

PROTEOLYSIS IN SAUSAGE FERMENTATION

SOSİS FERMENTASYONUNDA PROTEOLİZ

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ÖZET: Proteoliz, fermente sosislerin kalitesine katkıda bulunan temel biyokimyasal reaksiyonlardan biridir. Proteoliz reaksiyonları ürün yapısında doğal olarak bulunan enzimler ya da mikrobiyel orijinli enzimler tarafından katalize edilirler. Et proteinleri önce kas enzimleri tarafından polipeptitlere, daha sonra da peptidazlar vasıtasıyla küçük peptitlere hidrolize olur. Proteolizde son aşama, etin yapısında bulunan ya da bakteriyel orijinli aminopeptidazlar tarafından serbest amino asitlerin oluşmasıdır. Peptitler ve serbest amino asitler, fermente et ürünlerinde protein olmayan azotlu maddelerin temel bileşenleridir ve son üründe uçucu ve uçucu olmayan flavor bileşiklerinin oluşumuna katkıda bulunurlar.

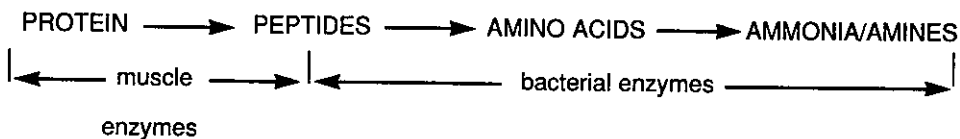
ABSTRACT: Proteolysis is one of the main biochemical reactions contributing to development of overall quality of fermented sausages. Proteolysis reactions are catalyzed by either endogenous enzymes inherent in the product or by enzymes from microbial origin. Meat proteins undergo hydrolysis first to polypeptides by endogenous muscle enzymes, and then further to small peptides by peptidases. Final step in proteolysis phenomena is free amino acid generation by bacterial aminopeptidases as well as aminopeptidases inherent in meat itself. Peptides and free amino acids are major components of the nonprotein nitrogen fraction in fermented meat products and contribute to the generation of volatile and nonvolatile flavor compounds in the final product.

INTRODUCTION

During sausage fermentation, many biochemical reactions involving proteins, carbohydrates and lipids take place, ultimately yielding volatile and nonvolatile compounds that contribute to the development of the specific aroma and taste characteristics of the products. Muscle protein is one of the more important components due to its nutritional role and the functional and structural contributions to the final product (IBANEZ et al., 1997). Proteolysis and insolubilization affect protein structure and conformation. The alteration of meat proteins during meat conditioning has been extensively studied because of the relation between protein degradation and meat tenderization. However, knowledge of proteolysis occurring during the fermentation process of meat products is only limited.

PROTEOLYSIS OCCURRED IN FERMENTED SAUSAGES

Proteolysis is an important phenomenon occurring during fermented sausage manufacture. It influences flavor development due to formation of several compounds of low molecular weight (small peptides, amino acids, aldehydes, organic acids, amines, etc.) in the final products (DIAZ et al., 1996). It has been noted by VERPLEATSE (1994) that both bacterial and muscle proteinases can contribute to proteolysis. Proteolysis affects aroma development in dry fermented sausages and the combined proteolytic activities may follow this pattern:



During the ripening of fermented sausages, several nonprotein nitrogen (NPN) compounds consisting of small peptides, amino acids, ammonia and amines are formed and responsible for the taste characteristics of the sausage. Acting as precursors of volatile compounds, free amino acids may be decarboxylated and deaminated to yield amines and organic acids, respectively, which may in turn lead to other changes, yielding volatile and nonvolatile flavor compounds (HIERRO et al., 1999).

ALTERATIONS IN THE NITROGEN FRACTION DURING FERMENTED SAUSAGE PROCESSING

There is no change or a slight increase in total nitrogen content of sausages during fermentation and drying according to the studies of DEMASI et al. (1990) and ASTIASARAN et al. (1990). DEMASI et al. (1990) reported a slight, but statistically insignificant, increase in total nitrogen content of sausages after fermentation, heating, and relatively short (12 days) drying periods. ASTIASARAN et al. (1990) found no significant increase in total nitrogen in dry fermented sausages. However, JOHANSSON et al. (1994) showed an increase in total protein (Nx6.25) content from 14.8% initially to 22.4% after 21 days of processing including maturation at 25 °C for 7 days and drying at refrigerated temperatures over 2 weeks. This increase in total protein can be attributed to drying because they did not present their data on a dry matter basis.

Heating of fermented sausages to 55 °C-63 °C resulted in a small amount of myofibrillar and sarcoplasmic proteins remaining soluble due to denaturation under conditions of low pH and heat application (ACTON, 1977). DE KETELAERE et al. (1974) showed substantial decreases in the sarcoplasmic and myofibrillar protein fractions of the total extractable protein as fermentation and drying time increased. The loss in extractability of these two fractions may vary from one product to another depending on product formulation and processing conditions (ASTIASARAN et al., 1990; GARCIA DE FERNANDO and FOX, 1991).

Data from several studies indicated that the nonprotein nitrogen (NPN) content consists of free amino acids, NH₃, other amines and peptide nitrogen increases during fermentation and drying and after heating. WARDLAW et al. (1973) demonstrated an increase of up to 60% in the NPN content after heating of fermented sausages to 63 °C. KLEMENT et al. (1974) observed a greater increase in the NPN content over time in acidified solutions of myofibrillar proteins than for sarcoplasmic proteins. DIERICK et al. (1974) also reported increases in the NPN fraction during the ripening of sausages without establishing its origin. Increases in most free amino acids, except glutamic, histidine, tyrosine, and ornithine, were observed during a 36-day overall ripening and drying period. Alanine, leucine, valine, serine, glycine and proline concentrations showed the greatest increase.

During fermentation and drying of sausages, proteolytic enzymes gradually degrade meat proteins. Fermentation of carbohydrates by lactic acid bacteria causes a significant drop in pH which favors proteolytic activity resulting in the partial fragmentation of proteins to form NPN compounds (LOIS et al., 1987; VERPLAETSE, 1994; IBANEZ et al., 1997; HIERRO et al., 1997). VERPLAETSE et al. (1989) demonstrated degradation of myosin, actin and troponin T during a 21 day processing period for dry sausages. A polypeptide with a molecular weight in the range 120 kDa-150 kDa from the heavy chain of myosin was generated, probably due to the activity of cathepsin. An increase in the concentration of polypeptides with a molecular weight varying from 14 kDa to 36 kDa was also observed resulting from proteolysis of meat proteins. Their data showed degradation in the heavy chain of myosin of about 49%, a decrease in actin and troponin T concentrations by about 30% during fermentation and an increase in the low molecular weight polypeptides of by 75.9% during the 21-day production period.

During the processing of fermented salami, DEMASI et al. (1990) reported significantly higher NPN content after heating and drying. The NPN fraction represented 10-11% of the total nitrogen in the initial mix and fermented mixes and increased to 13-14% in the heated and dried sausages. DEMASI et al. (1990) also reported an increase of more than 5 mg/100g dry sample in 14 of 20 amino acids in the fermented sausages when amino acid concentration changes were compared between the initial mixes and heated samples.

GARCIA DE FERNANDO and FOX (1991) found an increase in the amount of water-soluble nitrogen and free amino acids during processing of fermented sausages although the salt soluble nitrogen showed a decrease. VERPLAETSE et al. (1992) stated that proteolysis during sausage ripening is reflected an increase in the NPN concentration and amounts to approximately 20% of the total nitrogen content. He further stated that proteinases (endopeptidases) are mainly active during initial fermentation, involving the degradation of myosin and actin while exopeptidases which generate amino acids are more important in the later drying period. JOHANSSON et al. (1994) also indicated that the content of NPN in fermented sausages increased from 10.2 to 15.3% during the entire processing (fermentation up to 7 days and drying for 2 weeks) and storage period of approximately 2 months. The increase was most rapid during the first days of the maturation (fermentation) process at 25 °C. The greatest change in solubility for the water-soluble proteins, as well as the salt soluble proteins occurred between processing days 0 and 3. During this period, the pH dropped to 5.0 and the proteins were denatured. Water-soluble proteins with molecular weights between 20 and 30 kDa had almost disappeared by the day 7 as well as a salt soluble protein of 50 kDa.

RODRIGES-NUNEZ et al. (1995) investigated proteolysis in dry cured ham. They reported an increase in NPN content as a result of muscle protein degradation and they attributed these changes to the muscle cathepsins B, O, H, and L. These proteinases are located in the lysosomes and appear to remain active during processing. They pointed out that the last proteolytic step consisted of the conversion of peptides to free amino acids, and that this would be the result of activity by the aminopeptidases.

APPLICATIONS AFFECTING PROTEOLYSIS IN FERMENTED SAUSAGES

Induced changes prompting proteolysis by application of different technologies could have an important effect on the sensorial properties of fermented meat products (ZAPELENA et al., 1997). There are two types of processes assumed to provide better products in terms of proteolysis. The first is proteolytic enzyme addition to the product formulation and the other is proteolytic starter culture application.

Use of Proteolytic Enzymes

The potential role of enzymes in flavor generation suggests that their intentional application might contribute to an economical and sensorial improvement of the process of fermented sausage production. This application is a technology that is basically independent of the application of starter cultures (HAMMES and HERTEL, 1998). The addition of exogenous proteolytic enzymes in dry sausage manufacturing may be suitable for increasing the low molecular weight compounds and as a consequence, for shortening the overall process or, at least, to improve sausage flavor (DIAZ et al., 1996).

Proteolytic enzymes generated by bacterial metabolism are generally studied in fermented sausages. The effect of added pronase E (isolated from *Streptomyces griseus*) on proteolysis in dry fermented sausages was investigated by DIAZ et al. (1993). Increases in the NPN content were the highest during the first days of ripening. NAES et al. (1995) utilized a bacterial proteinase isolated from *Lactobacillus paracasei* and reported increased protein degradation and peptide formation in fermented sausages as compared to control sausages. Proteinase addition gave a specific increase in glutamate, serine and lysine contents. BLOM et al. (1996) selected proteinases from lactic acid bacteria to obtain accelerated ripening of fermented sausages. Enzymes from lactic acid bacteria were chosen since proteinases from this group of bacteria were assumed to be more suited and adapted to function during the fermentation process. When they were used in sausage fermentation with *Lactobacillus sake*, protein degradation products such as small peptides and amino acids showed higher concentrations in the proteinase-containing sausage, which might represent the stimulating components originating from the proteolytic activity of the enzyme. Increases in the amounts of glutamate, a well-known flavor enhancer, and serine and lysine were noted whereas total amino acid concentration showed little or no difference between samples.

ZAPELENA et al. (1997) examined the use of neutrase, a metalloproteinase from *Bacillus subtilis*, in a traditional fermented sausage (chorizo) that also contained a starter culture. They reported that the myofibrillar proteins had a higher loss of solubility than that found for the sarcoplasmic proteins. This may have been due to acidity rather than neutrase effects. However, sausages with neutrase showed an increase in NPN fraction concentration. In the peptide fraction, the α -NH₂ nitrogen increase was much higher in the product with neutrase (78.29%) than in the product without enzyme (20.93%).

DIAZ et al. (1996) reported that the addition of papain to dry fermented sausages resulted in higher NPN contents. According to their electrophoretic studies, proteolysis of high molecular weight myofibrillar and sarcoplasmic proteins was also more prominent in the protease-added sausage group. HAGEN et al. (1996) determined the effects of proteinases from *Lactobacillus paracasei* and from *Bacillus licheniformis* (Alcalase) and reported that the enzyme from *Lactobacillus paracasei* accelerated dry sausage ripening while Alcalase was not effective.

A partial hydrolysis of meat proteins apparently occurs with the addition of exogenous proteinases to sausage formulations. An increase of proteolysis above the level normally obtained from endogenous proteinases generally results. The production of peptides and amino acids may stimulate starter culture metabolism and create a more rapid and dramatic pH drop in the sausages. The stimulation effect when proteinases are added may explain the accelerated gel (texture) formation that results in a shortened fermentation and drying time while yielding flavor development comparable to ordinarily processed dry sausage (BLOM et al., 1996). The results obtained in practical investigations of the use of enzymes in sausage fermentation provide knowledge of the potential of those activities which may be employed in developing specifically, designed starter cultures. These cultures might, for example, produce the desired enzymes during the fermentation process which lead to product quality improvement (HAMMES and HERTEL, 1998).

Starter Culture Applications

The extent of proteolysis during ripening of dry fermented sausages depends on several factors including the nature of meat microflora and conditions during processing. The effects of different levels of curing ingredients and process parameters have a significant effect on inherent muscle proteases and peptidases involved in the dry curing process. However, the possible contributions of activities from microbial origin are not well-understood (SANZ and TOLDRA, 1997). In dry cured ham production, results of numerous studies are consistent with a collaborative and consecutive role in proteolysis by muscle cathepsin D and bacterial enzymes, the former preparing peptide substrates for the latter. As a result, the last proteolytic step in the conversion of oligopeptides to smaller peptides and free amino acids would be due to the action of microbial aminopeptidases (SANZ et al, 1998). DEMASI et al. (1990) and VERPLAETSE et al. (1992) noted that changes in the NPN fraction could be dependent on the starter culture used in fermented sausages. VERPLAETSE (1994) reported that the first step of fermented sausage ripening, the degradation of myosin and actin to fragments of 135 kDa and 38 kDa, respectively, is due to endogenous enzyme activity. However, further breakdown of polypeptides to smaller peptides and free amino acids was attributed partly to enzymes of microbial origin (40%) and to endogenous meat enzymes (60%). This sequence was based on examining sausages with antibiotics where enzyme activity was only 60% of the control sausages when microorganisms were not able to grow or metabolize.

MOLLY et al. (1997) emphasized the responsibility of both the meat and bacterial proteinases in the ripening and flavor generation in dry fermented sausages. FLORES et al. (1997) found the greatest increase in NPN content (from 11% to 12.5%) during fermentation occurring in samples with *Lactobacillus sake* and *Staphylococcus carnosus* as a mixed starter culture. IBANEZ et al. (1997) stated that during the first weeks of the slower ripening process, sausages inoculated with *Lactobacillus plantarum* and *Staphylococcus carnosus* (mixed culture) contributed significantly higher NPN generation than the control product without starter.

PROTEOLYTIC ACTIVITY OF STARTER CULTURES USED IN FERMENTED SAUSAGES

The physiology, biochemistry and genetics of proteolytic systems of dairy strains of lactic acid bacteria have been well studied because of their impact on the fermentation process and on textures and flavors of fermented milk products (SANZ et al., 1999). However, proteolytic activity by starter cultures of meat origin is not as well investigated even though a few studies have been conducted to determine the proteolytic characteristics of various starter cultures.

The data from several studies as shown in Table 1 indicate that *Staphylococcus* sp. and selected lactic acid bacteria, as starter cultures in fermented meat products, might possess proteolytic activity. However, more research on the proteolysis of meat proteins by starter cultures and their contribution to the flavor of the final product is needed.

Table 1. The Proteolytic or Presumed Proteolytic Activities of Some Starter Cultures Used in Fermented Sausages

Culture	Optimum Growth Temperature	Proteolytic Activity	Reference for Proteolytic Activity
<i>Lactobacillus casei</i>	30-40°C	+	FADDA et al. (1998)
<i>L. curvatus</i>	30-40°C	+	MONTEL et al. (1992); FADDA et al. (1998)
<i>L. plantarum</i>	30-40°C	+	MONTEL et al. (1992); FADDA et al. (1998)
<i>L. sake</i>	30-40°C	+	MONTEL et al. (1992); FADDA et al. (1998)
<i>Pediococcus acidilactici</i>	40°C	-	DEIBEL et al. (1961)
<i>P. pentosaceus</i>	28-32°C	+/-	DEMASI et al. (1990)/MONTEL et al. (1992)/ Bermel et al. (1992)
<i>Staphylococcus carnosus</i> (as <i>Micrococcaceae</i>)	30-40°C	+/-	MONTEL et al. (1992)/IBANEZ et al. (1997)
<i>S. xylosum</i>	25-35°C	+/-	JOHANSSON et al. (1994)/BERMELL et al. (1992)/ MONTEL et al. (1992)
<i>Streptomyces griseus</i>	25-30°C	+	EILBERG and LIEPE (1977)

FLAVOR DEVELOPMENT IN FERMENTED SAUSAGES RELATED TO PROTEOLYSIS

Conversion of meat with nearly no flavor into meat products with a distinctly recognizable aroma involves a system of interacting microbial, physical and biochemical reactions (VERPLAETSE, 1994). During the sausage fermentation and drying process, a number of changes in the carbohydrate, lipid and protein components of the sausage mix take place simultaneously and are likely interrelated with flavor development in the final product. However, the exact relationship between the characteristics of fermented, cured flavor generation and the factors that directly have an effect on specific flavor notes are not well known.

Main processes involved in typical flavor development in fermented sausages are glycolysis, lipolysis, lipid oxidation, proteolysis, seasonings and curing salts (VERPLAETSE, 1994). Proteolysis, which may originate from endogenous enzymes or from the microorganisms, is one of the processes in flavor generation and as such, also depends on processing factors such as temperature, relative humidity, pH, etc. (HAMMES and HERTEL, 1998). Flavor compounds generated from these factors can be divided into volatile compounds that are closely related to smell and nonvolatile compounds which influence taste (VERPLAETSE, 1994; DAINY and BLOM, 1995).

Volatile flavor compounds resulting from proteolysis have been reported to comprise only a small amount of the total volatiles (VERPLAETSE, 1994). JOHANSSON et al. (1994) reported that methyl aldehydes and methyl alcohols as well as sulfur compounds like dimethyl disulfide and methanthiol might be formed from amino acids liberated by proteolysis. However, many of the sulfur compounds may also originate from the spices and seasoning such as onion and garlic.

Other compounds generated from protein, peptide and amino acid hydrolysis appear to be the important factors in taste development. Amino acids produce amines by decarboxylation and also have an important role in Maillard reactions that result in the production of numerous flavor products (FLORES et al., 1997). A correlation between free amino acid and peptide concentrations in the sausage with some taste descriptors such as spicy, beefy, sweet, bitter and astringent was pointed out (VERPLAETSE, 1994). While a high concentration of small peptides results in a spicy taste, if the concentration is too high, a negative taste impression such as bitter and astringent notes occurs. Beefy and sweet flavors in sausages are due to low amounts of small peptides. An increase in the amount of ammonia resulting from deaminase activity by the microflora in fermented sausage can cause a slight increase in the pH during drying. This effect neutralizes final acidity of the product and thus enhances sausage taste (DEMEYER et al., 1979; DEMEYER et al., 1992; FLORES et al., 1997).

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