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PROCESSING TEMPERATURE AND HIS INFLUENCE REGARDING CHEMICAL PROPERTIES OF THE DRY SAUSAGES

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Abstact

In this paper, the effect of processing temperature on the formation of amines in dry sausages was studied in pilot and factory trials. Sausages made from the same batch of raw materials, were divided into two groups each fermented at different processing temperatures. Commercial preparations of starter cultures containing lactic acid bacteria and Staphylococcus carnosus were used, a lower processing temperature resulted in higher levels of amines and delayed pH decrease in comparison with the higher temperature. However, more amines were formed at the higher processing temperature when glucono-delta-lactone with S. carnosus was used. According to these results the selection of raw materials and also the choice of processing temperature for the amine-negative starter culture used, are critical control points in preventing the formation of high levels of biogenic amines.

KEYWORDS : dry sausages, processing temperature, biogenic amines

INTRODUCTION

Biogenic amines are organic bases formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketoncs.

Storage temperature has a clear influence on the formation of biogenic amines in foods. (Taylor, 1986).

In this study, higher levels of histamine were detected at higher ripening temperatures. In supplemented MRS broth. *Lactobacillus bulgaricus* showed maximum histamine, tyramine and tryptamine production at 37°C. The temperatures studied were 20°C, 30°C, 37°C and 45°C (Chancier *et al,* 1989).

There are reports of varying levels of biogenic amines in fermented sausages obtained from retail markets (Rice *et al.,* 1975; Vandekerekhove, 1977; Pechanek *et al.,* 1983; Pfannhauser & Pechanek, 1984; Bauer *et al.,* 1989; Tschabrun *et al.,* 1990). Fermentation temperatures for dry sausages are usually between 15 and 26°C, lower temperatures being preferred if a product of high quality and long shelf-life is desired. Undried, spreadable sausages are usually fermented at 22-26°C, and for semi-dry sausages mainly produced in the USA — even higher fermentation temperatures along with shorter fermentation times are applied (Lilcke, 1985). Although these widely varying temperatures

are used for dry sausage manufacture there are only a few publications concerning the effect of ripening temperature on the formation of biogenic amines in dry sausages. Kranner *et al.* (1991) reported more histamine formation at a higher ripening temperature (18^{G}C) compared with a lower one (7°C), especially with the addition of histaminc-producing microorganisms.

The purpose of this work was to study the effect of processing temperature on the formation of biogenic amines in dry sausages.

MATERIALS AND METHODS

The work was carried out as three pilot plant trials (trials 1-3) and one factory trial (trial 4). In each trial, sausages made from one batch of raw materials and starter culture were divided into two groups processed either starting from 24"C (A) or from 14°C (B) in trials I, 2 and 4 and in trial 3 starting from 24°C (A) or I8°C (C).

Pilot plant trials (trials 1-3)

Sausages were prepared from meat slaughtered 3 days previously and deep-frozen overnight (30% beef, 25% pork 30% pork back and 15% minced beef, which was only refrigerated at +2°C). The raw materials were chopped in a cutter and starter culture, spices, glucono-delta-lactone (GDL, for Baktoferment 61 sausages only), sodium ascorbate and NaCI with nitrite were added. For Flora-Carn SL fermented sausages 6g/kg glucose was used and 5g/kg for Baktoferment 61 + GDL fermented sausages. After chopping, the sausage mass was stuffed with a filler into 60-mm diameter regenerated collagen casings to a weight of 400—450g/sausage.

In trials 1-3, two kinds of starter combinations were used: (1) Flora-Carn SL (Chr. Hansen's Lab., Denmark, containing *Lactubacillus pentosus* and *Staphylococcus carnosus)* or (2) Baktoferment 61 (Rudolf Muller & Co., Germany, containing *S. carnosus):* with GDL. Sausages of both of the starter combinations 1 and 2 were divided into two groups, each fermented in different temperature. All the sausages were smoked on days 3, 6, 8 and 14.

The processing programs used in trials 1 and 2 were: (A) 24°C/88% relative humidity (rh) for 2 days, 20°C/86% rh for 5 days, 18X/84% rh for 5 days, 18°C/82% rh for 2 days; and (B) 14°C/90% rh for 1 day, 14X792% rh for 9 days and 14°C90% rh for 4 days. In trial 3 programs (A) and (C) (18°C/90% rh for 1 day, 18°C/92% rh for 1 day, 18X/94% rh for 1 day and 18°C/95% rh for 4 days) were used. All the sausages in trial 3 were ripened from the 8th to the 14th day at 18°C/95% rh. In all the trials 1 - 3 after 14 days of ripening the sausages were transferred to

ripened to a total of 28 days at 14°C/75% rh.

Factory trial (trial 4)

Dry sausages (diameter 70 mm, weight 1600g) were prepared in a meat factory from deep-frozen meat (33% beef, 40% pork and 27% pork fat), spices (0-7%), NaCl (2 5%), $NaNO_2$ (120 ppm, 10% solution), glucose and maltodextrin (0-6%). In trial 4, starter culture RM 2000 (Rudolf Muller & Co., Germany, containing *Pediococcus pentosaceus* and *S. carnosus)* was used. Two batches of raw material (each 213 kg) were manufactured. The sausages from both batches of raw material were divided into two groups. The first group was processed on the normal line of the meat factory - A. The other group was transferred to a ripening chamber - B. Processing program (A) was: 24°C/93% rh for 12h, 24°C/84% rh for 12 h, 23°C/81% rh for 16h, 21X789% rh for 24 h, 19X785% rh for 36 h, 18°C/85% rh for 36 h and 16°C/78% rh up to the 27th day. Processing program (B) was: 14°C/93% rh for 24 h, 14X790% rh for 24 h, 14°C/86% rh for 24h, 14°C/84% rh for 28h and 15X775-80% rh up to the 27th day. The sausages were smoked from the 1st to the 5th day.

Sampling

In trials 1-3, two samples from both of the batches of raw materials were analysed for pH, a_w, bacteriological counts and biogenic amines. During ripening, two sausages of each combination were taken as samples on days 6, 13 and 27. pH was followed every day up to 8 days (two duplicate samples) and then less frequently. Weight loss was followed from one sausage per combination every day during the lirsl week and thereafter less frequently until the 27th day. The sensory analysis was performed after 28 days by triangle test between the two different processing temperatures (not between the starter cultures). The sensory panel consisted of undergraduate students of food technology of Oradea University, Environmental Protection Faculty -17 people.

In trial 4, three replicate samples were taken from both batches of the raw materials. Two sausages of each batch during ripening (each further divided into two duplicate samples = 8 samples/processing program) were taken as samples on days 6, 13 and 27. Weight loss was followed from three sausages per combination almost every day during the first week and thereafter less frequently until the 27th day. The sensory analysis was performed by triangle test between the two different processing temperatures after 28 days. The sensory panel consisted of undergraduate students of food technology of Oradea University, Environmental Protection Faculty -17 people

Microbiological and chemical analyses

A 10-g sample of sausage was serially diluted with a diluent

containing 0-1% peptone and 0 85% NaCl in sterile deionized water. Coliform bacteria were enumerated on Violet Red Bile agar [VRB, Merck, ISO method No. 4832 (1991) (F.) incubated at 37°C for 24 h], enterococci on Slanetz-Bartley agar (SB, Merck, NCFA method No. 68 2nd edn, 1992, incubated at 44 5°C for 48 h), moulds and yeasts (only in trial 4) on malt extract agar (Oxoid) with added chlortctracycline (lOOmg/litrc) and chloramphenicol (lOOmg/litre) incubated at 22°C for 3 days (yeasts) and 5 days (moulds), lactic acid bacteria on de Man, Rogosa and Sharpe agar with sorbic acid (MRS-S, LabM MRS with sorbic acid of Fluka, Pharmacopoeia of Culture Media for Food Microbiology, 1987; incubated al 22°C for 7 days anaerobically) and hemolytic bacteria, micrococci and *Bacillus* spp. on blood agar base (BBL) containing 5% defibrinated horse blood (at 37°C for 48 h). The numbers of micrococci were confirmed by gram staining and a catalase test as well as by the typical appearance of the colonies.

pH-values were measured directly from the samples using a WTW pi I 537 meter (Germany) equipped with an Ingold DXK-S7 electrode (trials 1 3) or an Ingold LOT406-M6-DXK-S7/25 electrode (trial 4). After an equilibration period of 4h (trials 1-3) or 2h (trial 4), a_w values were obtained at 25°C using a Rotronic Hygroskop (Fattore Vitale & Co., Italy).

Biogenic amines were determined using the HPLC method of Eerola *et al.* (1993) with a diode array detector, using two duplicate analyses/sample. The detection limits were 1 mg/kg for tyramine, histamine, spermine, spermidine and cadaverine and 2 mg/kg for putrescine and phenylethylamine. The pure lactic acid bacterial strains of the starter cultures used had been previously found to be histamine- and tyramine-negative in fortified MRS broth (Maijala, 1993). The *Slaphylococci* strains of the starter cultures were studied with a similar method in fortified trypticase soy broth and were also found to be histamine and tyramine-negative (results not shown).

Statistics

Values of biogenic amines below the detection limits were considered as 0,5 ppm (for a detection limit of 1 ppm) or 1 ppm (for a detection limit of 2 ppm) in the statistical analyses. Multiple analysis of variance (ANOVA) was performed by Polifact (version 1.0) software on an Acer Extensa PC. The significance of the results of the sensory triangle tests was studied according to LMBG method 00-90 (7) Dreiecksprufung (1987).

RESULTS

Bacteriological results

The mean numbers of coliforms in the raw materials of trials 1, 2, 3 and 4 were 2-3 $\log_{10}$ cfu/g, 4-7 $\log_{10}$ cfu/g, 2-3 $\log_{10}$ cfu/g and 1-5 $\log_{10}$ cfu/g, respectively, the mean numbers of enterococci <2T $\log_{10}$ cfu/g, <2-l $\log_{10}$ cfu/g, 30 $\log_{10}$ cfu/g and <20 $\log_{10}$ cfu/g, the mean numbers of hemolytic bacteria 40 $\log_{10}$ cfu/g, 2-3 $\log_{10}$ cfu/g, 2-7 $\log_{10}$ cfu/g and <20 $\log_{10}$ cfu/g and the mean number of *Bacillus* spp. 4-2 $\log_{10}$ cfu/g, 3-6 $\log_{10}$ cfu/g, 3-7 $\log_{10}$ cfu/g and 2-2 $\log_{10}$ cfu/g, respectively. The numbers of moulds and yeasts in trial 4 were <2-5 $\log_{10}$ cfu/g.

The results of lactic acid bacteria (LAB) and micrococci are presented in Table 1. The highest levels of LAB in raw materials in pilot trials were detected in trial 3. In the factory trial 4 the initial levels of LAB were even higher but the bacterial flora consisted mainly of the starter culture used for previous batches on the same day (results not shown). In pilot trials the number of LAB increased more slowly at 14°C compared with 24°C during the first 6 days and the number of micrococci were lower at the lower temperatures compared with 24°C. However, due to the relatively small number of samples, these differences were not statistically significant.

In trial 4, temperature had a significant influence on the levels of LAB ($P<0.05$) and micrococci ($P<0.001$) in the final product. Eighty LAB strains were isolated from the MRS-S plates of the sausages in trial 4 after 27 days. Of the isolates from the higher processing temperature, 60% were Gram-positive cocci (identified as *P. pentosaceus* by the API50CHL system) compared with only one isolate (2-5%) from the lower temperature. The higher fermentation temperature (24°C) clearly favoured growth of the starter culture *P. pentosaceus* compared with the lower temperature at the end of ripening.

TABLE 1

The Mean Numbers of Lactic Acid Bacteria and Micrococci (log_{10} cfu/g) in Sausagesduring fermentation

Trial(s)	*Trials 1. 2*		*Trials 1, 2*		*Trial 3*		*Trial 3*		*Trial 4*	
Starter program	*Flora Cam SL*		*Baktoferment 61 + GDL*		*Flora Cam SL*		*Baktoferment 61 + GDL*		*KM 2000*	
	24° C	***we***	*24° C*	*14°C*	*24°C*	*18°C*	*24°C*	*I8°C*	*24° C*	*I4°C*
Lactic acidbacteria										
Day 0	3 7	3 7	3 5	3 5	4 7	4 7	4 4	4 4	5 7	5 7
Day 6	8 4	8 1	7 7	6 9	8 4	8 4	8 4	8 4	8 6	8 4
Day 13	8 0	8 4	7 8	8 1	8 4	8 4	8 3	8 1	8 6	8 6
Day 27	7 8	8 1	7 3	7 2	8 0	8 3	8 0	8 1	8 3	8 5
Micrococci										
Day 0	4 2	4 2	4 5	4 5	2 5	2 5	2 5	2 5	4 4	4 4
Day 6	6 5	6 1	6 9	5 9	6 7	6 1	6 1	5 7	4 6	4 7
Day 13	6 2	5 8	6 8	5 7	6 3	5 6	5 7	5 4	4 8	4 7
Day 27	5 7	5 6	6 5	5 6	5 6	5 7	5 6	5 5	4 1	4 5

24°C, 18°C and 14°C, processing programs starting from 24°C (A), 18°C (C) and 14°C (B), respectively.

The levels of other bacteria studied remained at the normal level for this type of product during ripening and no great differences were detected between the processing programs (A) and (B) or (A) and (C). With two exceptions, the levels of coliforms remained in all samples < 10 cells/g, the levels of enterococci <2-9 log_{10} cfu/g, the levels of hemolytic bacteria <4 0 $log_{]0}$ cfu/g in trials 1-3 and <2 1 log_{10} cfu/g in trial 4, the levels of *Bacillus* spp. <4-8 $log_{1(l}$ cfu/g in trials 1-3 and < 2 1 $log_{,n}$ cfu/g in trial 4 and the levels of moulds and yeast in trial 4 < 2-4 log_{10} cfu/g.

Biogenic amines, In the raw materials of the pilot plant trials 1-3 the levels of biogenic amines were relatively low : tryptamine < 7 ppm, phenylethylamine < 10 ppm, histamine <4 ppm, spermidine <4 ppm and spermine <31 ppm. During the ripening there were no significant differences in the concentrations of these amines and the levels at the end of ripening were tryptamine < 43 ppm, phenylethylamine < 25 ppm, histamine < 5 ppm, spermidine < 7 ppm and spermine < 35 ppm.

In trials 1 and 2 the combined effect of temperature and starter combination was significant according to the results of ANOVA (F<0001), concentrating especially on putrescine (P<001) and tyramine (/'><0 01) (Table 2). The temperature used for processing the sausages significantly influenced the levels of putrescine (F<001), cadaverine (P<0 05) and tyramine (P<001). This influence was highly dependent on the starter combination (/"< 0-001), resulting in lower levels of amines in sausages fermented by Baktoferment 61 + GDL at lower temperatures and in higher levels of amines in sausages fermented by Flora-Carn SL

at lower temperatures. The temperature also had a significant effect on the biogenic amines in trial 3 (P< 0-001), but for the individual amines this effect was not statistically significant due to the small number of samples.

TABLE 2

The Mean Levels of Putrescinc, Cadaverine and Tyramine (mg/kg) in Sausages during Fermentation

Trial(s)	Trials 1, 2		Trials 1, 2		Trial 3		Trial 3	
Starte program	Flora Carn SL		Baktoferment 61 +GDL		Flora Carn SL		Baktoferment 61 + GDL	
	24°C	14°C	24°C	14°C	24°C	18°C	24°C	18°C
Putrcscine								
Day 0	<2	<2	<2	<2	<2	<2	<2	<2
Day 6	2	2	<2	3	36	23	43	20
Day 13	< 2	2	4	4	82	85	100	81
Day 27	6	8	22	12	157	161	163	143
Cadaverine								
Day 0	1	1	<1	<1	1	1	1	1
Day 6	1	1	3	1	1	1	1	1
Day 13	1	2	4	4	1	1	1	1
Day 27	4	3	12	2	5	3	4	3
Tyramine								
Day	1	1	1	1	1	1	1	1
Day 6	9	9	11	8	54	59	60	49
Dav 13	13	13	18	10	87	4	82	77
Day 27	13	27	29	19	166	153	109	161

24°C, 18°C and 14°C, processing programs starting from 24°C (A), 18°C (C) and 14°C (B), respectively.

The levels of putrescine, tyramine, spermine and spcrmidine in the sausages after 27 days in trial 4 were significantly lower at the higher processing temperature compared with the lower temperature.

The clear influence of the raw material can be seen by comparing the results for putrescine and tyramine during ripening in trials 1-3 using temperature program (A). The batch of raw material also had a significant influence on the levels of putrescine, cadaverine and tyramine (P<000l) and spermidine (/^{J}<001) in trial 4.

Weight loss, pH, water activity (a_w) and sensory analysis

The pH decreased more rapidly at higher fermentation temperatures fermented with LAB-containing starter culture and no GDL. In sausages fermented by Baktoferment 61 and GDL the pH decrease was already rapid during the first 24 h and was independent of the processing temperature.

Weight losses were almost equal and constant between the processing temperatures (A) and (B) in trials 1, 2 and 4, resulting in final losses of 29-1--32-9%. In trial 3, greater difficulties were encountered in maintaining an equal weight loss in both fermentation chambers, resulting in a 3% difference between the chambers. However, after the 20th day of ripening the weight losses were close to each other, with final losses of 32-5-33-7%. This difference can also be seen in the a^ values during ripening in trial 3 (Table 3). No major differences were detected between the processing programs (A) and (B) in other trials.

In triangle tests for sensory analysis the different processing programs could be statistically distinguished only in trial 1 of pilot plant trials (Flora-Carn SL P<0 05 and Baktoferment 61 + GDL P<001). No ranking order could be expressed in these results. In the triangle test made with sausages of factory trial 4 the two processing programs were well distinguished and the higher fermentation temperature was ranked better (P< 0,001).

TABLE 3

The Mean Values of Water Activity in Sausages during Fermentation

Trial(s)	*Trials 1, 2*		*Trials 1, 2*		*Trial 3*		*Trial 3*		*Trial 4*	
Starter program	*Flora Carn SL*		*Baktoferment 61 +GDL*		*Flora Carn SL*		*Baktoferment 61 + GDL*		*RM 2000*	
	24°C	*14°C*	*24°C*	*14°C*	*24°C*	*18°C*	*24°C*	*18°C*	*24°C*	*14°C*
Day 0	0 95	0 95	0 94	0 94	0 95	0 95	0 95	0 95	0 96	0 96
Day 6	0 94	0 93	0 94	0 94	0 94	0 94	0 95	0 92	0 95	0 95
Day 13	0 93	0 93	0 93	0 93	0 93	0 94	0 93	0 91	0 94	0 94
Day 27	0 89	0 89	0 90	0 90	0 88	0 88	0 88	0 86	0 92	0 93

24°C, 18°C and 14°C, processing programs starting from 24°C (A). 18°C (C) and 14°C (B), respectively.

DISCUSSION

According to these results, raw material has a clear influence on the levels of putrescine, tyramine, cadaverine and spermidine in dry sausages. This conforms with the results of Tschabrun *et al.* (1990), who could reduce the histamine content in dry sausages by using very fresh meat, and also with our own previous results (Maijala *et al*). The selection of raw material for the manufacture of dry sausages seems to be one of the most critical control points in reducing the formation of high levels of biogenic amines in the final product.

Processing temperature had an influence on the formation of biogenic amines, depending on the type of starter combination used.

With LAB-containing amine-negative starter cultures (Flora-Carn SL and RM 2000), lower levels of amines were detected in the final product processed at a higher temperature. However, this difference was no longer evident with a smaller temperature difference (24°C compared to 18°C). One explanation for the influence of processing temperature is that the higher fermentation temperature gives the starter culture the opportunity to outgrow non-starter lactic acid bacteria. Furthermore, the much lower numbers of the starter culture strain *P. peniosaceus* in the final product of the factory trial after processing at the lower temperature compared with the higher temperature supports this hypothesis. The better growth of LAB at the higher fermentation temperature could also be seen in the more rapid pH decrease at the beginning of fermentation at higher processing temperatures.

These results suggest that a starter culture should be selected bearing in mind not only the amine-negative properties of the culture but also its ability to compete and grow well at the temperature intended for processing of the product. In this way the product quality can be guaranteed with regard to the formation of biogenic amines. As pH was decreased rapidly by the addition of GDL and only *S. carnosus* as starter culture, more putrescine, cadaverine and tyramine were formed at higher temperatures. This was probably due to increased proteolysis at higher temperatures. Temperature also has an effect on the activity of decarboxylasc enzymes. Joosten & van Boekel (1988) reported that the activity of histidine decarboxylase of *L. buchneri* increased as temperature increased from 15°C to 30°C. Kranner *et al.* (1991) showed that histamine formation in raw sausages requires both a sufficient number of histidine-dccarboxylating microorganisms and available histidine and is further increased by increased storage temperature and age of the raw material. They detected lower levels of histamine in sausages fermented at a lower temperature (7°C) with or without added histamine-positive *L. buchneri* compared with a higher temperature (18°C). They did not use any starter culture. The question of the formation of histamine at somewhat higher processing temperatures and with starter culture was one of the main interests of this study. According to our earlier studies, a rapid pH decrease reduces the histamine levels formed in minced meal and dry sausages (Maijala *et at.* 1993, Maijala *et al.* 1992). In this trial LAB grew better at a higher processing temperature, resulting in a more rapid pH decrease compared with the lower temperature. Unfortunately the levels of histamine were very low in this trial (< 5 ppm), probably due to the high quality raw materials used.Therefore no differences could be detected between the processing temperatures and this question still remains unanswered.

According to these results, not only the selection of raw materials, but also the choice of optimal processing temperature for the amine-negative starter culture used, are important critical control points in preventing the formation of high levels of biogenic amines in dry sausages. The ability of the starter culture to compete with and outgrow amine-positive bacteria originating from the raw materials is also important.

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