REHYDRATION OF FREEZE-DRIED PORK AS RELATED TO pH AND PROTEIN DENATURATION

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Joan Ruth Sudon

1963

This is to certify that the

thesis entitled

REHYDRATION OF FREEZE-DRIED PORK AS RELATED TO

pH AND PROTEIN DENATURATION

presented by

Joan Ruth Suden

has been accepted towards fulfillment
of the requirements for

M.S. degree in Food Science

A. M. Pearson
Major professor

Date May 13, 1963

O-169

ABSTRACT

REHYDRATION OF FREEZE-DRIED PORK AS RELATED TO

pH AND PROTEIN DENATURATION

by Joan Ruth Suden

With the advent of World War II, extensive investigations occurred in the preparation of dehydrated foods for the armed forces. Of all the dehydration methods studied, freeze-drying produced dehydrated foods of the highest quality.

Despite all the advantages of freeze-drying, freeze-dried meat rehydrates to only 80-90% of the original water content, is tougher, and has a drier texture than that of the control. The objectives of this study were: (1) To investigate the effect of pH on the percentage rehydration of freeze-dried pork; and (2) To determine the degree of protein denaturation and its relationship to rehydration.

Results indicated that there was no significant correlation between percentage rehydration and either pH of the rehydrating solution or pH of the rehydrated meat. Freeze-dried pork showed no optimum pH for rehydration.

Freeze-dried pork was found to rehydrate to a much lower level than beef. The percentage rehydration of freeze-dried pork ranged from 48.54% to 92.41% with a mean percentage rehydration of 73.75% ± 9.26. Fat content did not influence rehydration.

An increase in the pH of freeze-dried pork occurred when the fillets were rehydrated in deionized water. A loss of acidic volatiles during dehydration was indicated. If the volatiles were trapped and utilized in the reconstitution of the dried meat solids, the original pH of the fresh meat slurry was regained.

Results showed that no significant correlations existed between percentage rehydration and sarcoplasmic protein nitrogen, 0.53 μ (KCl-bicarbonate) extractable protein nitrogen, soluble fibrillar protein nitrogen or non-protein nitrogen content. However, as the percentage of rehydration increased, there was a marked increase in the protein content of the rehydrating solution.

The sarcoplasmic protein nitrogen content of freeze-dried and rehydrated pork decreased from that of the fresh control. Thus denaturation of the sarcoplasmic proteins of pork occurred during the freeze-dehydration process.

Rehydrating solutions of similar ionic strength had identical, qualitative amino acid compositions. The qualitative amino acid composition of the rehydrating solutions was not influenced by pH. However, a change in ionic strength varied the qualitative amino acid composition of the rehydrating solution.

REHYDRATION OF FREEZE-DRIED PORK AS RELATED TO pH AND PROTEIN DENATURATION

By

Joan Ruth Suden

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1963

ACKNOWLEDGMENTS

The author wishes to express her sincere gratitude and appreciation to Dr. A. M. Pearson for his continuous guidance and encouragement during the course of this study and preparation of this manuscript.

Sincere thanks are extended to Dr. Leroy R. Dugan for his guidance and assistance in the use of the freeze-drying facilities in his laboratory, to Mrs. Dora Spooner for her aid in the statistical analysis and to Mrs. Beatrice Eichelberger for typing this manuscript.

Most of all, the author wishes to acknowledge her husband, Edward, for his patience, encouragement and unending faith.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

The ideal dehydrated food was defined by Gooding and Rolfe (1955) as one having the appearance, palatability, and nutritional quality of the freshly prepared food. The dehydrated food would reconstitute rapidly when water was added. It would also have a long storage life under a range of conditions, a high packaging density, low processing losses and economy in manufacture.

With the advent of World War II, extensive investigations occurred in the preparation of dehydrated foods for the armed forces. Of all the dehydration methods studied, freeze-drying produced dehydrated foods of the highest quality.

The advantages of freeze drying have been outlined by Flosdorf (1949). The low temperatures of operation avoided chemical changes in labile components and the loss of volatile constituents was minimal. No bubbling, foaming, or shrinkage of the material occurred. The tendency for coagulation was at a minimum and no case hardening was apparent. Under the frozen conditions of drying, neither bacterial growth nor enzymatic changes occurred.

Despite all of the above factors, freeze-dried meats only rehydrate to 80-90% of the original water content. The reconstituted freeze-dried meat is tougher and has a drier texture than that of the control. The water-holding capacity of the meat has been altered.

The objectives of this study are twofold:

(1) To investigate the effect of pH on the percentage rehydration of freeze-dried pork.

(2) To determine the degree of protein denaturation and its relationship to rehydration.

LITERATURE REVIEW

Physical properties of freeze-dried meat

The physical characteristics of freeze-dried beef (biceps femoris) were outlined by Tappel et al. (1955). The composition was determined as 80-85% protein and 13-17% lipid. These workers found a 3% moisture content in 1-inch thick pieces, which had been dried for 24 hours. The biceps femoris retained a structure similar to balsa wood with no volume change due to the freeze-drying process. Its density (0.33 g/cc.), porosity (80%) and thermal conductivity (0.02 B. Th. U./h./ft./°F) were determined after dehydration. The low thermal conductivity was accounted for by the wood-like physical structure of the meat. The muscle was pink in color but changed to tan on storage. Harper and Tappel (1957) have explained the color change as being due to the low oxygen tensions in the freeze-dryer. They suggested that oxymyoglobin is deoxygenated to form myoglobin which is labile to oxidation on storage.

According to Harper and Tappel (1957), freeze-dried pork was very similar to freeze-dried beef in structure and texture. They reported that the color of pork was initially a light pink, which soon changed to light tan. The color remained unchanged until active browning deterioration developed.

Hankins et al. (1946) reported that dehydrated pork actually contained less fat and more protein than was calculated from the composition of the raw meat. They also showed that the chemical composition of the

dehydrated product was influenced by the composition of the raw material, extent of the drying, and any losses in constituents in addition to moisture, that occurred during processing. The investigations of Doty et al. (1953) indicated that the histological changes found in freeze-dried beef were very slight and the gross chemical composition of meat on a moisture-free basis was apparently little changed by dehydration.

Factors influencing rehydration

Wang et al. (1953) studied the effect of various freezing methods on the reconstitution of freeze-dried beef. Reconstitution of the freeze-dried samples was compared by measuring the increase in moisture content and of muscle fiber diameter. Meat pre-frozen at -17°C, -80°C and -150°C was reconstituted. Samples prefrozen at -17°C had the largest amount of interfibral space, the greatest degree of recovery of muscle fiber diameter, and the fastest initial water penetration.

Investigations by Wang et al. (1954) have shown that the rate of freezing had a marked effect on the size of the ice crystals formed and their location in the foodstuff. With rapid freezing, the ice crystals were extremely small and mostly inside the cells. As freezing rate decreased, the size of ice crystals increased and the frequency of loci decreased. The freezing changed from an intracellular to an intercellular pattern, and eventually quite severe mechanical damage occurred within the cell structure. As the ice was sublimed, pores of large diameter remained in the dry tissue. The material that had been frozen

slowly offered a reduced resistance to the escape of the water vapor from the ice surface as compared with rapidly frozen material.

Luyet (1962) compared the effects of various freezing rates on the structure of freeze-dried muscle. The experimental results of Luyet corraborated those of Wang et al. (1954).

According to Greaves (1954), freeze-dried serum went into solution at a much faster rate, if prepared from rapidly frozen serum. Experimental results of Gooding and Rolfe (1957) indicated that rapidly frozen meat failed to give a product which could be easily reconstituted. The Ministry of Agriculture, Fisheries and Food (1961) recommended the use of an intermediate rate of freezing, such as that obtained by blast freezing, in order to gain an optimum crystal size. Harper and Tappel (1957) suggested that meat be frozen by pre-freezing in external freezing equipment, since case-hardening occurred during evaporative freezing within the drying cabinet.

The rehydration of freeze-dried meat has been investigated by Auerbach et al. (1954). These investigators reported that meat cut across the grain reconstituted more rapidly and more completely than that cut longitudinal to the grain. The thickness of the sample was found to influence both the rate and level of rehydration. A 1-inch section rehydrated for 3 hours did not reach the same level of rehydration that a 1/2 inch section achieved in 3 minutes.

Auerbach et al. (1954) also reported that reconstitution was unaffected by water in the temperature range 22-55°C. The optimum pH of

rehydration was reported as 7.00. These workers stated that reconstitution was more rapid and more complete in a vacuum. Although the percentage rehydration varied from 80-90%, after cooking the texture usually was drier than that of the controls.

According to Turner (1956) increased hydration of raw dehydrated meat occurred when salts were added to the rehydration water. He reported that the addition of 1.5% to 2% NaCl with 0.1% to 0.15% tetrasodium pyrophosphate ($Na_4P_2O_7$. 10 H_2O) improved the texture and overall acceptability of the final product.

Protein denaturation

Taylor (1953) reported that many proteins have been freeze-dried without obvious denaturation and appear to be stable indefinitely in the dry state. At least two food proteins, egg albumen, according to Bull (1944), and rabbit myosin, according to Bailey (1956), cannot be freeze-dried without denaturation.

The changes caused by freeze-drying in the histological micro-structure and in the molecular structure of the fiber substance were studied by Connell (1957). He reported that the fibers lost their close contact and it was possible to separate them easily. There were also aggregations of fibers, and areas of fused fibers separated by large spaces. The investigator suggested that the change in molecular structure of the fibers was due to denaturation of actomyosin during dehydration and was accompanied by a loss of the gel-forming ability of protein and water.

Connell (1958) studied the effect of drying on fish muscle proteins. He reported that the protein-gel system of the dried fish was more disorganized than that of fresh fish, even though the microscopical appearance of the muscle cell was unchanged. The true water-binding capacity of the proteins was greatly reduced. The solubility of the proteins of freshly prepared dehydrated fish in 0.5 M KCl (pH 7.00) was found to be very much less than that of the proteins of fresh fish.

Hunt and Matheson (1958) reported a decrease in the actomyosin ATP-ase activity of cod and beef muscle on dehydration. The results of Connell (1958) and of Hunt and Matheson (1958) indicated that the main structural protein complex of muscle, actomyosin, had been denatured on drying.

Brooks (1958) attributed the dry, tough texture of freeze-dried meat to the loss of water-holding capacity by the muscle proteins. He suggested that protein denaturation during drying may be responsible.

The results of Hamdy et al. (1959) confirmed those of Brooks (1958). They added various solutes to the water used for rehydration in order to investigate the water-holding properties of freeze-dried beef. These investigators showed that the rehydration of freeze-dried meat was not greatly improved as a result of changing the ionic atmosphere in meat.

Hamdy et al. (1959) reported that freeze dehydration of beef at a 43°C plate temperature and 1500 μ Hg resulted in a considerable decrease in the concentration of the water soluble nitrogen. Upon heating the freeze-dried samples, the amount of juice released was much more than that of the respective controls prior to freeze-drying. At a plate temperature of 22-30°C, 300-400 μ Hg, these workers detected no effect on

the pH of meat. Similarly, there was no measurable effect on the water soluble proteins or the 4% TCA soluble proteins of both the control and those samples to which salt was added. They also detected no changes in pH as a result of frozen storage or freeze-dehydration of the meat samples. The evidence indicated that denaturation of the proteins had resulted in changes in permeability of the fibers.

According to Deatherage and Hamm (1960), quick freezing of muscle tissue did not decrease the hydration of muscle nor cause protein denaturation. On the other hand, slow freezing caused a small but significant decrease in the water-holding capacity of meat. It was postulated that some alteration of protein structure was caused by the formation of the large ice crystals between the cells. With slow freezing, the liklihood of denaturation increased as the proteins were in the presence of a concentrated salt solution for a longer period of time.

The denaturation of proteins during freeze-drying was studied by Hamm and Deatherage (1960) using water-holding capacity and buffering capacity at different pH values, as well as measurement of the dye binding ability of the free acidic and basic groups of muscle proteins. In general fresh meat exhibited a greater water-holding capacity than freeze-dried at a pH of 5.6 (the natural pH of meat). Over a range of pH, freeze-dried meat was found to exhibit greater water-binding capacities than fresh meat at a pH higher than 6.5 but less at a pH lower than 6.5. The minimum water binding capacity for both products was at pH 5.0, where differences were greatest between the fresh and freeze-dried meat.

Hamm and Deatherage (1960) also found that differences existed between fresh and freeze-dried meat in their buffering capacities. The difference was greatest between pH 6 and 7, where for both the water extract and structural proteins, freeze-dried meat exhibited a higher buffering capacity. Results obtained from dye binding by free acidic and basic groups supported the assumption that more free acidic groups were liberated on the basic side of the isoelectric point.

Deatherage and Hamm (1960) also concluded that the removal of water gives rise to a decreased number of protein groups available to bind water after reconstitution. They further suggested that drying resulted in a more closed protein structure due to the salt and/or hydrogen bridge type of bonds, which can be reversed at high or low pH.

Hamm and Deatherage (1960) also reported a drop of muscle hydration with an increase of temperature. However, the rehydrated samples had the same moisture content. They also studied the influence of the shape of meat on the water-holding capacity. They showed that the water-holding capacity of all freeze-dried beef samples was less than that of fresh meat. The hydration of the powdered ground meat was greater than that of the rehydrated and ground cubed meat. This in turn was somewhat higher than hydration of the ground dehydrated meat, which was rehydrated without powdering. These results indicated that the grinding of meat before drying was disadvantageous.

According to Hamdy et al. (1959) the electrophoretic patterns of the myosin extract of beef muscle appeared to be greatly affected during freeze dehydration.

Cole and Smithies (1960) investigated the electrophoretic patterns of a 0.15 μ extract of freeze-dried beef. Their results indicated that few changes had been introduced through freeze drying. In addition, actomyosin extracts from freeze-dried beef sedimented at a faster rate than actomyosin from frozen beef. There was no marked decrease in the level of specific ATP-ase activity.

A more intensive and extensive investigation of the ATP-ase activities of a beef actomyosin extract was performed by Cole (1962). It was found that stimulation of ATP-ase by 2,4-dinitrophenol was less for both freeze-dried and frozen tissues than it was for fresh meat extract. No significant difference between frozen and freeze-dried extracts was reported.

EXPERIMENTAL METHODS

Fat and moisture analysis

Moisture was determined by drying 5 g. samples of ground meat in a disposable aluminum dish for 24 hours in a 105°C oven. The dried samples were used for fat extraction with the Goldfish apparatus according to the procedure described by Hall (1953).

Nitrogen analysis

All nitrogen analyses were performed, in duplicate, by the micro-Kjeldahl method as outlined by the American Instrument Company (1961). Nitrogen contents were reported as mg. of protein nitrogen or non-protein nitrogen per ml. of solution, or per g. of solids.

Non-protein nitrogen determination

Non-protein nitrogen was determined after precipitating the proteins by adding 5 ml. of 10% trichloroacetic acid to 15 ml. of all extracted protein solutions. After 15 minutes the material was filtered through Whatman No. 1 filter paper and the filtrate was analyzed for nitrogen content. The value was multiplied by 1.33 in order to obtain the non-protein nitrogen content per ml. of the original solution.

Measurement of pH

All pH measurements were made with a Beckman Model G pH meter. The electrodes were placed directly into the ground meat sample or protein solution, and the observed values were recorded to the nearest hundredth unit.

Centrifugation

Centrifuging was performed at 2500 rpm (1400 x gravity) in a model PR-2 refrigerated International Centrifuge at 4°C.

Reagents

American Chemical Society reagent grade chemicals and deionized distilled water were used throughout the experiment.

Statistical analysis

Simple correlation coefficients, means, and standard deviations were calculated as described by Snedecor (1956).

Experimental meat

All meat samples were obtained from carcasses of hogs slaughtered in the Michigan State University abattoir. No attempt was made to relate treatment effects to the previous history of the animal, since meat from the same animal served as the untreated control in all studies.

Sample preparation

The longissimus dorsi muscle was used in all studies. All separable fat and visible connective tissue were removed from the excised muscle samples.

Fresh meat

One hundred g. samples were removed from the posterior, middle and anterior portions of the muscle. The composite sample was ground twice through a 1 cm. plate and twice through a 2 mm.plate of a Hobart grinder.

The grinder head and plates were pre-chilled to 4°C in all cases to prevent heat denaturation of the sample. The pH of the fresh meat was recorded. A portion was frozen and analyzed later for total nitrogen, fat and moisture content.

Freeze-dehydration

The remainder of the longissimus dorsi muscle was sliced into 26-28 fillets about 1/4 inch in thickness, weighed to the nearest tenth of a gram and prefrozen in aluminum foil at -28.9°C blast for 3 hours. The frozen samples were freeze-dried for 20-24 hours in a Stokes Freeze-Drier, Laboratory Model 2003 F-2 using a vacuum of 150 μ Hg., with a plate temperature ranging from 28-30°C. Upon removal from the Stokes apparatus, the samples were immediately reweighed. The fillets were wrapped individually in aluminum foil and stored under nitrogen in a desiccator at room temperature. Length of storage never exceeded four weeks.

Rehydration of fillets

Fillets of known weight were immersed in 150 ml. of rehydrating solution in covered casserole dishes. Detailed composition of all rehydrating solutions is contained in appendix A. In order to submerge the samples, glass weights were utilized. The fillets were rehydrated 4 1/2 hours at 4°C. All rehydration procedures were carried out in duplicate.

The rehydrated pork was blotted with Whatman No. 1 filter paper for 1/2 minute on each side to remove excess moisture. Samples were weighed

and the percentage rehydration was calculated according to the formula, $\frac{W_R}{W_L} \times 100$, where W_R = g. moisture regained in rehydration, W_L = g. moisture lost in freeze-dehydration. The pH of the rehydrated fillet was recorded. The protein nitrogen and non-protein nitrogen of the rehydrating solution were measured.

Protein fractionation

The protein fractionation procedure was adapted from that of Hegarty (1963), with modifications according to the methods of Cole and Smithies (1960) and of Seagran (1958), and is described below.

All fractionation procedures were carried out in duplicate at 4°C. The scheme of analysis is shown in figure 1. It was used for the quantitative determination of sarcoplasmic protein nitrogen, non-protein nitrogen, and total fibrillar protein nitrogen. The scheme shown in figure 2 was used for the determination of fibrillar protein solubility.

Five g. of fresh or rehydrated pork were weighed into a 250 ml. centrifuge tube. Eighty ml. of a phosphate buffer, pH 7.6, μ = 0.05, (0.156 M K_2HPO_4; 0.0035 M KH_2PO_4) was used to transfer the sample to a micro blender container. Protein denaturation due to excessive foaming was avoided by comminuting with the Waring blendor, in which the speed was adjusted by means of a Powerstat transformer setting of 40. The samples were blendorized for a 10 second burst followed by a 3 minute rest period. This process was repeated three times. After blendorizing, the suspension was transferred back to its original tube. The container was rinsed with 20 ml. of extracting solution. After one hour the

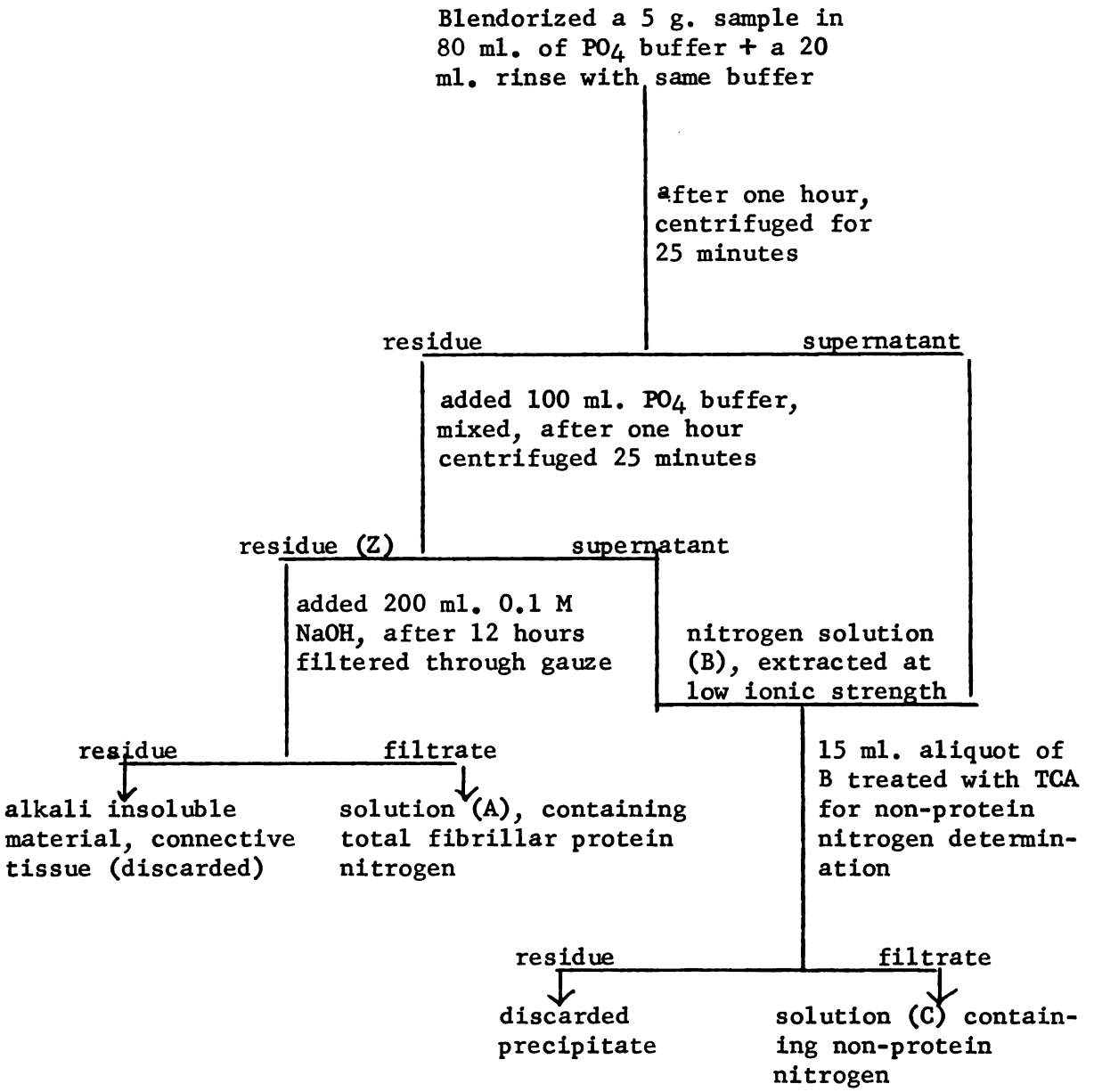


Figure 1. Scheme of analysis for the quantitative determination of sarcoplasmic protein nitrogen, non-protein nitrogen and total fibrillar protein nitrogen.

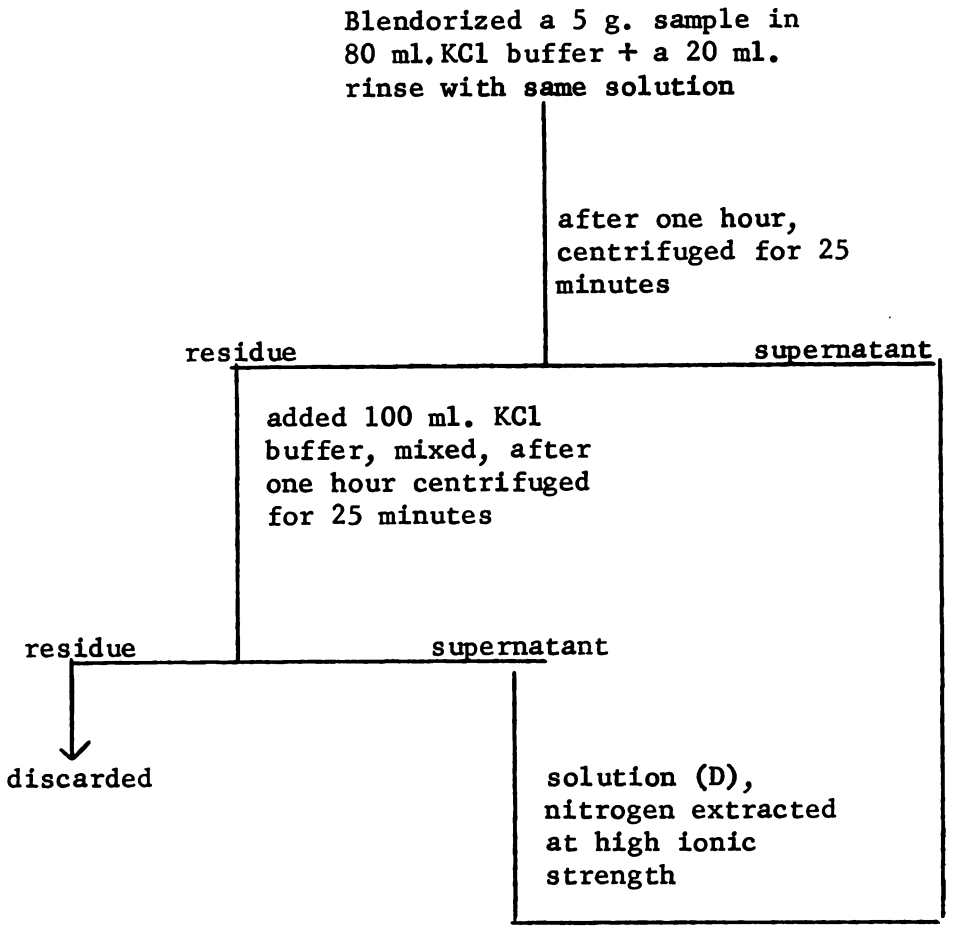


Figure 2. Scheme of analysis for the quantitive determination of fibrillar protein nitrogen solubility.

material was centrifuged for 25 minutes and the supernatant was decanted. One hundred ml. of extracting solution was added to each tube. Complete dispersion of the precipitate was achieved by stirring with a glass rod.

After one hour the material was centrifuged and the supernatant decanted as before. The two decanted solutions were combined and designated as B, or the protein solution extracted at low ionic strength. Two aliquots of 15 ml. were taken for nitrogen and non-protein nitrogen analyses. The filtrate resulting from the TCA precipitation was designated as C. The residue (Z) resulting from extraction with phosphate buffer was extracted with 200 ml. of 0.1 M NaOH for 12 hours at room temperature. The volume of the tube contents was measured after filtration through gauze. A very small amount of residue (alkali insoluble material, i.e., collagen and elastin) was removed by filtration. An aliquot of the filtrate (A) was taken for nitrogen analysis. The procedure in figure 2 is exactly the same as the first two steps outlined in figure 1, except that the extracting solution was a KCl-carbonate buffer, pH 8.25, $\mu = 0.53$, (0.5 M KCl; 0.03 M $NaHCO_3$). The solution extracted by this scheme was designated D.

Solutions A, B, C, and D were analyzed for nitrogen and results designated as A^n, B^n, etc. which represent the following fractions:

A^n = total fibrillar protein nitrogen

B^n = nitrogen extractable at low ionic strength

C^n = non-protein nitrogen

D^n = nitrogen extractable at high ionic strength

$B^n - C^n$ = sarcoplasmic protein nitrogen

$D^n - B^n$ = soluble fibrillar protein nitrogen

$D^n - (C^n + B^n)$ = connective tissue protein nitrogen.

The above values were averages of duplicate analyses recorded to the second decimal place. Variation between duplicates for B^n, C^n, D^n was normally 0 - .02 mg. per ml. Variation in the second decimal place in the case of A^n was found to be 0 - .04 mg. with an extreme range in one or two cases of .09 mg. per ml.

Amino acid composition of rehydrating solution

The scheme of analysis outlined in figure 3 was utilized for the qualitative determination of the amino acid composition of the rehydrating solutions.

Forty ml. of rehydrating solution were hydrolyzed with 40 ml. 12 N HCl for 24 hours and then filtered. The resultant hydrolyzate (I) represented the total amino acid content of the rehydrating solution. Forty ml. of rehydrating solution were mixed with 160 ml. of 100% ethyl alcohol to precipitate the proteins. After 30 minutes, the material was filtered through Whatman No. 1 filter paper. The residue was discarded. The filtrate was concentrated to 16 ml. with a Rinco rotary evaporator at 40°C. One ml. of the concentrated solution (F) was removed. This solution represented the free amino acid content of the original rehydrating solution. Fifteen ml. of 12 N HCl were added to the remaining 15 ml. of concentrate. The solution was hydrolyzed for 24 hours and then filtered. The hydrolyzate (II) contained the total non-protein nitrogen amino acids.

The amino acids were separated on a one-dimensional descending paper chromatograph. A 1-n-butanol-acetic acid-water (upper layer) of a 4:1:5 by volume solvent system was used. The ninhydrin-cupric nitrate spray of

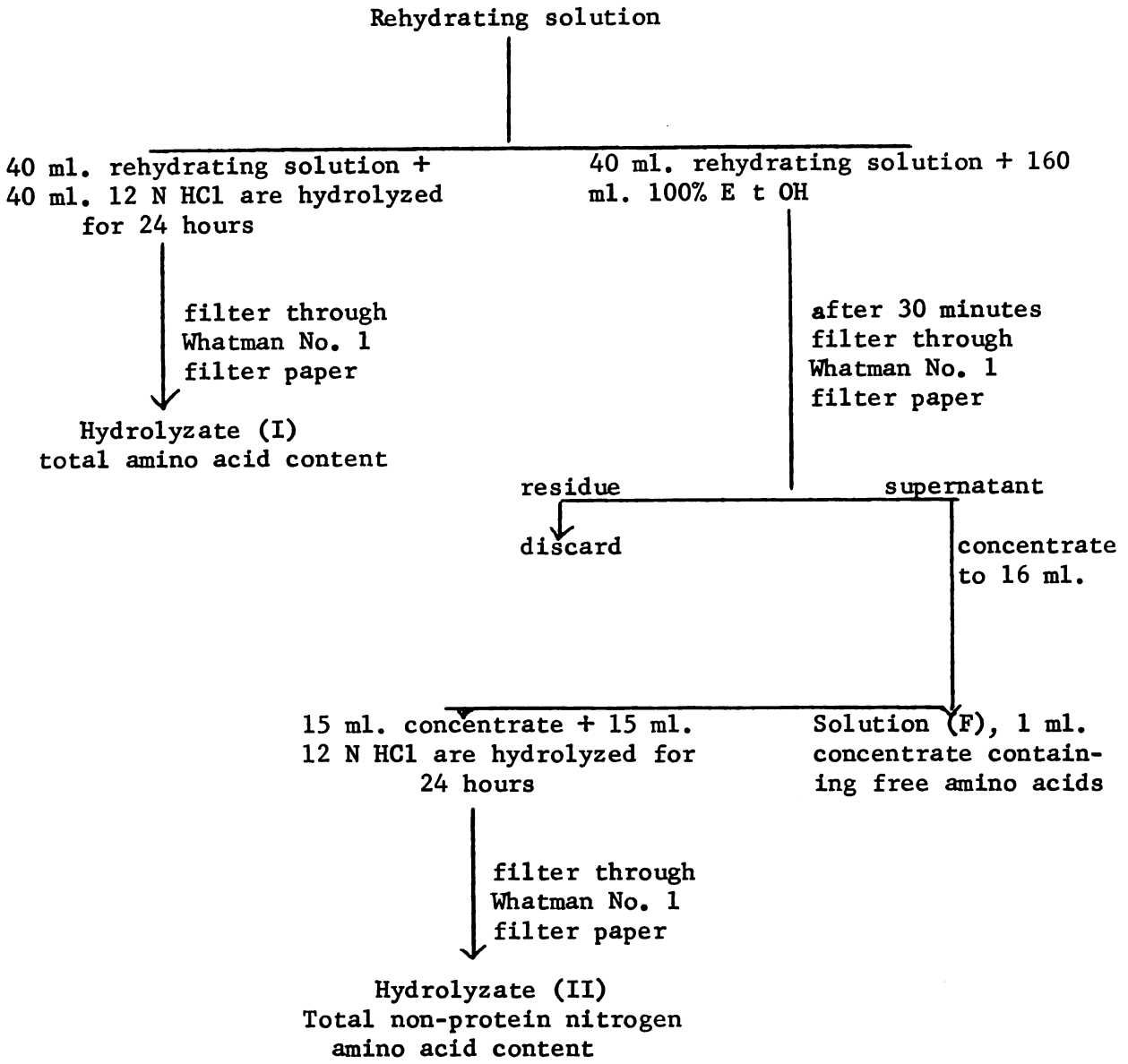


Figure 3. Scheme of analysis for the qualitative determination of amino acid composition of the rehydrating solution.

Moffat and Lytle (1959) permitted complete resolution of the incompletely separated amino acid spots. The three solutions (Hydrolyzate I, II, and solution F) were placed on Whatman No. 1 paper in 100 microliter aliquots. The chromatogram was developed until the solvent front had advanced 32 to 35 cm. beyond the point at which the sample was applied. The paper was removed from the apparatus and dried in a 105°C oven for 5 minutes. The chromatogram was sprayed with the ninhydrin-cupric nitrate indicator and dried in a 105°C oven for 2 minutes. The colors given with this reagent are listed in appendix B.

Volatile loss detection

A 20 g. portion of the ground fresh meat was blendorized with 80 ml. of deionized water for one minute in a Waring blender adjusted with a Powerstat transformer setting of 60. The pH of the slurry was taken. The meat slurry was transferred to a 1000 ml. round bottom flask, which was then slowly rotated in an ethanol-dry ice bath. This caused the meat slurry to be frozen as a thin shell on the flask's surface. The flask was attached to a vacuum distillation apparatus, which consisted of one ethanol-dry ice trap and two liquid nitrogen traps. A Welch Duo-Seal vacuum pump created a vacuum ranging from 170 μ to 50 μ Hg. Complete dehydration occurred within 6-9 hours. The ethanol-dry ice trap contained all of the water removed from the meat slurry. The water was thawed and its pH was recorded.

The volatiles lost in the dehydration process were trapped in the two liquid nitrogen traps. The volatiles were distilled into the thawed water using nitrogen to flush out the containers. After each trap had been distilled, the pH of the solution was taken.

The solution consisting of all volatile constituents removed during the dehydration of the slurry was added to the dehydrated meat solids. The pH of the reconstituted meat slurry was then measured.

RESULTS AND DISCUSSION

Influence of pH on percentage rehydration

A total of 70 fillets taken from the longissimus dorsi of three hogs was used in this study. Duplicate samples were rehydrated in either 10 or 11 different buffers of 0.05 μ covering a pH range of 3.62 to 9.05. Four samples were rehydrated in a buffer of 0.1 μ, pH 3.05 and 2 samples were rehydrated in a buffer of 0.1 μ, pH 2.55.

The means and standard deviations for pH and percentage rehydration are presented in table 1. There were no significant differences between the means for the three loins in either the pH of the rehydrating solution or the pH of the rehydrated meat.

Table 1. Means and standard deviations for pH of rehydrating solution, pH of rehydrated meat and percentage rehydration

	Means and standard deviations			
Item	Loin 1	Loin 2	Loin 3	Pooled data
pH of rehydrating solution	6.40 ± 1.81	6.35 ± 1.74	6.38 ± 1.85	6.38 ± 1.80
pH of rehydrated meat	5.55 ± .51	5.47 ± .44	6.41 ± .48	5.47 ± .48
Percentage rehydration	67.41*± 8.86	80.61**±7.38	72.71*± 6.18	73.75 ± 9.25

**Significantly different at the 1% level from the underlined observations.
* Significantly different at the 5% level.

Table 2 summarizes the correlation coefficients of pH versus percentage rehydration. The association between percentage rehydration and either

pH of the rehydrating solution or pH of the rehydrated meat was not statistically significant. Thus, freeze-dried pork showed no optimum pH for rehydration. This is in contrast to the work of Auerbach et al. (1954), who reported that the highest level of rehydration for freeze-dried beef occurred at pH 7.00. This indicates that a difference between freeze-dried pork and beef exists, which, perhaps, can be attributed to different physiological characteristics of the two species.

Table 2. Correlation coefficients between pH of rehydrating solution, pH of rehydrated meat and percentage of rehydration

Loin	Correlation coefficients	
	pH rehydrating solution vs percentage rehydration	pH rehydrated pork vs percentage rehydration
1	-.217	-.064
2	-.081	-.027
3	0.091	-.067
Pooled data	-.071	-.078

A direct correlation between pH of the rehydrating solution and the pH attained by the rehydrated pork was observed for all three loins in this study. The correlation coefficients are shown in table 3. This straight line relationship was not surprising as the pH of meat would be expected to change on the addition of an acidic or basic solution. The amount of change in pH would be dependent on the buffering capacity of the proteins. Sherman (1961) reported that the pH of fresh pork is effected

Table 3. Correlation coefficients between the pH of rehydrating solution and pH of rehydrated pork

Loin	Correlation coefficients
1	+.916**
2	+.721**
3	+.846**
Combined data	+.824**

**Significant at 1% level.

by the addition of neutral salts and polyphosphates. In the present study, no attempt was made to determine the effect of neutral salts and polyphosphates on the pH of freeze-dried pork.

The analysis of variance between percentage rehydration for different loins is summarized in table 4. The F ratio of 17.27 was highly significant at the 1% level. The Studentized range test indicated that there was a significant difference between the means for percentage rehydration of the loins from the three different hogs utilized in this study. The occurrence of a significant deviation between loins may have been caused by breed differences or possibly by an individual reaction to the dehydration process.

The experimental data indicates that freeze-dried pork rehydrates to a much lower level than beef. Percentage rehydration of freeze-dried pork ranged from 48.54% to 92.41%. The mean percentage rehydration of 70 samples is 73.75% and the standard deviation is 9.26. The distribution of percentage rehydration is summarized in the histogram shown in figure 4.

Table 4. Analysis of variance of percentage rehydration and pigs

Source	d.f.	Sum of squares	Mean square	F
Percentage rehydration	69	5996.43		
Loins	2	2039.96	1019.98	17.27**
Individuals	67	3956.47	59.05	

**Highly significant at 1% level.

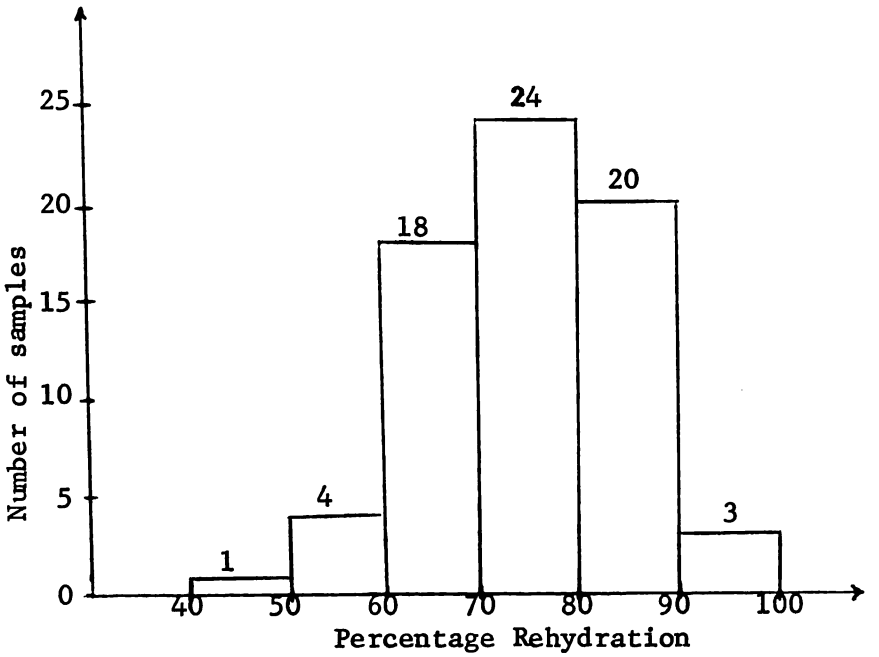


Figure 4. Histogram of percentage rehydration for freeze-dried pork fillets.

According to Tappel et al. (1955) 1-inch pieces of the biceps femoris of beef attained an 80-90% level of rehydration. Harper and Tappel (1957) have stated that freshly prepared freeze-dried beef rehydrates to a maximum level of 80-100% of its original water content. Although freeze-

dried pork is very similar to freeze-dried beef in structure and texture, there is a noticeable difference in their rehydration characteristics.

The conditions of this investigation varied from those of Tappel, who utilized a -17.8°C freezing temperature, a 0.1 to 0.2 mm. Hg. pressure and a plate temperature of 45°C. A freezing temperature of -28.9°C, a 0.15 mm. Hg. pressure and a plate temperature of 25-30°C were used in this study. A rapid freezing rate tends to increase product quality, while a lower plate temperature during the freeze-drying process tends to reduce protein denaturation. Thus, the experimental conditions of this investigation should have caused an equivalent or greater percentage rehydration than that reported for freeze-dried beef by Harper and Tappel (1957). However, the results of this study indicated that freeze-dried pork will usually rehydrate in the range of 64-83% which is considerably lower than the 80-100% reported by Harper and Tappel (1957) for freeze-dried beef.

Results outlined in table 5 show that loin 1, which had a low fat content, did not rehydrate to a higher level than loin 3, which had a high fat content. These results are in direct opposition to those of Harper and Tappel (1957), who stated that the high fat content of pork was responsible for a decreased percentage of rehydration. According to Orme et al. (1958), the fat content from beef longissimus dorsi ranged from 1.90 to 11.21%. The mean percent fat averaged 4.25 and 8.59% for good and prime steers, respectively. Harrington and Pearson (1962) reported that the intramuscular fat content of pork longissimus dorsi muscle differing greatly in marbling averaged 3.45% with a range from

Table 5. Mean percentage rehydration and percent fat content of fresh *longissimus dorsi*

Loin	% fat	Mean percentage rehydration
1	2.40	67.41 ± 8.86
2	2.94	80.61 ± 7.38
3	4.26	72.71 ± 6.18

1.1 to 7.4%. Pearson *et al.* (1962) using another group of pigs found the percentage fat of pork *longissimus dorsi* ranged from 2.14 to 8.14%. Thus, the percentage intramuscular fat in beef and pork *longissimus dorsi* does not differ greatly. Therefore, if the fat content influenced rehydration, no marked difference in rehydration would be expected between beef and pork. It is also interesting to note that loin 2, which had an intermediate fat content, rehydrated more completely than either of the others.

The percentage fat reported in this study was obtained from a composite sample taken from the whole loin. It is known that the fat content of the *longissimus dorsi* varies with sampling position. Since the fat content of the individual rehydrated fillets of loins 1, 2, and 3, had been calculated from the fat content of the fresh control, a fourth loin was freeze-dried and rehydrated. Results showed that the correlation ($r = .168$) between fat content and percentage rehydration was not statistically significant. Therefore, fat content does not greatly influence percentage rehydration.

The differences in percentage rehydration between loins from different pigs may be caused by a combination of factors including pre-slaughter treatment, electrolyte content of muscle and over-all muscle composition.

One of the outstanding features of this series of experiments was the increase in the pH of freeze-dried pork when rehydrated in deionized water. The influence of freeze-drying on the pH of rehydrated pork is shown in table 6. Seven out of eight rehydrated fillets achieved a higher pH than that of the controls.

Table 6. Influence of freeze-drying on the pH of rehydrated pork

	pH		
Loin	Fresh	Rehydrated duplicates[1]	
1	5.32	5.69	5.65
2	5.35	5.25	5.45
3	5.51	5.75	5.75
4	5.29	5.45	5.45

[1]Samples rehydrated in deionized water at 4°C.

In order to determine whether the change in pH was caused by protein denaturation or a loss of volatile constituents, the volatiles were collected and added back during rehydration. Results indicate that the volatile losses are responsible for some of the changes in pH, and if trapped and utilized in reconstitution of the dried meat solids, the original pH of the fresh meat slurry could be regained. Table 7 presents a summarization of the results of the volatile-loss determination.

Table 7. Influence of freeze-drying on the loss of volatiles from pork.

Conditions	Trial (1)	Trial (2)	Δ pH = $pH_1 - pH_2$
Pressure	170-70 μ Hg.	50 μ Hg.	
Dehydration time	9 hours	6 hours	
pH			
Meat slurry, fresh	5.23	5.15	
Volatile Fractions			
Trap 1: Solution 1 (thawed water)	6.45	5.62	+0.83
Trap 2: Solution 2 (Solution 1 + volatiles of 1st liquid N_2 trap)	5.68	5.30	+0.38
Trap 3: Solution 3 (Solution 2 + volatiles of 2nd liquid N_2 trap)	4.49	5.62	-1.13
Meat slurry, reconstituted (dehydrated solids + solution 3)	5.28	5.18	

The differences observed between trials in the pH of solutions 1, 2, and 3 (table 7) may be explained by the varying experimental conditions. A much higher evacuation of the flask in trial 2 was achieved. Thus, the meat slurry in trial 2 could be completely dehydrated in a shorter period of time. A complete equilibration of the volatiles between the traps in trial 2 could not be accomplished due to the shorter dehydration time. The total change in pH between trials was calculated as +.08 units, which was within the experimental error of ± .15. Although the volatiles were

not found in exactly the same traps in the two trials, the total change in pH was equivalent.

The change in pH indicated that volatiles which are acidic in nature are removed during the freeze-drying process. The removal of acidic volatiles during the freeze-drying process, accounts for the rise in pH found to occur on rehydration of freeze-dried pork. The nature of the volatiles was not studied in this investigation, however, it is likely that CO_2, H_2S, and short-chained fatty acids would be among the volatile constituents.

Flosdorff (1949) stated that the loss of volatile constituents during freeze-drying was minimal. Hamm and Deatherage (1960) refer to an occasional but not significant shift of pH in freeze-dried beef on rehydration. The significant pH change of freeze-dried pork on rehydration again indicates that freeze-dried pork is dissimilar to freeze-dried beef in its rehydration characteristics. This may be due to the inherent differences in the structure of the two species.

Protein denaturation

Seventy rehydrated fillets taken from the longissimus dorsi muscle of three pigs were used to investigate the degree of protein denaturation caused by the freeze-drying process. Duplicate samples of each rehydrated fillet and the fresh control were fractionated according to the schemes outlined in figures 1 and 2. The protein content, expressed as mg. nitrogen per g. of solids, was determined for the following fractions: (1) sarcoplasmic protein nitrogen, (2) 0.53 μ (KCl-bicarbonate) extractable protein

nitrogen, (3) soluble fibrillar protein nitrogen, and (4) non-protein nitrogen. The protein and non-protein nitrogen contents of the various rehydrating solutions were also determined.

The means and standard deviations for the nitrogen content of the various protein fractions extracted from the freeze-dried loins are presented in table 8. There were no significant differences between the means for the three loins in the sarcoplasmic protein nitrogen and 0.53 μ (KCl-bicarbonate) soluble protein nitrogen. The differences between the means for the three loins in the protein and non-protein nitrogen contents of the rehydrating solution were not statistically significant.

Table 8. Means and standard deviations for the nitrogen content of the protein fractions extracted from freeze-dried loins expressed as mg. N/g. solids.

Item	Means and standard deviations			
	Loin 1	Loin 2	Loin 3	Pooled data
Sarcoplasmic protein nitrogen	26.93 ± 4.01	27.23 ± 4.79	28.04 ± 7.24	27.41 ± 5.58
0.53 μ soluble protein nitrogen	35.07 ± 6.15	33.48 ± 5.03	35.99 ± 7.89	34.84 ± 6.56
Soluble fibrillar protein nitrogen	8.29 ± 6.21	6.57 ± 4.62	8.08 ± 4.97	7.63 ± 5.34
Non-protein nitrogen	12.88 ± 1.82	10.72*± 1.69	13.91 ± 3.05	12.63 ± 2.53
Rehydrating solution protein nitrogen	2.25 ± .654	2.95 ± .981	2.63 ± .615	2.62 ± .821
Rehydrating solution non-protein nitrogen	4.49 ± .556	5.34 ± .686	5.62 ± .740	5.17 ± .820

*Significantly different at 1% level from the underlined observations. All other values were not significantly different.

A large standard deviation for the soluble fibrillar protein content was obtained for all three loins. It is impossible to arrive at direct conclusions on the basis of these results due to the wide range in the data. The analysis of variance for the soluble fibrillar protein content among the three loins indicated that there was no statistical difference between loins. The range in data obtained from freeze-dried loins could have resulted from incomplete extraction of the fibrillar proteins.

Bailey (1954) stated that extractability of proteins was not solely determined by solubility. He concluded that extractability of the intracellular protein fraction appeared to be determined by pH, ionic strength of the extracting solution, type of extractant, and by adequacy of grinding. Dyer et al. (1950) also concluded that the most important point in the extraction of protein was a sufficiently fine subdivision of the muscle fibrils. In the present study, the freeze-dried fillets were hand-minced, thus, the size of the mince varied. In further studies, the experimental error could probably be reduced by increasing the size of the fillets in order to permit mechanical grinding, and thereby, obtain a more uniform particle size.

The analysis of variance for non-protein nitrogen content among three freeze-dried loins is summarized in table 9. The F value of 9.11 was highly significant at the 1% level. The Studentized range test indicated that the non-protein nitrogen content of loin 2 was significantly different from that of loins 1 and 3. The non-protein nitrogen contents of loins 1 and 3 did not differ significantly. The variation in non-protein nitrogen content of the three loins could possibly arise from different amounts of decomposition products of metabolism in the three animals.

Table 9. Analysis of variance for non-protein nitrogen content among three freeze-dried loins.

Source	d.f.	Sums of squares	Mean square	F value
Non-protein nitrogen content	69	443.28		
Loins	2	94.71	47.36	9.11**
Individuals	67	348.57	5.20	

**P < .01

The nitrogen content of the protein fractions extracted from the fresh longissimus dorsi controls is shown in table 10. The sarcoplasmic protein nitrogen, the 0.53 μ (KCl-bicarbonate) extractable protein nitrogen and the soluble fibrillar protein nitrogen fractions of loin 2 contained more nitrogen than either loin 1 or 3. Loins 1 and 3 did not differ significantly in the nitrogen content of the various protein fractions. The differences observed in the non-protein nitrogen content may be due to individual animal differences.

Table 10. The nitrogen content of the protein fractions extracted from the fresh longissimus dorsi controls expressed as mg. N/g. solids

Item	Nitrogen content		
	Loin 1	Loin 2	Loin 3
Sarcoplasmic protein nitrogen	30.45	47.51	35.04
0.53 μ extractable protein nitrogen	36.98	57.74	39.11
Soluble fibrillar protein nitrogen	6.53	10.23	4.07
Non-protein nitrogen	14.31	13.95	16.47

The correlations between percentage rehydration and the nitrogen contents of the extracted protein solutions are presented in table 11. There was no significant correlation between percentage rehydration and sarco-

plasmic protein nitrogen, 0.53 μ (KCl-bicarbonate) extractable protein nitrogen, soluble fibrillar protein nitrogen or non-protein nitrogen of the three freeze-dried loins. Direct correlations of 0.541 for loin 2 ($P < .01$) and 0.413 for loin 3 ($P < .05$) were obtained between percentage rehydration and the protein content of the rehydrating solution. The correlation between percentage rehydration and the protein content of the rehydrating solution for loin 1 was positive, but was not significant. The pooled data, however, had a correlation of 0.461, which was significant at the 1 percent level.

Table 11. Correlations between percentage rehydration and the nitrogen content of the extracted protein solutions expressed as mg. N/g. solids

	Correlation coefficients			
Protein fractions	Loin 1	Loin 2	Loin 3	Pooled data
Sarcoplasmic protein nitrogen	+.224	-.071	+.072	+.059
0.53 μ extractable protein nitrogen	-.001	-.096	+.095	-.061
Soluble fibrillar protein nitrogen	-.152	+.024	+.055	-.116
Rehydrating solution protein nitrogen	+.062	+.541**	+.413*	+.461**
Non-protein nitrogen	+.19	+.14	+.21	-.083

*Significant at 5% level
**Significant at 1% level

The trend established by loins 2 and 3 indicated that the greater the percentage rehydration, the more proteins would be leached from the

freeze-dried fillets. The larger the amount of water reabsorbed by the meat, the more proteins that could be extracted and transported into the rehydrating solution. No statistically significant correlations were found between the pH and the protein content of the rehydrating solution for the three loins. However, when the data were pooled for analysis, a correlation of +.274, ($P < .05$) was obtained. It may be concluded that an interaction between percentage rehydration and pH of the rehydrating solution may effect the protein nitrogen content of the rehydrating solutions.

A comparison of the nitrogen content of the protein fractions extracted from fresh and freeze-dried loins is shown in table 12. The sarcoplasmic protein fraction for all three loins noticeably decreased in nitrogen content on freeze dehydration. As shown in table 3, a certain amount of protein nitrogen was leached out by the rehydrating solutions. If one assumes the nitrogen content of the rehydrating solution to be composed entirely of sarcoplasmic protein nitrogen, a decrease in sarcoplasmic protein content on freeze dehydration is still evident. The non-protein nitrogen content of all three loins decreased after freeze-dehydration and rehydration had occurred. However, when the non-protein nitrogen content of the rehydrating solution (table 3) is considered, the total non-protein nitrogen content of all three freeze-dried loins was greater than that of the fresh controls.

Hamdy et al. (1959) reported a decrease in the water soluble nitrogen content of reconstituted beef, which had been freeze-dried at a plate temperature of 43°C and a pressure of 1500 μ Hg. Freeze-drying at a

Table 12. A comparison of the nitrogen content (mg. N/g. solids) of the protein fractions extracted from fresh and freeze-dried loins[1]

	Nitrogen content (mg. N/g. solids)					
	Loin 1		Loin 2		Loin 3	
Protein fractions	Fresh	Freeze-dried	Fresh	Freeze-dried	Fresh	Freeze-dried
Sarcoplasmic protein nitrogen	30.45	26.93	47.51	27.23	35.04	28.04
0.53 μ extractable protein nitrogen	36.98	35.07	57.74	33.48	39.11	35.99
Soluble fibrillar protein nitrogen	6.53	8.29	10.23	6.57	4.07	8.08
Non-protein nitrogen	14.31	12.88	13.95	10.72	16.47	13.91

[1]Mean value of nitrogen content for freeze-dried loins is recorded.

plate temperature of 22-30°C and 300-400 μ Hg. chamber pressure resulted in no detectable effect on the water soluble nitrogen content. Kronman and Winterbottom (1960) stated that freezing of beef resulted in a decreased extractability of water soluble proteins, as well as in a loss of specific electrophoretic and ultracentrifugal components.

Results of the present study indicated that the sarcoplasmic protein nitrogen content of pork decreased, when the fillets were freeze-dehydrated at a 28-30°C plate temperature and a pressure of 150 μ Hg. Denaturation of the sarcoplasmic proteins of pork appear to result from freeze-drying as evidenced by a decrease in the concentration of the nitrogen content of the sarcoplasmic protein fraction. The freeze-dehydration process may in some way effect the bonds that are due to electrostatic interaction between polar groups and to van der Waals forces between non-polar groups

of the meat proteins. Irreversible structural changes may occur which influence percentage rehydration and the water holding properties of pork.

The amount of 0.53 μ (KCl-bicarbonate) extractable protein nitrogen of reconstituted pork also decreased. This is a composite protein fraction consisting of both sarcoplasmic and soluble fibrillar proteins. Since the sarcoplasmic protein fraction decreased, the decrease in the 0.53 μ extractable protein nitrogen was expected.

Due to the large standard deviation of the values obtained for the soluble fibrillar protein nitrogen content of all three freeze-dried loins, conclusions concerning the possible influences of freeze-drying on the fibrillar proteins can not be resolved.

Correlations between the sarcoplasmic protein nitrogen content and pH of the rehydrating solution or pH of the meat are expressed in table 13. A highly significant direct correlation between the pH of the rehydrating solutions and the nitrogen content of the sarcoplasmic protein fraction was obtained for loins 1, 2 and for the pooled data. Although possitive correlations were found for loin 3, they were not significant. The trend established for the pooled data, however, is not surprising as the effect of pH on protein extractability is well-known.

Investigation of the influence of pH on the amino acid composition of the rehydrating solutions was performed on loin 4. The qualitative amino acid composition of the rehydrating solutions at pH 2.55, 5.85, 9.05 and deionized water was determined. The amino acids in the various rehydrating solutions were fractionated (figure 3) into total amino acid

content (Hydralyzate I), total non-protein nitrogen amino acid content (Hydrolyzate II), and free amino acids (solution F).

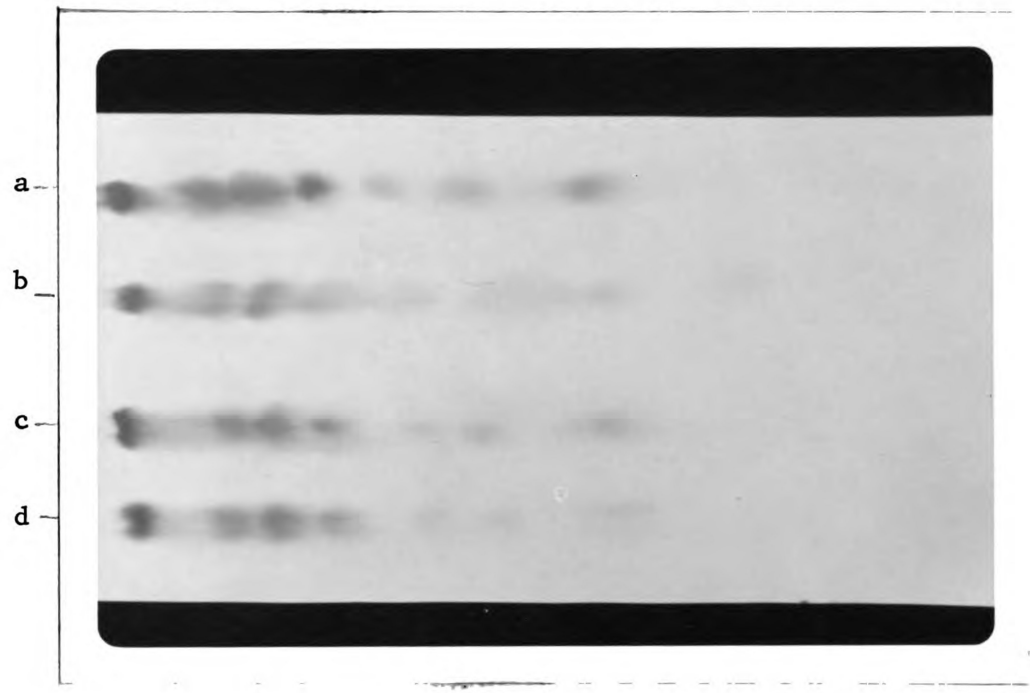
Table 13. Correlations between the sarcoplasmic protein nitrogen content and pH

	Correlation coefficients			
	Loins			
pH	1	2	3	Pooled data
Rehydrating solution	+.574**	.646**	+.370	+.483**
Meat	+.719**	+.517**	+.030	+.316**

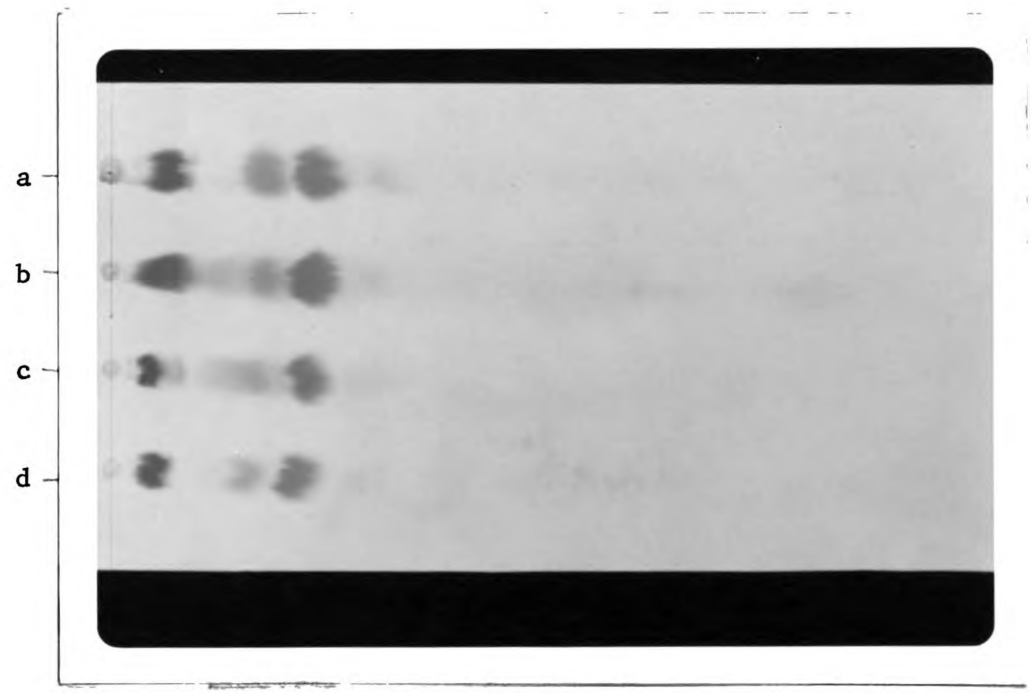
**Significant at 1% level

A qualitative separation of the amino acids was achieved by utilizing one-dimensional descending paper chromatography. Photographs of the chromatograms were taken and the results of this study are shown in figures 5 and 6. The chromatograms indicated that the rehydrating solutions of deionized water (a), pH 5.15 (c) and pH 9.05 (d) were similar in their qualitative amino acid composition. Thus, pH did not influence the qualitative amino acid composition of rehydrating solutions. No attempt was made in the present study to determine the quantitative amino acid composition.

At pH 2.55 the rehydrating solution (b) contained a distinctly different qualitative amino acid composition than the other rehydrating solutions. The ionic strength of the pH 2.55 rehydrating solution was 0.1, while all other rehydrating solutions investigated had an ionic strength of 0.05. Therefore, a change in ionic strength of the rehydrating solution greatly influenced the fingerprinting of amino acids obtained

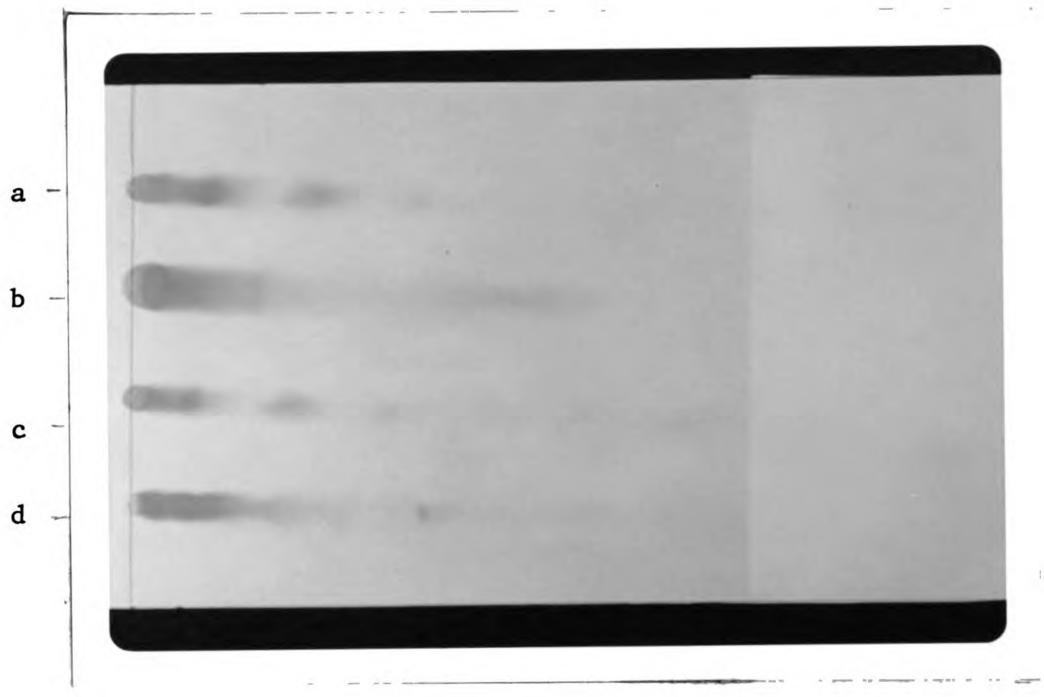


Hydrolyzate I

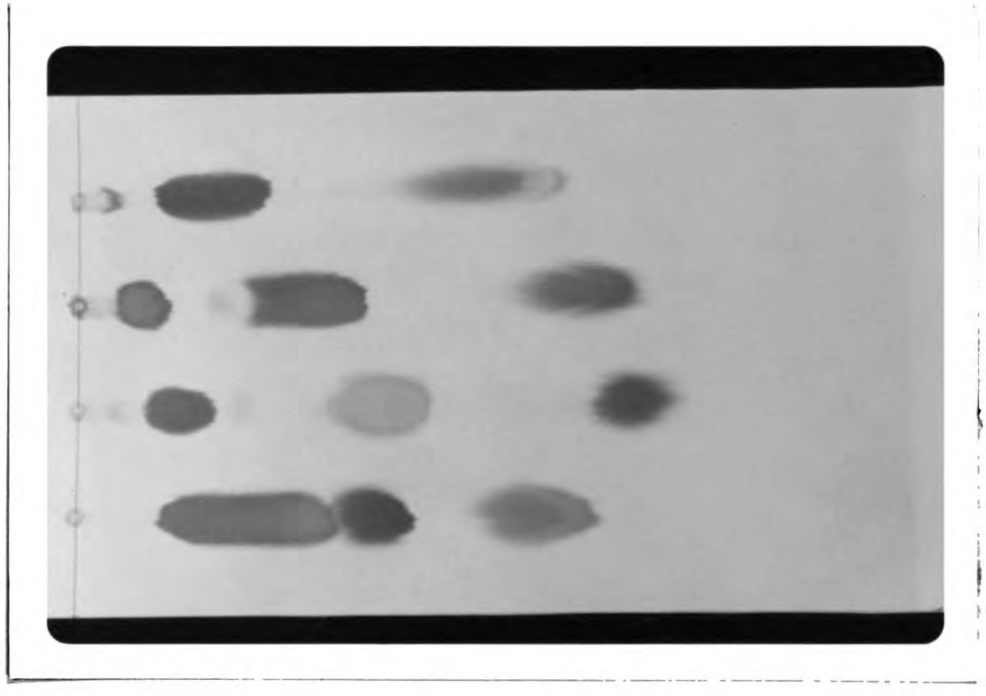


Hydrolyzate II

Figure 5. Chromatograms of the qualitative amino acid composition of total amino acid content (Hydrolyzate I) and total non-protein nitrogen amino acid content (Hydrolyzate II). (a, b, c, and d represent the rehydrating solutions: deionized water, pH 2.55, pH 5.85, and pH 9.05, respectively.)



Solution F



Knowns

Figure 6. Chromatograms of the qualitative amino acid composition of free amino acid content (Solution F) and known amino acids (Knowns). (a, b, c, and d represent the rehydrating solutions: deionized water, pH 2.55, pH 5.85, and pH 9.05, respectively.)

for the total amino acid content (Hydrolyzate I), the total non-protein nitrogen amino acid content (Hydrolyzate II), and free amino acids (Solution F). This is an expected result as ionic strength influences protein solubility and thus, also influences the qualitative amino acid composition.

SUMMARY AND CONCLUSIONS

Seventy freeze-dried fillets taken from the longissimus dorsi muscle of three hogs were utilized in this investigation. The fillets were rehydrated in buffers varying in pH in order to determine the influence of pH on percentage rehydration. The degree of protein denaturation caused by freeze-dehydration was studied by comparing the nitrogen content of the extracted protein fractions of the freeze-dried, rehydrated fillets with the respective protein fractions of the fresh controls.

The percentage rehydration of freeze-dried pork ranged from 48.54% to 92.41% with a mean percentage of 73.75% ± 9.26. Freeze-dried pork was found to rehydrate to a much lower level than beef. There was a significant difference between the means for percentage rehydration of the loins obtained from the three different hogs used in this study.

A direct correlation between pH of the rehydrating solution and the pH attained by the rehydrated pork was observed for all three loins in this investigation. However, percentage rehydration was not significantly influenced by either pH of the rehydrating solution or pH of the rehydrated meat. Thus, freeze-dried pork showed no optimum pH for rehydration.

An increase in the pH of freeze-dried pork was noted when the fillets were rehydrated in deionized water. Acidic volatile losses during the freeze-dehydration process were investigated and found to be responsible

for most of the changes in pH. If these volatiles were trapped and utilized in reconstitution of the dried meat solids, the original pH of the fresh meat slurry could be regained. The significant pH change of freeze-dried pork on rehydration again indicated that freeze-dried pork is dissimilar to freeze-dried beef in its rehydration characteristics.

A correlation of only +.168 between fat content and percentage rehydration indicated that fat content had little effect on percentage rehydration in pork.

Results indicated that there was no significant correlation between percentage rehydration and sarcoplasmic protein nitrogen, 0.53 μ (KCl-bicarbonate) extractable protein nitrogen, soluble fibrillar protein nitrogen or non-protein nitrogen content. A positive correlation existed between percentage rehydration and the protein content of the rehydrating solution. Thus, the greater the percentage rehydration, the larger the amount of proteins that are leached into the rehydrating solution.

The non-protein nitrogen content of freeze-dried loin 2 was significantly different from that of freeze-dried loins 1 and 3. The non-protein nitrogen content of the fresh controls also varied. On freeze-dehydration, the total non-protein nitrogen content increased.

The sarcoplasmic protein fraction noticeably decreased in nitrogen content on freeze dehydration and rehydration. Thus, apparent denaturation of the sarcoplasmic proteins of pork resulted on freeze-drying. Due to the large standard deviation for the soluble fibrillar protein

fraction, no conclusions concerning the possible influences of freeze-drying on the denaturation of fibrillar proteins could be formulated.

The qualitative amino acid composition on reconstitution with deionized water, pH 5.85, or pH 9.05 rehydrating solutions was similar. A dissimilar composition was indicated for the pH 2.55 rehydrating solution. The pH 2.55 buffer had an ionic strength of 0.1, while all others studied had an ionic strength of 0.05. Therefore, pH did not influence the qualitative amino acid composition of the rehydrating solutions. A change in ionic strength, however, greatly influenced the qualitative amino acid composition of rehydrating solutions.

LITERATURE CITED

American Instrument Co. 1961. The determination of nitrogen by the Kjeldahl procedure including digestion, distillation and titration. American Instrument Co., Reprint No. 104.

Auerbach, E., Wang, H., Maynard, N., Doty, D. M., and Kraybill, H. R. 1954. A histological and histochemical study of beef dehydration. V. Some factors influencing the rehydration level of frozen-dried muscle tissue. Food Res. 19, 557.

Bailey, K. 1954. Structure proteins. II. Muscle. The Proteins. Vol. II B, 854. H. Neurath and K. Bailey, eds., Academic Press, New York.

Bailey, K. 1956. Muscle Proteins. Brit. Med. Bull. 12, 183.

Biochemists' Handbook. 1961. Cyril Long, editor. D. Van Nostrand Company, Inc., New York.

Brooks, J. 1958. The structure of the animal tissues and dehydration. Fundamental Aspects of the Dehydration of Foodstuffs. Society of Chemical Industry, London, p. 8.

Bull, H. B. 1944. Adsorption of water vapor by proteins. J. Amer. Chem. Soc. 66, 1499.

Cole, L. J. N. 1962. A comparison of the effects of freezing and drying on the rehydratability of freeze-dried beef. p. 217. Fisher, F. R., ed., Freeze-Drying of Foods, National Academy of Sciences - National Research Council. Washington 4, D. C.

Cole, L. J. N. and Smithies, W. R. 1960. Methods of evaluating freeze-dried beef. Food Res. 25, 363.

Connell, J. J. 1957. Some aspects of the texture of dehydrated fish. J. Sci. Food and Agric. 8, 526.

Connell, J. J. 1958. The effect of drying and storage in the dried state on some properties of the proteins of food. Fundamental Aspects of the Dehydration of Foodstuffs. Society of Chemical Industry, London, p. 167.

Deatherage, F. E. and Hamm, R. 1960. Influence of freezing and thawing on hydration and charges of the muscle proteins. Food Res. 25, 623.

Doty, D. M., Wang, H., and Auerbach, E. 1953. Dehydrated foods: Chemical and histological properties of dehydrated meat. J. Agr. and Food Chem. 1, 664.

Dyer, W. J., French, H. V. and Snow, J. M. 1950. Proteins in fish muscle. I. Extraction of protein fractions in fresh fish. J. Fish. Res. Bd., Can. 7, 585.

Flosdorf, E. W. 1949. Freeze-drying (Drying by Sublimation) Reinhold Publishing Co., New York.

Gooding, E. G. B. and Rolfe, E. J. 1955. The vacuum contact-plate dehydration of foodstuffs. I. A first appraisal. J. Sci. Food and Agric. 6, 427.

Gooding, E. G. B. and Rolfe, E. J. 1957. Some recent work on dehydration in the United Kingdom. Food Tech. 11, 302.

Greaves, R. I. N. 1954. Theoretical aspects of drying by vacuum sublimation. p. 87. Harris, R. J. C., Ed., Biological Applications of Freezing and Drying, Academic Press, New York.

Hall, J. L. 1953. Methods of estimating degree of fatness in carcasses and cuts. Ether extraction method of estimating degree of fatness in carcasses and cuts. Proc. Recip. Meat Conf. 7, 122.

Hamdy, M. K., Cahill, V. R., and Deatherage, F. E. 1959. Some observations on the modification of freeze dehydrated meat. Food Res. 24, 79.

Hamm, R. and Deatherage, F. E. 1960. Changes in hydration and changes of muscle proteins, during freeze-dehydration of meat. Food Res. 25, 573.

Hankins, O. G., Ernst, A. J., Kauffman, W. R. 1946. Chemical composition of raw and dehydrated meat. Food Res. 11, 501.

Harper, J. C. and Tappel, A. L. 1957. Freeze drying of food products. Academic Press, New York. Advances in Food Res. 7, 172.

Harrington, G. and Pearson, A. M. 1962. Chew count as a measure of tenderness of pork loins with various degrees of marbling. J. Food Sci. 27, 106.

Hegarty, G. R. 1963. Solubility and emulsifying characteristics of intracellular beef muscle proteins. Unpublished Ph.D. thesis, Michigan State University.

Hunt, S. M. V. and Matheson, N. A. 1958. The effects of dehydration on actomyosin in fish and beef muscle. Food Tech. 12, 410.

Kronman, M. J. and Winterbottom, R. J. 1960. Post mortem changes in the water-soluble proteins of bovine skeletal muscle during aging and freezing. J. Agric. Food Chem. 8, 67.

Luyet, B. J. 1962. Effect of freezing rates on the structure of freeze-dried materials and on the mechanism of rehydration. p. 194. Fisher, F. R., ed., Freeze-Drying of Foods, National Academy of Sciences - National Research Council. Washington 4, D. C.

Ministry of Agriculture, Fisheries and Food. 1961. The Accelerated Freeze-Drying (AFD) Method of Food Preservation. London.

Moffat, E. D. and Lytle, R. I. 1959. Polychromatic technique for the identification of amino acids on paper chromatograms. Anal. Chem. 31, 926.

Orme, L. E., Pearson, A. M., Bratzler, L. J., and Magee, W. T. 1958. Specific gravity as an objective measure of marbling. J. of Animal Sci. 17, 693.

Pearson, A. M., Harrington, G., West, R. G. and Spooner, M. E. 1962. The browning produced by heating fresh pork. I. The relation of browning intensity to chemical constituents and pH. J. Food Sci. 27, 177.

Seagran, H. L. 1958. Contribution to the chemistry of the king crab. Comm. Fisheries Rev. 11, 15.

Sherman, P. 1961. The water binding capacity of fresh pork. I. The influence of sodium chloride, pyrophosphate, and polyphosphate on water absorption. Food Tech. 15, 79.

Snedecor, G. W. 1956. Statistical Methods. 5th ed. The Iowa State College Press, Ames, Iowa.

Tappel, A. L., Conroy, A., Emerson, M. R., Regier, L. W., and Stewart, G. F. 1955. Freeze-dried meat. I. Preparation and properties. Food Tech. 9, 401.

Taylor, J. F. 1953. The isolation of proteins. The Proteins IA, p. 29. H. Neurath and K. Bailey, eds., Academic Press, New York.

Turner, E. W. 1956. The future of dehydrated meat as a convenience food item. Proc. of the 8th Res. Conf., American Meat Institute, Chicago, p. 37.

Wang, H., Andrews, F., Rasch, E., Doty, D. M., and Kraybill, H. R. 1953. A histological and histochemical study of beef dehydration. I. Rate of dehydration and structural changes in raw and cooked meat. Food Res. 18, 351.

Wang, H., Auerbach, E., Bates, V., Doty, D. M., and Kraybill, H. R. 1954. A histological and histochemical study of beef dehydration. IV. Characteristics of muscle tissues dehydrated by freeze-drying techniques. Food Res. 19, 543.

APPENDIX

Appendix A. Composition of rehydrating solutions.

Reference: Biochemists Handbook 1961

1. Hydrochloric acid - potassium chloride

25°C, I = 0.1 A ml. 0.2 M - HCl + C ml. 0.2 M - KCl, diluted to 1 l.

pH	A	C
2.20	42	458
2.41	25	475
2.80	10	490
3.11	5	495

2. Acetic acid - sodium acetate

25°C, I = 0.05. A ml. M - acetic acid + 50 ml. M - NaOH diluted to 1 liter.

pH	A
3.6	650
3.8	428
4.0	288
4.2	200
4.4	145
4.6	110
4.8	87.7
5.0	73.8
5.2	65.0
5.4	59.5
5.6	56.0
5.8	53.8

Appendix A. Composition of rehydrating solutions. (continued)

3. Potassium dihydrogen phosphate - disodium hydrogen phosphate

25°C, I = 0.05. A ml. 0.5 M - KH_2PO_4 + B ml. 0.5 M - Na_2HPO_4 diluted to 1 l.

pH	A	B
6.0	74.2	8.58
6.2	64.6	11.8
6.4	53.4	15.5
6.6	42.0	19.3
6.8	31.4	22.8
7.0	22.4	25.8
7.2	15.4	28.2
7.4	10.3	30.0
7.6	6.74	31.0
7.8	4.36	31.8
8.0	2.80	32.4

4. Sodium bicarbonate - sodium carbonate

25°C, I = 0.05. A ml. M - $NaHCO_3$ + B ml. M - Na_2CO_3 diluted to 1 l.

pH	A	B
9.0	39.8	3.41
9.2	35.5	4.83

Appendix B. Colors given with the ninhydrin-cupric nitrate spray of Moffat and Lytle (1959). (The order in which the amino acids are listed is also the order in which they appear on descending chromatograms.)

Cystine	Gray
Lysine	Reddish brown, pink ring forms on standing
Histidine	Light brown with dark brown ring inside a yellow ring
Asparagine	Golden
Arginine	Dark purple
Serine	Greenish brown, red ring forms on standing
Aspartic acid	Light blue (if removed from the oven too soon, the aspartic acid spot will be bright green.)
Glycine	Orange brown with bright orange ring
Threonine	Greenish brown, changes to purplish brown on standing
Glutamic acid	Purple, fades slightly on standing
Alanine	Dark purple
Proline	Light green with yellow ring
Cysteine	Gray
Tyrosine	Light brown
Valine	Purple
Methionine	Grayish purple with yellow ring
Tryptophan	Brown with bright blue ring, ring fades rapidly
Isoleucine	Light blue
Phenylalanine	Greenish yellow
Leucine	Light purple with yellow ring

Appendix C. Formulas used in calculations.

Percentage moisture in rehydrated samples: M_r

$$\% \text{ moisture } (M_r) = \frac{(M_f \times W_f) - (W_f - W_d) + (W_r - W_d)}{W_r} \times 100$$

$$= \frac{(M_f \times W_f) - W_f + W_r}{W_r} \times 100$$

where: M_f = percentage moisture in fresh loin

W_f = weight of fillet prior to freezing (g)

W_d = weight of fillet after dehydration (g)

W_r = weight of fillet after rehydration (g)

Percentage fat in rehydrated samples: F_r

$$\% \text{ fat } (F_r) = \frac{(W_f \times F_f)}{W_r} \times 100$$

where: F_f = percentage fat in original loin

W_f = weight of fillet prior to freezing (g)

W_r = weight of fillet after rehydration (g)

Weight of solids in rehydrated or fresh sample: W_s

$$\text{Weight of solids } (W_s) = S - S(M_r) - S(F_r)$$

$$\text{(rehydrated)} = S\,(1 - M_r - F_r)$$

where: S = sample weight (g)

M_r = percentage moisture in rehydrated sample

F_r = percentage fat in rehydrated sample.

Weight of solids (fresh) is obtained by substituting M_f and F_f in the above formula where

M_f = percentage moisture in fresh sample

F_f = percentage fat in fresh sample

Appendix C. Formulas used in calculations. (continued)

Percentage rehydration of freeze-dried meat.

$$\% \text{ rehydration} = \frac{W_r - W_d}{W_f - W_d} \times 100$$

where: W_r = weight of fillet after rehydration (g)

W_d = weight of fillet after dehydration (g)

W_f = weight of fillet prior to freezing (g)

Total fibrillar protein nitrogen content of solution A.

Total fibrillar protein nitrogen = A^n =

$$X_f \frac{\text{mg } N_2}{\text{ml}} \times \frac{V \text{ ml}}{S \text{ g.}} \times \frac{S \text{ g.}}{W_s \text{ g}} = \frac{X_f V}{W_s} \left(\frac{\text{mg } N_2}{\text{g solids}} \right)$$

where: X_f = fibrillar protein nitrogen content of solution A (mg N_2/ml.)

V = volume of 0.1 N NaOH (ml.)

S = sample weight (g)

W_s = weight of solids in rehydrated (or fresh) sample (g)

Total water soluble protein nitrogen content of solution B.

Total water soluble protein nitrogen = B^n =

$$X_w \frac{\text{mg } N_2}{\text{ml}} \times \frac{200 \text{ ml extracting solution}}{S \text{ g}} \times \frac{S \text{ g}}{W_s \text{ g}} = \frac{X_w (200)}{W_s} \left(\frac{\text{mg } N_2}{\text{g solids}} \right)$$

where: X_w = water soluble protein nitrogen content of solution B (mg N_2/ml)

S = sample weight (g)

W_s = weight of solids in rehydrated (or fresh) sample (g)

Appendix C. Formulas used in calculations. (continued)

Non-protein nitrogen content of solution C.

Non-protein nitrogen = C^n =

$$X_c \frac{\text{mg } N_2}{\text{ml}} \times 1.33 \times \frac{200 \text{ ml extracting solution}}{S \text{ g}} \times \frac{S \text{ g}}{W_s \text{ g}}$$

$$= \frac{X_c (266.67)}{W_s} \left(\frac{\text{mg } N_2}{\text{g solids}} \right)$$

where: X_c = non-protein nitrogen content of solution C (mg N_2/ml)

S = sample weight (g)

W_s = weight of solids in rehydrated (or fresh) sample (g)

Total salt soluble protein nitrogen content of solution D.

Total salt soluble protein nitrogen = D^n =

$$X_s \frac{\text{mg } N_2}{\text{ml}} \times \frac{200 \text{ ml extracting solution}}{S \text{ g}} \times \frac{S \text{ g}}{W_s \text{ g}}$$

$$= \frac{X_s (200)}{W_s} \left(\frac{\text{mg } N_2}{\text{g solids}} \right)$$

where: X_s = salt soluble protein content of solution B (mg N_2/ml)

S = sample weight (g)

W_s = weight of solids in rehydrated (or fresh) sample (g)

Total protein content of the rehydrating solution (R^n)

Total protein content of the rehydrating solution = R^n =

$$X_r \frac{\text{mg } N_2}{\text{ml}} \times \frac{[150 - (W_r - W_d)] \text{ ml}}{W_r \text{ g}} \times \frac{S_r \text{ g}}{W_s \text{ g}} = \frac{X_r S_r (150 - W_r + W_d)}{W_r W_s}$$

$$\left(\frac{\text{mg } N_2}{\text{g solids}} \right)$$

Appendix C. Formulas used in calculations. (continued)

where: X_r = rehydrating solution protein content (mg N_2/ml)

S_r = sample weight of rehydrated meat (g)

W_r = weight of fillet after rehydration (g)

W_d = weight of fillet after dehydration (g)

W_s = weight of solids in rehydrated sample (g)

Non-protein nitrogen content of the rehydrating solution: R^{np}

Non-protein nitrogen content of the rehydrating solution: R^{np} =

$$X_{np} \frac{\text{mg } N_2}{\text{ml}} \times 1.33 \frac{[150 - (W_r - W_d)]}{W_r \quad g} \text{ ml} \times \frac{S_r \text{ g}}{W_s \text{ g}} =$$

$$\frac{1.33 \ (X_{np} \ S_r)(150 - W_r + W_d)}{W_r \ W_s} \left(\frac{\text{mg } N_2}{\text{g solids}}\right)$$

where: X_{np} = rehydrating solution non-protein content (mg N_2/ml)

S_r = sample weight of rehydrated meat (g)

W_r = weight of fillet after rehydration (g)

W_d = weight of fillet after dehydration (g)

W_s = weight of solids in rehydrated sample (g)

Actual protein content of rehydrating solution.

$$\text{Actual protein content} = (R^n - R^{np}) \left(\frac{\text{mg } N_2}{\text{g solids}}\right)$$

where R^n = total protein nitrogen content of the rehydrating solution (mg N_2/g solid)

R^{np} = non-protein nitrogen content of the rehydrating solution (mg N_2/g solids)

Appendix D. Correlations between all investigated factors of loin 1.

	pH buffer	pH meat	% Rehydration	Sarcoplasmic protein nitrogen	0.53 μ extractable protein nitrogen	Soluble fibrillar protein nitrogen	Rehydrating solution protein nitrogen	Total fibrillar protein	Total protein
pH meat	+.916*	....							
% rehydration	-.217	-.064	....						
Sarcoplasmic protein nitrogen	+.574**	+.719**	+.224	....					
0.53 μ extractable protein nitrogen	+.095	+.188	-.001	+.258	....				
Soluble fibrillar protein nitrogen	-.273	-.263	-.152	-.362	+.805**	....			
Rehydrating solution protein nitrogen	+.422	+.489*	+.062	+.673**	-.130	-.547**	....		
Total fibrillar protein	-.447*	-.397	+.170	-.084	+.074	+.132	+.107	....	
Total protein	-.254	-.149	+.266	+.295	+.068	-.107	+.360	+.868**	....
Rehydrating solution non-protein nitrogen	+.363	+.376	+.002	+.589**	+.209	-.165	+.448*	+.118	+.351

* P (< .05)
** P (< .01)

Appendix F. Correlations between all investigated factors of loin 3.

	pH buffer	pH meat	% Rehydration	Sarcoplasmic protein nitrogen	0.53 μ extractable protein nitrogen	Soluble fibrillar protein nitrogen	Rehydrating solution protein nitrogen	Total fibrillar protein	Total protein
pH meat	+.846**	....							
% rehydration	+.091	-.067	....						
Sarcoplasmic protein nitrogen	+.370	+.030	+.072	....					
0.53 μ extractable protein nitrogen	+.142	-.077	+.095	+.764**	....				
Soluble fibrillar protein nitrogen	-.321	-.183	+.055	-.208	+.468*	....			
Rehydrating solution protein nitrogen	+.401	+.279	+.413*	-.072	-.151	-.130	....		
Total fibrillar protein	-.463*	-.316	+.129	-.265	+.004	+.333	-.270	....	
Total protein	-.205	-.258	+.152	+.292	+.425*	+.208	-.227	+.816**	....
Rehydrating solution non-protein nitrogen	-.031	+.053	+.036	-.300	-.208	+.103	+.512*	+.008	-.075

* P (< .05)
** P (< .01)

Appendix H. Complete calculated data for the longissimus dorsi of freeze-dried loin 1.

Sample Number	1	2	5	21	11	18	3	7
pH buffer	3.05	3.05	3.73	3.73	4.35	4.35	6.1	6.1
pH meat	4.65	4.68	4.85	4.98	5.09	5.03	5.65	5.62
1. % rehydration	76.78	83.10	76.30	53.59	61.25	70.34	61.17	69.78
2. Sarcoplasmic protein nitrogen*	22.23	21.35	26.47	23.09	23.21	20.92	29.00	30.18
3. Salt soluble protein nitrogen (0.53 μ)*	28.60	42.34	34.03	40.98	30.23	29.29	27.02	36.57
4. Soluble fibrillar protein nitrogen*	6.37	20.99	7.56	17.89	7.14	8.37	--	6.39
5. Rehydrating solution protein nitrogen*	1.14	1.00	2.36	2.21	2.00	2.12	2.31	2.72
6. Total fibrillar protein nitrogen*	111.51	104.70	84.52	105.24	91.12	109.04	92.59	87.52
7. Total protein nitrogen*	156.69	143.49	133.77	140.88	147.50	149.95	142.57	138.90
8. Rehydrating solution non-protein nitrogen*	3.92	4.11	4.68	4.41	4.00	4.26	4.43	5.16
9. Non-protein nitrogen*	17.89	12.33	15.74	12.55	14.52	13.61	14.24	13.32

*(mg. N_2/g. solids)

Appendix H. Complete calculated data for the longissimus dorsi of freeze-dried loin 1. (continued)

Sample number	24	13	23	4	9	15	6	10
pH buffer	6.7	6.7	7.19	7.19	7.3	7.3	7.65	7.65
pH meat	5.82	5.80	5.9	6.0	5.48	5.50	5.72	5.60
1. % rehydration	66.08	80.77	66.40	81.41	62.95	61.36	70.09	71.43
2. Sarcoplasmic protein nitrogen*	27.00	34.07	31.63	33.10	28.17	27.25	26.98	23.04
3. Salt soluble protein nitrogen (0.53 μ)*	36.55	50.23	31.05	32.78	33.68	33.97	30.37	23.51
4. Soluble fibrillar protein nitrogen*	9.55	16.16	--	--	5.51	6.72	3.39	--
5. Rehydrating solution protein nitrogen*	2.86	2.48	2.35	3.22	2.45	2.04	2.81	2.33
6. Total fibrillar protein nitrogen*	91.87	103.62	89.56	91.63	91.97	88.08	94.86	85.36
7. Total protein nitrogen*	136.32	159.77	139.14	143.34	139.65	134.57	140.04	128.72
8. Rehydrating solution non-protein nitrogen*	3.77	4.98	4.63	3.84	5.08	5.21	4.36	4.44
9. Non-protein nitrogen*	11.82	14.62	10.97	11.55	11.98	11.99	11.03	13.55

*(mg. N_2/g. solids)

Appendix H. Complete calculated data for the longissimus dorsi of freeze-dried loin 1. (continued)

Sample number	20	26	8	12	22	17
pH buffer	7.72	7.72	9.18	9.18	Deionized	H_2O
pH meat	5.61	5.65	6.68	6.55	5.69	5.65
1. % rehydration	58.99	59.05	68.41	73.85	48.54	61.33
2. Sarcoplasmic protein nitrogen*	26.17	26.07	28.73	35.02	26.53	22.19
3. Salt soluble protein nitrogen (0.53 μ)*	40.93	36.74	44.39	34.40	40.99	32.90
4. Soluble fibrillar protein nitrogen *	14.76	10.67	15.66	--	14.46	10.71
5. Rehydrating solution protein nitrogen *	2.16	1.62	1.30	3.96	2.39	1.73
6. Total fibrillar protein nitrogen *	93.35	92.06	72.65	108.15	100.61	78.27
7. Total protein nitrogen*	138.48	136.12	118.04	165.33	148.37	115.75
8. Rehydrating solution non-protein nitrogen *	4.27	4.28	4.70	5.96	4.80	3.46
9. Non-protein nitrogen *	12.53	12.09	10.59	12.24	14.04	10.10

*(mg. N_2/g. solids)

Appendix I. Complete calculated data for the longissimus dorsi of freeze-dried loin 2.

Sample number	18	25	4	5	8	2	1	26
pH buffer	3.05	3.05	3.79	3.73	4.35	4.35	5.85	5.85
pH meat	4.55	4.20	4.99	4.95	5.40	5.30	5.75	5.75
1. % rehydration	66.47	86.06	84.24	81.08	82.47	83.52	92.41	91.54
2. Sarcoplasmic protein nitrogen*	20.43	23.61	22.97	19.61	28.85	22.82	31.60	26.99
3. Salt soluble protein nitrogen (0.53 μ)*	24.26	29.56	24.69	25.76	30.29	33.73	29.77	42.64
4. Soluble fibrillar protein nitrogen*	3.83	5.95	1.72	6.15	1.44	10.91	--	15.65
5. Rehydrating solution protein nitrogen*	1.64	1.65	2.80	2.81	2.75	3.72	2.57	5.39
6. Total fibrillar protein* nitrogen	79.23	88.80	66.77	71.44	86.05	90.94	80.00	74.16
7. Total protein nitrogen*	137.19	132.72	109.68	109.06	133.60	134.97	133.47	123.45
8. Rehydrating solution non-protein nitrogen*	5.23	6.52	4.80	4.49	4.72	5.22	4.81	6.70
9. Non-protein nitrogen*	10.66	12.14	12.34	10.71	12.23	12.27	14.49	10.21

*(mg. N_2/g. solids)

Appendix I. Complete calculated data for the longissimus dorsi of freeze-dried loin 2. (continued)

Sample number	15	14	7	23	10	19	12	13
pH buffer	6.10	6.10	6.7	6.7	7.19	7.19	7.30	7.30
pH meat	5.86	5.86	5.80	5.83	5.60	5.63	5.30	5.32
1. % rehydration	84.72	79.08	73.96	70.35	81.23	75.43	84.19	85.80
2. Sarcoplasmic protein nitrogen*	26.01	27.00	23.67	23.49	34.48	30.87	29.34	28.04
3. Salt soluble protein nitrogen (0.53μ)*	35.57	40.53	33.28	33.77	38.17	30.54	37.87	34.09
4. Soluble fibrillar protein nitrogen*	9.56	13.53	9.61	10.22	3.69	--	8.53	6.05
5. Rehydrating solution protein nitrogen*	3.63	2.45	2.15	2.33	2.31	2.56	2.79	4.83
6. Total fibrillar protein nitrogen*	90.92	84.91	74.37	68.74	86.23	78.85	74.48	73.70
7. Total protein nitrogen*	138.48	131.14	113.65	110.02	139.66	129.09	121.74	123.05
8. Rehydrating solution non-protein nitrogen*	5.96	5.52	5.10	3.97	5.20	4.44	5.15	5.34
9. Non-protein nitrogen*	11.96	11.26	8.36	11.49	11.44	12.37	10.08	11.14

*(mg. N_2/g. solids)

Appendix I. Complete calculated data for the longissimus dorsi of freeze-dried loin 2. (continued)

Sample number	3	11	6	9	21	24	16	17
pH buffer	7.65	7.65	7.72	7.72	9.18	9.18	Deionized H_2O	
pH meat	5.83	5.80	5.35	5.39	6.20	5.80	5.25	5.45
1. % rehydration	90.07	62.07	72.50	82.07	75.00	85.07	82.58	82.70
2. Sarcoplasmic protein nitrogen *	20.97	32.86	30.72	27.98	36.27	36.58	22.94	25.47
3. Salt soluble protein nitrogen (0.53 μ)*	30.34	40.79	39.31	28.38	37.54	31.05	34.81	36.89
4. Soluble fibrillar protein nitrogen *	9.37	7.93	8.59	0.40	1.27	--	11.87	11.42
5. Rehydrating solution protein nitrogen *	3.54	1.89	2.91	2.96	2.27	2.88	5.21	2.79
6. Total fibrillar protein nitrogen *	59.75	69.43	76.60	74.62	83.77	88.53	77.00	77.66
7. Total protein nitrogen *	97.34	120.92	125.91	120.31	139.91	148.17	120.97	121.09
8. Rehydrating solution non-protein nitrogen *	5.35	5.40	5.88	6.18	4.87	5.87	6.50	4.93
9. Non-protein nitrogen *	7.73	11.34	9.80	8.57	12.73	14.31	9.32	10.24

*(mg. N_2/g. solids)

Appendix J. Complete calculated data for the longissimus dorsi of freeze-dried loin 3.

Sample number	11	8	2	28	27	10	9	19
pH buffer	2.55	2.55	3.62	3.62	4.28	4.28	5.85	5.85
pH meat	4.79	4.90	4.90	4.99	4.62	4.60	5.15	5.10
1. % rehydration	83.25	58.46	66.47	70.98	80.12	74.47	71.88	75.00
2. Sarcoplasmic protein nitrogen*	21.07	24.21	20.86	23.94	20.69	27.78	30.37	37.64
3. Salt soluble protein nitrogen (0.53 μ)*	37.33	37.65	37.88	31.02	25.72	35.05	40.99	34.41
4. Soluble fibrillar protein nitrogen *	16.26	13.44	17.02	7.08	5.03	7.27	10.62	--
5. Rehydrating solution protein nitrogen*	1.83	1.36	2.32	2.55	3.42	1.82	2.01	2.73
6. Total fibrillar protein nitrogen*	95.94	98.21	88.25	89.57	87.84	84.82	65.71	41.91
7. Total protein nitrogen*	138.63	144.17	137.93	133.36	129.37	134.64	112.81	101.86
8. Rehydrating solution non-protein nitrogen *	6.42	5.13	5.78	5.42	7.18	4.34	4.23	5.51
9. Non-protein nitrogen *	13.67	15.26	10.72	11.88	10.24	15.88	10.49	14.07

*(mg. N_2/g. solids)

Appendix J. Complete calculated data for the longissimus dorsi of freeze-dried loin 3. (continued)

Sample number	7	15	21	25	13	24	3	14
pH buffer	6.68	6.68	6.95	6.95	7.15	7.15	7.58	7.58
pH meat	5.20	5.15	5.82	5.85	5.32	5.29	5.95	5.92
1. % rehydration	65.71	77.61	69.19	64.55	74.47	70.05	67.76	82.94
2. Sarcoplasmic protein nitrogen*	31.51	29.88	20.87	21.84	34.77	30.30	32.11	24.27
3. Salt soluble protein nitrogen (0.53 μ)*	40.99	41.79	22.58	31.96	36.81	35.02	33.04	32.71
4. Soluble fibrillar protein nitrogen *	9.48	11.91	1.71	10.12	2.04	4.72	0.93	8.44
5. Rehydrating solution protein nitrogen*	2.98	3.12	2.90	2.78	2.71	2.78	2.40	4.45
6. Total fibrillar protein nitrogen*	43.74	70.54	62.51	51.91	87.53	64.54	77.55	78.58
7. Total protein nitrogen*	96.21	119.72	103.03	101.20	153.78	127.39	132.82	130.36
8. Rehydrating solution non-protein nitrogen *	6.16	5.19	5.79	6.30	4.83	6.03	5.83	6.42
9. Non-protein nitrogen*	11.82	9.99	10.96	9.29	16.40	14.93	16.64	16.22

*(mg. N_2/g. solids)

Appendix J. Complete calculated data for the longissimus dorsi of freeze-dried loin 3. (continued)

Sample number	18	26	12	16	17	20	5	23
pH buffer	7.61	7.61	7.85	7.85	9.05	9.05	Deionized H_2O	
pH meat	5.60	5.71	5.35	5.48	6.19	6.35	5.75	5.75
1. % rehydration	77.47	65.56	80.00	68.84	74.74	70.04	78.95	76.51
2. Sarcoplasmic protein nitrogen*	30.75	28.39	52.05	33.11	29.60	29.54	18.98	18.47
3. Salt soluble protein nitrogen (0.53 μ)*	33.80	32.91	65.67	37.62	40.09	38.55	24.64	35.56
4. Soluble fibrillar protein nitrogen*	3.05	4.52	13.62	4.51	10.49	9.01	5.66	17.09
5. Rehydrating solution protein nitrogen*	2.70	2.63	2.62	2.49	2.65	2.10	2.30	3.51
6. Total fibrillar protein nitrogen*	63.48	68.87	75.39	88.89	69.07	74.79	75.24	81.35
7. Total protein nitrogen*	116.64	121.74	158.38	147.42	121.89	124.12	116.34	122.25
8. Rehydrating solution non-protein nitrogen*	4.34	6.53	5.46	5.91	5.70	4.98	5.10	6.31
9. Non-protein nitrogen*	15.37	15.32	22.86	17.02	14.87	12.71	14.72	12.61

*(mg. N_2/g. solids)