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# THE ASSOCIATION OF PROTEIN SOLUBILITY WITH PHYSICAL PROPERTIES IN A FERMENTED SAUSAGE

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## SUMMARY

Batches of summer sausage (a fermented semi-dry variety) were prepared under commercial conditions. Samples were withdrawn from the smokehouse at various time intervals and tested for pH, shear force and solubility of the sarcoplasmic and myofibrillar protein fractions. With increasing process time, pH declined and the solubility of the nigrogen containing compounds in the myofibrillar fraction decreased markedly, whereas the solubility of the sarcoplasmic proteins decreased less and at lower a pH. Non-protein nitrogen remained relatively constant except for an increase at the end of the process under condition of high temperature and acidity. Shear force data showed that an increase in firmness developed simultaneously with decreasing pH; this occurred at the same time that solubility of the two protein fractions (mainly myofibrillar) decreased. Two complete experimental runs were conducted and the results were similar.

## INTRODUCTION

The semi-dry fermented variety of sausage known as summer sausage is characterized by distinctive properties of flavor, texture and firmness. A major portion of the firmness development occurs during the fermentation. Variations in the firmness of product are sometimes experienced as a problem in the industry. The mechanism responsible for the development of firmness

must be understood in order to carefully control the process.

Sokolov and Tchekhovskaya (1971) investigated the development of structure during drying of a fermented sausage. They found an aggregation of myofibrillar proteins accompanied with the appearance of electrostatic, hydrogen and disulfide bonds. The aggregation was greater in the periphery of the sausage possibly being due to the acid compounds in smoke which aided in the charge formation on the proteins.

The objective of this study was to determine the changes in solubility of various classes of muscle proteins during processing of a summer sausage (semi-dry fermented meat product) and to relate these results to changes in pH and firmness of the product.

## EXPERIMENTAL MATERIAL AND METHODS

Sausage preparation. Coarse ground pre-salted beef (70% lean) and fine ground pre-salted beef trim (50% lean) were chopped in a silent cutter to a desired consistency at which time seasoning ingredients were added followed by starter culture (AC-1 Hanson Laboratory, Milwaukee, Wisconsin). The mix was then stuffed into No. 2 fibrous casings (Union Carbide, Chicago, stuffing diameter 62 mm) fermented in an air-conditioned smokehouse at 37°C and then heated up to 55°C internal temperature. Sample 1 was taken immediately after stuffing and was at a temperature of approximately 6-8°C. Sample 7 was the finished product at a temperature of 55°C. Samples 3 through 6 were withdrawn from the smokehouse at intervals of 3-6 hr during the fermentation and ranged between 34-37°C in temperature. All samples withdrawn from the smokehouse were chilled down to 20°C in a cold water shower before they were placed in a cooler at 3°C. All processing took place under commercial conditions. A typical

proximate analysis of the sausage was 27% fat, 50% moisture and 17% protein.

Two complete experimental runs were conducted at an interval of 45 days and are referred to as Experiment I and Experiment II; the formulations and procedures were identical. Three different batches were manufactured for each experiment as follows: batch A had 2.5% NaCl and starter culture, batch B had 3.0% NaCl and starter culture and batch C had 3.0% salt but no starter (control).

Sample preparation. The samples were cooled to an internal temperature of 3°C and were then shipped under refrigeration to the University of Wisconsin facilities where further tests were conducted. The samples were held an additional day at 3°C before firmness analyses were conducted.

A sample with standardized dimensions was obtained as follows: The sausage was chilled in an ice bath and then a 2.54 cm slice was obtained by slicing with a sharp knife in a template device. A carefully sharpened 5 cm diameter stainless steel tube was used to core a standardized diameter sample from the 2.54 cm thick slice so that the surface layer could be discarded. The 5 cm diameter core was used for shear analysis. This method was designed to yield uniformly sized samples and to circumvent the firmness effect due to case hardening of the surface during thermal processing, which would not be the same firmness that resulted from the constant low temperature fermentation.

A paired sample was frozen in liquid nitrogen (Liq-$N_2$) until analysis of protein extractability which took place during the following three weeks.

Firmness analysis. The previously described standardized samples

were sheared at a rate of 30 second down stroke with a L.E.E. Kramer shear press. Five replicates of each of the 21 samples per experiment were sheared and the average was used as the firmness value. The shearing apparatus was located at room temperature, but all the samples were stored in an ice bath at 0°C before shearing.

Extractable protein. Samples were removed from Liq-$N_2$ storage and powdered in a previously cooled aluminum blender cup (Waring) at top speed according to the procedure of Westenbrink and Krabbe (1936). The samples were extracted at 2°C according to the modified method of Helander (1957). Sarcoplasmic protein was extracted with 0.03 N potassium phosphate, pH 7.4 and total soluble protein was extracted with 1.1 N potassium iodide in 0.1 N potassium phosphate, pH 7.4. Non-protein nitrogen (NPN) was measured after tricholoroacetic acid (20% w/v) precipitation. Triplicate extractions were performed on each of the 42 samples (21 per experiment). Myofibrillar protein was calculated as the difference between the amounts of total soluble protein and sarcoplasmic protein (including NPN). Sarcoplasmic protein was calculated on the basis of nitrogen difference between the 0.03 N potassium phosphate extract and its TCA precipitation filtrate. Nitrogen quantity was determined on the original sausages in triplicate by Marco-Kjeldahl method. The triplicate extracts and NPN nitrogen quantities each were determined in duplicate by the micro-Kjeldahl method (ADAC, 1960).

The following modifications were made to the Helander method: (1) 2 gram samples were used instead of 1 gram because of smaller quantities of soluble protein, (2) centrifugation was conducted at 10,000 g instead of 1,500 g, (3) a third 2 hr extraction was added to the 0.03 N potassium

phosphate fraction instead of just two 3 hr extractions.

pH measurements. Five grams of sample was homogenized in 45 ml of distilled water. The subsequent mixture was filtered and the pH of the filtrate was measured with a combination glass-reference electrode.

Statistical analysis. All data were subjected to an Analysis of Variance and Duncan's Multiple Range Test for significant differences as described by Steel and Torrie (1960).

## RESULTS AND DISCUSSION

pH value and internal temperature. The pH values for sausage from both experiments at time intervals throughout processing are shown in Fig. 1. The results from the two experiments were similar. pH, in general, declined to a final value of 4.6 to 4.9. An interesting pattern was established which showed that batch A had a more rapid pH decline than batch B. This could perhaps be explained by the fact that batch A had a lower salt level; salt is inhibitory to growth of the starter culture which is responsible for acid production. The control batch (C) which did not include starter culture did not show a pH decline, and, in fact, the pH actually rose from 5.3 to 5.6 during processing. Other investigators have reported the same phenomena of increasing pH during the heating of meat (Hamm and Deatherage, 1960; Kauffman et al., 1964; Paul et al., 1966). Fox et al. (1966) found a pH increase from 5.4 to 5.9 in the processing of frankfurters. A possible explanation of this increase is the loss of free acidic groups by the formation of new stable cross linkages (Hamm and Deatherage, 1960).

The internal temperature of the sausage during processing is shown in Fig. 2. As the product was moved into the smokehouse for fermentation the

temperature increased to the range of 34-37°C and remained at that approximate level until it was finished at 55°C.

Firmness. The results for the shear analysis are given in Fig. 3. The controls showed little firmness development until they were subjected to the heating change from the 34-37°C plateau to 55°C. In both experiments, sample 7 of the controls was significantly firmer ($P \leq .01$) than sample 6; there were no significant differences from samples 1 through 6. We interpret these results as demonstrating no formation of a characteristic summer sausage structure during the slight increase in pH that occurred in the control. Firmness development was more rapid in the A batches than the B batches which is probably a reflection of the faster development of acid in the A batches (see Fig. 1). Although firmness development was more rapid in the A batches than the B, the final firmness values were not significantly different. This points out that either 2.5% or 3.0% salt is adequate for satisfactory development of firmness. All samples that had a pH below 5.0 were significantly different ($P \leq .01$) from the control sample at that same time of incubation. From the general shape of the curves, it is clear that the sausage became firmer as the pH of the sausage dropped. For example, from Experiment I, sample 3 from batch A (pH = 5.0) was significantly different ($P \leq .01$) from sample 3 of the control (pH = 5.4) in shear value. It should be noted that the three batches of sausage from an experiment were in the same smokehouse for exactly the same amount of time. The difference between values from the two experiments probably represent actual differences in firmness.

Myofibrillar protein extractability. The change in solubility of the myofibrillar proteins during processing is shown in Fig. 4. There were no

significant differences ($P \geq .05$) for A, B and C batches for either experiments in the sample taken immediately after chopping. The values of 29.2% to 35.5% extractability for both experiments are for post-rigor meat and are within the range reported by Sayre and Briskey (1963). They found an extractability of myofibrillar proteins of 10% to 40% (35% average for the six conditions they studied) for the myofibrillar proteins in pork muscle 24 hr postmortem; the percent extractability was dependent upon temperature and pH at the onset of rigor mortis. They also postulated that under conditions of high temperature and medium or low pH (5.3-5.6), loss of solubility was more severe for the myofibrillar proteins than it was for the sarcoplasmic fraction which also agrees with the findings of this experiment (see Fig. 5 and discussion).

The significant ($P \leq .01$) drop of solubility for all samples from the first to the second sampling time demonstrates the effect of the heat change from 6°C to about 37°C. Paul *et al.* (1966) found a decrease of about 40% in solubility of myofibrillar protein by a heat treatment of 40°C for 10 hr. We found a decrease in extractability of from 35% to 42% as temperature was raised from about 6°C to 34-37°C. There was a 60% reduction in solubility in samples held 10 hr at 50°C (Paul *et al.*, 1966), whereas in this experiment there was a reduction of 80% by the time samples had reached 55°C, even in the samples where no pH drop occurred.

It should be noted also that the final pH of the samples did not change the amount of extractable myofibrillar protein in sample 7. If the pH was 5.6 or 4.6, the amount that was extractable was not significantly different, pointing out the heat susceptibility of this type of protein, regardless of pH influence. Although the amount of extractable protein was

the same, these samples possessed a difference in shear value that was significant, showing the importance of acid development during the fermentation.

The change in solubility of myofibrillar proteins during the fermentation when pH is declining but temperature is constant, is interesting. The percent decrease in protein solubility was calculated as:

$$\frac{\text{\% solubility sample 2 - \% solubility sample 6}}{\text{\% solubility sample 2}}$$

In Experiment I, the percent decrease was 52% for batch A and 50% for batch B while in Experiment II it was 62% for A and 62% for B. The control batches failed to show any reduction in solubility of myofibrillar protein even though the mix was exposed to a pH of 5.3-5.5 and a constant temperature of 34-37°C. In contrast, batches A and B for both experiments showed a marked decrease in solubility; these samples were exposed to a decreasing pH at a temperature of 34-37°C. The results indicate that the declining pH at constant temperature decreases the solubility of myofibrillar proteins and in turn increases the shear value (firmness) of the sausage. The difference between experiments (a decrease of about 50% in Experiment I compared to 60% in Experiment II) may have been due to differences in pH and temperature between the two experiments. The percent decrease in solubility of the myofibrillar proteins is greater in this type of sausage than that of the sarcoplasmic proteins (see Fig. 5).

Sarcoplasmic protein extractability. The change in solubility of the sarcoplasmic proteins during processing is presented in Fig. 5. Sample 1 for all batches ranged from 16.1% to 18.8% soluble sarcoplasmic protein as a percentage of total protein nitrogen. There was no significant difference among samples. Sayre and Briskey (1963) worked with 24 hr postmortem pork

muscle pH of 5.3-5.6 with different temperatures at onset of rigor and found an extractability of the sarcoplasmic protein of 18-23%.

Paul et al. (1966) revealed a 20% decrease in solubility of sarcoplasmic proteins of rabbit on exposure to 40°C for 10 hr at constant pH. Our work revealed a 16-22% decrease in solubility of sarcoplasmic protein in samples heated from 6°C to about 37°C for 4 hr.

Although there was some fluctuation, the control batches showed little variation between samples 2 and 6.

Batches A and B, from samples 2 to 6, both show decreases in solubility which were significantly different from the control batch. For example, in Experiment II, batch A was significantly different from the control sample at sample 4 ($P \leq .05$) and samples 5 and 6 ($P \leq .01$) and batch B was different at sample 6 ($P \leq .05$). Also, in Experiment I both batches A and B were significantly different from the control at sample 6 ($P \leq .01$). The decrease in solubility of these sarcoplasmic proteins was not as drastic as experienced with the myofibrillar proteins, but rather showed a gradual decrease to sample 6. This suggests that the sarcoplasmic proteins were less susceptible to insolubilization at pH 5.2-5.0 than were the myofibrillar proteins which showed significant decreases in solubility ($P \leq .01$) at 5.2-5.0 (see sample 3 for batch A of Experiment I).

The solubility at sample 7 was significantly different ($P \leq .01$) from sample 6 in all cases except in Experiment II, batch A, where the pH of 4.65 and temperature had already lowered the solubility level to 7.2% so that heat treatment to 55°C did not further decrease solubility.

The percent decrease in solubility of sarcoplasmic proteins during the constant temperature fermentation period was also calculated. In

Experiment I, batch A decreased 36% and batch B decreased 24% while in Experiment II, batch A decreased 47% and batch B decreased 21%. There was little decrease in the control batches.

We concluded that the myofibrillar proteins are more important than the sarcoplasmic proteins in development of the firmness that is characteristic of summer sausage.

Non-protein nitrogen. The results for non-protein nitrogen (NPN) determination on the various extracts is presented in Fig. 6. The higher non-protein nitrogen in batch A of both experiments could be due to high acid-high temperature condition altering protein structure, because in both experiments the acidity developed more rapidly in batch A than in batch B. In Experiment I there were no significant differences except sample 1 of batch A and sample 7 of batch B, which were different ($P \leq .05$).

In Experiment II, batch A showed the same trend of a significant increase in sample 7. In the low-acid control batches, a significant increase was not noted. This indicates that acid at high temperatures may increase NPN; an exception occurred in Experiment II batch B, however.

Hamm and Deatherage (1960) reported a slight increase in NPN with increasing temperature at slightly rising pH conditions. They offered the suggestion that at higher temperatures smaller nitrogen containing molecules (e.g., nucleotides, ammonia) bound by proteins are released. Paul et al. (1966) also found significant increases in NPN by heating muscle protein systems for several hours at 60°C. They attribute this increase to enzymatic activity naturally present in the meat tissue that could change solubility. Sajber et al. (1971) found an increase of NPN due to an increase of certain amino acids during the fermentation of "Stajer"

sausages. Results from our work resembles all of these findings although the heat treatment was not as drastic as the Paul et al. (1966) example. In their samples heated at lower temperature (40°C), they found little change of NPN level.

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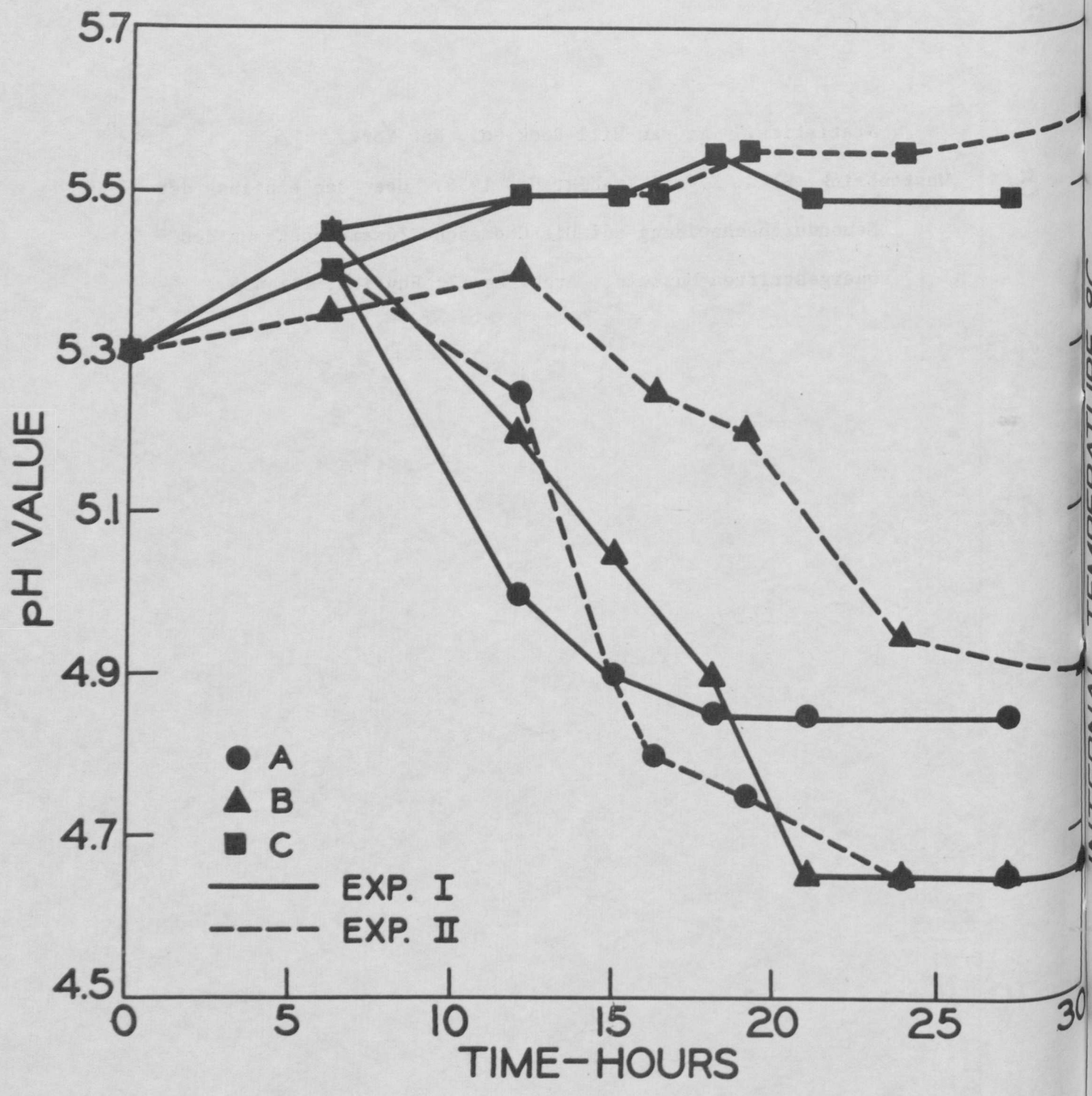


Figure 1 pH values of the sausage during processing. A contains 2.5% NaCl and starter culture, B contains 3.0% NaCl and starter culture, C contains 3.0% NaCl.

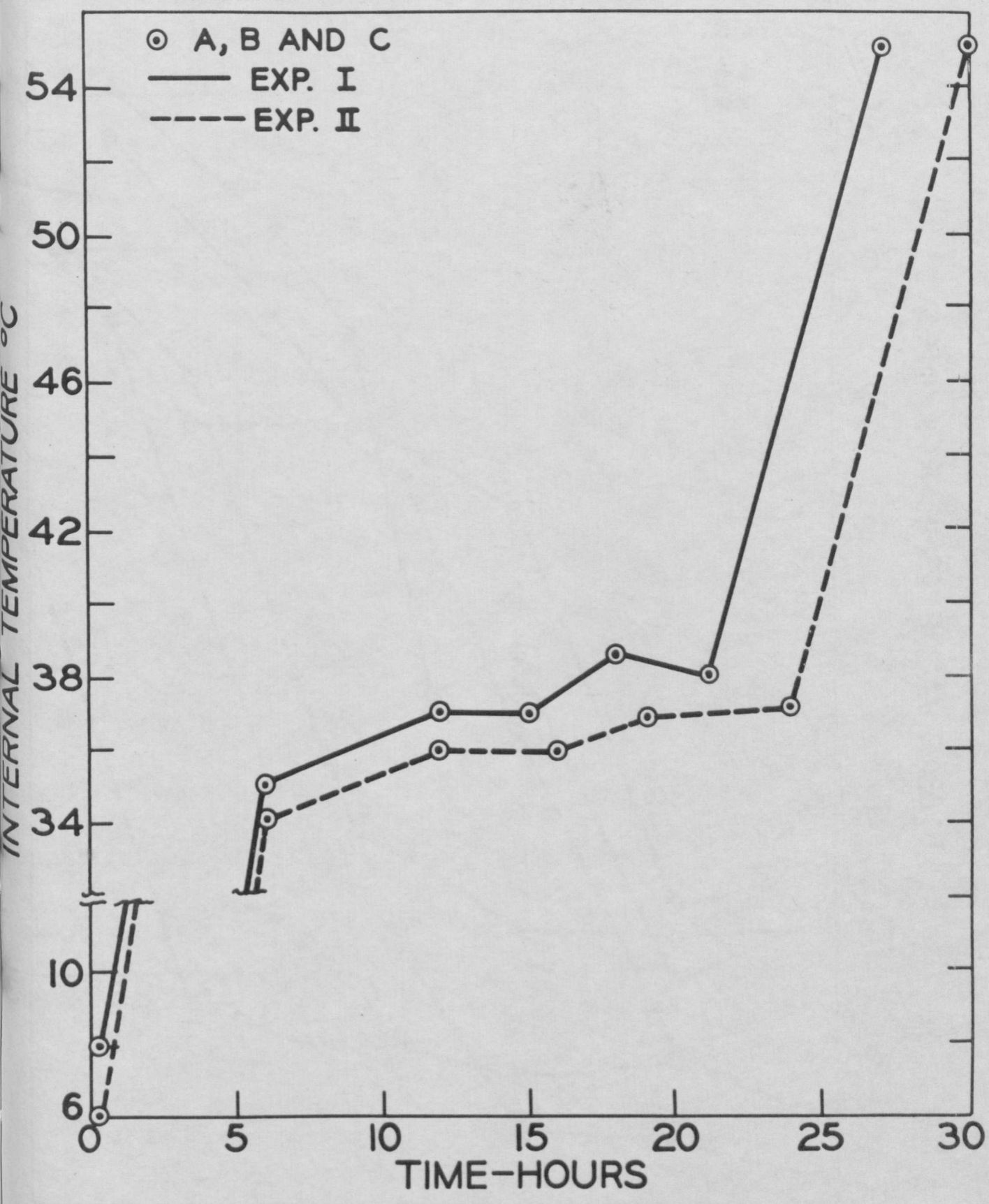


Figure 2 Internal temperature of the sausage during processing. Batches representing salt levels and starter culture combinations are pooled.

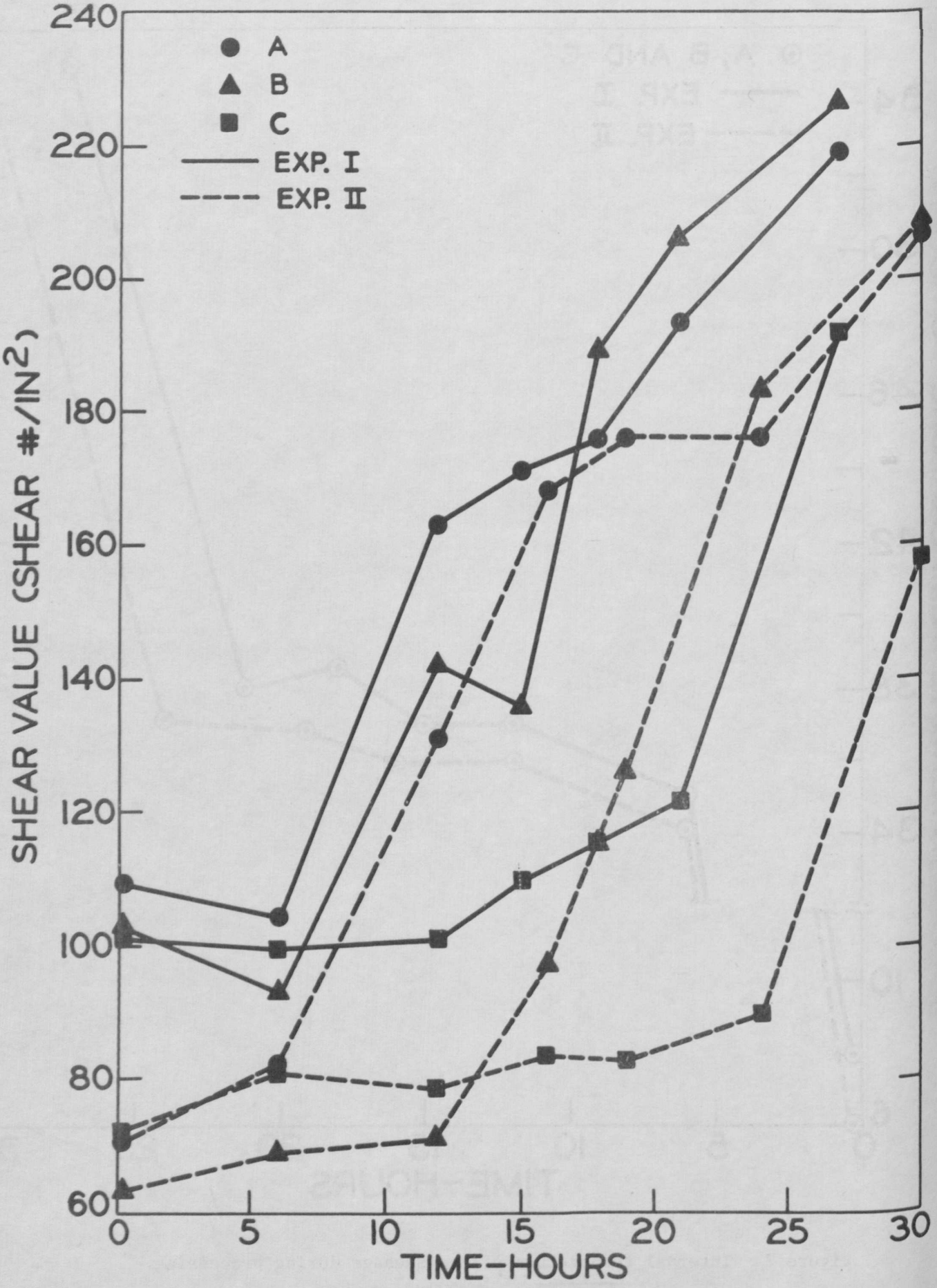


Figure 3 Shear press values of the sausage during processing. A contains 2.5% NaCl and starter culture, B contains 3.0% NaCl and starter culture, C contains 3.0% NaCl.

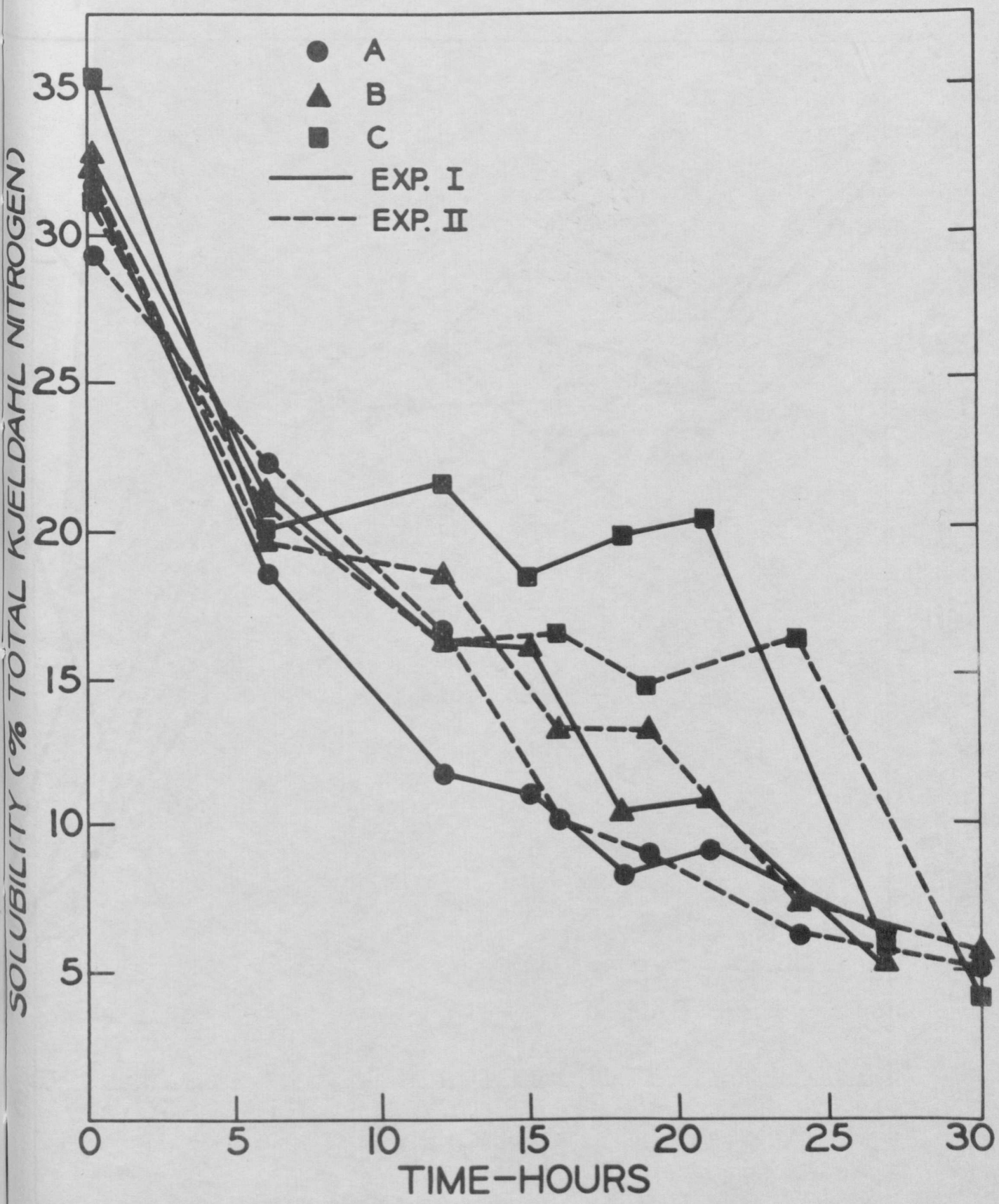


Figure 4 Solubility of myofibrillar proteins in the sausage during processing. Results are expressed as a percentage to total protein nitrogen. A contains 2.5% NaCl and starter culture, B contains 3.0% NaCl and starter culture, C contains 3.0% NaCl.

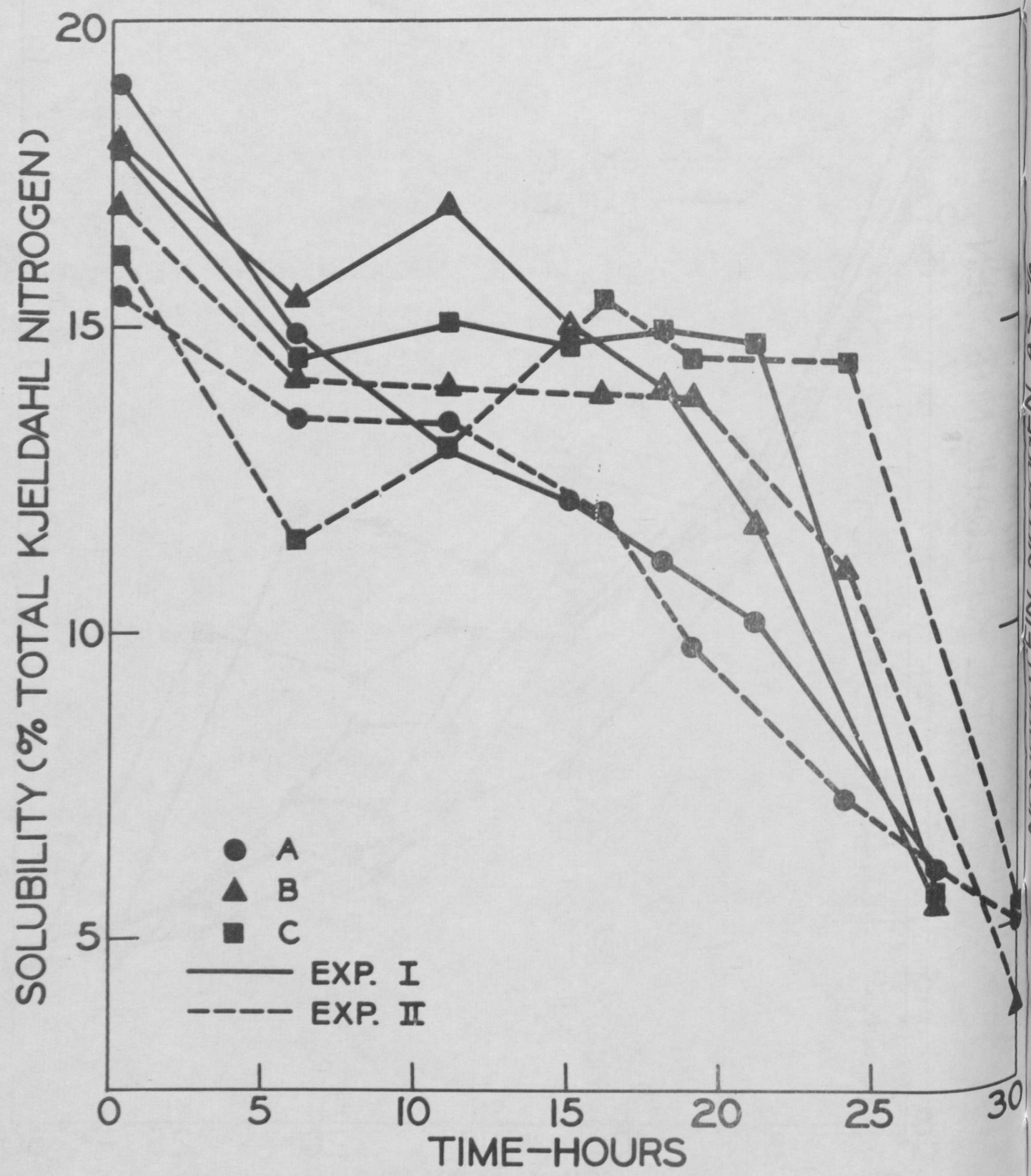


Figure 5 Solubility of sarcoplasmic proteins in the sausage during processing. Results are expressed as a percentage of total protein nitrogen. A contains 2.5% NaCl and starter culture, B contains 3.0% NaCl and starter culture, C contains 3.0% NaCl.

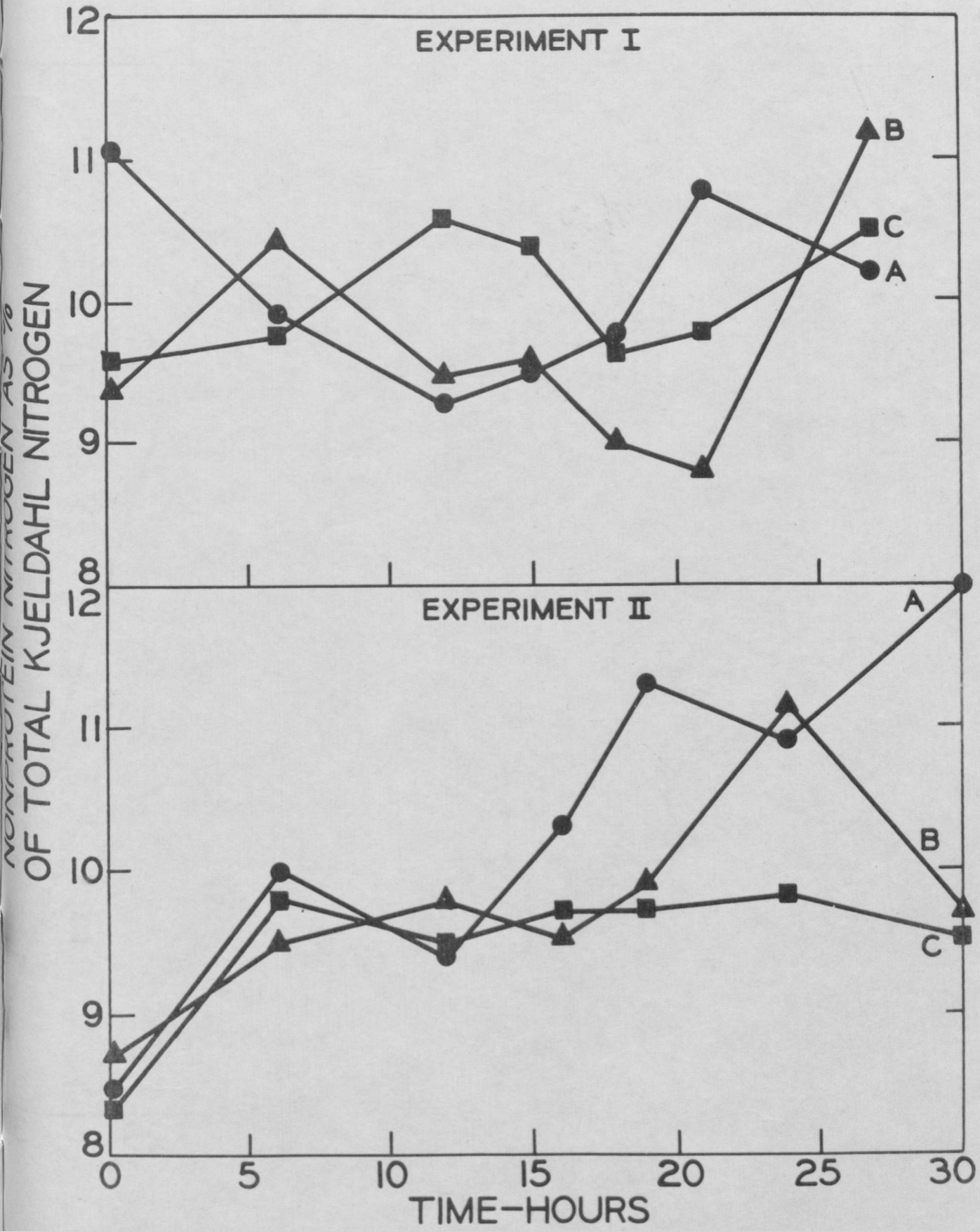


Figure 6 Non-protein nitrogen levels in the sausage during processing. Results are expressed as a percentage of total protein nitrogen. A contains 2.5% NaCl and starter culture, B contains 3.0% NaCl and starter culture, C contains 3.0% NaCl. All were heated to 55°C except A in Experiment II which was heated to 60°C.

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Summary.

Concentration changes for ammonia, total and individual free amino acids, total peptides, nucleotides, nucleosides and som individual amines were followed during ripening of dry sausage, with an without added "Starter culture". A decrease was observed for peptides, nucleotides, glutamic acid, histidine, tyrosine and ornithine, an increase for all other compounds, being most intense for total free amino acids during the first days of ripening. The rate of free amino acid production exceeded the rate of ammonia production. The presence of a starter culture intensified free amino acid production and peptide disappearance. A tenfold increase in the concentrations of histamine, tyramine and putresceine was observed in the presence of a starter culture.

Résumé.

La concentration de l'ammoniaque, d'acides aminés totaux et individuels, de peptides totaux, de nucléotides, nucléosides et de quelques amines a été suivie dans le saucisson cru, préparé avec et sans culture d'ensemencement. Une diminution a été observée pour les peptides, les nucléotides, l'acide glutamique, la histidine, la tyrosine et l'ornithine et une augmentation pour les autres composés, la plus intense étant trouvée pour les acides aminés libres totaux, pendant les premiers jours du mâturage.

La vitesse de production d'acides aminés libres, surpassait celle de l'ammoniaque. La présence d'une culture d'ensemencement intensifiait la production d'acides aminés libres, et la disparition des peptides. Une augmentation décuple a été obseyée pour la concentration de la histamine, de la tyramine et de la putrescéine, en présence d'une culture d'ensemencement.

Zusammenfassung.

Die Gehaltsveränderung von Ammoniak, totalen und individuellen freien Aminosäuren, totalen Peptiden, Nucleotiden, Nucleosiden und von einigen aminen, wurde bestimmt in schnellreifender Rohwurst, mit und ohne Starterkultur hergestellt. Eine Verminderung wird beobachtet für Peptiden, Nukleotiden, Glutaminsäure, Histidin, Tyrosin und Ornithin, und eine Anreicherung von allen anderen Bestandteilen. Diese Bereicherung war am grössten für die freien Aminosäuren während die ersten Tage der Herstellung und starker mit einer Staterkultur. Die Produktionsgeschwindigkeit der freien Aminosäuren übertraf diese des Ammoniaks. Die Anwesenkeit einer Starterkultur erhöhte die Produktion von freien Aminosäuren und das Verschwinden von Peptiden. Eine zehnfache Zunahme der Konzentartion wurde beobachtet für Histamin, Tyramin und Putrescein, in Anwesenheit einer Starterkultur.