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INFLUENCE OF A BACTERIAL PROTEINASE ON RIPENING OF DRY SAUSAGE

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INTRODUCTION

The sensory characteristics of dry sausage depends, in addition to the various ingredients used, on the accumulation of fermentation products from degradation of carbohydrates, proteins and lipids. Non-enzymatic reactions as well as endogenous enzymes and enzymes of bacterial origin may be important in flavour formation as a result of proteolysis and lipolysis (Selgas *et al.*, 1988). The effect of a proteinase from Aspergillus oryzae in dry sausage production has been investigated in order to shorten the ripening period (Diaz *et al.*, 1992). However, the sausages containing high amounts of proteinase had an overall lower quality as judged by a test panel.

The aim of this study was to investigate the effect of addition of a bacterial proteinase on the fermentation and ripening of dry sausage .

MATERIALS AND METHODS

Preparation of sausages

The initial sausage mixture contained (%w/w): beef (51.3), pork (18.7), lard (25.6), salt (0.4), dextrin (0.3) and glucose (0.4). Smoking and addition of spices were excluded. Lactobacillus sake L45 was used as starter culture and supplied at $4x10^6$ cells/g sausage mixture. The sausage components were mixed and divided into two batches (10kg each). Proteinase was added to batch 1. The second batch was the control in which no enzyme was added. Twenty sausages (0.5kg) were prepared from each batch. After the initial fermentation phase (two days at 24°C and 92% relative humidity (rh), two days at 20°C and 88%rh, two days at 18°C and 85%rh) the sausages to which proteinase was added and the control sausages were ripened at 15°C and 85%rh for 14 and 35 days respectively. Thereafter they were vacuum-packed and stored at 4°C.

All the results obtained were corrected for differences in weight loss.

Preparation of proteinase from L. paracasei subsp. paracasei NCDO151

Cell wall bound proteinase was extracted according to Næs and Nissen-Meyer (1992) with one exception; 50mM sodium phosphate buffer pH6.0, containing 10mM EDTA (buffer A) was used as extraction buffer. Crude proteinase extract (300ml) from 6L of cell culture was added to sausage mixture 1. This corresponds to an enzyme concentration of 12U/g sausage, as measured by degradation of ^{14}C-methylated casein (Næs and Nissen-Meyer, 1992).

Buffer A (300ml) was added to the control sausage mixture.

Determination of D-and L-lactic acid

Two grams sausage was homogenized in 10ml H2O for one minute. The suspension was heated at 70°C for 20 minutes in closed-capped tubes and centrifuged at 10000xg for 20 minutes. The supernatant was analyzed for D- and L-lactic acid using an enzymatic food analysis kit (Boeringer Mannheim, Mannheim, FRG).

Analysis of fluorescamine reactive amino groups

Five grams of sausage was homogenized for 15 seconds in 40ml 25mM potassium phosphate buffer, pH7.4. The homogenate was passed through a 0.45µm filter (Milex HA, Millipore S.A., France) and appropriate dilutions were made. The filtrate was mixed with two volumes of 15% trichloroacetic acid (TCA) (w/v), and then centrifuged. TCA soluble compounds, containing amino groups, in the extract were determined by a fluorescamine based assay (Pacifici and Davis, 1990). Fluorescence was measured at 394nm exitation and 475nm emission in a luminescence spectrophotometer (Perkin Elmer LS 50). Leucine was used to obtain a standard curve. The results were expressed as mg leucine equivalents.

Electrophoresis of proteins

Extraction of water soluble proteins and SDS-olyacrylamide gel electrophoresis were carried out as described by Næs *et al.* (1992).

Water activity

Sausage samples were cut into approximately 5mm cubes and placed in a sample holder. Water activity (Aw) was measured by an electronic hygrometer (NOVASINA Aw-center, sensor: enRSK-4/CT-4, Novasina AG, Switzerland).

Sensory analysis

Standard procedures for sensory descriptive profiling according to Amerine *et al.* (1965) and Risvik (1985) were used. Sample preparation and .sensory parameters were examined as described previously (Næs *et al.*, 1991). Statistical analysis was performed using the SYSTAT-program for univariate analysis (Wilkinson, 1990). Sensory analyses were performed 14 and 35 days after the production day.

RESULTS AND DISCUSSION

L. sake L45, which is a fast growing and bacteriocin producing organism (Mørtvedt and Næs, 1990) was selected as starter organism in order to minimize the contribution of other microorganisms during the fermentation and ripening period. A rapid pH drop (Figure 1) and a corresponding increase in viable counts (data not shown) during the first three days of sausage production indicated a normal fermentation process for the sausages.

The pH dropped more rapidly and reached a lower final value in the sausages to which proteinase was added than in the control sausages. The more extensive pH drop in the sausages containing proteinase can be explained by a corresponding increase in D-lactic acid. L-lactic acid remained constant during the production period (data not shown). Since proteinase addition had no effect on the viable counts, the increased D-lactic acid production may be caused by an increased bacterial metabolism. It appeared that the starter bacteria in the sausages with proteinase had a higher availability of fluorescamine reactive compounds (Figure 2).

An increased degradation of water soluble meat proteins in the proteinase containing sausages were also observed (Figure 3). These observations, indicating a higher level of free amino acids and peptides, may explain the higher metabolic rate in the sausages with proteinase. The SDS-PAGE protein pattern after seven days of climatization of the control sausages was reached within three days by addition of proteinase. The sausages to which proteinase had been added showed a more rapid loss of weight and a corresponding decrease in Aw in comparison to the control sausages (Figure 4). The control sausages needed one extra week of ripening to obtain similar values in Aw and weight loss. In the case of the sausages with proteinase, the ripening time was terminated by chilled storage 14 days after production. The control sausages were maturated for additional 21 days which is reflected in a continuous drying throughout the whole period.

Addition of proteinase showed an effect on various sensory attributes during the first 14 days of dry sausage ripening (Table 1a). An increased rate of aroma development (taste intensity and maturity) caused by addition of proteinase, indicated an accelerated ripening process. The more acidic taste of the proteinase containing sausages was due to the increased amount of D-lactic acid.

When comparing the proteinase containing sausages to the control sausages, after 14 days of ripening, the control sausages were softer (Table 1a). Proteolytic enzymes degrade proteins into peptides and amino acids and a less firm product would perhaps be expected (Diaz *et al.*, 1992). However, proteolytic activities early in the fermentation period resulted in less water binding capacity as seen by the changes in weight loss and Aw during the ripening period, and thus a faster drying of the sausage occurred.

After 14 days of ripening the sausages containing proteinase were vacuum-packed and stored at 4 °C, while maturation of the control sausages continued. A second sensory analysis was performed after 35 days (Table 1b). The sensory differences of the taste attributes observed after 14 days of maturation had now disappeared. However, after 35 days differences in colour attributes appeared. An extensively drying of the control sausages, reflected in weight loss and Aw, may explain the colour development.

Addition of proteinase, therefore, seems to induce changes similar to those observed during a normal fermentation process (without enzyme added). Moreover, the changes appeared earlier in the fermentation and ripening period.

CONCLUSION

Addition of proteinase to sausage mixture shorten the dry sausage production time. The proteinase containing sausages appeared mature after 14 days of ripening, whereas the control sausages required one additional week to reach the same stage of maturity. These findings may be of economical importance for the fermentation industry.

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Table 1. Effect of proteinase addition on sensory attributes of dry sausage after 14 days (A) and 35 days (B) of ripening.

A	14 days of ripening	
	Control	Proteinase#
Intensity taste	6.09*	6.58
Maturity taste	4.41	5.56
Acidic taste	2.65	3.73
Bitter taste	2.70	3.28
Hardness	3.09	3.80

B	35 days of ripening	
	Control	Proteinase#
Hardness	5.40	4.60
Fat colour tone	5.56	4.83
Whiteness of cross-section	3.89	5.18
Colour tone of cross-section	6.18	5.08
Colour intensity of cross-section	5.96	5.21

* All values are average intensities for 10 assessors and 3 replicates and are significant different ($P<0.05$).
# The ripening of the proteinase containing sausages were terminated by chilled storage (40°C) in vacuum 14 days after the production day.