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THE EFFECT OF TWO BACTERIAL PROTEINASES ON DRY SAUSAGE RIPENING

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SUMMARY

The effect of two bacterial proteinases, from *Lactobacillus paracasei* subsp. *paracasei* NCDO151 and Alcalase from *Bacillus licheniformis* (manufactured by NOVO Nordisk, Copenhagen, Denmark), on the ripening of dry sausages were compared. Increased levels of starter bacteria, D-lactic acid, NPN-nitrogen and meat protein breakdown and a higher pH-drop indicated that the enzyme from *Lactobacillus paracasei* accelerated the dry sausage ripening, whereas this effect could not by detected in sausages with the Alcalase enzyme. Sensory analyses confirmed that sausages containing proteinase from *L. paracasei* showed the same degree of maturity after 14 days as the sausages without enzymes or with the Alcalase enzymes showed after 28 days.

INTRODUCTION

The northern type of dry sausage normally has a ripening time of three to four weeks. During this period the water activity and the pH of the product are lowered significantly, the texture changes from a soft batter to a hard, sliceable sausage and various flavour compounds are formed. The flavour compounds generated during ripening may be peptides and amino acids arising from proteolytic activity, lactic and acetic acids from breakdown of carbohydrates and free fatty acids, aldehydes, ketones and esters from catalysis of lipids. The accelerating effect of the proteinase from *L. paracasei* subsp. *paracasei* NCDO151 on dry sausage ripening has already been shown (Næs *et al.*, 1995). In this follow-up study the effect of this enzyme is compared with that of a commercially available proteinase.

MATERIALS AND METHODS

Proteinase preparation: The serine proteinase from *L. paracasei*. subsp. *paracasei* NCDO151 (=NCDO151 proteinase) was extracted according to Næs *et al.*(1994). 300 ml crude proteinase extract (sterilized by filtration) from 15 l cell culture was added to the sausage mixture, corresponding to 5 Arbitrary Units (AU) per gram sausage mixture as measured by degradation of ^{14}C-methylated casein. The commercial proteinase extract from *B. licheniformis* (Alcalase) was sterilized by filtration and diluted with the extraction buffer above (50 mM sodium phosphate with 10 mM EDTA, pH 6.0) to 300 ml, to give an enzyme activity corresponding to 10 AU/g sausage mixture. The proteinase activities were determined at conditions comparable to those existing under sausage fermentation; pH=5.6 and temperature=20°C.

Preparation of sausages: A model salami without spices and not subjected to smoking was made which contained (% w/w): beef (51.3), pork(18.9), lard (25.7), sodiumchloride (3.3), nitrite (0.02) dextrin (0.3), glucose (0.4) and ascorbic acid (0.04). *Lactobacillus sake* L45 was used as a starter culture and supplied at 6 x 10^5 cells/g sausage mixture. The ingredients were mixed and divided into 4 batches (12.5 kg each). The experiment was performed following a full factorial design, with two factors (NCDO151 proteinase and Alcalase) at two levels (no and full level of enzyme addition). Batch 1 was the control batch into which no enzyme was added, only 300 ml extraction buffer. Proteinase extract from NCDO151 was added to batch 2, Alcalase extract to batch 3 and extracts of both proteinases to batch 4. 30 sausages (400 g) were prepared from each batch. A replicate of this production was made on the same day so that in total there were 8 batches of sausages. The sausages were stuffed in casings and placed in a climatisation cabinet under the following conditions: 2 days at 24°C, 92% relative humidity (RH), 2 days at 20°C, 88% RH, 2 days at 18°C, 85% RH and finally the sausages were allowed to ripen at 15°C, 85 % RH until they were considered mature.

Analytical methods
Samples were collected for various analysis after 0, 1, 2, 3, 7, 14, 21 and 28 days. The weight of each sausage was determined before and after the time in the climatisation cabinet and the weight loss calculated. All analyses were performed on duplicate samples.

pH was assayed on 10 g sausage homogenised in 40 ml distilled water and a_w was measured by an electronic hygrometer (NOVASINA a_w-centre, sensor: enRSK-4/CT-4, Novasina AG, Switzerland) on 10 g sausages cut in 5 mm cubes. Microbial growth was evaluated on 10 g samples by plating 10-fold dilutions onto blood agar (Difco) for total counts and MRS agar (Oxoid) for lactobacilli count. The plates were incubated at 30°C for 2 days. To determine differences in D- and L-lactic acid levels, 2.0 g sausage were homogenised in 10 ml. H_2O for 1 min. with an Ultra Turrax. The suspension was heated at 70°C for 20 min. and centrifuged at 10.000 x g for 20 min. The supernatant was analysed for D-and L-lactic acid using an enzymatic analysis kit (Boeringer-Mannheim, Germany).Extraction of water- and salt soluble meat proteins and SDS-polyacrylamide electrophoresis were performed as described by Næs *et al.*(1992) and analysis of non-protein nitrogen according to Kjeldahl as described by DeMasi *et al.* (1990).

Sensory descriptive profiling was carried out by 11 trained assessors at 14 and 28 days after production. The flavour profile of the sausages was determined using an unstructured line with end points (1-9) where 1 denoted low intensity and 9 high intensity for each of the following 18 characteristics: Odour intensity, acidic odour, colour tone of fat, whiteness, overall colour tone, colour intensity, flavour intensity, maturity flavour, fresh flavour, acidic taste, sour taste, salty taste, bitter taste, rancid flavour, hardness, fattiness, juiciness and stickiness. Differences in sensory score were evaluated using Tukey's test.

All data were treated using an analysis of variance to detect the effect of the two treatments.

RESULTS AND DISCUSSION

Microbial fermentation was observed in all sausages, as registered by a drop in pH, an increase in lactobacilli count and in D-lactic acid formation. However, these effects were much more pronounced in the sausages into which were added proteinase extract from *Lactobacillus paracasei*, i.e. batches 2, 4, 6 and 8 (fig. 1 and 2). In all instances the sausages with Alcalase added followed the control closely.

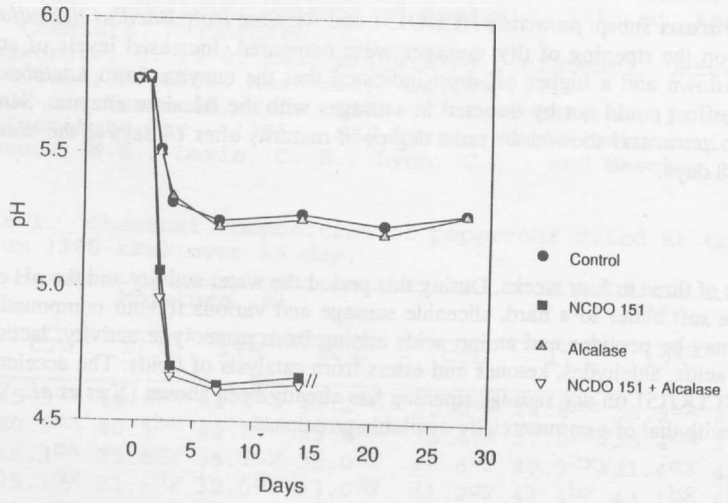

Figure 1: pH drop in sausages. Each curve is the mean of 4 replicates. // denotes that these sausages were considered mature after 14 days and therefore removed from the climatisation cabinet.

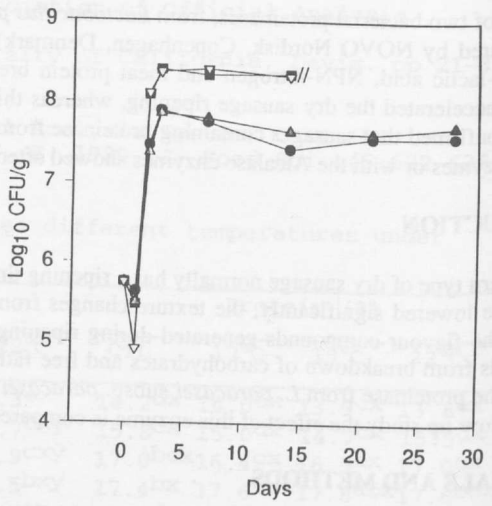

Figure 2: Lactobacilli count in the sausages. Each curve is the mean of 4 replicates.

The addition of NCDO151 proteinase influenced the degradation pattern of proteins on SDS-PAGE in that several meat proteins were degraded more rapidly in the sausages with this enzyme added (results not shown). This degradation is also illustrated by a higher NPN-value after 14 days in the sausages with NCDO151 proteinase added compared with the control and the sausages with Alcalase added ($p<0.001$) (results not shown).The differences detected by chemical analysis were verified by sensory evaluation. After 14 days of

NPN-value after 14 days in the sausages with NCDO151 proteinase added compared with the control and the sausages with Alcalase added ($p<0.001$) (results not shown).The differences detected by chemical analysis were verified by sensory evaluation. After 14 days of maturation, the sensory analysis showed differences in 11 out of 18 attributes between the sausages with and without NCDO151 proteinase ($p<0.01$). At the next sensory evaluation (after 28 days maturation) these differences were smaller or had altogether disappeared

It is obvious that the action of the NCDO151 proteinase stimulates the metabolism of the starter organism as is illustrated by increased viable counts, higher pH drop and a corresponding increased amount of D-lactic acid, and that this proteinase degrades meat proteins under real sausage maturation conditions. This effect promotes the maturation of the sausages as documented by sensory analysis, in such a way that after two weeks, these sausages display almost all the characteristics of a mature product that the control sausages or the sausages with only Alcalase added first show after four weeks.

CONCLUSION

The accelerating effect of proteinase from *Lactobacillus paracasei* subsp. *paracasei* NCDO151 on dry sausage ripening has been firmly established. The exact specificity of the enzyme is essential for the desired effect, as the commercial proteinase Alcalase did not show similar effects on the ripening. Further investigations into this phenomenon will include evaluation of the aroma profiles of the sausages with and without enzymes added by GC/MS analyses.

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