

THE INFLUENCE OF NITRITE ON THE FORMATION OF BIOGENIC AMINES IN DRY SAUSAGES

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SUMMARY

Dry sausages were manufactured in the pilot plant with three different levels of nitrite (1= 50 mg/kg NaNO₂, 2= 120 mg/kg NaNO₂ and 3= 200 mg/kg NaNO₂). The fermentation was performed with five commercial starter cultures: Baktoferment 62, Duploferment 66, Pentoferment 85, Condi-Rasant 820/10 and Flora Carn SL. Biogenic amines, nitrite, nitrate, pH, a_w and microbial counts were analysed during ripening of the sausages. Nitrite was eliminated during fermentation process. After 49 days of ripening the nitrate concentrations were less than 10 mg/kg in all the samples. The levels of biogenic amines were compared to each other by calculating Biogenic Amine Index (=BAI) for each sausage. Addition of nitrite had no effect on amine formation when fermentation was performed with other starter cultures than Pentoferment 85. With this starter culture the lowest BAI was achieved as 200 mg/kg NaNO₂ was added.

INTRODUCTION

Biogenic amines are of considerable importance in foods, particularly in fermented foods, in view of their pronounced physiological effects in humans and animals (e.g. migraine, histamine intoxication). They are mainly formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (MAGA, 1978; ASKAR and TREPTOW, 1986). There are several reports of high levels of biogenic amines in fermented sausages obtained from retail markets (RICE et al., 1975; VANDEKERCKHOVE, 1977; 1982; PECHANEK et al. 1983; TSCHABRUN et al., 1990). The effect of raw material, starter cultures and GDL on the biogenic amine formation have been previously studied in our laboratory (MAIJALA et al. 1992a,b). Nitrite is known to affect on the fermentation process (LEISTNER et al., 1973; PUOLANNE, 1977). Therefore the purpose of this work was to study the effect of three different levels of NaNO₂ addition on the formation of biogenic amines in dry sausages.

MATERIALS AND METHODS

Sausages

The basic formula, the sizes of sausages and the process used as well as the handling of samples were the same as described in our previous work (MAIJALA et al., 1992a). Variations between the different parts were the starter cultures together with the combinations of three different

levels of NaNO_2 . Three parts were fermented by Baktoferment 61 (B1= 50 mg/kg NaNO_2 , B2 = 120 mg/kg NaNO_2 , B3 = 200 NaNO_2). These sausages were fermented with the combination of 0.7% GDL + 0,15% glucose. The other 12 parts of the sausage mass were fermented without GDL (0,6% glucose) and with the same levels of NaNO_2 as (B1, B2, B3) but using Duploferment 66 (D1, D2, D3), Pentoferment 85 (P1, P2, P3), Condi-Rasant 820/10 (C1, C2, C3) or Flora-Carn SL (F2, F2, F3) as starter culture.

Methods of analysis

Nitrate and nitrite were analysed by the liquid chromatographic method of EGGERS and CATTLE (1986) with some minor modifications. Biogenic amines were determined as their dansylderivatives by liquid chromatography (EEROLA et.al., 1992). Microbial studies were carried out from 10 g of mixed sausage, which was serially diluted with sterile 0.1% peptone and 0.8% NaCl water. Each dilution was applied to Violet Red Bile Agar (NCFA, method no. 44, 1990), Slanetz-Bartley agar (NCFA, method no. 68, 1978), Malt extract agar, blood agar base containing 5% defibrinated bovine blood and de Man, Rogosa and Sharpe agar with sorbic acid (NCFA, method no. 140, 1991). pH-values were measured using an Orion Research Incorporated SA 520 pH/mV-meter, with a RossTM pH-electrode no.8163 (Switzerland). a_w -values were obtained at 25 °C using a Rotronic Hygroskop (Fattore Vitale & Co, Italy).

RESULTS AND DISCUSSION

The levels of nitrite and nitrate were analysed after 1, 21 and 49 days after fermentation. After 1 day of fermentation the levels of nitrate were higher when more NaNO_2 was added and the amounts of nitrate were decreased below 10 mg/kg in all samples after 49 days of fermentation (Table 1). The nitrite was almost totally eliminated in all samples during fermentation process. The pH values and the levels of lactic acid bacteria are presented in table 2. The levels detected during ripening were typical for the starter cultures used. There were no big differences between the samples of different NaNO_2 addition concerning pH, a_w and the microorganisms studied.

The biogenic amines were studied five different times during fermentation.. The amounts of tryptamine, phenylethylamine, cadaverine, spermidine and spermine remained rather low during all the fermentations. Mean values at the end of ripening were: tryptamine 31 mg/kg \pm 6, phenylethylamine 28 mg/kg \pm 15, cadaverine 9 mg/kg \pm 7, spermidine 5 mg/kg \pm 1 and spermine 4 mg/kg \pm 4. The tyramine concentrations increased during all the fermentations. The amounts of tyramine were in raw material 3 mg/kg and at the end of the ripening the concentrations varied from 125 to 302 mg/kg (Table 4).

KARMAS (1981) proposed the Biogenic Amine Index (BAI) for the measurement of the quality of raw and processed seafood: $\text{BAI} = (\text{Histamine} + \text{Putrescine} + \text{Cadaverine}) / (1 + \text{Spermine} + \text{Spermidine})$

BAI was used to compare the results of different sausages in this study (Table 3). No remarkable differences between the different addition levels of NaNO_2 were detected except when fermented with Pentoferment 85. The lowest BAI was achieved with this starter culture as 200 mg/kg NaNO_2 was added. Some differences between starter cultures were observed: the lowest BAI was achieved with Flora-Carn SL and the highest BAI with Bactoferment 61. It is possible that the starter culture affect on the formation on biogenic amines by influencing on the other microbes of raw material (MAIJALA et al. 1992b).

CONCLUSIONS

There were no clear differences between the three different levels of NaNO_2 added on the formation of biogenic amines during ripening in dry sausages.

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Table 1. The levels of nitrite and nitrate (mg/kg) found in sausages during ripening prepared with three different NaNO₂ concentrations and five different starter cultures.

	day	D1	D2	D3	P1	P2	P3	C1	C2	C3	F1	F2	F3	B1	B2	B3
nitrite	1	8.6	6.1	9.2	<1	2.0	3.5	<1	1.0	1.7	1.5	1.6	8.1	<1	2.4	4.7
	21	12.5	12.7	11.6	14.2	7.0	13.3	12.1	10.7	9.9	12.8	12.9	12.2	12.4	11.2	11.2
	49	<1	<1	3.0	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
nitrate	1	38.9	39.6	118	32.2	62.9	91.0	31.5	48.5	74.9	29.4	60.4	79.1	31.8	43.3	45.0
	21	6.9	6.9	6.2	9.2	5.4	5.9	5.2	6.2	11.4	6.5	6.5	6.6	5.9	8.8	13.8
	49	<1	6.1	7.0	5.8	5.7	6.9	<1	2.6	6.0	5.7	6.0	7.7	4.6	6.9	9.0

Table 2. The pH values and lactic acid bacteria (log CFU/g) in dry sausages during ripening prepared with three different NaNO₂ concentrations and five different starter cultures.

	day	D1	D2	D3	P1	P2	P3	C1	C2	C3	F1	F2	F3	B1	B2	B3
pH	0	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
	1	5.5	5.5	5.6	5.6	5.6	5.6	5.5	5.3	5.3	5.5	5.5	5.4	5.1	5.1	5.2
	7	4.7	4.7	4.7	4.7	4.7	4.7	4.8	4.7	4.8	4.8	4.7	4.7	4.8	4.7	4.8
	21	4.7	4.8	4.7	4.7	4.6	4.6	4.4	4.7	4.7	4.8	4.8	4.7	4.9	4.9	4.7
	49	4.9	4.9	4.9	5.0	5.0	4.9	5.0	5.0	5.0	5.0	4.9	4.9	4.5	4.9	5.0
lactic acid bacteria	0	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
	1	6.8	6.9	6.8	7.0	6.5	6.5	7.9	8.6	8.4	7.8	8.0	8.4	4.6	4.6	4.4
	7	8.7	8.3	8.2	8.5	7.9	8.1	8.9	9.1	9.0	8.1	8.4	8.1	8.7	8.7	8.9
	21	8.2	7.6	7.7	8.0	7.8	8.0	7.6	7.3	7.5	8.6	8.7	8.8	8.6	8.6	8.6
	49	7.5	7.6	7.1	7.7	7.3	7.7	8.6	8.4	8.6	7.2	7.1	7.0	8.1	8.1	8.2

Table 3. The BAI-results during fermentation of sausages; calculated from the amounts of histamine, putrescine, cadaverine, spermidine and spermine (mg/kg).

	day	B1	B2	B3	D1	D2	D3	P1	P2	P3	C1	C2	C3	F1	F2	F3
BAI	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	2	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1
	7	1.2	1.1	0.8	0.3	0.3	0.3	0.5	0.4	0.3	0.3	0.3	0.1	0.2	0.2	0.1
	21	5.3	4.4	28.0	1.0	1.0	0.8	1.1	1.3	0.6	3.1	2.7	3.9	0.3	0.3	0.2
	35	6.8	7.1	5.0	1.9	1.5	1.5	1.7	2.3	1.0	3.8	4.5	4.0	0.3	0.4	0.6
	49	6.9	7.3	6.3	3.1	2.4	2.8	3.0	3.4	1.0	4.4	4.4	4.8	0.8	0.6	0.6

Table 4. The amounts of tyramine (mg/kg) during fermentation of sausages

	day	B1	B2	B3	D1	D2	D3	P1	P2	P3	C1	C2	C3	F1	F2	F3
TYR	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	2	8	7	7	9	8	6	11	9	25	7	7	6	7	6	6
	7	93	85	87	32	46	35	57	34	35	31	49	54	23	21	21
	21	178	207	175	128	121	153	158	142	87	182	175	201	69	62	69
	35	241	272	217	227	210	184	273	226	160	183	209	202	75	120	143
	49	281	302	288	297	253	259	275	235	153	232	230	239	125	172	141