured on transversal sausage slices (1 cm) as CIELAB coordinates L* (luminosity), a* (redness) and b* (yellowness). The latter are related to tristimulus values as described by Hunter (1975). Surface colour was measured on longitudinal slicess (1.5 cm). In preliminar experiments, it was found that visual preference for colour by a non trained taste panel was higly correlated to a* (R^2 = .96). Colour stability was determined by repetition of reflectance measurements after exposure of vacuum packed

transversal slices (1 cm) at 8 °C and 90% r.h.(refrigerator) to a 75 Watt lamp at 1 m. (light flux = .7 W/m²) for up to 24 hrs.

RESULTS

pH : Initial pH for all experiments was 5.78 ± .07 (mean value ± S.E. of 14 measurements), dropping on average after 2 days with .72; .75; .64; .67 and .57 units in the presence of 74; 86; 150; 171 and 296 mg/Kg $NaNO_2$ respectively. The use of a starter sausage was required for acification.The data suggest an inhibitory effect of nitrite on fermentation, confirming earlier work (Zaika et al,1976).

Nitrite and nitrate changes : Nitrite was rapidly depleted in all experiments, whereas nitrate accumulated. No clear effect of ascorbate was observed on these changes. From molar changes, it can be calculated that net nitrate production accounts for 62% ± 15% (mean ± S.E.) of nitrite disappearance up to 24 hrs. after stuffing (Typical results shown in table 2). At later stages, other reactions clearly become more important. Such reactions are numerous and complex : generalizing Casssens et al (1979) stated that myoglobin accounted for 5-15% of the nitrite originally added, nitrate 1-10%, residual nitrite 5-20%, gas 1-5%, sulfhydryl 5-15%, lipid 1-5% and protein 20-30%. Clearly, only nitrate production from nitrite is involved in net nitrate changes, as nitrate, when added alone, does not disappear: up to 17 d after stuffing, nitrate

Table 2. Nitrite and nitrate changes.

	Expt.1				Expt.2			
	s.1		s.3		s.3		s.7	
µmoles/Kg added								
$NaNO_2$	2478		1239		4290		2145	
KNO_3	-		1239		-		2145	
Total	2478		2478		4290		4290	
Recovered (% of total)	$NaNO_2$	KNO_3	$NaNO_2$	KNO_3	$NaNO_2$	KNO_3	$NaNO_2$	KNO_3
after 0 hrs.	100	0	50	50	100	0	50	50
4-6 hrs.	76	21	36	53	71	5	34	66
24-28 hrs.	78	28	33	62	68	18	26	54
48-52 hrs.	14	36	8	72	40	28	13	60
4 days					18	28	11	43
12 days							11	31
17 days							5	48

recovery was 97 ± 3 % and 90 ± 5 % (mean values ± S.E. of 8 determinations) in s.5 and s.6 of Expt.2 respectively. These data suggest that Micrococci present in the starter sausage are not sufficiently active, competitive or numerous to induce nitrate reduction, before pH becomes inhibitory.

Colour and colour stability: The substitution of nitrite for nitrate clearly brightens the surface colour (increased L*)(underlined values in table 3), whereas it is clear that surface is always considerably darker than interior. Brightening of the surface is however not an effect of nitrate in se , but rather the reflection of lower nitrite addition, as evidenced in expt.3: increasing both nitrite and nitrate addition lowered L* from an average value of 47.5 to 46.5 (brighter sausage than in other Expts.!). It should be remembered that such small differences in colour coordinates as discussed here, are much more apparent for the eye. Surface darkening because of higher nitrite addition is most probably due to an extensive formation of MMb during cuttering and immediately after stuffing. Further acidification and surface drying fixes the MMb colour on the surface. Dry matter content was 73.1 ± 1.0 % and 54.6 ± .3 % for the 1 cm sausage edge and for the center respectively (mean values ± S.E. for s.1, 2, 3 & 4 of expt.1). Results on colour formation in expt.3 support that hypothesis: series with lower nitrite addition (s.1, 2 & 3: 74 mg/Kg) showed bright red surface colour 3d. after stuffing, whereas at higher nitrite addition (s.4,5 & 6 : 148 mg/Kg) surface colour was still greyish. These differences were also apparent for the

Table 3. Effect of nitrate and ascorbate on colour.

Added (mg/Kg)	Expt.1				Expt.2			
$NaNO_2$	171		86		296		150	
KNO_3	-		125		-		162	
Ascorbate	-	600	-	600	-	600	-	600
Colour coordinates								
L* surface	<u>35</u>	35	<u>38</u>	37	<u>37</u>	37	<u>39</u>	31
interior	53	53	52	51	44	50	51	49
a* surface	15	14	16	17	13	12	12	17
interior	12	13	15	15	16	15	12	16

interior (Table 4). The presence of a Micrococci starter seemed to improve reddening at high concentrations, but luminosity was lower (underlined values in Table 4).Addition of ascorbate does

not seem to affect these changes to any clear extent. In this respect it should be noted that 50 mg/Kg of nitrite is considered to be sufficient for both colouring and anti-microbial purposes in dry sausage (Lücke,1985) (Demeyer et al,1986).

Table 4. Effects of nitrite and nitrate on colour formation.

Expt.3: added (mg/Kg)				Colour Coordinates			
				3d after stuffing		21 days after stuffing	
$NaNO_2$	KNO_3	Asc.	Microc.	L*	a*	L*	a*
74	108	-	-	47	13	56	12
74	108	600	-	44	13	54	13
74	108	600	+	46	12	55	13
148	216	-	-	46	9	54	11
148	216	600	-	46	9	52	10
148	216	600	+	43	13	52	13

Colour stability as measured here is mainly affected by the use of ascorbate and is reflected by the decrease in a* value following illumination. Average interior a* value was 13.0 ± 1.0 and 13.5 ± 1.0 for sausages prepared without and with ascorbate respectively. These values were respectively reduced however by 7.9 ± 1.0 and 5.5 ± .7 after 24 hrs. of illumination, this difference being clearly significant (mean values of 6 determinations ± S.E.).

Redox potential and ascorbate depletion : Nitrite levels clearly affected early values of redox potential and ascorbate recovery as illustrated in Table 5. Increasing nitrite concentrations clearly decreases ascorbate recovery. Obviously, reduction of MMb, probably involved in ascorbate consuming reactions, is not sufficient to

Table 5. Nitrite addition, redox potential and ascorbate recovery.

Expt.2	Ascorbate recovery (%)[1]	Redox potential (Eh') (mV)[2]
$NaNO_2$ (mg/Kg)		
0 (s.2)	70	- 54
150	33	8
296	11	102

[1] determined immediately after cuttering [2] after 2 days

improve surface colour (Table 3). The establishment of a more reducing environment however promotes colour stability (see also

Ranken, 1981).

Conclusions

- Nitrate is not reduced to nitrite in sausages prepared using a starter sausage (" back slopping ") probably because of lack of (active ?) Micrococci.

- Nitrite is rapidly depleted and nitrate accumulates in such sausage fermentation. In the initial stages of fermentation, nitrate accumulation accounts for over 50 % of nitrite disappearance.

- In such sausages, oxydation of nitrite to nitrate is coupled to rapid oxydation of MbO_2 and Mb to MMb, giving a greyish surface and interior colour. Later MMb is reduced and NOMB is formed, responsible for the bright red colour. At high nitrite concentrations (say over 100 mg/Kg) surface MMb formation exceeds the potential for subsequent reduction, hampered by surface drying. Rate of reddening (NOMb formation) is not affected by the presence of nitrate or ascorbate. A slight improvement in the presence of a Micrococci starter was observed, but such effect was negligible compared to that of nitrite concentration. Substitution of nitrate for nitrite does not affect colour, except for the lowering of nitrite concentration.

- Colour stability is mainly promoted by residual ascorbate and not affected by substitution of nitrate for nitrite. Ascorbate depletion and redox potential are increased by increasing nitrite levels.

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