FERMENTATION OF DRY SAUSAGE - THE EFFECT OF ADDED PROTEINASE AND LIPASE FROM LACTOBACILLI

H. Næs, B.O. Pedersen, A.L. Holck, L. Axelsson, V. Holten and H. Blom

MATFORSK, Norwegian Food Research Institute, Oslovn. 1, 1430 Ås, Norway

SUMMARY

Proteinase (from *Lactobacillus paracasei* ssp. *paracasei* NCDO 151) and lipase (from *Lactobacillus plantarum* MF 32) were added to the sausage mix and dry sausages were made. Colour development (L*,a*,b*), pH, degradation of water soluble proteins, NPN, individual amino acids and total fatty acid composition were followed for 42 days. With the addition of proteinase, colour developed differently as compared to control and lipase sausages, although after 42 days all three colour coordinates were equal to those found in the control and lipase sausages. Proteinase also caused a rapid decrease in pH, down to 4.5 in three days as compared to 4.95 for the control and lipase sausages. NPN showed no differences between the three sausages at any stage of the fermentation and maturation period. Degradation of water soluble proteins as determined from SDS-PAGE revealed differences when comparing the proteinase sausages with both the control and lipase sausages. Analyses of fatty acid content showed that unsaturated fatty acids were the group most influenced by addition of lipase.

INTRODUCTION

Fermented meats have a flavour which is product specific, derived from the different ingredients used. The formation of aroma and taste is based on enzymatic as well as non-enzymatic reactions, both contributing to the wealth of components which together make up the characteristic flavour of a fermented meat product. The flavour is composed of volatiles with odour properties, nonvolatiles with taste and tactile properties, together with enhancers and synergists (DVIWEDI, 1975). Apart from the contribution of spices employed, flavour components are principally derived from protein, fat and carbohydrate breakdown, a process dependent on exo- as well as endogenous enzyme activities where climate and pH represents the parameters easiest controllable. Calpain is active at neutral pH and is reported to be responsible for specific degradation of tropomyosin, C-protein, troponin T and troponin I during the meat aging process (KINSELLA, 1988 & GOLL et al 1983). Calpain activity probably ceases shortly after slaughter due to the metabolization of glycogen to lactic acid causing the pH to drop well below 6.0. According to VERPLATSE et al(1989), myosin, actin and troponin T are the proteins that undergo the most extensive hydrolysis, 48.8 %, 32.7 % and 26.5 % reduction, respectively, after 21 days fermentation. They also concluded that the breakdown of proteins in fermented meat was caused either by endogenous lysosomal or by bacterial proteinases similar in specificity to cathepsins B and D, or both. The fermenting sausage provides optimal conditions for cathepsin activity with a pH of 4.8-5.2 combined with a temperature of 15-22 °C. The peptides thus produced are subject to further breakdown by peptidases, reflected by an increase in free amino acids and other non protein nitrogen (NPN). The lipid phase plays a double role in the flavour picture. I. Hydrolytic and autoxidative degradation of triglycerides giving rise to a wilderness of flavour components. In a resent work by BERDAGUE et al (1991) they found that aldehydes alone, derived from lipid degradation constituted 22 % of the relative area in their gas chromatograms. II. Many of the flavour compounds are of non polar nature. Thus they solubilize in the lipid phase and are contained here. The amount of lipids in dry sausages varies greatly, but the bulk is in the order of 25-55 % measured as fat in total weight percentage. Porcine backfat dominates. This fat contains approximately 1,5 % Myristic (14:0), 22 % Palmitic (16:0), 13 % Stearic (18:0), 4 % Palmitoleic (16:1), 48 % Oleic (18:1) and 10 % Linoleic (18:2) (KINSELLA, 1988).

Lipases are known to hydrolyse preferentially the outer fatty acids of the triglyceride molecule. DEMEYER et al (1974) have shown that in fermented sausage the rate of lipolysis decreased in the order: linoleic- oleic- stearic- palmitic acid, thereby demonstrating a lipolytic preference for position 1 and 3 of the triglycerides, the position most frequently occupied by unsaturated fatty acids. The liberated unsaturated fatty acids undergo nonenzymatic oxidation, thus producing lipid peroxides and carbonyls, compounds which

contributes to flavour both in terms of rancidity and pleasant odours. The present investigation was undertaken to study the effect of lactobacillus proteinase and lipase on the biochemistry of ripening of dry sausage.

MATERIALS AND METHODS

Preparation of sausages, preparation of lipase extract, determination of lipase activity and proteolytic activity were performed as described by NÆS et al (1991). L, a and b colour coordinates were measured employing a Hunter Lab, Lab Scan II instrument (Hunter Ass. Lab. Inc., Reston, Va., USA.).

Preparation of proteinase from *L. paracasei* subsp. paracasei NCDO151: Cells were harvested, washed and cell wall bound proteinase extracted according to NÆS and NISSEN-MEYER (1992). Crude proteinase extract from 700 ml culture was used for production of 7 kg sausage mix.

Analyses of nonprotein nitrogen (NPN). Extraction of NPN from sausage samples were done essentially as described by DEMASI (1990). The sample was homogenized for 10 s in Ultra Turrax. Addition of LiCl and LiOH during the acid precipitation step was excluded. NPN concentration was determined according to Kjeldahl.

Amino acid analysis. The amino acid extraction was performed as follows: 5 g of dry cured sausage was diluted 1:10 with 0.1 N HCl and homogenized for 10 s in Ultra Turrax. The samples were centrifuged at 10000 x g for 20 min and the supernatant filtered through a nylon membrane of pore size 45 µm (Nytal DIN130-40, SST Thal, Switzerland). Deproteinization and amino acid derivatization of standard samples were performed as described by ARISTOY and TOLDRA (1991). The samples were analyzed on a Spectra-Physics SP8700XR liquid chromatograph equipped with a Biotronic UV detector, BT3030 (254 nm). The column used was Supelcosil LC-18 DB, 25 cm x 4.6 mm (5 µm particle size) (Supelco, Bellafonte, PA.USA). The column temperature was kept constant at 40 °C with a column Heater (BAS Temperature Controller LC-22A). The solvent system was as described by ARISTOY and TOLDRA (1991), with a flow rate of 0.8 ml/min.

Extraction of proteins for SDS-polyacrylamide gelelectrophoresis. 5 g of sausage was diluted 1:8 with 40 ml 25 mM K-phosphate buffer, pH 7.4. Samples were homogenized for 15 s using an Ultra Turrax and centrifuged at 10000g for 20 min. This supernatant was designated the water soluble protein extract. The pellets were further treated for extraction of salt soluble proteins in the following way: The pellet is washed twice with the K-phosphate buffer and centrifuged at 10000 g for 10 min each time. The pellet was resuspended in 25 ml of a KI/K-phospate solution (1.1 M KI solution is mixed with an equal volume of 0.1 M K-phosphate buffer, pH 7.4 just before addition to the pellet). The samples were allowed to stand at 4 °C over night. Thereafter, the samples are filtrated through a nylon membrane, 45 µm and stored at -18 °C before electrophoresis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was carried out at a constant voltage of 250 V on 8-18 % gradient SDS-PAGE gels (ExcelGel, Pharmacia), using ExcelGel SDS buffer strips (Pharmacia). The proteins were visualized by staining with Coomassie Brilliant Blue G-250. Finally the gels were scanned using LKB Ultroscan XL densitometer (LKB, Bromma, Sweden).

Analysis of fatty acid composition.

Total lipid extraction was performed according to FOLCH et al (1957). The procedure of MASON and WALLER (1964) was employed for transmethylation. The resulting fatty acid methyl Esters were subjected to gas chromatography using a polar, fused silica capillar column, DB 23 (30 m, 0.25 mm, 0.25 µm) in a Hewlett-Packard (HP) 5890 gas chromatograph, with FID detector and HP 3365 Chemstation software (Hewlett-Packard, Palo Alto, CA., USA). Results were calculated as percentage of the total peak area of each analytical run.

RESULTS AND DISCUSSION

Effect of added proteinase: As shown in Figure 1, the effect of the proteinase addition could be followed by changes in lightness,(L) and pH, which developed different from the reference sausages.

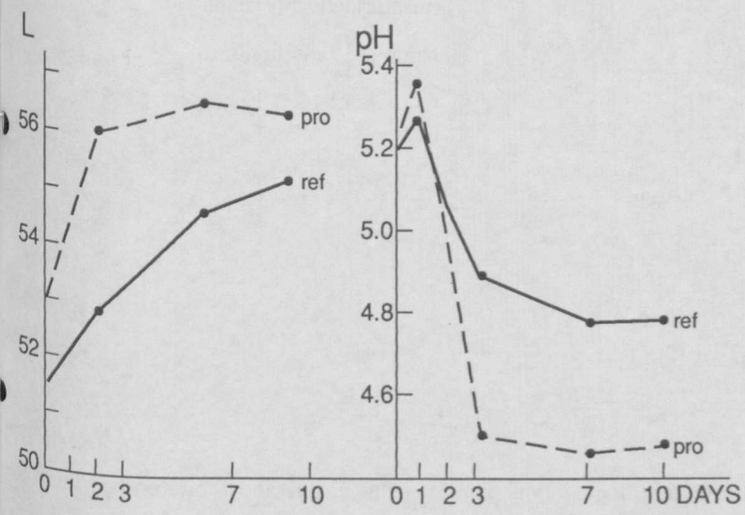

Figure 1. Development of lightness (2) and pH in reference (ref) and proteinase (pro) sausages. After 42 days, no significant difference in L or pH could be demonstrated.

These differences, however, were not reflected by the NPN analysis (results not shown), where an increase with time but no differences between proteinase and reference sausages could be demonstrated. Individual amino acid analyses were performed to quantify the fractions of acidic and basic amino acids. There was a slight difference in the amounts of Met., Leu., Ileu., Ser., Ala., Val. (all neutral), Gln. (basic) and Asp. (acidic), but the differences were to small to explain the sudden pH drop measured in the proteinase sausages. For the other amino acids no difference could be demonstrated (results not included).

Regarding all the above mentioned amino acids, the proteinase sausages have lower content of each amino acid analyzed except for

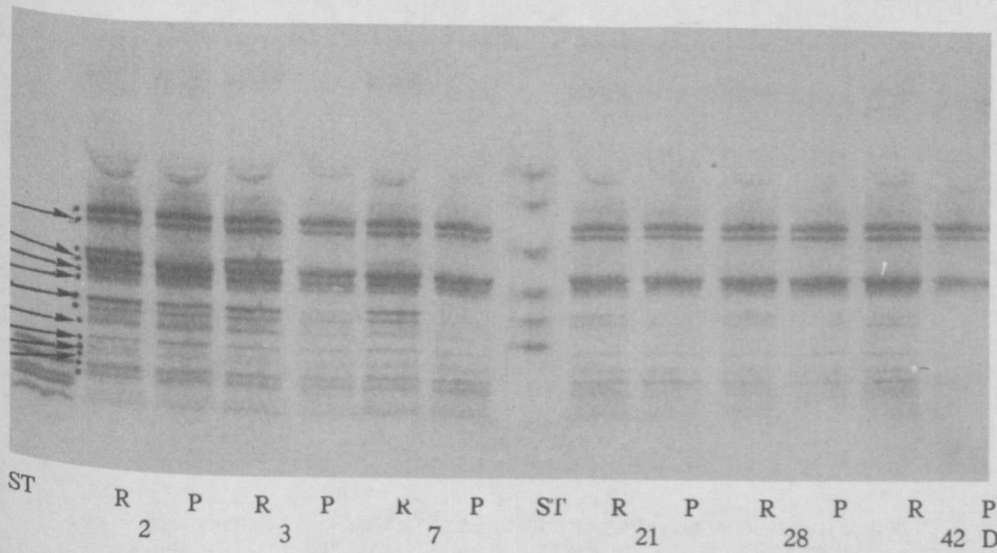

Figure 2. SDS-PAGE of water soluble proteins from dry sausage. R:reference, P:proteinase, ST:standard 2:desmin, 4:actin, 5:troponin T, 6:tropomyosin; 7: 30000D, 9:myosin light chain 1, 10:troponin I, 11: troponin C/myosin light chain 2, 12: myosin light chain 3.

serine (results not shown). Thus the observed drop in pH may be caused by an increase in the amount of acidic oligopeptides.

With regard to protein degradation the water extracts of proteins from the reference and the proteinase sausages were clearly different, as demonstrated in Figure 2. The salt soluble protein extracts did not show the same effect of the proteinase addition.

The protein pattern after 42 days of climatization of the reference sausages were reached within two to three days by addition of proteinase. Due to the rapid pH development and the protein degradation pattern demonstrated, this enzyme may have a potential for a reduction of the production time of fermented dry sausages, as was also indicated by the development in lightness (L).

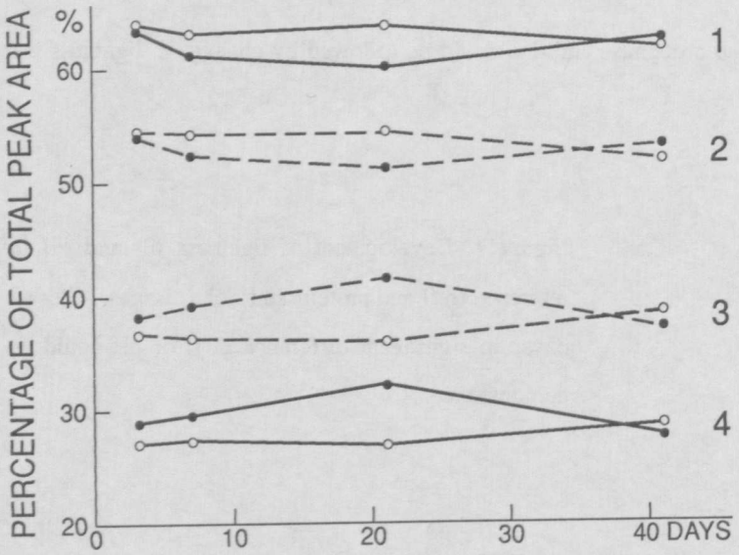

Figure 3. Development in relative amounts of fatty acids:O:reference, ●:lipase.
1: Σ C-18 fatty acids
2: Σ unsaturated fatty acids
3: Σ saturated fatty acids
4: Σ C-14 & C-16 fatty acids

Effect of added lipase

Colour coordinates, pH, NPN, individual amino acids and the protein degradation pattern of the lipase and the reference sausages disclosed no differences (results not shown). Analysis of the fatty acid composition (C-14 to C-18) are presented in Figure 3. The results show that the C-18 fatty acids (18:0, 18:1, 18:2 and 18:3) are more labile in the first three weeks of the ripening period when lipase is added. This is due to a relatively large breakdown of 18:1 and a somewhat smaller breakdown of 18:2, while the relative changes in the percent distribution of the fatty acids in the reference sausages only show minor variations with time. Addition of lipase brings about a more extensive turnover of the unsaturated fatty acids, in accordance with the expected affinity of this bacterial lipase towards the outer positions of the triglyceride molecule.

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