Journal of FOOD PROCESSING and PRESERVATION

Edited by
T. P. LABUZA

FOOD & NUTRITION PRESS, INC.
WESTPORT, CONNECTICUT 06880
USA

VOLUME 6, NUMBER 2 JUNE 1982

JOURNAL OF FOOD PROCESSING AND PRESERVATION

All articles for publication and inquiries regarding publication should be sent to Prof. T. P. Labuza, University of Minnesota, Department of Food Science and Nutrition, St. Paul, MN 55108 USA.

All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc., 265 Post Road West, Westport, Connecticut USA.

One volume of four issues will be published annually. The price for Volume 6 is $60.00 which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $72.00 per year via surface mail, and $80.00 per year via airmail.

Subscriptions for individuals for their own personal use are $40.00 for Volume 6 which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $52.00 per year via surface mail, and $60.00 per year via airmail. Subscriptions for individuals should be sent direct to the publisher and marked for personal use.

The *Journal of Food Processing and Preservation* is listed in *Current Contents/ Agriculture, Biology & Environmental Sciences (CC/AB).*

The *Journal of Food Processing and Preservation* (USPS 456-470) is published quarterly by Food & Nutrition Press, Inc.—Office of Publication is 1 Sherwood Square, Westport, Connecticut 06880 USA.

Second class postage paid at Westport, CT 06880.

POSTMASTER: Send address changes to Food & Nutrition Press, Inc., 1 Sherwood Square, Westport, CT 06880.

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FOOD & NUTRITION PRESS, INC.
WESTPORT, CONNECTICUT 06880 USA

ISSN 0145-8892

Printed in the United States of America

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PRODUCTION OF ENRICHED PROTEIN FRACTIONS OF β-LACTOGLOBULIN AND α-LACTALBUMIN FROM CHEESE WHEY

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Received for Publication December 17, 1981

ABSTRACT

A method for the fractionation of enriched fractions of β-lactoglobulin and α-lactalbumin from whey has been developed. To accomplish this separation, the whey proteins are concentrated by ultrafiltration, the concentrate is adjusted to pH 4.65 and demineralized by electrodialysis. The demineralized concentrate is readjusted to pH 4.65, if necessary, and a precipitate consisting primarily of β-lactoglobulin is formed. This precipitate is separated from the enriched α-lactalbumin fraction by centrifugation. Protein yields and purities were determined gravimetrically and by gel electrophoresis. Some functional properties of each fraction were evaluated.

INTRODUCTION

Whey proteins consist mainly of β-lactoglobulin, α-lactalbumin, bovine serum albumin, and immunoglobulins (Table 1). The main purpose of this study was to develop a method to produce enriched fractions of β-lactoglobulin and α-lactalbumin from cheese whey. In addition, functional property studies were undertaken for each of the fractions.

Journal of Food Processing and Preservation **6**(1982)55-71.

Table 1. Components of cheese whey proteins

Cheese Whey Proteins	Concentration in Milk (gm/100 ml)
Total serum proteins	0.40-0.80[a]
β-lactoglobulin	0.20-0.40[b]
α-lactalbumin	0.07-0.15[b]
Bovine serum albumin	0.02-0.05[b]
Immunoglobulins	0.05-0.11[b]
Other components	0.06-0.17[b]

[a]From Rowland nitrogen distribution (Rowland 1938)
[b]From electrophoretic method (Rolleri *et al.* 1955)

EXPERIMENTAL

Demineralization

A pilot-scale electrodialysis unit with a 9 in. × 10 in. membrane stack (Ionics Inc., Watertown, MA) was used for demineralization. The membrane stack consisted of 18 cell pairs of alternating 111 EZL-216 anion and 61 CZL-183 cation selective membranes along with alternating diluent and concentrate compartments with polyethylene spacers between the membranes. The anode electrode was a platinum coated tantalum (Tirrelay B) while the cathode electrode was a silver finished platinum (Hastelloy C). The top and the bottom end blocks were drilled and tapped for external hydraulic connections. The direct current electricity needed in electrodialysis was produced by the control panel/rectifier combination unit which converted 220 volt, 3 phase, 60 cycle AC input power to 150 volt DC at 70 amp output power (Johnson *et al.* 1976). During demineralization, the pretreated whey was used as both diluent and concentrate solutions and 0.1 N NaCl solution acidified to pH 2.5 with concentrated HCl was used as electrode solution. The diluent and concentrate solutions were circulated through the system at a constant flow rate of 50 ml/s until temperature became constant at 20 ± 1°C. The flow rate in the electrode stream was adjusted until the pressure of the electrode stream was the same as the diluent and concentrate streams. The conductivity of the diluent stream was measured before the rectifier was turned to a constant voltage of 50 volts across the membrane stack. The current dropped from 4.6-3.0 amp at the beginning to 0.3-0.2 amp at the end of the demineralization process. The mode of operation was batch recirculation.

Preconcentration

Preconcentration was accomplished by ultrafiltration on an Abcor tubular unit using membranes with a molecular weight cut-off of 20,000 daltons and a surface area of 0.102 m^2 (Matthews *et al.* 1976). During ultrafiltration, the whey temperature was 25°C and the inlet and outlet pressures were adjusted to 20 and 10 psi, respectively. The mode of operation was batch recirculation.

Fractionation of Enriched Fractions of β-lactoglobulin and α-lactalbumin

Figure 1 is a flow diagram of the β-lactoglobulin and α-lactalbumin fractionation process. The whey proteins were concentrated by ultrafiltration partially removing water, salt, lactose, and other compounds of low molecular weight. The resulting concentrate was adjusted to pH 4.65 with concentrated HCl or NaOH. The concentrate was electrodialyzed to remove low molecular weight ions such as Na^+, K^+, Ca^{+2}, Mg^{+2}, and Cl^-. The demineralized whey was readjusted to pH 4.65 with 0.1 N HCl or NaOH, and a precipitate of β-lactoglobulin resulted. The precipitate was separated at 9,000 X g for 30 min at 20°C with a laboratory RC-5 superspeed refrigerated centrifuge. The supernatant contained the enriched α-lactalbumin fraction.

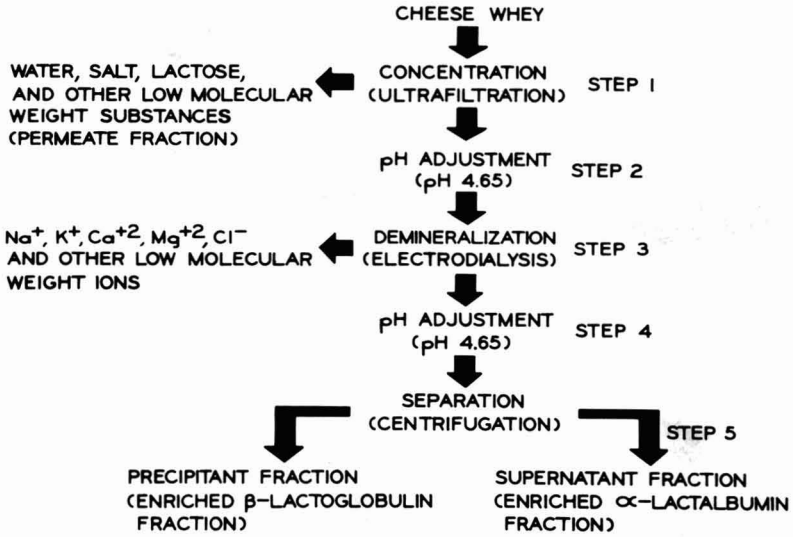

FIG. 1. FLOW DIAGRAM FOR FRACTIONATION OF β-LACTOGLOBULIN AND α-LACTALBUMIN

Chemical Analysis

Total solids and fats were determined by the Mojonnier method (Mojonnier and Troy 1965); total noncombustible ash by the AOAC gravimetric method (AOAC 1970); and total nitrogen by the micro-Kjeldahl method (Bradstreet 1965). 6.38 was used to convert nitrogen to protein.

FUNCTIONAL PROPERTY STUDIES

Solubility

The effect of pH and salt on β-lactoglobulin solubility was determined. Two gram samples of dried enriched β-lactoglobulin were dissolved in distilled water, in 0.1 M and in 0.3 M NaCl solution to make 100 ml solutions. The pH of the solutions were then adjusted with 0.1 N HCl or NaOH. Each of the solutions was mixed for 30 min and then centrifuged at 1000 X g for 10 min (Sternbury *et al.* 1975). The amount of nitrogen in both fractions was determined. The protein solubility is expressed as the ratio of the nitrogen in the supernatant portion to the nitrogen in the original solution.

Water Holding Capacity

The amount of water retained in the precipitated pellet of the enriched β-lactoglobulin fraction after centrifugation has been defined as the water holding capacity (Lin and Humbert 1974). The demineralized whey, in which the final conductivity was 100-200 υmho/cm, was adjusted to pH 4.65 and then centrifuged at 7000 X g for 15 min. After the enriched α-lactalbumin fraction was removed, the amount of water retained in the enriched β-lactoglobulin fraction was determined. The water holding capacity was reported as the ratio of the number of grams of water in the enriched β-lactoglobulin fraction to the number of grams dry weight of the enriched β-lactoglobulin fraction.

Whippability

Egg white, demineralized whey, enriched α-lactalbumin, and enriched β-lactoglobulin were made into four separate solutions by adding sucrose and water to give 5.6% protein and 55% total solids. These solutions were adjusted to pH 8.5 with 5 N $Ca(OH)_2$ and then whipped at full speed to prepare meringue (Harris and Withers 1966). The percentage overrun was calculated using the formula:

$$\text{PERCENTAGE OVERRUN} = \frac{(V_1 - V_2) \times 100}{V_2}$$

Where V_1 = initial weight of solution
V_2 = weight of same volume of foam

The whipped products were transferred to funnels to measure stability, defined as the time required for the first drop of liquid to drain (Haggett 1976).

The original solution of demineralized whey, the enriched α-lactalbumin, and the enriched β-lactoglobulin were each mixed with the original egg white solution to study the meringue quality. The mixtures were adjusted and whipped, spread in small aluminum foil cans with diameters of 3 cm and heights of 2 cm, then baked in an oven at 178°C for 6 min. The textures of the top and longitudinal cross sectional surfaces and the shrinkage of the meringues were evaluated.

Polyacrylamide Gel Electrophoresis

The patterns of whey proteins were determined by following the Melachouris procedure (Melachouris 1969). The EC-470 slab gel electrophoresis (E-C Apparatus Corporation, St. Petersburg, FL) was used.

RESULTS AND DISCUSSION

Fractionation Of β-Lactoglobulin And α-Lactalbumin From Whey

A white precipitate of β-lactoglobulin occurred in low ash content whey at pH 4.65. The supernatant or the enriched α-lactalbumin fraction became a clear yellow solution after the precipitate had been centrifuged. There were several factors which affected the fractionation process: ash content, concentration, and pH.

Effect Of pH On Fractionation Efficiency

Raw Cheddar cheese whey, pH 5.8, without prior concentration or pH adjustment was demineralized to 0.060-0.051% ash content (wet weight basis). The demineralized whey was split into two lots, the pH adjusted to 4.65 and 5.20, respectively, and centrifuged. The pH values of 4.65 and 5.20 were selected for the following reasons: (1) In initial experiments we observed that a precipitate of β-lactoglobulin occurred

readily in demineralized whey at 4.65. Further, this pH value has been reported as the optimal pH for maximum formation of the octamer of β-lactoglobulin A (Townend and Timasheff 1960); (2) The isoionic point of β-lactoglobulin has been reported to vary from pH 5.1 to 5.3 depending upon salt concentration (McKenzie 1970). We found that the yield of protein recovered at pH 4.65 was 2.1 times greater than at pH 5.20.

Effect Of Ash Content On Fractionation Efficiency

Raw cottage cheese whey, pH 4.39, without prior concentration or pH adjustment was subjected to demineralization. During demineralization, samples were taken periodically, the ash content was determined and, if necessary, the pH was adjusted to 4.65 prior to centrifugation. The effect of ash content on protein recovery is shown in Fig. 2, where the slope of the curve is -0.102 at ash contents between 0.185 and 0.316% and -0.474 at ash contents between 0.023 and 0.117. At the lower ash content, the amount of protein recovered by centrifugation increased 4.5 times.

Effect Of Initial pH On Demineralization And Fractionation Efficiency

Cheddar cheese wheys with an initial pH of 5.97, 5.10, 4.65 and cottage cheese whey with an initial pH of 4.39, without prior concen-

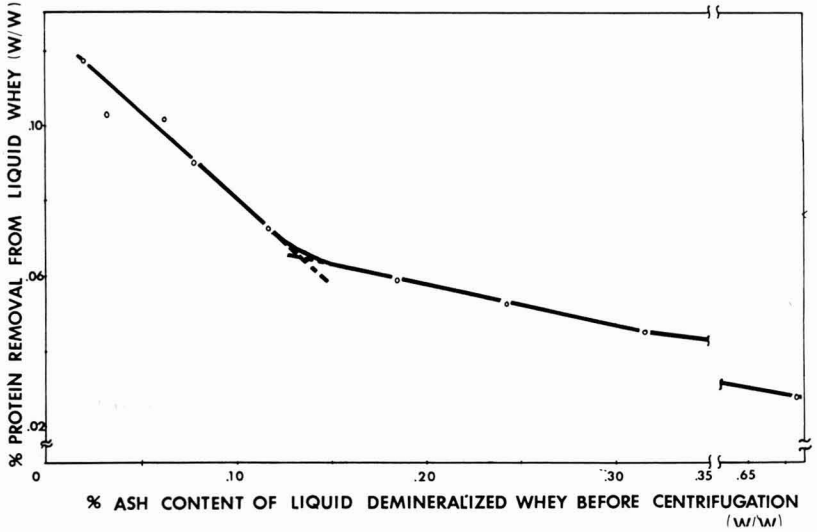

FIG. 2. EFFECT OF ASH CONTENT ON PROTEIN REMOVAL
(The demineralized whey was adjusted to pH 4.65 before centrifugation.)

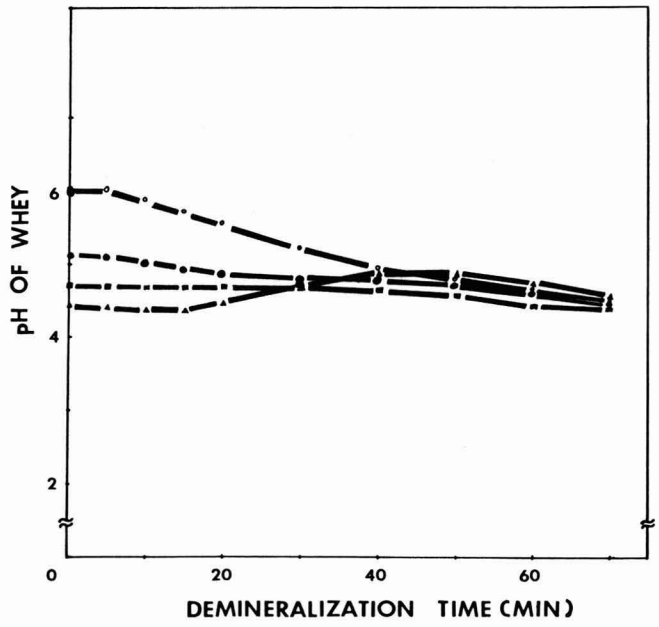

FIG. 3. EFFECT OF INITIAL WHEY pH BEFORE DEMINERALIZATION ON pH CHANGE DURING DEMINERALIZATION

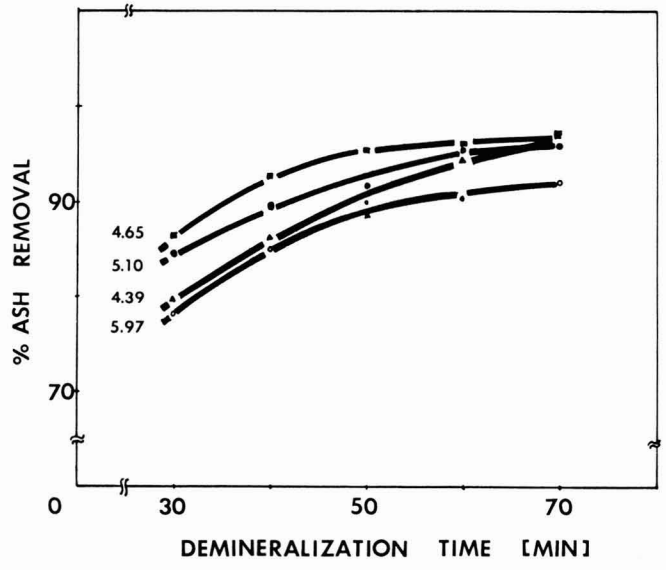

FIG. 4. EFFECT OF WHEY pH PRIOR TO DEMINERALIZATION ON ASH REMOVAL

(The demineralized whey was adjusted to pH 4.65 before centrifugation.)

tration, were subjected to demineralization (Fig. 3). As can be seen, those wheys with an initial pH above 4.65 decreased in pH during demineralization and the cottage cheese whey with an initial pH of 4.39 increased in pH during demineralization. The whey with an initial pH of 4.65 remained relatively constant through 40 min of demineralization.

The demineralization causes a shift of pH towards a constant value as well as a decrease in buffer capacity and lactic acid.

Figure 4 shows the effect of pH on the rate of ash removal during electrodialysis. Five ash determinations were made on each of the four wheys being demineralized, with the first samples being taken after 30 min and the last taken at 70 min. During the 70 min demineralization, the whey with the initial pH of 4.65 showed the greatest ash reduction and the whey with the initial pH of 5.97 showed the least.

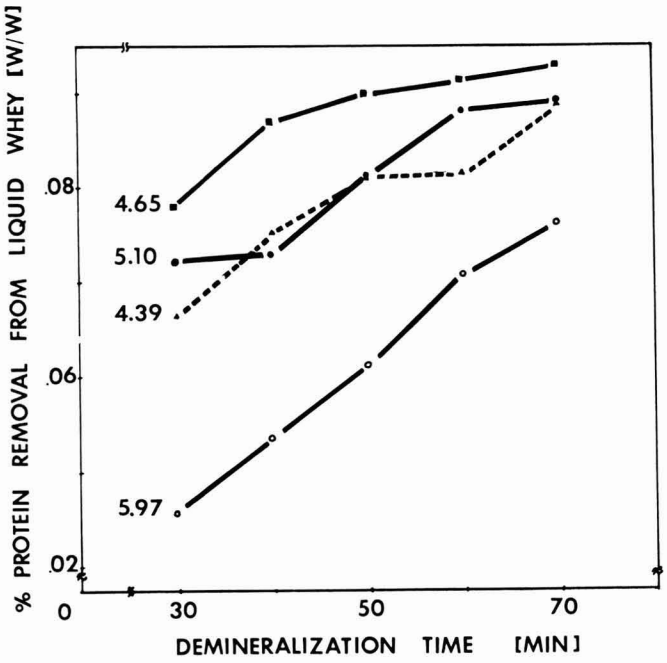

FIG. 5. EFFECT OF INITIAL WHEY pH PRIOR TO DEMINERALIZATION ON PROTEIN REMOVAL

(The protein contents of whey with initial pH of 4.39, 4.65, 5.10, and 5.97 were 0.923, 0.935, 0.935, and 0.940 gm/100 gm liquid whey. The demineralized whey was adjusted to pH 4.65 before centrifugation.)

Each of the twenty samples were then centrifuged to determine the effect of initial pH on protein removal (Fig. 5). As anticipated, the percentage of protein removal increased with increased ash removal (Fig. 6).

These experiments show that the initial pH of the whey affects both the amount of β-lactoglobulin precipitated and the change in pH

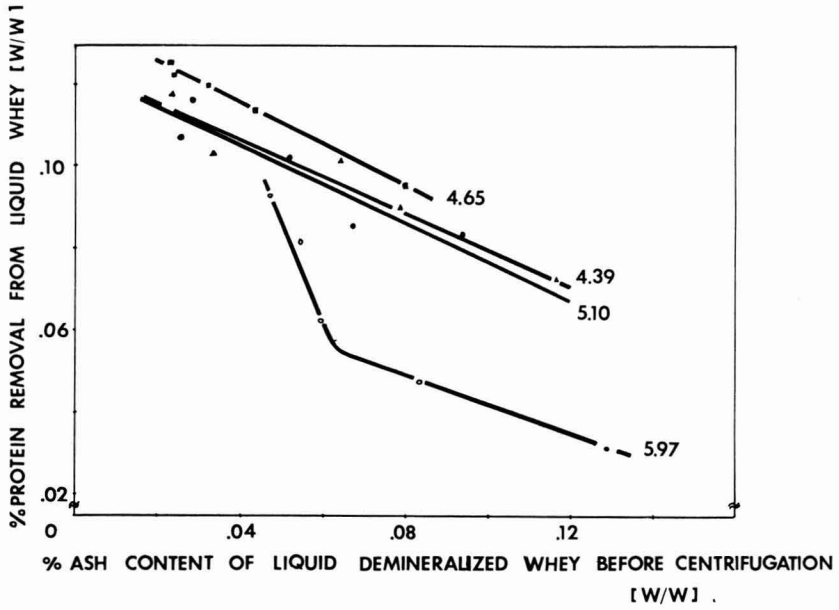

FIG. 6. EFFECT OF INITIAL WHEY pH PRIOR TO DEMINERALIZATION ON PROTEIN REMOVAL AT VARIOUS ASH LEVELS

(The protein contents of whey with initial pH of 4.39, 4.65, 5.10, and 5.97 were 0.923, 0.935, 0.935, and 0.940 gm/100 gm liquid whey. The demineralized whey was adjusted to pH 4.65 before centrifugation.)

during demineralization. Whey with an initial pH of 4.65 showed both highest percentage of ash removal and protein recovery.

Effect Of Preconcentration Of Whey

Cheddar cheese whey, after 0, 60, and 80% volume reduction by ultrafiltration, was adjusted to pH 4.65, demineralized, readjusted to pH 4.65 and centrifuged (Fig. 1). The effect of concentration on protein recovery is shown in Table 2. As can be seen, there is only a slight increase in the percentage of protein removal when the whey is

Table 2. The effect of preconcentration and demineralization on protein fractionation

	% Volume Reduction of Whey By Ultrafiltration		
	0	60	80
% ash content of whey[a] (WWB)[1]	0.568	0.557	0.638
% ash content of whey[b] (WWB)[1]	0.035	0.035	0.032
% protein content of whey[b] (WWB)[1]	0.920	1.908	2.880
% protein removal from whey[b] (WWB)[2]	0.133	0.371	1.650
% protein removal from whey protein[b] (WWB)[3]	14.78	19.47	57.34
% protein remainder in whey[b] (WWB)[4]	85.22	80.53	42.66
% total solids of whey[b] (WWB)[1]	5.84	6.84	8.98

[a] Cheddar cheese whey before demineralization and/or after ultrafiltration, pH 4.65
[b] Demineralized Cheddar cheese whey before centrifugation, pH 4.65
[1] Wet weight basis, gm per 100 gm liquid whey
[2] Wet weight basis, gm protein in the enriched β-lactoglobulin fraction from 100 gm liquid whey
[3] (gm protein in the enriched β-lactoglobulin fraction from 100 gm liquid whey × 100) ÷ gm protein in 100 gm liquid whey
[4] (gm protein in the enriched α-lactalbumin fraction from 100 gm liquid whey × 100) ÷ gm protein in 100 gm liquid whey

reduced in volume from 0 to 60%. However, a substantial increase in protein removal occurs when the whey volume is further reduced to 80% with 57.34% of the total protein being removed from the whey. This represents a β-lactoglobulin recovery of approximately 90%.

Polyacrylamide Gel Electrophoresis

The distribution of whey proteins in each of the enriched protein fractions was determined by discontinuous polyacrylamide gel electrophoresis (Fig. 7). The enriched β-lactoglobulin fraction consisted primarily of β-lactoglobulin A and B when compared with the gel pattern for β-lactoglobulin A and B from Sigma Chem Corp. The enriched β-lactoglobulin fraction also showed several light bands of other whey proteins. The gel pattern for the enriched α-lactalbumin fraction was similar to the gel pattern for the concentrated whey except for the decrease in the intensity of the β-lactoglobulin band. Each protein band in the gel patterns for both enriched fractions and for the control samples of β-lactoglobulin and the concentrated whey shows the same mobility. This indicates that the proteins in both fractions were unaltered.

The results of the gravimetric method and the gel electrophoretic pattern indicate that most of the β-lactoglobulin can be successfully separated from α-lactalbumin.

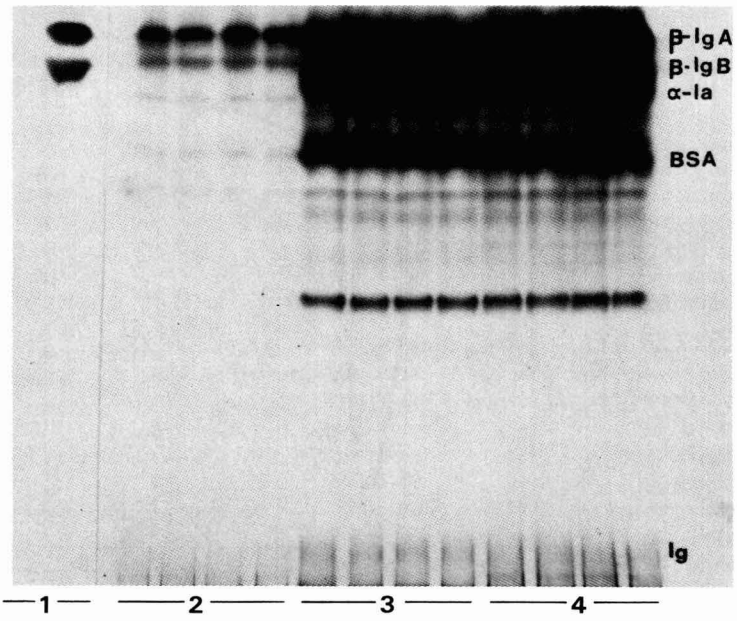

FIG. 7. POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERNS

1) β-lactoglobulin A and B from Sigma Chem Corp. 2) Enriched β-lactoglobulin from whey with 80% volume reduction by ultrafiltration. 3) Enriched α-lactalbumin from whey with 80% volume reduction by ultrafiltration. 4) Cheddar cheese whey with 80% volume reduction by ultrafiltration.

FUNCTIONAL PROPERTIES

Solubility

A 1% solution of the β-lactoglobulin fraction in water was prepared and percent solubility as a function of pH determined (Fig. 8). Minimum solubility was found at pH 4.42 rather than 4.65, which is probably due to the NaOH added to adjust pH. The solubility is greater than 85% at pH 3 and below and at pH 5 and above. In addition, the effect of pH was determined when the 1% β-lactoglobulin was prepared using 0.1 and 0.3 M NaCl (Table 3). The protein solubility at pH 4.42 increased from 7.5% to 74.5% in the 0.1 M NaCl solution and to 84.1% in the 0.3 M NaCl solution. At pH 7, the addition of salt did not improve the solubility of the protein.

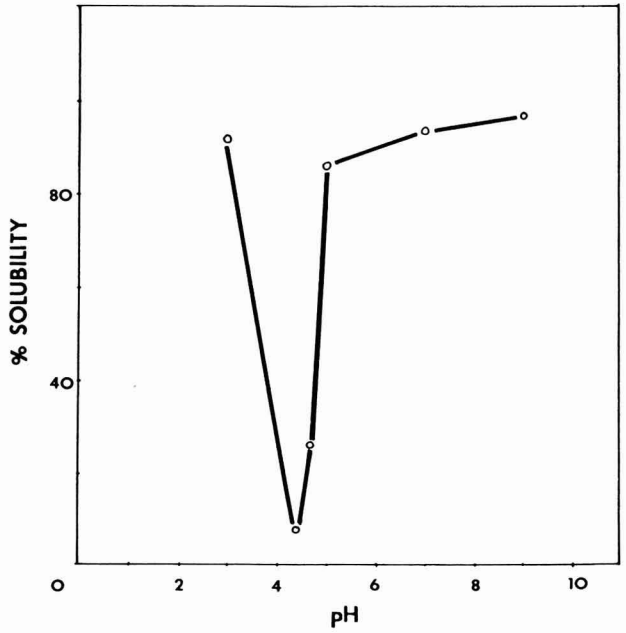

FIG. 8. EFFECT OF pH ON β-LACTOGLOBULIN SOLUBILITY

Table 3. The effect of salt and pH on the solubility of β-lactoglobulin

Conditions	% Solubility[c]
1. Dissolve in water	
pH 4.42[a]	7.50
pH 7.00[b]	93.10
2. Dissolve in 0.1 M NaCl solution	
pH 4.42[a]	74.50
pH 7.00[b]	91.00
3. Dissolve in 0.3 M NaCl solution	
pH 4.42[a]	84.10
pH 7.00[b]	92.10

[a] Without any pH adjustment
[b] With a pH adjustment
[c] The values are expressed as percent of the nitrogen in soluble fraction to the total nitrogen in the solution before centrifugation

Water Holding Capacity

One gram of the precipitated pellets of the enriched β-lactoglobulin fraction from Cheddar cheese whey held between 2.19 and 2.88 grams of water. The water holding capacity of this fraction indicates that it might find application in such things as processed meat and cheese products.

Whippability

Five solutions were prepared as indicated below, adjusted to pH 8.5 with 5 N Ca $(OH)_2$ and each contained 5.6% protein and 55% total solids.

Solution A dried enriched α-lactalbumin, sucrose powder, and water
Solution B demineralized whey powder, sucrose powder, and water
Solution C dried enriched β-lactoglobulin, sucrose powder, and water
Solution D raw egg white and sucrose powder
Solution E a 50:50 mixture of solutions D and A

The foam stability and percent overrun for solutions A, B, C, and D are shown in Table 4. The values were determined after whipping until stiff, glossy peaks appeared. However, the demineralized whey

Table 4. Whippability of egg white, demineralized whey, enriched α-lactalbumin, and enriched β-lactoglobulin

Samples	% Overrun	Stability (min)
Enriched α-lactalbumin (A)	450	120[b]
Demineralized whey powder (B)	100	<1[b]
Enriched β-lactoglobulin (C)	0	<1[b]
Egg white (D)	300	60[a]

[a]The first drop from the whipped egg white sample was light yellow and transparent
[b]The first drop from the samples were milky

(B) and the enriched β-lactoglobulin (C) did not show glossy peaks even after doubling the whipping time used for egg white (D).

The solution containing α-lactalbumin showed the highest overrun and greatest foam stability. The whipped samples of α-lactalbumin and egg white were transferred to funnels and photographed (Fig. 9). After standing 24 h, the α-lactalbumin foam had a dried texture inside and out while the whipped egg white did not. This would indicate that the egg white has superior moisture holding ability.

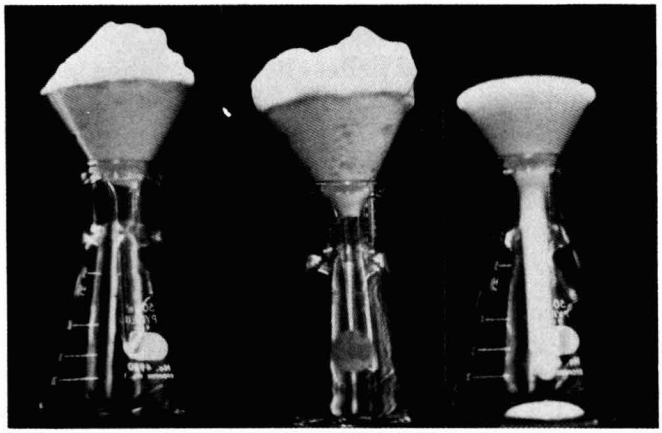

FIG. 9. FRESHLY WHIPPED (1) EGG WHITE, (2) ENRICHED α-LACTALBUMIN, AND (3) DEMINERALIZED CHEDDAR CHEESE WHEY

Meringues were prepared from Solutions D (egg white) and E (mixture of α-lactalbumin and egg white) (Fig. 10). The α-lactalbumin egg white mixture had low shrinkage and a texture similar to the egg meringue except for slight tackiness which could be due to the presence of lactose (Jelen 1973).

Lipid Content

The enriched β-lactoglobulin had a higher lipid content than either the enriched α-lactalbumin or demineralized whey (Table 5). This is probably due to the reaction of β-lactoglobulin with lipid molecules. The enriched β-lactoglobulin fraction has a protein lipid ratio of approximately 1:0.6. This high lipid holding ability of the β-lactoglobulin indicates that it might have potential use as an emulsifier.

It was also observed in one sample that the enriched β-lactoglobulin formed on the surface of the whey following centrifugation as well as on the bottom of the tube. One possible explanation of this is that in instances where the density of the "lipid-β-Lactoglobulin complex" is less than that of water, it floats to the surface, but when the reverse is true, it settles to the bottom. In addition, long chain lipid molecules are more likely to cause the "complexes" to float to the surface than short chain ones. The presence of β-lactoglobulin especially in the floating precipitates makes the complete removal of β-lactoglobulin from whey more difficult.

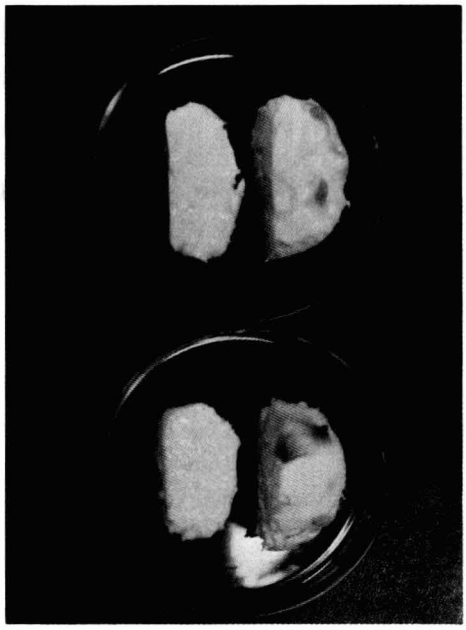

FIG. 10. MERINGUES FROM (1) EGG WHITE, AND (2) A 50:50 MIXTURE OF EGG WHITE AND ENRICHED α-LACTALBUMIN

Table 5. Protein and lipid content of demineralized Cheddar cheese whey, enriched α-lactalbumin, and enriched β-lactoglobulin

Samples	% Protein[a]	% Lipid[b]	Lipid:Protein
Demineralized Cheddar cheese whey	14.09	2.07	0.15
Enriched α-lactalbumin	12.10	0.64	0.05
Enriched β-lactoglobulin	51.66	30.99	0.60

[a]% protein content of dried sample
[b]% lipid content of dried sample

CONCLUSION

From this study it can be concluded that this process is a potentially useful method for separating β-lactoglobulin from α-lactalbumin without any damage to the proteins. The two undernatured fractions have different functional properties; therefore, it may be a beneficial way of increasing whey protein utilization by taking advantage of their unique functional properties.

Further experimentation is still needed with this process and the two fractions in order to determine its feasibility for the food industry.

ACKNOWLEDGMENTS

This work was supported in part by the Food Engineering Pilot Plant and the College of Agricultural and Life Sciences, University of Wisconsin-Madison. The authors also wish to thank Ionics, Inc., Watertown, MA. for making available an electrodialysis unit and Abcor, Inc., Wilmington, MA. for making available an ultrafiltration unit and membranes.

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SOME FACTORS INFLUENCING THE PRODUCTION OF DIMETHYLAMINE AND FORMALDEHYDE IN MINCED AND INTACT RED HAKE MUSCLE

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Received for publication December 23, 1981

ABSTRACT

Mincing stimulated the breakdown of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (HCHO) in red hake muscle at both above and below freezing temperatures. Aging of red hake muscle on ice as fillets (skin on or off) prior to frozen storage resulted in a greater extent of DMA production as compared to fillets derived from fish aged in the whole or gutted form.

Frozen storage temperature influenced the extent of DMA production, yet the freezing nadir had no effect on the rate of its formation during storage at -5°C. Additive studies indicated that reducing conditions in the tissue exert considerable influence on the reaction rate in frozen minced tissue. Trimethylamine (TMA), DMA, dimethylaniline and citrate were found to inhibit the reaction in frozen minced tissue.

INTRODUCTION

Recently, much effort has been directed toward utilization of non-conventional marine resources. The red hake (*Urophycis chuss*) fish stock in the North Atlantic provides good potential for exploitation in the fish block trade (Regenstein *et al*, 1980). A factor hindering present utilization of this species is the extensive toughening of the tissue during frozen storage (Gendron 1980; Dyer and Hiltz 1974; Gill *et al.* 1979). This problem, common to frozen muscle of many gadoid fishes, is thought to manifest itself through the breakdown of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde; the latter product serving to cross-link proteins and causing

Journal of Food Processing and Preservation 6(1982)73-97.

the toughening (Amano and Yamada 1964; Yamada *et al.* 1969; Castell *et al.* 1971; Tokunaga 1974; Dingle and Hines 1975; Castell *et al.* 1973a,b; Dingle *et al.* 1977; Crawford *et al.* 1979). Of the gadoid fishes, the muscle from red hake is particularly susceptible to this reaction (Castell *et al.* 1971).

The greater the disruption of the tissue, the greater the rate of DMA production during frozen storage (Tokunaga 1964). Other laboratories have also observed enhanced rates of DMA production upon mincing and freezing of gadoid muscle tissue (Hiltz *et al.* 1976; Babbit *et al* 1972; Crawford *et al.* 1979). This reaction is reported to be enzymic in nature (Tomioka *et al.* 1975; Tokunaga 1964; Lall *et al.* 1975; Yamada and Amano 1965a,b), although there is evidence for nonenzymic steps under some conditions (Spinelli and Koury 1979, 1981). In our laboratory we have observed enzyme-catalyzed production of DMA in both soluble (Phillippy 1981) and particulate (Parkin and Hultin 1981) fractions isolated from red hake muscle. Studies that have explored the temperature dependency of the reaction during frozen storage report the maximal rate of DMA production at -5 to -10°C (Licciardello *et al.* 1981; Tokunaga 1974). It has also been shown that added compounds can affect the rate of DMA production in minced gadoid tissue (Tomioka *et al.* 1975).

Our interest in the utilization of red hake began with a study of frozen red hake blocks (Kelleher *et al.* 1981). Here we observed that textural toughening was minimal in blocks derived from 'aged' red hake (held on ice 5 days prior to filleting and block forming) and maximal for blocks derived from fillets dipped in polyphosphate/erythorbate, as determined by sensory panelists. Chemically, we found DMA (and other parameters) to be a reliable index of textural deterioration of red hake tissue as evaluated by a sensory panel, agreeing with conclusions of an earlier study (Gill *et al.* 1979).

In this paper we report our studies on the effects of tissue disruption (mincing), freezing rates/nadir, temperature and the effect of several additives on the rate and extent of DMA formation in red hake fillets and minced tissue.

MATERIALS

Red hake were obtained from Gloucester fishermen on the same day they were caught. The fish were transported to the laboratory on ice and were either processed immediately (fresh) or held on ice under refrigeration and processed the following day (one-day-old fish). Minc-

ing was accomplished with a Rival electric meat grinder. Unless otherwise stated, when filleting is discussed, it includes removal of the skin.

METHODS

Chemical Analyses

Preparation of Trichloroacetic Acid (TCA) Extract. Fifty grams of each thawed fish sample was homogenized for 1 min in a Waring Blender with 50 ml of deionized, distilled water and 100 ml of 10% TCA. Upon filtering of the extract through Whatman #1 paper, aliquots from each sample were taken for the following analyses:

Dimethylamine (DMA). DMA was analyzed according to the method of Dyer and Mounsey (1945) with the exception that the aqueous layer was partitioned with 12 ml of 5% carbon disulfide in benzene. Shaking was accomplished with a Cole-Palmer Roto-Torque Model 7637 using the maximum speed available.

Trimethylamine (TMA). TMA in the TCA extracts was analyzed by the procedure of Dyer (1945), replacing the 50% K_2CO_3 with 45% KOH as suggested by Shewan *et al.* (1971).

Trimethylamine-oxide (TMAO). TMAO in the TCA extract was determined by the procedure of Yamagata *et al.* (1969) using a 60°C water bath for 10 min to achieve reduction of TMAO to TMA rather than 80°C. This procedure employs $TiCl_3$ to reduce TMAO to TMA. To ensure complete reduction of TMAO a final concentration of 0.67% $TiCl_3$ was used rather than the 0.33% level outlined in the original procedure. The TMAO-reduced extract was then subjected to the TMA analysis outlined above. TMAO content was then computed as the difference between the TMA content of the reduced and unreduced (those assayed directly for TMA) extracts.

Free Formaldehyde (HCHO). Free HCHO in the TCA extract was determined by the procedure of Nash (1953).

Bound Formaldehyde. Bound HCHO was calculated as the difference between the values for DMA and free HCHO for each sample extract. This calculation is based on the assumption that DMA and HCHO are produced in a stoichiometry of 1:1 from TMAO.

Extractable Protein (EP). A protein extract for each sample was prepared according to the method of Dyer *et al.* (1950), and protein content in the extract was determined by the biuret procedure of Gornall *et al.* (1949). Extractable protein is defined here as that

extracted in 5% NaCl/0.02 M $NaHCO_3$, pH 7.0 at 0°C for 5 min. The blender used for the extraction of protein was fitted with a baffle to prevent foaming of the protein. The percentage of EP was calculated throughout the storage periods on the basis of the extractable protein content of the fresh or 'zero-time' fish tissue being set equal to 100%.

Statistical Analysis. For all of the storage studies, statistical significance was determined by analysis of variance and Duncan's Multiple Range Test. Statistical analyses was performed only on the DMA data (except where otherwise noted) in each study since it is believed that DMA is one of the more reliable indices of textural deterioration in red hake muscle (Gill *et al.* 1979).

EXPERIMENTAL DESIGN

Storage Studies

Minced Fish Study. Fresh fish was immediately treated in the following manners: (a) filleted, minced and immediately frozen (minced/unaged); (b) filleted, minced, held on ice 6 days prior to freezing (minced/aged); (c) whole fish held on ice 6 days prior to filleting, mincing and freezing (aged-whole/minced); and (d) gutted fish held on ice for 23 days after a 30 sec dip in 10 ppm streptomycin (gutted/dipped). All processed samples (except gutted/dipped) were packed in Whirl-Pak bags (approximately 100 g each), frozen and stored at -6°C.

Aging Study. One-day-old fish were treated in the following manners: (a) filleted, minced and immediately frozen (minced/unaged); (b) filleted, minced, held on ice 4 days prior to freezing (minced/aged); (c) filleted and immediately frozen (filleted/unaged); and (d) whole fish held on ice 4 days prior to filleting and freezing (aged/filleted). All processed samples (approximately 100 g) were packed in Whirl-Pak bags, frozen and stored at -6°C.

Processing Study. One-day-old fish were treated in the following manners: (a) filleted, held on ice 4 days prior to freezing (filleted); (b) filleted, leaving skin on, held on ice 4 days prior to skinning and freezing (filleted w/skin); (c) gutted fish held on ice 4 days prior to filleting and freezing (gutted); and (d) whole fish held on ice 4 days prior to filleting and freezing (whole). The filleted treatment groups (skin on or off) were packed (approximately 100 g) in Whirl-Pak bags during the aging and frozen storage periods. The whole and gutted

fish were held on ice and after aging and filleting, the fillets (approx. 100 g) from each group were packed in Whirl-Pak bags prior to freezing. Frozen storage was at -20°C.

For all of the storage studies, duplicate samples were thawed in water at room temperature and representative portions were obtained for each duplicate sample. For intact fillets, representative sampling involved excising muscle from several locations of the fillet to comprise the total sample. Analyses of TMAO, DMA, HCHO and EP were performed at each interval of storage on duplicate samples of fish.

Additive Studies

One-day-old fish were filleted, minced, mixed until homogeneous and separated into 200 g portions. To each 200 g portion, 4 ml of an additive solution was introduced and the mixture was made homogeneous by manually mixing the contents (100 strokes). Good homogenity of the additive-minced fish mixture was assured by observing that dye solutions were well distributed throughout the minced fish as a result of this procedure. Fifty g samples of the additive-fish mixtures were packed in Whirl-Pak bags and were sealed prior to freezing and storage at -12°C. At each point of analysis, duplicate samples were removed from storage, thawed in room temperature water and extracted with TCA. The TCA extract was then utilized for the determination of DMA contents of the fish-additive mixtures. DMA contents are reported on a percentage basis relative to a control sample (4 ml of deionized, distilled water per 200 g) and calculated on the basis of the amount of fish (49 g) comprising the 50 g mixture.

Effect of Freezing Rate on DMA Production During Frozen Storage

One-day-old fish were filleted, minced, and mixed until homogeneous; 50 g samples were packed in Whirl-Pak bags. Equal portions of the samples were frozen under one of the following conditions:

(a) frozen in air at -5°C;
(b) frozen in air at -20°C;
(c) frozen by immersion in acetone equilibrated to -80°C; and
(d) frozen in air at -80°C and held for 4 days.

Subsequent to the differential freezing processes, all samples were transferred to a -5°C freezer and were held until analysis at 3 or 7 and 14 days of storage. Each sample was analyzed for DMA content after thawing and preparation of a TCA extract.

Freeze-Thaw Study

Fifty gram samples of a homogeneous mixture of two-day-old minced fish muscle were packed in Whirl-Pak bags, frozen and held at -6°C. After 7 days of frozen storage, the samples were thawed and half of each sample was analyzed for DMA after preparation of a TCA extract. The remaining portion of each sample was held at 4°C for 5 days, after which the remaining half of each sample was assayed for DMA after extraction with TCA.

RESULTS AND DISCUSSION

Minced Fish Study

This study examined the effect of aging and tissue disruption on the production of DMA during frozen storage of minced red hake muscle. Freezing and frozen storage were carried out at -6°C and an 'aging' period of 6 days prior to freezing was employed.

The rates of DMA formation in the tissue as influenced by the various treatments are shown in Fig. 1. It is clearly shown (and verified by several subsequent experiments) that DMA production is stimulated as a result of tissue disruption alone and that freezing the tissue is not an absolute requirement. Three times as much DMA was formed in minced tissue (minced/aged) as compared to the gutted/dipped fish when both samples were held on ice for 6 days. Furthermore, only 0.4 mmoles DMA per 100 g of fish were produced in the gutted/dipped fish held for 23 days on ice. Under these conditions of storage the extent of DMA production appears to be limited, possibly due to competition of another pathway utilizing TMAO or due to physical barriers imposed by intact muscle. The limitation on DMA production cannot be attributed to TMA formation in this case since the amount of TMA was not significant until after 14 to 17 days of storage, well after DMA production had ceased. Apparently, mincing (tissue disruption) serves to overcome the restriction on DMA formation, most likely by allowing a more complete mixing of the reaction components than occurs in intact tissue.

During frozen storage itself, the minced tissue that was immediately frozen (minced/unaged) produced DMA at a rapid rate during the entire storage period. On the other hand, the minced tissue that had been held on ice for 6 days (minced/aged) reached a peak of DMA production after 8 days of frozen storage. The DMA content then decreased, indicating that the tissue may metabolize this compound.

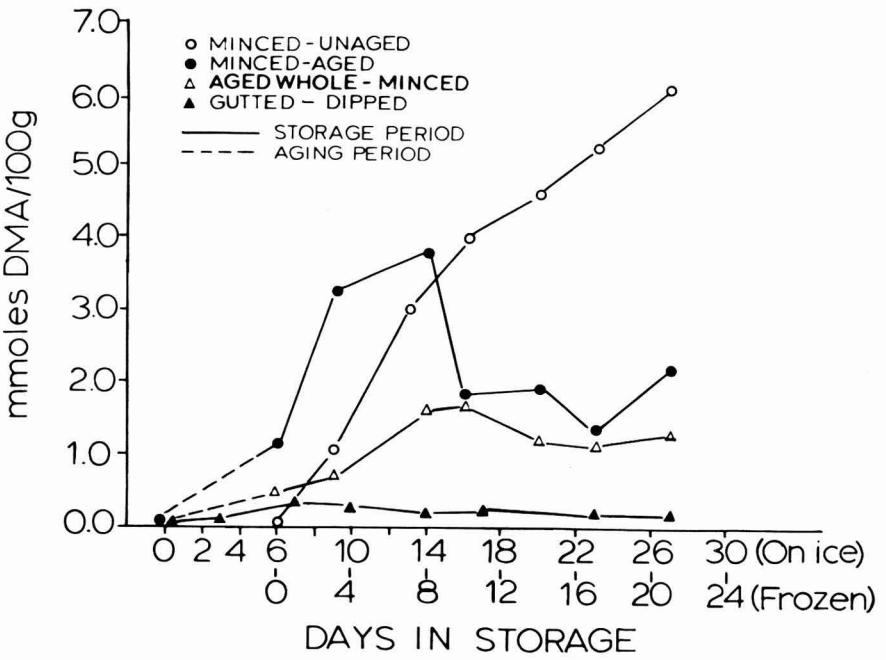

FIG. 1. MINCED FISH STUDY: DMA PRODUCTION IN MINCED RED HAKE MUSCLE HELD FROZEN AT -6°C

Duncan's Multiple Range Test ($p < 0.001$)[1]

Treatment	Treatment Mean (mmoles DMA/100 g)
Minced/Unaged	3.49a
Minced/Aged	1.92b
Aged Whole/Minced	1.08c

All of the minced treatments were stored at -6°C with or without prior aging. For other details see Experimental Design.

[1]Values followed by the same letter are not significantly different

The limitation of the extent of DMA formation may well be due to the depletion of precursor (TMAO) since this compound was depleted after only 3 days of frozen storage (Table 1). Most of the TMAO depletion can be attributed to TMA formation which is probably a competitive pathway with DMA production. Another possible factor is that TMA inhibits the DMA reaction (see Additive Study Results Section). The formation of significant amounts of TMA during frozen storage is contrary to common belief (Castell *et al.* 1970, 1972). We suggest that the formation of TMA during frozen storage of red hake occurred via microbial enzymes which were not inhibited by the

Table 1. Minced fish study: chemical parameters for storage of minced and gutted red hake held at -6°C or in ice

Treatment	Parameter	Time (days)										
		Pre-Aged	0	3	7	8	10	14	17	21	23	27
Minced/Unaged	TMAO	—	10.5	9.01	10.4	—	8.38	8.59	7.66	7.30	—	—
	DMA	—	0.05	1.06	3.03	—	4.00	4.63	5.26	6.10	—	—
	TMA	—	0.09	0.08	0.21	—	0.19	0.15	0.23	0.24	—	—
	Σ Amines	—	10.6	10.2	13.6	—	12.6	13.4	13.2	13.6	—	—
	Free HCHO	—	0.04	0.29	0.81	—	1.09	1.37	0.92	1.11	—	—
Minced/Aged	TMAO	10.5	8.11	0.00	—	0.00	0.00	1.50	0.00	0.00	—	—
	DMA	0.05	1.14	3.26	—	3.83	1.83	1.94	1.34	2.23	—	—
	TMA	0.09	5.20	11.5	—	9.94	12.4	7.54	9.83	12.2	—	—
	Σ Amines	10.6	14.4	14.8	—	13.8	14.2	11.0	11.2	14.3	—	—
	Free HCHO	0.04	0.04	0.11	—	0.26	0.02	0.12	0.16	0.35	—	—
Aged-Whole/Minced	TMAO	10.5	11.3	8.06	—	7.93	7.16	7.60	5.78	8.43	—	—
	DMA	0.05	0.48	0.71	—	1.60	1.68	1.20	1.11	1.31	—	—
	TMA	0.09	1.00	1.15	—	1.48	1.51	2.06	2.11	1.66	—	—
	Σ Amines	10.6	12.8	9.92	—	11.0	10.4	10.9	9.00	11.4	—	—
	Free HCHO	0.04	0.06	0.09	—	0.20	0.13	0.06	0.14	0.23	—	—
Gutted/Dipped	TMAO	—	10.5	12.9	8.77	—	4.78	1.49	0.47	—	0.00	0.00
	DMA	—	0.04	0.11	0.34	—	0.28	0.19	0.34	—	0.17	0.18
	TMA	—	0.09	0.11	0.66	—	0.28	0.91	2.68	—	5.60	5.26
	Σ Amines	—	10.6	13.1	9.82	—	5.34	2.59	3.49	—	5.77	5.44
	Free HCHO	—	0.04	0.03	0.10	—	0.06	0.03	0.04	—	0.03	0.03

All minced treatments were stored at -6°C with or without aging and the gutted-dipped fish were held in ice
All values are expressed in mmoles/100g. Σ amines is the sum of TMAO, DMA and TMA

freezing process. Aging of the tissue allowed the microorganisms to grow in sufficient numbers to produce the effect.

Another important result of this study is that during storage of frozen minced tissue derived from whole fish that had been aged on ice (aged-whole/minced), DMA was limited to 44% and 20% of the peak amount produced for the minced/aged and minced/unaged treatment groups, respectively. Since the TMA content was lower and the TMAO content was higher for the aged-whole/minced group than for the minced/aged group, some factor associated with the former treatment must be accountable for the additional limitation placed on DMA production during frozen storage. This suggests that maintaining the structural integrity of the muscle during the aging period is beneficial (with respect to DMA production) in the event that the tissue is to be subsequently stored frozen as minced fish. Statistically, the difference between each of the three minced fish treatments is significant ($p < 0.001$) as determined by analysis of variance and Duncan's Multiple Range Test. Statistical treatment of the data from this study included only those data points that represent common intervals of analysis among each of the three minced fish treatments.

Table 1 also indicates that there was relatively good conservation of amines (Σ TMAO, DMA, TMA) between the frozen treatment groups considering sample variability, whereas the amount of these amines in the gutted/dipped fish held on ice for 23 days declined. This may indicate that another pathway of TMAO depletion may be operable in red hake tissue or that DMA is further degraded.

Of the other parameters investigated, both free and bound HCHO (Table 1) patterns followed closely the trend of DMA production for all of the treatments. The percentage of HCHO bound was found to range from 70% to 100%, with only minor differences existing between treatments. However, there was some indication that the percentage of HCHO bound in tissue that had been aged was consistently slightly higher than that for unaged fish. Such a relationship between aging and percentage of HCHO bound could have important ramifications concerning the textural deterioration of frozen hake muscle.

Aging Study

This study also examined the effects of 'aging' and tissue disruption on the production of DMA during frozen storage but under different parameters. In this study, the 'aging' period was limited to 4 days, the storage period was extended to 10 weeks with comparisons being

drawn between filleted and minced tissue as well as between aged and unaged samples for each tissue form.

DMA production for these treatment groups are presented in Fig. 2. Dimethylamine was produced more rapidly in minced tissue than in fillets, indicating again that tissue disruption is a factor affecting TMAO breakdown by this pathway. As was seen in the previous study, the minced tissue produced more DMA during the 'aging' (unfrozen) period than did the whole fish. The data in Fig. 2 again verifies that freezing itself is not an absolute requirement for stimulation of TMAO breakdown to DMA and HCHO. Mincing greatly increased the rate of DMA production during the initial stages of frozen storage prior to slowing down to a rate that was comparable to

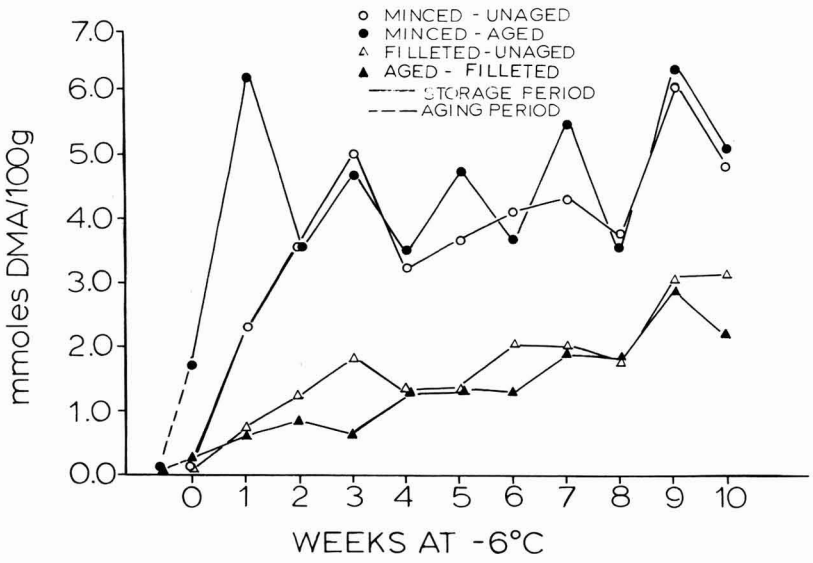

FIG. 2. AGING STUDY: DMA PRODUCTION IN MINCED AND FILLETED RED HAKE MUSCLE HELD AT -6°C

Aged samples were held in ice for 4 days prior to freezing. Duncan's Multiple Range Test ($p < 0.001$)[1]

Treatment	Treatment Mean (mmoles DMA/100 g)
Minced/Unaged	4.41a
Minced/Aged	3.76a
Filleted/Unaged	1.38b
Aged/Filleted	1.70b

[1]Values followed by the same letter are not significantly different

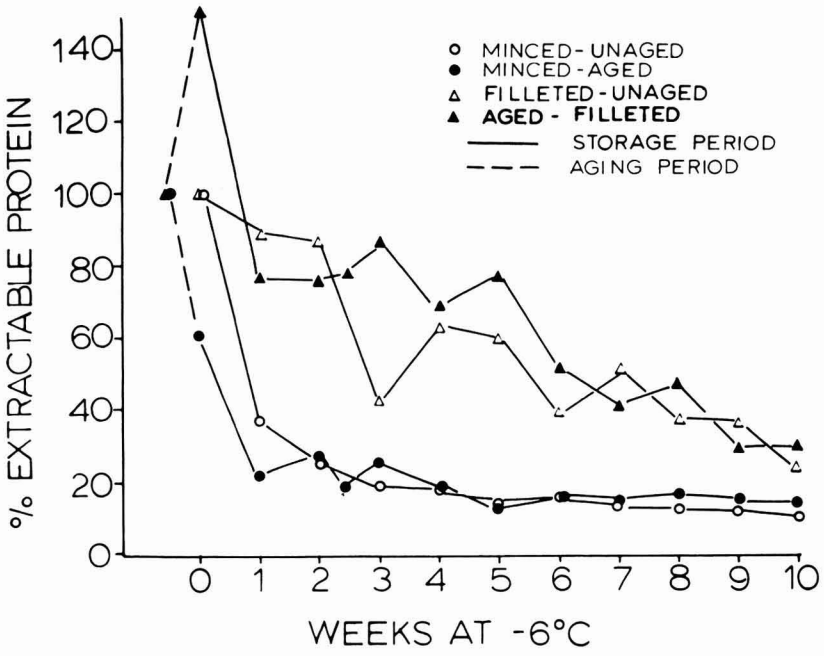

FIG. 3. AGING STUDY: EXTRACTABLE PROTEIN FOR MINCED AND FILLETED RED HAKE MUSCLE HELD AT -6°C

Aged samples were held in ice for 4 days prior to freezing

the rate observed in the frozen fillets. This slowing down of the reaction rate may be due to the depletion of the components that were involved in the reaction, either substrate (TMAO) or cofactors. Differences between the minced and filleted tissue groups in DMA content were statistically significant at $p < 0.001$ by analysis of variance and Duncan's Multiple Range Test with no significant differences existing between the aged and unaged samples for each tissue form.

Differences in EP values reflected those found for DMA contents for the treatments involved (Fig. 2 and 3), indicating an inverse relationship between these parameters. Furthermore, only minor differences in EP values were observed for the aged versus unaged samples for either the mince or the fillet groups. According to the EP data, a maximal loss in protein extractability occurred at about 5 weeks for the minced samples, corresponding to 10-15% EP. The EP values for the filleted samples displayed a fairly linear decrease as a function of storage time. Over the 10 week storage period, the EP of the filleted samples did not reach the minimum value the minced samples reached after 5 weeks. A good correlation between EP and DMA was

previously reported in studies involving frozen red hake blocks (Kelleher *et al.* 1981) and frozen red hake fillets (Gill *et at.* 1979).

The differences in DMA contents for the minced/aged versus the minced/unaged samples that were observed in the minced fish study were not observed in this experiment. TMA analysis indicated that there was little TMAO breakdown via TMA during the 'aging' period as well as throughout the frozen storage period (Table 2). TMAO levels at onset of the frozen storage period were twice as high for the unaged treatment groups compared to the aged groups. For the minced/aged samples, TMAO loss could be accounted for by DMA production, whereas for the aged/filleted group, TMAO depletion could not be attributed to either TMA or DMA production.

TMAO levels were greatly depleted for the minced fish treatments after 1-2 weeks of frozen storage. Concomitantly, DMA production was restricted after this initial period of storage. Only minor differences were observed between the filleted treatment groups with respect to DMA production. This seems to contradict our earlier findings (Kelleher *et al.* 1981). However, the aging period involved may be the most critical factor. An aging period of 4 days was employed for the study at hand, whereas 5 days was used in our previous report (Kelleher *et al.* 1981). The extra day of holding on ice may be critical regarding the natural loss or destruction of cofactors that may be required for TMAO breakdown to DMA and HCHO, accounting for this discrepancy on the effects of aging.

As was seen in the previous study, HCHO followed a pattern similar to that of DMA production for all of the treatments (Table 2). The percentage of HCHO bound was consistently between 60 and 80% for all of the treatments, with the aged samples yielding slightly higher values than their unaged counterparts. The sum of the related amines (Σ TMAO, DMA, TMA) appears to be conserved throughout storage, although there was considerable variation in the data.

Processing Study

This study examined the effects of 'aging' red hake along with various processing procedures prior to frozen storage with regard to production of DMA. The samples were frozen and stored at -20°C for 8 months; the aging period was limited to 4 days.

Figure 4 shows the accumulation of DMA in the fillets as a function of the various treatments. The samples aged as fillets (skin-on or skin-off) displayed almost twice as much DMA production as did the fillets originating from fish that were aged with the muscle intact (whole or

Table 2. Aging study: chemical parameters for the storage of minced and filleted red hake stored at -6°C

Treatment	Parameter	Time (Weeks)										
		Pre-Aged	0	1	2	3	4	5	6	7	8	9	10
Minced/Unaged	TMAO	—	5.27	2.74	1.06	0.57	0.87	0.30	0.38	0.20	0.03	0.00	0.04
	DMA	—	0.14	2.60	3.56	5.02	3.22	3.68	4.11	4.33	3.75	6.08	4.63
	TMA	—	0.01	0.14	0.20	0.21	0.18	0.20	0.26	0.20	0.31	0.34	0.26
	Σ Amines	—	5.42	5.48	4.82	5.80	4.27	4.18	4.75	4.73	4.09	6.42	5.13
	Free HCHO	—	0.04	1.18	1.58	1.36	1.72	1.64	1.33	1.42	1.32	1.19	1.40
Minced/Aged	TMAO	5.27	2.51	1.30	1.04	1.01	0.47	0.19	0.09	0.08	0.00	0.13	0.00
	DMA	0.14	1.72	6.20	3.52	4.70	3.48	4.78	3.65	5.53	3.55	6.34	5.05
	TMA	0.01	0.07	0.17	0.24	0.40	0.21	0.27	0.24	0.21	0.29	0.37	0.27
	Σ Amines	5.42	4.30	7.67	4.80	6.11	4.16	5.24	3.98	5.82	3.84	6.84	5.32
	Free HCHO	0.04	1.02	1.41	1.41	1.29	1.27	1.12	0.98	1.04	0.69	0.76	1.29
Fillet/Unaged	TMAO	—	5.28	4.37	4.77	3.99	3.44	3.58	4.06	3.81	3.21	3.54	2.98
	DMA	—	0.08	0.77	1.24	1.85	1.34	1.34	2.04	2.01	1.78	3.05	3.15
	TMA	—	0.01	0.05	0.09	0.09	0.08	0.08	0.13	0.15	0.21	0.18	0.16
	Σ Amines	—	5.37	5.09	6.10	5.93	4.86	5.00	6.23	5.97	5.20	6.77	6.29
	Free HCHO	—	0.03	0.42	0.58	0.76	0.54	0.56	0.61	0.76	0.76	0.79	0.88
Aged/Filleted	TMAO	5.28	2.58	3.94	2.46	3.79	3.71	2.78	2.74	2.54	1.52	2.80	2.73
	DMA	0.08	0.26	0.61	0.84	0.64	1.31	1.36	1.30	1.93	1.80	2.89	2.22
	TMA	0.01	0.18	0.14	0.23	0.65	0.24	0.28	0.20	0.16	0.33	0.80	0.43
	Σ Amines	5.37	3.02	4.69	3.53	5.08	5.26	4.42	4.24	4.63	3.65	6.49	5.38
	Free HCHO	0.03	0.05	0.17	0.16	0.30	0.41	0.37	0.34	0.39	0.27	0.52	0.84

All values are expressed in mmoles/100 g. Σ Amines is the sum of TMAO, DMA and TMA
Aged samples were held in ice four days prior to freezing

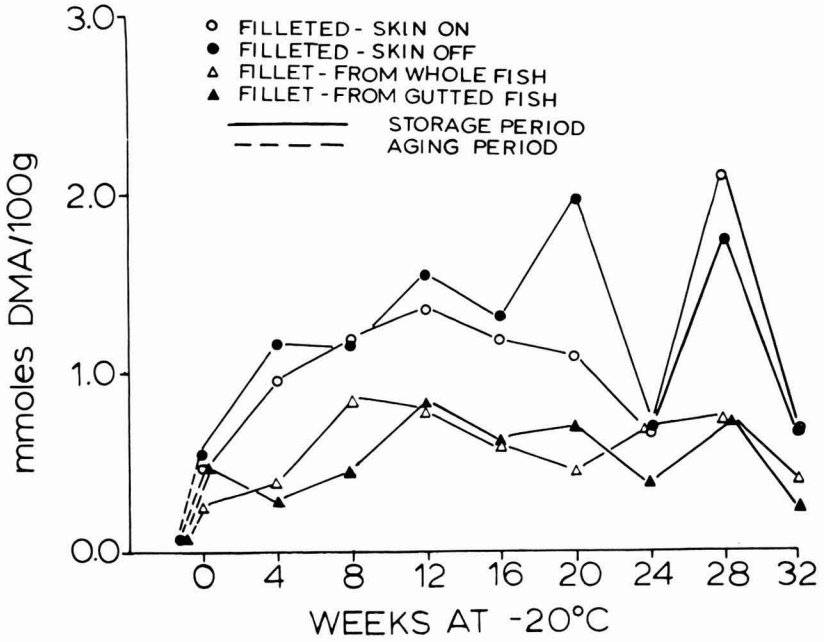

FIG. 4. PROCESSING STUDY: DMA PRODUCTION IN SKIN-OFF FILLETS HELD AT -20°C

Aging of all samples was for 4 days in ice prior to processing all groups into skin-off fillets and subsequent frozen storage. Duncan's Multiple Range Test ($p < 0.001$)[1]

Treatment	Treatment Mean (mmoles DMA/100 g)
Skin-off Fillet	1.09a
Skin-on Fillet	0.99a
Fillet from Whole	0.54b
Fillet from Gutted	0.49b

[1]Values followed by the same letter are not significantly different

gutted), even though all samples were processed into skinless fillets prior to frozen storage. This differential pattern of DMA formation was established in spite of the fact that there was little difference in DMA content between treatments at the end of the aging period. The type of treatment prior to frozen storage influences the rate and extent of DMA (and HCHO) formation during frozen storage of aged red hake muscle. Perhaps the filleting operation disrupts enough tissue to allow for more mixing of substrates during the aging period beyond that which takes place as a result of freezing and ice crystal damage. The freezing process concentrates these substrates, possibly leading to enhanced rates of DMA formation.

Another important feature of the data in Fig. 4 is that maximal formation of DMA takes place early in the storage period. Only 8-12 weeks elapsed before maximal DMA production was accomplished in the fillets derived from whole or gutted fish. Although the scattering of data obscured the interpretation of the pattern of DMA production in the samples aged as fillets (skin-on or skin-off) the extent of DMA production in these fillets was limited to about 2 mmoles/100 g. This pattern of DMA production at -20°C contrasts with that which takes place in fillets stored at -6°C (Fig. 2) in two ways. The rate is faster (as expected) at -6°C than at -20°. More importantly, the extent of DMA production appears to be less for the fillets at -20° compared to those at -6°C. Comparable samples are fillets that were derived from aged whole fish and held at -6°C and those that were prepared from aged whole and gutted fish and held at -20°C. Therefore, temperature of storage seems to have an effect on the extent of DMA production, possibly by limiting the extent of mixing of the reaction components. Analysis of variance and Duncan's Multiple Range Test revealed that the only significant differences in DMA content between treatments was for samples aged as fillets (filleted or filleted with skin) compared to those aged with the muscle intact (whole or gutted) ($p < 0.001$).

Analysis of the samples for HCHO indicated that the amount of free and bound HCHO was consistent with the DMA data (Table 3). As was found in the previous studies, the percentage of HCHO bound by the tissue was consistently between 60 and 80% with no differences existing between treatments. TMAO and TMA contents for all of the samples were similar, with little TMA produced during the aging period. However, analysis of variance indicated that a significant difference ($p < 0.01$) existed in TMAO content between the filleted treatments (skin-on versus skin-off) indicating that the skinning treatment alone influenced the disposition of some of the tissue TMAO. TMAO breakdown may occur by some pathway other than DMA formation, since there was no significant difference between these two treatments with regard to this latter parameter.

Additive Studies

Observations made previously during a study on textural deterioration of red hake muscle stored as fish blocks indicated that fish subjected to a dip containing sodium tripolyphosphate and erythorbate yielded a tougher product than was produced from fish that was not so treated (Kelleher *et al.* 1981). This led us to investigate the effects of some commonly used additives on TMAO breakdown. In

Table 3. Processing study: chemical parameters for storage of skin-off fillets held at -20°C

Treatment	Parameter	Time (Weeks)									
		Pre-Aged	0	4	8	12	16	20	24	28	32
Fillet Skin-off	TMAO	5.28	4.97	3.29	3.28	3.12	2.61	2.64	3.21	3.19	2.01
	DMA	0.08	0.56	1.17	1.16	1.55	1.31	1.98	0.71	1.75	0.66
	TMA	0.01	0.13	0.30	0.09	0.10	0.46	0.41	0.34	0.10	0.10
	Σ Amines	5.37	5.66	4.78	4.53	4.77	4.38	5.03	4.26	5.04	2.77
	Free HCHO	0.03	0.20	0.56	0.42	0.59	0.47	0.51	0.27	0.40	0.18
Fillet Skin-on	TMAO	5.28	5.50	4.26	4.13	3.82	4.02	3.20	4.63	4.36	3.19
	DMA	0.08	0.47	0.95	1.19	1.37	1.19	1.11	0.68	2.13	0.70
	TMA	0.01	0.32	0.29	0.48	0.32	0.19	0.34	0.19	0.21	0.29
	Σ Amines	5.37	6.29	5.50	5.80	5.51	5.30	4.65	5.50	6.70	4.18
	Free HCHO	0.03	0.12	0.18	0.28	0.43	0.34	0.32	0.19	0.39	0.12
Fillet from Gutted Fish	TMAO	5.28	3.03	3.74	4.13	3.90	3.25	3.17	3.33	3.66	3.98
	DMA	0.08	0.48	0.28	0.47	0.86	0.64	0.73	0.39	0.75	0.25
	TMA	0.01	0.09	0.21	0.06	0.12	0.12	0.34	0.27	0.18	0.08
	Σ Amines	5.37	3.60	4.23	4.66	4.88	4.01	4.24	3.99	4.59	4.31
	Free HCHO	0.03	0.10	0.09	0.12	0.16	0.15	0.17	0.09	0.16	0.07
Fillet from Whole Fish	TMAO	5.28	2.58	4.44	1.70	3.65	3.66	3.24	3.42	3.51	3.29
	DMA	0.08	0.26	0.40	0.87	0.80	0.60	0.47	0.70	0.76	0.41
	TMA	0.01	0.18	0.24	2.42	0.19	0.13	0.12	0.08	0.08	0.12
	Σ Amines	5.37	3.32	5.08	4.99	4.64	4.39	3.83	4.20	4.35	3.82
	Free HCHO	0.03	0.05	0.09	0.11	0.16	0.09	0.14	0.11	0.07	0.26

Aging of all samples was for 4 days in ice prior to processing all groups into skinless fillets and subsequent frozen storage. All values are expressed in mmoles/100 g. Σ Amines is the sum of TMAO, DMA and TMA.

addition, substances were examined that might be predicted to accelerate or inhibit the reaction. These studies were accelerated by the use of high frozen storage temperatures (-12°C) and through the effect(s) of mincing. Mincing was also necessary to allow for homogeneous distribution of the additives. DMA analysis was performed as an index to the extent of TMAO breakdown. Storage times of 2-3 weeks were involved.

The influence on DMA production exerted by each component was compared to that of the control (control = 100) after computation of an average value derived from all of the data points taken throughout the storage periods. The range of % DMA formation is also reported (Table 4) and represented the highest and lowest values encountered during the storage period for each additive. The ranges are indicative of the extent of fish sample variation rather than variation in the chemical analyses (which was $\leqslant 8\%$). For compounds undergoing only a single trial, the range is composed of the values that reflect each of the two points of analysis (2 and 3 weeks). The effects of the tested compounds are provided in Table 4 and are classified as stimulatory, slightly stimulatory, no effect, slightly inhibitory or inhibitory. Operationally, we defined these categories as $> 140\%$, 120-140%, 90-120%, 70-90% and $< 70\%$ of the control, respectively. For borderline cases, the range of values for DMA production was also considered in categorizing the compound. Of the stimulatory compounds, the most potent appear to be the redox potentiators (phenazine methosulfate, menadione and methylene blue), suggesting the importance of the oxidation-reduction potential to TMAO breakdown in hake muscle. In fact, methylene blue has often been used as an assay component for partially purified preparations of 'TMAOase' (Yamada and Amano 1965b; Harada 1975; Yamada *et al.* 1969). Reducing agents (ascorbate and erythorbate) also greatly stimulated DMA production over a wide range of concentrations in the tissue. We have shown that ascorbate can contribute to the breakdown of TMAO to DMA in an isolated membrane system derived from red hake skeletal muscle (Parkin and Hultin 1981). Sodium tripolyphosphate had no effect on DMA production, and it is therefore assumed that erythorbate was responsible for the greater DMA production and toughening observed in our earlier study (Kelleher *et al.* 1981).

EDTA and ferrous iron both stimulated DMA production when each was added separately to the minced tissue. One might expect opposite effects from these compounds, yet this anomaly has also been observed for frozen minced Pacific whiting (Spinelli and Koury 1981). Perhaps EDTA preferentially binds to an inhibitory cation naturally

Table 4. Additive study: the effect of additives on the production of DMA in minced red hake held at -12°C

Additive Compound(s)	Concentration	Trials	Effect	Range of % DMA Formation	$\bar{X}$ % DMA Formation
Ferrous iron	1 mM	2	Stimulatory	123-201	154
EDTA	1 mM	2	Stimulatory	125-148	138
Phenazine methosulphate	0.1 mM	2	Stimulatory	223-344	274
Menadione	0.1 mM	1	Stimulatory	109-202	156
Methylene Blue	0.1 mM	1	Stimulatory	259-359	309
Ascorbate	0.5 mM	2	Stimulatory	128-195	162
Ascorbate	14 mM	1	Stimulatory	356	356
Ascorbate	57 mM	1	Stimulatory	449	449
Erythorbate	0.5 mM	2	Stimulatory	141	141
Erythorbate	14 mM	1	Stimulatory	496	496
Erythorbate	57 mM	1	Stimulatory	544	544
Cysteine/Ferrous iron	2.0 mM/1.0 mM	1	Stimulatory	119-210	164
Cysteamine/Ferrous iron	2.0 mM/1.0 mM	1	Stimulatory	172-230	201
Choline/Ferrous iron	10 mM/1.0 mM	1	Stimulatory	126-175	150
Betaine/Ferrous iron	10 mM/1.0 mM	1	Stimulatory	170-188	179
Taurine/Ferrous iron	1.0 mM/1.0 mM	1	Stimulatory	125-174	149
Cysteine	2.0 mM	2	Sl.Stimulatory	110-152	125
Glutathione (Red.)	2.0 mM	2	Sl.Stimulatory	103-141	130
Choline	10 mM	3	Sl.Stimulatory	94-179	132

Ferric iron	1.0 mM	2	No Effect	94-116	108
Cysteamine	2.0 mM	3	No Effect	71-160	89
Mercaptoethanol	2.0 mM	2	No Effect	84-140	98
Thiourea	2.0 mM	2	No Effect	71-115	94
Dithiothreitol	2.0 mM	1	No Effect	91-137	114
Betaine	10 mM	1	No Effect	94-98	96
Taurine	1.0 mM	2	No Effect	92-114	98
Hypotaurine	1.0 mM	2	No Effect	93-156	114
Dithionite	1.0 mM	1	No Effect	100-101	100
Sodium Tripolyphosphate	0.2 mM	3	No Effect	82-129	104
NADPH	0.01 mM	1	No Effect	93-112	102
Cupric copper	1.0 mM	2	Sl. Inhibitory	47-143	81
DMA	10 mM	3	Sl.Inhibitory	50-102	78
Citrate	1.0 mM	3	Sl.Inhibitory	50-102	73
Deoxycholate	0.1%	3	Sl.Inhibitory	68-131	88
Deoxycholate	1.0%	1	Inhibitory	31-48	40
Dimethylaniline	10 mM	2	Inhibitory	25-76	42
TMA	10 mM	3	Inhibitory	18-76	50

Compounds are classified operationally as stimulatory ($\geq$ 140%), slightly stimulatory (120-140%), no effect (90-120%), slightly inhibitory (70-90%) or inhibitory ($\leq$ 70%) relative to the control (100%). The range is defined as the highest and lowest values encountered during any of the trials for each compound(s). For each compound(s) with a single trial, the % DMA formation was represented by values corresponding to each of the two intervals of analysis during the storage period

present in red hake muscle, thus relieving some of the inhibition and being perceived as stimulating the reaction. Sulfhydryl reagents were found to either slightly stimulate (glutathione and cysteine) or have no effect on DMA production. Cysteine has previously been reported to stimulate this reaction in Pacific whiting (Spinelli and Koury 1981), and it has been found to take part in the membrane-catalyzed TMAO breakdown previously noted (Parkin and Hultin 1981). Of those sulfhydryl reagents having no effect on the reaction rate, mercaptoethanol and dithiothreitol appeared to slightly stimulate the reaction over the first stages of storage and slightly inhibit the reaction at later stages. Furthermore, the stimulatory effects of cysteine and glutathione were also more pronounced in the earlier stages of storage. Perhaps this observation is related to the instability of reduced sulfhydryls as a function of time (Fluharty 1974). Choline and betaine, two indigenous amine compounds in fish muscle, had a slightly stimulatory and negligible effect on the reaction rate, respectively. Other common amines in fish muscle, taurine and hypotaurine, had a negligible effect on the reaction rate.

When betaine, cysteine, cysteamine, taurine and choline were added to the minced tissue in the presence of Fe^{+2}, the stimulatory effect of these combinations could be accounted for by ferrous iron alone. It is interesting to note that whereas ferrous iron is stimulatory, ferric iron appeared to have no affect on the reaction rate. This observation again suggests the importance of the reducing conditions in the tissue.

Dimethylaniline and TMA appeared to be the most potent inhibitors of DMA production of the compounds tested. The fact that TMA inhibits the reaction reflects upon the results of the minced fish storage study where it was found that aging (accompanied by an accumulation of TMA) served to limit the extent of DMA production during frozen storage. Due to the structural similarities of TMA, TMAO and DMA, one might anticipate that TMA acts as a competitive enzyme inhibitor and that sufficient concentrations could serve to limit the production of DMA from TMAO. It is interesting that citrate serves to slightly inhibit the reaction whereas EDTA was found to be stimulatory, even though both compounds are chelating agents. The observation that cupric copper is an inhibitor has also been reported in frozen minced Pacific whiting (Spinelli and Koury 1981).

It is acknowledged that these additive experiments offer only crude information and conclusions must be drawn carefully for several reasons. First of all, mincing the flesh destroys the natural structures and compartments that normally exist in the tissue; the integrity of

the muscle has already been observed to exert considerable influence on the reaction as it takes place prior to and during frozen storage. Secondly, the amount of additive administered to the minced fish can only be controlled at the point of addition, since there are undoubtedly several chemical and enzymic pathways in the fish in which the added components may take part. Likewise, the amount of additive already present in the fish has not been considered in these experiments. However, we feel that these studies provide valuable information on the nature of the DMA and HCHO producing systems in red hake muscle, and gross comparisons of the effect of certain compounds on these systems may be made.

Freezing Study

The freezing method and temperature nadir were examined for their effect on the production of DMA (in minced tissue stored at -5°C). The results are shown in Table 5. A rate constant for production of DMA was computed from the initial data points (3 or 7 days), and is provided in the Table. Analysis of variance indicated that no significant differences existed between treatments with respect to DMA formation over the 14 day storage period. Minced red hake was utilized in this study in an effort to accelerate the reaction; perhaps the results would be different for fish fillets frozen by this differential regime.

Freeze-Thaw Study

The minced fish and aging studies demonstrated the stimulation of DMA production in red hake tissue without the requirement of its being frozen. Consistent with a tissue disruption theory for the stimulation of this reaction is that upon thawing of frozen tissue, the breakdown of TMAO to DMA and HCHO should not cease. This study was to determine whether the reaction is truly 'freeze-activated' or is merely stimulated by tissue damage. The results are presented in Table 6. DMA production took place in the minced tissue during frozen storage, and upon thawing, DMA production continued. Furthermore, the rate of DMA production upon thawing is similar to the rate during frozen storage. One could expect concentration effects of freezing to activate the systems, but these results dispute the premise that 'freeze-activation' of the systems requires that the tissue be in the frozen state for DMA (and HCHO) formation to occur. The results of this study coupled with those from the storage studies provide a strong case that tissue disruption stimulates the breakdown of TMAO to DMA and HCHO, as was implied by the results of an earlier study (Tokunaga 1964).

Table 5. Freezing study: production of DMA in minced red hake held at -5°C as a function of freezing nadir

Treatment	Time (Days)				Rate Constant (k) mmoles DMA/day-100 g
	0	3	7	14	
Frozen at -5°C	0.14±0.05	—	2.60±0.03	2.52±0.17	0.35
Frozen at -20°C	0.14±0.05	—	2.40±0.14	2.74±0.08	0.32
Frozen at -80°C	0.14±0.05	0.99±0.02	—	2.78±0.16	0.28
Frozen at -80°C	0.14±0.05	—	2.46±0.23	3.66±0.45	0.33

All values are expressed in mmoles/100 g. Rate constants are derived from the rate of DMA production over the initial period of analysis (0 to 3 days or 0-7 days)

Table 6. Freeze thaw study: production of DMA in minced red hake held at -6°C and then at 4°C

Sample	DMA (mmoles/100 g)		
	0 Days	7 Days at -6°C	5 days at 4°C
A	0.03	0.27	0.55
B	0.03	0.29	0.52

ACKNOWLEDGMENTS

This work was supported in part by NOAA and the New England Fisheries Development Task Force, by the Massachusetts Agricultural Experiment Station, and by the Graduate School of the University of Massachusetts at Amherst.

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AN INVESTIGATION INTO INSECT PROTEIN[1]

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Received for Publication August 3, 1979

ABSTRACT

*Due to its easy availability, cleanliness and relative ease of cultivation, Mexican fruit fly larvae (*Anastrepha ludens*) were selected to study insect protein. The larvae were found to contain 79.5% moisture, 9.8% protein, 6.2% fat, 2.3% ash and 2.2% nitrogen-free extract. Larvae protein was found to be high in lysine and methionine, but low in valine, isoleucine and tryptophane, deficiencies which were reflected by a corrected PER value of 1.63. Protein concentrate and isolate prepared from larvae contained 65% and 87% protein, respectively, with corresponding recoveries of 94% and 85%. The protein solubility curve was typical, exhibiting minimum solubility of 8% at pH 5 and maximum solubility of 95% at pH 10. The protein was found to possess negligible foaming and emulsifying properties, while larvae RNA content was 1.9%, a lower value than those reported for yeasts, but higher than those reported for microscopic algae.*

INTRODUCTION

It is known that highest quality protein foods for human consumption generally come from the animal kingdom, notably mammals, fish and shellfish. Since insects also belong to this kingdom and are known to be related to crustaceans (both are arthropods) it was

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Journal of Food Processing and Preservation **6**(1982)99-110.

believed that they might be possessors of similar high quality protein. Furthermore, since insects have short life cycles, they should be fairly easy to cultivate, thus making their protein relatively abundant and of low cost, a good potential source of food for the world's malnourished.

Consumption of insects by humans is not unknown. In some parts of Mexico, for example, ants, grasshoppers and certain types of larvae are eaten as delicacies by segments of the population. This is also known to occur in other countries of Latin America as well as in Africa (Conconi 1976). Contrary to what might be expected, the idea of consuming insects is not necessarily repugnant to humans. Conconi (1976) conducted a survey among 30,000 persons including housewives, chefs and scientists, and obtained an 82% affirmative reply on the possibility of including insects in their diets.

Flies constitute one of the largest families of insects and are found all over the world; some species serve as food for higher animals. The total number of fly species is approximately 86,000, of which 16,000 occur in the United States and Canada (Borror and White 1970). Flies have a large reproductive capacity; in four months (April to August), descendants of a pair of typical flies, if all survive and reproduce normally, can attain a total of 2×10^{20} individuals (USDA 1952). The reproduction of suitable types of flies, therefore, if adequately controlled, could lead to an abundant and renewable food supply.

For this work, Mexican fruit fly larvae (*Anastrepha ludens*) were selected due to their availability, cleanliness, ease of cultivation and high yields.

MATERIALS AND METHODS

Larvae were obtained from the U.S. Department of Agriculture Station in Monterrey, Mexico. Arrangements were made so that each shipment was received the same day it was sent. After being received, most samples were dried 12 h in a cabinet dryer at 35-40°C, after which they were packed in polyethylene bags and stored at 5°C. Fresh samples, however, were used for proximal analysis, protein solubility curve and protein concentrate and isolate determinations.

Proximal Chemical Analysis

Larvae moisture, protein, fat and ash contents were obtained by the usual methods: drying to constant weight in an oven (approximately

24 h at 100°C) for moisture, micro-Kjeldahl for proteins, Soxhlet extraction for fat, and burning in a muffle furnace (approximately 550°C for 12 h) for ash (AOAC 1965). Nitrogen-free extract was determined by difference. All determinations were run in triplicate.

Protein Solubility Curve

Protein solubility was obtained as a function of pH using a modification described by Mattil (1970). Fresh larvae were first ground in a mortar at low temperature (15°C) in order to prevent protein denaturation. Two grams of sample was then dispersed in 40 ml of distilled water whose pH had previously been adjusted to the desired value by addition of either 0.1N NaOH or HCl. The sample was placed on a magnetic stirrer and continuously agitated at room temperature (27°C) for 30 min. Extraction pH was verified and adjusted several times during, and at the end of the period. Following agitation, the sample was centrifuged at 5000 rpm for 20 min, after which the supernatant liquid was filtered through Whatman No. 1 filter paper, and 20 ml aliquots were taken for nitrogen determination. Sample nitrogen content was determined, and protein solubility calculated, as described by Mattil (1970). Solubility was measured and plotted as a function of pH for the pH range 2 to 12 inclusive.

Amino Acid Analysis

Amino acid analysis, with the exception of tryptophane and cystine, was quantitatively determined by the procedure of Spackman *et al.* (1958). The sample was hydrolyzed in boiling hydrochloric acid for 24 h under nitrogen flush. Tryptophane and cystine determinations, including hydrolytic procedures, were carried out by the methods of Kohler and Palter (1967) and Schramm *et al.* (1954), respectively.

Protein Quality

Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) of larvae protein were determined using the method described by Del Valle and Perez Villa-señor (1974), using casein as a reference protein.

Protein Concentrate and Isolate

Procedures utilized for obtention of larvae protein concentrate and isolate followed methods commonly used for obtention of similar products from protein sources, as described by Anson (1959) and also by Hagenmeier *et al.* (1972, 1973). Briefly, the concentrate was

obtained by washing fresh larvae with water, under conditions of minimum protein solubility (pH 5, 60°C), in order to remove water soluble substances and fat. On the other hand, the isolate was obtained by dissolving proteins from fresh larvae in water under conditions of maximum solubility (pH 10, room temp.), followed by precipitation of the protein under conditions of minimum solubility (pH 5). Initial sample size was 50 g of fresh larvae for both concentrate and isolate runs, the two of which were conducted in triplicate.

All fractions resulting from the concentrate and isolate runs were dried to constant weight in an oven at 100°C, and their proximate chemical analyses were determined (AOAC 1965). Data obtained were used for making material balance calculations on protein and fat recoveries.

Functional Properties

Functional properties of larvae proteins measured were emulsifying capacity, according to the method of Carpenter and Saffle (1964) and Webb (1970), and foaming capacity, using the procedure described by Richert *et al.* (1974). Cottonseed and safflower oils were used in the determination of emulsifying capacity which, together with foaming capacity, was measured as a function of pH. Egg white was used as a reference protein in the foaming capacity determinations.

Nucleic Acid Content

Ribonucleic acid (RNA) content of larvae was determined according to the method of Munro and Allison (1964).

RESULTS AND DISCUSSION

Proximal Analysis

Table 1 reports larvae proximal analysis on both wet and dry bases. On a wet basis the protein content was rather low, similar to that found in mollusks (10%), and considerably below that of most common fresh animal protein foods, (except milk) which vary from approximately 12% for pork flesh and eggs, to approximately 27% for the muscle of certain species of fish (perciform scombrids such as tuna, mackerel, swordfish, etc.) (FAO 1970). On a dry basis, however, protein content was fairly high. Significantly, the fat content was also high, almost equal to the protein content. It may be concluded that quantitatively, fly larvae would probably not be a good protein source, and that the large fat content would also pose a problem in their utilization.

Table 1. Proximal analysis of mexican fruit fly larvae (*Anastrepha ludens*)

Component	Percent, Wet Basis	Percent, Dry Basis
Protein	9.8	46.0
Moisture	79.5	—
Ash	2.3	7.4
Fat	6.2	37.0
Nitrogen Free Extract	2.2	9.6

Protein Solubility Curve

Figure 1 shows that the larvae protein solubility curve was typical, with a minimum solubility of 8% at pH 5, the isoelectric point, and maximum solubility of 95% at pH 10. This data suggested that it would be possible to obtain a protein concentrate and isolate from larvae fairly easily, and with fairly good yields, as was indeed found to be the case.

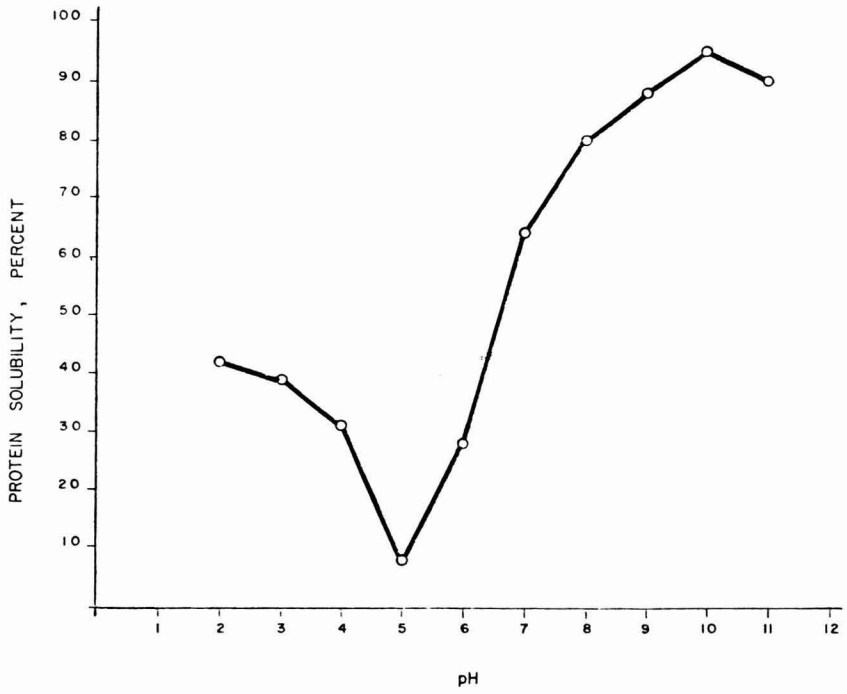

FIG. 1. SOLUBILITY CURVE FOR PROTEIN OF MEXICAN FRUIT FLY LARVAE, *ANASTREPHA LUDENS*.

Amino Acid Analysis

Table 2 reports the amino acid analysis of larvae protein. To complement this information, the FAO (1957) reference pattern was included and the chemical score for each amino acid, based on the FAO pattern, was also calculated. It may be seen that larvae protein was high in phenylalanine, leucine, lysine and methionine, but quite low in tryptophane, histidine, cystine and isoleucine, and somewhat low in threonine. The chemical score was 67, based on the most limited amino acid which was tryptophane.

It was concluded that, due to the large number of limited amino acids, larvae alone would probably not be a good protein source. On the other hand, due to their high lysine and methionine contents, they could be used for complementation of cereal and legume proteins, which are limited in lysine and methionine, respectively, and which, with the exception of corn, generally contain adequate or more than adequate amounts of those amino acids found to be limited in larvae. In the case of corn, which is also limited in tryptophane, complementation with larvae proteins would probably be more difficult.

Table 2. Amino acid analysis of protein of mexican fruit fly larvae (*Anastrepha ludens*)

	Grams Per 16 Grams Nitrogen		
Amino Acid	Fly Larvae	FAO Reference Protein	Score
Valine	3.80	4.20	90
Isoleucine	2.97	4.20	71
Threonine	4.05	5.20	78
Tryptophane	0.94	1.40	67
Phenylalanine	3.75	2.80	134
Leucine	7.13	4.80	149
Lysine	6.03	4.20	144
Methionine	2.26	2.20	103
Cystine	1.26		
Histidine	1.61		
Aspartic Acid	11.00		
Sarine	4.53		
Glutamic Acid	18.79		
Proline	5.58		
Glycine	5.63		
Alanine	9.12		
Tyrosine	6.87		
Arginine	4.68		

Protein Quality

Table 3 shows, as might have been expected from the large number of limiting amino acids, that larvae protein quality was not too high, although it was neither too low, as reflected by both PER and NPU values. Generally, quality of larvae proteins was comparable to that of proteins of common oilseeds and legumes, whose corrected PER values vary from approximately 1.0 for lentils to 2.0 for soybeans, but lower than that for proteins of common animal foods, with reported corrected PER values varying from 2.2 for beef to 3.8 for eggs (FAO 1970).

With respect to casein, both indices were quite similar, being 65% for PER and 74% for NPU.

Results of protein quality determinations and amino acid analysis were therefore consistent and mutually verified each other. It was concluded that, although Mexican fruit fly larvae belong to the animal kingdom, they were definitely inferior to common animal foods, as much in quality as in quantity of proteins.

Table 3. Quality of protein of mexican fruit fly larvae (*Anastrepha ludens*)

System	Corrected Protein Efficiency Ratio	% Per To Casein	Net Protein Utilization	% NPU to Casein
Fruit fly larvae	1.63 ± 0.22	65%	38.0 ± 4.2	75%
Casein	2.50 ± 0.20	100%	50.8 ± 4.7	100%

Protein Concentrate and Isolate

Table 4 reports proximal analyses for larvae protein concentrate and isolate on a dry basis, while Table 5 summarizes data on material balance calculations for both products. It may be seen from Table 4 that, as might be expected, the isolate contained more protein and less fat than the concentrate. On the other hand, protein contents of both products were somewhat, although not much, lower than values for corresponding products from vegetable sources, which are of the order of 65-70% for concentrates and greater than 90% for isolates (Wolf and Cowan 1971).

Concerning protein recoveries from the original larvae, Table 5 shows that both were fairly high and comparable with those obtained from vegetable sources (Wolf and Cowan 1971). Table 5 also shows clearly that protein was concentrated in both concentrate and isolate due to elimination of fat, nitrogen-free extract and ash.

The large protein recoveries obtained, and the fairly high protein

Table 4. Proximal analyses for protein concentrate and isolate from mexican fruit fly larvae (*Anastrepha ludens*), dry basis

Component	Protein Concentrate Percent Dry Basis	Protein Isolate Percent Dry Basis
Protein	65.4	86.6
Ash	6.8	2.9
Fat	20.6	10.5
Nitrogen free extract	7.2	0.0

Table 5. Results of material balance calculations in obtention of protein concentrate and isolate from mexican fruit fly larvae (*Anastrepha ludens*)

	Larvae	Concentrate	Isolate
Component content			
Protein, grams	4.91	4.64	4.18
Ash, grams	1.14	0.49	0.27
Fat, grams	3.09	1.48	0.52
Nitrogen free extract, grams	1.12	0.39	0.01
Total, grams	10.26	7.00	4.98
Percent with respect to individual component in larvae			
Protein	100	94.4	85.1
Ash	100	42.7	23.3
Fat	100	47.9	16.8
Nitrogen free extract	100	34.8	0.9
Total	100	68.2	48.5

[1]In both concentrate and isolate runs, starting material was 50 grams of fresh larvae
[2]All data are reported on a moisture-free basis
[3]Although components of other product streams from concentrate and isolate runs were also calculated, they are not reported here because they are considered to be not relevant

and low fat contents of both products indicate that, if larvae protein were indeed to be used for human food, it might be more desirable to utilize it in the form of concentrate or isolate, rather than in the form of larvae.

Functional Properties

Emulsifying and foaming capacity data for larvae proteins is reported in Tables 6 and 7, respectively. It may be seen that both properties were negligible. Since larvae used for these determinations were dried and not fresh, it is possible that drying might have adversely affected protein functionality. On the other hand, because drying was conducted under very mild conditions, precisely with the purpose of minimizing protein denaturation, it is doubtful that it alone could have affected functional properties to a very large extent.

Table 6. Results of determination of emulsifying capacity of proteins of mexican fruit fly larvae (*Anastrepha ludens*)

	Centrifuged Extract (Visual Method)		
pH	5.0	7.0	10.0
Milliliters of oil required to break emulsion	0.0	0.0	0.0
	(Emulsion was not formed in any case)		

Table 7. Results of determination of foaming capacity of proteins of mexican fruit fly larvae (*Anastrepha ludens*)

System	A	B	C	D
Egg white	720	30	98.3	38
Fruit fly larvae				
pH 5	18	42	0.6	0
pH 7	20	43	0.7	0
pH 10	23	45	0.9	0

A: Time required for separation of first drop of liquid from foam (sec)
B: Total beating time for end point (sec)
C: Percent Overrun
D: Percent liquid separated after 30 min

It may be concluded that larvae protein functional properties were probably low *per se*, and not as a result of drying.

Nucleic Acid Content

Concerning nucleic acid content, Table 8 shows that fly larvae contained less RNA than yeasts, but somewhat more than microscopic algae. It is known that consumption of yeasts by humans in large amounts poses problems due to their high nucleic acid content, but that this problem is encountered much less often with microscopic

Table 8. Ribonucleic acid (RNA) content of mexican fruit fly larvae (*Anastrepha ludens*) and other protein sources (Waslien 1970)

Source	Percent RNA
Yeasts	
Saccharomyces cerivisiae (Commercial sample)	7.0
Saccharomyces cerivisiae (Laboratory sample)	4.2
Saccharomyces carlsbergensis	3.5
Candida utilis	4.4
Candida tropicalis	3.6
Algae	
Spirulina maxima	0.6
Mexican Fruit Fly Larvae	
Anastrepha ludens	1.9

algae (Nugent 1965; Young 1970; Waslien 1971). Based on this data, it is not clear whether utilization of fly larvae as a human food would pose a problem, so that further research would be required.

SUMMARY AND CONCLUSIONS

It has been established that on a wet basis, Mexican fruit fly larvae contain less protein than do most common fresh animal protein foods. On a dry basis, however, the protein content is fairly high. Mexican fruit fly larvae exhibit a large amount of fat, which is nearly equal to the protein content.

Larvae protein is only of intermediate quality, comparable to that of common oilseeds and legumes, but lower than that of most common animal protein foods. This intermediate protein quality is reflected by PER and NPU values and also by amino acid analysis, which shows the protein to be limited in a number of essential amino acids. Fly larvae protein, on the other hand, is high in lysine and methionine, which are two essential amino acids which are limited in most cereals and legumes, respectively.

The protein solubility curve is typical in its shape, and exhibits minimum solubility at pH 5 and maximum solubility at pH 10. Protein concentrates and isolates can be prepared from larvae with good protein recoveries and fairly high protein contents on a wet basis.

Larvae protein show negligible foaming and emulsifying capacities. Finally, larvae contain less RNA than yeasts, but more than microscopic algae.

It may be concluded that as a food, Mexican fruit fly larvae would be a source of protein of intermediate quality and quantity, and should probably not be used as such and alone. On the other hand, they could be advantageously used to complement cereal and legume foods with lysine and methionine, respectively. In this regard, it would probably be more desirable to use the protein concentrate or isolate, rather than the original larvae. If this were done, however, it would be necessary to conduct more research on larvae nucleic acid content which, although not too high, is not low enough to exclude the possibility of a problem.

Finally, it is also concluded that as a source of functional protein, Mexican fruit fly larvae would definitely be poor.

ACKNOWLEDGMENT

The authors wish to express their gratitude to the United States Department of Agriculture Regional Laboratory in Monterrey, Mexico, for providing all fly larvae used in this work.

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MOISTURE MOBILITY IN FRANKFURTER DURING THERMAL PROCESSING: ANALYSIS OF MOISTURE PROFILE

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Received for Publication February 9, 1982

ABSTRACT

This paper presents a determination of the moisture distribution in meat emulsion sausage of cylindrical geometry at several process times as a function of composition, process temperature and relative humidity, with a technique and model for determining the corresponding moisture mobility. Simultaneous heat and mass transfer model with Fickian type liquid diffusion predicted the temperature and moisture profiles. The combined normalized standard deviation (σ_p) for four moisture profiles and five temperature histories vary between 0.08 and 0.25. The moisture diffusivity follows Arrhenius type relationship and was a function of concentration, temperature and composition of the product.

INTRODUCTION

Analysis of the fundamental physical processes and the determination of those physical properties important for the water removal process, provide valuable information as to how to prevent undesirable processes leading to impairment of quality. Carefully controlled thermal processes are necessary to develop the initial color, characteristic skin and internal texture, and eliminate trichinella and microbial populations without emulsion destabilization, excessive moisture loss or texture changes. Little work has been conducted on

[1]Salaries and research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, the Ohio State University, Journal Article 82-

the mobility and states of water during thermal processing of meat emulsion products.

In a previous paper (Mittal *et al.* 1981), the mobility of moisture in meat emulsion was reported for low temperatures ($< 58°C$) and a slab geometry. It was not possible to investigate the movement of moisture during cooking at high temperatures because of the substantial amount of moisture loss from the bottom and middle of the slab in addition to that at the top surface. This was due to heat associated changes and shrinkage of the product in contact with aluminum ring. Therefore, a cylindrical shape (frankfurter), in which the flux of moisture would be unidirectional radially outward, was used to study moisture mobility at higher temperatures.

The objective of this work was to assess and predict moisture and temperature distribution in frankfurters as a function of composition, relative humidity, process time and temperature above 55°C.

MATERIALS AND METHODS

A central composite rotatable design was employed for the experiments (Cochran and Cox 1957). For three factors this design consisted of 20 experiments i.e. 8 vertices of a cube (2^3 factorial), 6 center points for replication and 6 star points to give the design the property of being rotatable. Since heat and mass transport parameters follow Arrhenius relationship, the linear coordinate for temperature was chosen as the inverse of absolute temperature. Similarly, the linear coordinates for FP and RH were their logarithmic values. Table 1 presents the variables and their actual levels obtained after transforming them for these investigations. The order of experiments was completely randomized.

Fresh emulsion was prepared before starting each experiment in the laboratory in a modified Waring Blendor (Mittal 1979). The formulation included nonfat dry milk (3%), salt (2.5%), sucrose (0.5%), corn syrup solids (2%), spices and curing agents. Protein, fat and water were added according to the desired fat protein ratio (FP). Most of the fat was taken from the pork meat and protein from the lean beef. The viscosity of the emulsion was used as an index of the extent of chop.

A closed loop wind tunnel was used to provide the controlled environment. The wind tunnel consisted of a fan, heating and cooling sections, steam injection system, drying column and a test section (Mittal *et al.* 1981). The temperature was controlled by proportional rate and integral modes to within ±1°C. The humidity was controlled

Table 1. Variables and their levels for experiments

Variable	Variable Levels				
	-1.68	-1.0	0.0	1.0	1.68
Temperature (°C)	58	63	69	76	81
Relative humidity (%)	41	48	60	75	87
Fat-protein ratio	1.2	1.4	1.9	2.5	3.0

to within ±1% by injecting steam and venting the circulating air. The airflow was controlled within ±0.01 m/s with an orifice, a pressure transmitter and a flow recorder-controller to regulate a butterfly valve.

Meat emulsion was stuffed into polyethylene casing of 2.5 cm diameter with the help of a hand stuffer. Five nearly identical test frankfurters of about 15.2 cm length and of same weight were suspended in the test section with a frame of galvanized wires. Into one frankfurter, four copper constantan thermocouple probes were inserted axially at different locations. The probes were extended 7.6 cm through a plastic stopper which was inserted at the top of the casing as a thermocouple position guide. To prevent moisture losses from the sides of plastic stopper, the sides and holes were coated with a high vacuum grease.

Four other frankfurters were used for the determination of moisture profiles and associated dimensional changes during the different stages of cooking. These moisture profiles were measured at about 4, 7, 10 and 13% moisture losses. The dimensional changes were determined by measuring the length and diameter.

After the loss of desired moisture, the entire frankfurter was chilled immediately into the acetone dry ice solution for quick freezing. The acetone dry ice solution was maintained below -45°C in a Cryocool cooler. In preliminary tests, we checked that no mass transfer occurred under the freezing method used. This seems to be due to the presence of sausage casing and minimal freezing time. A frozen disk from the central 2 cm was then divided into ten thin radial sections with stainless steel cork borers of different diameters. Six sections were of R/8 thickness and taken from the inner portion of the frozen disk. Four more sections from outer layer were of R/16 thickness. The moisture content of these thin rings were then determined by oven method employing ASAE standard S353 (Baxter and Hahn 1979). The

moisture profile was developed by assigning the mean moisture content (dry basis) to the mean location on the respective slice.

The equilibrium moisture contents of the meat emulsion spread in thin aluminum foil discs were recorded for different processing conditions and formulations.

MATHEMATICAL MODELING

The following assumptions were made to define the process mathematically:

1. An infinitely long (L/(2R) ratio $>$ 5.5), and homogeneous cylinder.
2. Uniform initial temperature and moisture distributions.
3. Constant thermal properties of emulsion.
4. A transportation mechanism of liquid diffusion followed by a vaporization at the surface was considered. Earlier pure vapor diffusion and the liquid diffusion followed by vaporization at various nodes were also considered. The comparison of predicted and observed moisture profile values provided the base for above assumption.
5. Negligible shrinkage: The data on shrinkage were collected. The changes in length were 0.0 to 5.0%, and the changes in diameter were ±7%. The shrinkage data were found to have no correlation with the composition of the product and processing conditions. This might be due to the uncontrolled stuffing pressure applied by hand stuffing. However, the density of the raw frankfurters was kept at 965±6 kg/m^3.
6. Ambient air temperature and moisture to be a step function of time.
7. An infinite mass transfer coefficient at the surface. The mass transfer Biot number $\geqslant 10$; the air velocity was 1.7 m/s in the test section for this purpose.

Based on these assumptions, the mathematical model characterizing simultaneous heat and mass transfer can be represented as follows:

$$\frac{\delta m}{\delta t} = \frac{1}{r} \frac{\delta}{\delta r} \left(D_m \cdot r \frac{\delta m}{\delta r}\right) \tag{1}$$

$$\frac{\delta T}{\delta t} = \alpha\left(\frac{\delta^2 T}{\delta r^2} + \frac{1}{r} \frac{\delta T}{\delta r}\right) \tag{2}$$

for the initial conditions:

$$T(0,r) = T_o$$
$$m(0,r) = m_o \tag{3}$$

and the boundary conditions:

$$\left.\frac{\delta T}{\delta r}\right|_{r=0} = 0, \quad \left.\frac{\delta m}{\delta r}\right|_{r=0} = 0$$
$$\left.\frac{\delta T}{\delta r}\right|_{r=R} = \frac{-h_T}{K_h}(T_s - T_a) + \frac{D_{m,10}\,\rho_D}{K_h} \cdot L_v \left.\frac{\delta m}{\delta r}\right|_{r=R} \tag{4}$$
$$m(R,t) = m_e$$

where $D_{m,10}$ is the mass diffusivity at the surface node. Other symbols are defined at the end of the paper.

Nondimensional form of the model

Taking as nondimensional variables:

$$C = \frac{m-m_e}{m_o-m_e} \qquad \theta = \frac{T-T_o}{T_a-T_o} \tag{5}$$

$$\psi = r/R. \qquad Bi_H = \frac{h_T R}{K_h} \tag{6}$$

$$\lambda_1 = \frac{D_{m,10}\rho_D L_v}{K_h} \frac{m_o-m_e}{T_a-T_o} \tag{7}$$

and substituting in Eq. 1 to 6, one can obtain:

$$\frac{\delta\theta}{\delta t} = \frac{\alpha}{R^2}\left(\frac{\delta^2\theta}{\delta\psi^2} + \frac{1}{\psi}\frac{\delta\theta}{\delta\psi}\right)$$
$$\frac{\delta C}{\delta t} = \frac{1}{\psi R^2}\frac{\delta}{\delta\psi}\left(D_m\psi\frac{\delta C}{\delta\psi}\right) \tag{8}$$

or in expanded form

$$\frac{\delta C}{\delta t} = \frac{1}{R^2}\left[\frac{\delta D_m}{\delta\psi}\cdot\frac{\delta C}{\delta\psi} + \frac{D_m}{\psi}\frac{\delta C}{\delta\psi} + D_m\frac{\delta^2 C}{\delta\psi^2}\right] \tag{9}$$

$$\theta(0,\psi) = 0, \qquad \left.\frac{\delta\theta}{\delta\psi}\right|_{\psi=0} = 0 \tag{10}$$

$$\left.\frac{\delta\theta}{\delta\psi}\right|_{\psi=1} = -Bi_H(\theta_{\psi=1}-\theta_a) + \lambda_1 \left.\frac{\delta C}{\delta\psi}\right|_{\psi=1} \tag{11}$$

$$\left.\frac{\delta C}{\delta\psi}\right|_{\psi=0} = 0, \qquad C(1,t) = 0 \tag{12}$$

In the above equations, time (t) was kept in dimensional form to simplify the equations. To solve with digital computer employing the Continuous System Modeling Program (CSMP), the space coordinate was eliminated as a variable by dividing the cylinder into ten concentric shells. The moisture and temperature ratio profiles within each shell were approximated by a parabola (Hamdy and Barre 1969).

The thickness of shells were taken variable to accomodate real slice thickness with smaller thicknesses at the outer portion of the cylinder to take care of computational instabilities due to the sharp slope of concentration curve.

The nondimensional parabolic approximations for moisture concentration and the corresponding moisture ratios of the shells are given by Mittal (1979). The latter was used to calculate average concentration. The derivative of diffusivity with respect to distance was solved by a backward difference method.

The thickness of shells for temperature simulation were taken to be of equal size. Only four thermocouples were inserted in the frankfurter at different locations, as temperature gradients were relatively small.

The following set of equations was obtained after substituting above mentioned equations in 8 and 9. The equations of temperature ratios were same as derived by Agrawal (1976):

$$\frac{dC_1}{dt} = \frac{D_{m,1}}{R^2}\, 128(C_2 - C_1) \tag{13}$$

$$\left.\frac{dC_i}{dt}\right|_{i=2 \text{ to } 5} = \frac{32}{R^2}\left\{D_{m,i-1}(C_{i+1}-C_{i-1}) + D_{m,i}\left(\frac{2i+1}{2i-1}\, C_{i+1} - 4C_i + \frac{6i-5}{2i-1}\, C_{i-1}\right)\right\} \tag{14}$$

$$\frac{dC_6}{dt} = \frac{64}{231R^2}\left\{11\, D_{m,5}(16C_7-9C_5-7C_6) + D_{m,6}\cdot (208C_7 + 345C_5 - 553C_6)\right\} \tag{15}$$

$$\frac{dC_7}{dt} = \frac{2048}{375R^2}\left\{8.333D_{m,6}\,(2.25C_8-C_6-1.25C_7) + D_{m,7}(21C_8 + 32.3C_6 - 53.3C_7)\right\} \quad (16)$$

$$\frac{dC_8}{dt} = \frac{128}{27R^2}\left\{27D_{m,7}\,(C_9-C_7) + D_{m,8}\,(29C_9 + 79C_7 - 108C_8)\right\} \quad (17)$$

$$\frac{dC_9}{dt} = \frac{128}{29R^2}\left\{29D_{m,8}\,(C_{10}-C_8) + D_{m,9}\,(31C_{10} + 85C_8 - 116C_9)\right\} \quad (18)$$

$$\frac{dC_{10}}{dt} = \frac{256}{93R^2}\left\{-31\,D_{m,9}\,(C_9+3C_{10}) + D_{m,10}\,(153C_9-285C_{10})\right\} \quad (19)$$

$$\frac{d\theta_1}{dt} = \frac{200\alpha}{R^2}\,(\theta_2 - \theta_1) \quad (20)$$

$$\left.\frac{d\theta_i}{dt}\right|_{i=2-9} = \frac{100\alpha}{R^2}\left\{\frac{2i}{2i-1}\,\theta_{i+1} - 2\theta_i + \frac{2i-2}{2i-1}\,\theta_{i-1}\right\} \quad (21)$$

$$\frac{d\theta_{10}}{dt} = \left\{\frac{100}{R^2}\;\frac{160}{57}\,\theta_s - \frac{234}{57}\,\theta_{10} + \frac{74}{57}\,\theta_9\right\} \quad (22)$$

$$\theta_S = (9\theta_{10} - \theta_9 + 0.3Bi_H + \lambda_1(8-9C_{10} + C_9))/(8 + 0.3\,Bi_H) \quad (23)$$

$$\bar{\theta} = \frac{1}{2400}\left\{22\theta_1 + 72\theta_2 + 120\theta_3 + 168\theta_4 + 216\theta_5 + 264\theta_6 + 312\theta_7 + 360\theta_8 + 391\theta_9 + 475\theta_{10}\right\} \quad (24)$$

$$\bar{C} = \frac{1}{11520}\left\{165C_1 + 540C_2 + 900C_3 + 1260C_4 + 1633C_5 + 1854C_6 + 1254C_7 + 1212C_8 + 1250C_9 + 1452C_{10}\right\} \quad (25)$$

To analyze moisture profiles, the diffusivity was considered to be a function of product temperature and moisture concentration. The

following Arrhenius model was considered appropriate based on the experience with the emulsion slab moisture profile analysis (Mittal *et al.* 1981).

$$D_m = \text{Exp}\ (-A_1 + A_2/T_{abs} + A_3C) \tag{26}$$

To determine the coefficient of this model (A_1, A_2 and A_3) giving the best fit of the predicted values to observed data, the criterion was taken as the minimum sum of normalized standard deviation σ_p between computed and observed values of moisture and temperature profiles. It was calculated by:

$$\sigma_P = \frac{1}{S.N}\sum_{i=1}^{S}\sum_{n=1}^{N}(C_{n,i,exp} - C_{n,i})^2 + \left(\frac{1}{NT.t}\int_0^t \sum_{n=1}^{NT}(\theta_{n,exp} - \theta_n)^2 dt\right) \tag{27}$$

Initially, the multi-dimensional optimization program (Hooke and Jeeves 1961) was applied to determine the constants of Eq. 26, for three central experimental conditions. For all other conditions, the average values of A_2 and A_3 were used to reduce computational costs. The values of these constants were approximately equal to the values obtained from the slab moisture profile analysis (Mittal *et al.* 1981). A one dimensional optimization program was then used to calculate the optimum values of A_1 for other test conditions.

RESULTS AND DISCUSSION

Equilibrium Moisture Content

Equilibrium moisture contents of the meat emulsion at various conditions and compositions were recorded. These values were used to calculate moisture ratios from average moisture contents. The Chung and Pfost (1967) equation with a term for FP gave the best fit. The following model was obtained with Nonlinear Regression Analysis Procedure of the Statistical Analysis System (SAS) (Barr *et al.* 1976), giving a coefficient of determination of 0.965.

$$m_e = -0.102\ ln(-R_g\ .\ FP\ .\ (T+5.665)\ .\ ln(RH)/1.132E7) \tag{28}$$

Iglesias and Chirife (1976) described the literature data for 220 food isotherms comprising 69 different materials. Desorption and adsorp-

tion isotherms were described for a few meats such as beef, pork, chicken and trout for constant FP. However, no data was available for meat products at various FP.

Moisture Profile

The moisture profile plot during four stages of cooking in a non-dimensional moisture ratio and distance format is illustrated in Fig. 1. This is for the central point of the design. These moisture profiles indicate that most of the water was lost from outer layer rather than the remote interior of the product, since the processing time does not normally exceed that associated with time ratio ($\pi D_m t/r^2$) of 0.1. The maximum value of time ratio in these experiments was 0.12. However, it is not possible to calculate this ratio accurately due to the variation in D_m with time.

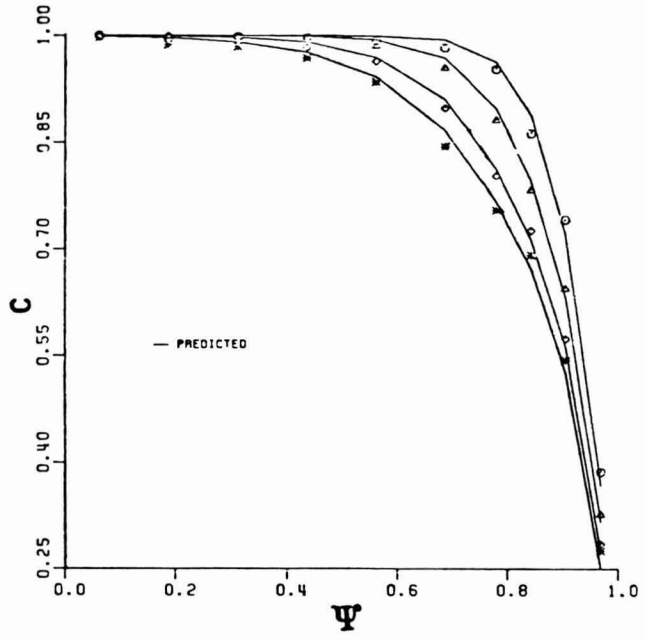

FIG. 1. COMPARISON OF EXPERIMENTAL AND PREDICTED MOISTURE PROFILES IN FRANKFURTER OF 1.9 FP DURING COOKING AT 69°C AND 60% RH

o — o 4% of moisture loss after 0.7 h, △ — △ 7% points of moisture loss after 1.2 h, ◇ — ◇ 10% points of moisture loss after 2.2 h, and * — * 13% points of moisture loss after 3.0 h.

The moisture profiles are very steep and large moisture gradients exist near the surface. It may be associated with the surface skin formation in frankfurters. Migration of solutes primarily due to the bulk flow of the solvent to the surface of frankfurter might also be associated with case hardening. The concentration of moisture at the center of frankfurter has changed little even after the removal of about 13% moisture (wet basis).

Figure 2 shows the nondimensional moisture ratio history at different nodes. These also indicate that the concentration profiles are quite steep in the first 4% moisture loss period especially nearer the surface. The slopes of these curves are decreasing with time. No appreciable moisture was lost from the central node of the frankfurter during the whole cooking process.

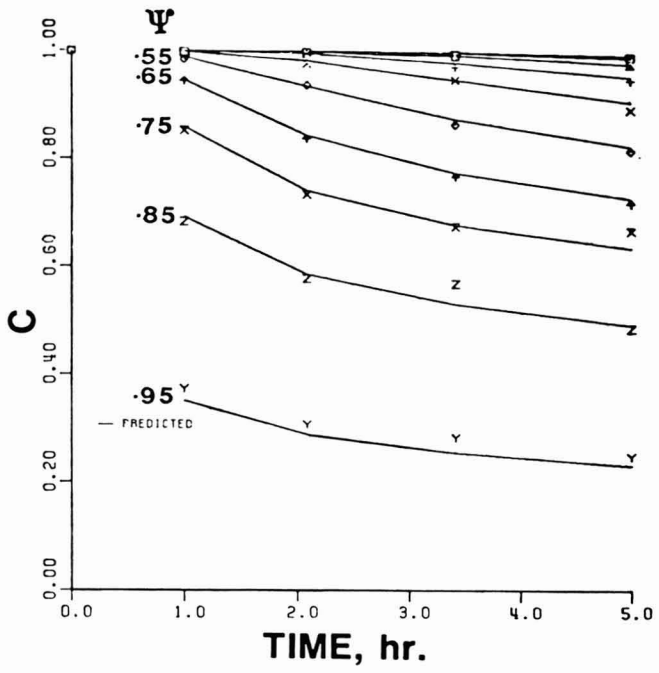

FIG. 2. EXPERIMENTAL AND PREDICTED MOISTURE RATIOS AT DIFFERENT LOCATIONS IN FRANKFURTER OF 1.9 FP DURING COOKING AT 58°C AND 60% RH

The 4% points of moisture loss after 1.0 h, 7% points of moisture loss after 2.1 h, 10% points of moisture loss after 3.6 h, and 13% points of moisture loss after 5.1 h.

The work of Hamm (1966) indicates that the water is released from protein at temperatures around 50°C. However, water that is released from the protein network will not necessarily flow to the surface of the muscle sample unless there is some mechanical or thermodynamic force applied to the protein structure: e.g. capillary, gravitational, diffusional and mechanical forces. Not enough water was transported from the central portion of the frankfurter due to low moisture diffusivity and short processing time.

Some water soluble protein and ions are carried by the moisture as it moves from the emulsion to the surface of the sausage. When these are left behind by the evaporating moisture, the insouble matrix shrinks due to reduction in moisture. Thus a protein skin is formed at the surface. The skin formation is also responsible for a larger moisture gradients near the surface of the product. This skin is desired for less moisture loss and better peeling of the casing.

Moisture Diffusivity

The model of simultaneous heat and mass transfer was used to predict moisture and temperature profiles. The values of A_1 giving minimum values of σ_p were determined for different processing conditions and composition of the product. The A_1 was found to be a function of the FP. The complete model of moisture diffusivity is given below:

$$D_m = \text{Exp}(8.6787 + 0.1348FP - 4341.5/T_{abs} + 8.55C) \qquad (29)$$

The rationale for selecting this model was discussed in detail by Mittal *et al.* (1979). This was based on Absolute Reaction Rate Theory and Arrhenius relationship. It is known that moisture diffusivity depends strongly on temperature and often very strongly indeed on the moisture concentration. Earlier Palumbo *et al.* (1977), and Jason (1958) found that fat is hydrophobic and retards the movement of moisture.

From the moisture diffusivity model it is clear that the values of diffusivity are increasing with the increase in moisture ratio exponentially. These diffusivity values are increasing with the increase in temperature, which is in accordance with the Arrhenius relationship. The activation energy is 36.1±2.3 kJ/mole for the moisture diffusion process. This activation energy is higher than 30.1 kJ/mole obtained by Jason (1958) for the dehydration of fish muscle and 26.4 kJ/mole value computed by Fish (1958) for the diffusion of water in starch gel.

Bruin and Luyben (1980) quoted the values of the activation energy for diffusion of a few food materials. For skim milk, it varies from 22 to 37 kJ/mole for the moisture content of 1.0 and 0.2, respectively. However, it is difficult to compare these values with the values obtained in this study due the variation in product characteristics.

Figures 1 and 2 show that the predicted moisture profiles compare well with the experimental values. The σ_p values computed for four moisture profiles and five temperature histories vary from 0.084 to 0.246.

Temperature Ratio Profile

The temperature ratios at experimental nodes were simulated by the simultaneous heat and mass transfer model. The computed temperature ratio at experimental node, n, falling in the ith shell was determined from computed temperature ratio at theoretical nodes with the parabola equation for ith shell.

Figure 3 illustrates the observed and predicted temperature ratios at four locations in a frankfurter during cooking at the central point of the design. The normalized standard deviation between predicted and observed temperature ratios varied between 0.05 and 0.14 for 20 experiments. Some deviations are due to the assumption of constant thermal diffusivity and latent heat of vaporization. Variation in environment conditions, i.e. temperature and relative humidity during experiment and the conduction of heat through temperature probe, are also responsible for these deviations. Dislocation of temperature probes in the frankfurter can also change temperature observations.

The temperatures at all the four locations increase at a rate of about 1.5°C/min in the beginning. After a steep rise, the temperatures increased very little to a level above wet bulb temperature, but below the air dry bulb temperature. There was negligible temperature gradient inside the frankfurter after the steep rise in temperature.

The temperatures inside the frankfurter as a function of time and radius were defined by a polynomial of third degree by Rao and Webb (1976) for one composition. They did not solve the heat and mass transfer equation to describe temperature profiles.

The values of the thermophysical properties of meat emulsion used in the simulation are tabulated in Table 2. The assumption of constant thermal properties with respect to moisture concentration seems reasonable in view of the small range of moisture concentration involved in the frankfurter processing.

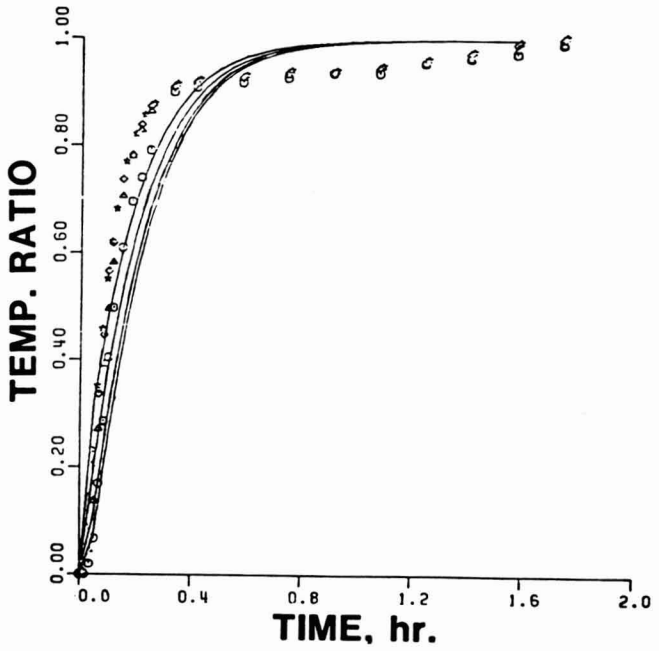

FIG. 3. PREDICTED AND EXPERIMENTAL TEMPERATURE RATIOS IN FRANKFURTER OF 1.9 FP DURING COOKING AT 81°C AND 60% RH

ψ = 0.0, 0.4, 0.6, and 0.9 for lower to upper curves, respectively.

Table 2. Thermophysical properties of frankfurter

Thermal Conductivity	1.55 KJ/(hr.m.K)	Agarwal 1976)
Specific Heat	3.39 KJ/(kg.K)	(Riedel 1956)
Latent Heat of Vaporization	2326 KJ/kg	
Heat Transfer Coefficient	Function of Temperature	(Kern 1950)
Density	965. kg/m^3	
Thermal Diffusivity	$4.76\times10^{-4}m^2/h$	

For the moisture profile analysis, ten samples of moisture concentration were taken and thickness of these concentric cylindrical samples were smaller at the outer side of the frankfurter. The moisture diffusivity values obtained here should give a better understanding of the moisture mobility in the meat emulsion at high temperatures processing. The techniques developed here can be used for analyzing the moisture transport behavior in other food materials during thermal processing.

CONCLUSIONS

1. Simultaneous heat and mass transfer model with liquid diffusion predicts the temperature and moisture profiles in the product during cooking.
2. The moisture diffusivity is a function of concentration, composition and temperature of the product.
3. The moisture profiles are very steep and large moisture gradients exist near the surface of the frankfurter and are associated with the surface skin formation. There is a negligible temperature gradient inside the frankfurter after the steep rise in temperature.
4. The moisture diffusivity follows the Arrhenius type of relationship with respect to product temperature.

LIST OF SYMBOLS

Bi_H	Biot modulus of heat transfer, h/K
C	concentration of water, dimensionless
D_m	diffusivity of water, m^2/h
FP	fat protein ratio
K_h	thermal conductivity, kJ/(h.m.K.)
L_v	latent heat of vaporization of water, kJ/kg
N	number of stages of cooking
NT	number of experimental nodes in temperature ratio profiles
R	radius of frankfurter, m
R_g	gas constant, J/(mole.K)
RH	relative humidity, %
S	number of stages of cooking
T	temperature, °C
h_T	heat transfer coefficient, $kJ/(h.m^2.K)$
m	moisture content, fraction dry basis
r	radial distance, m
t	time, h
α	thermal diffusivity, m^2/h
ρ_D	density of dry matter, kg/m^3
θ	temperature ratio
σ_p	normalized standard deviation between computed and observed values
λ_1	dimensionless ratio of latent heat to sensible heat
ψ	nondimensional distance, r/R

Subscript

1-10	node number
a	air
abs	absolute
e	equilibrium
exp	experimental
o	initial
s,S	surface

Superscript

—	average

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BOOK REVIEW

Developments in Meat Science—2 Edited by R. A. Lawrie. Applied Science Publishers Ltd. London. 1981.

Developments in Meat Science—2 is a welcome edition to the meat literature. The eight different chapters in the volume represent the following topics and authors: (1) doubling muscleing by Boccard; (2) connective tissue by Sims and Bailey; (3) stress by Lister, Gregory and Warriss; (4) postmortem changes affecting comminuted meat products by Hamm; (5) freezing by Calvelo; (6) intermediate moisture meats by Ledward; (7) determination of connective tissue free muscle protein by Olsman and Slump; (8) meat and health by Pearson.

As a general comment, it must be said that the articles are readable and informative and the overall volume is of very high quality. As a mild criticism, one might suggest that the authors could have included more information on the extremely important area of poultry products technology and also fish technology in their papers. Meat science, as defined by some researchers anyway, is meant to encompass both poultry and fish foods as well as red meats.

In his article on doubling muscling in cattle, Boccard comprehensively reviews the literature concerning this condition. An interesting point brought out by the author is that the stress susceptibility of double muscled animals results in higher levels of plasma creatine phosphokinase and this fact has been suggested as a possible test for the evaluation of the genetic predisposition for double muscling. The subject of connective tissue is given a rigorous review by Sims and Bailey; however, the authors might have considered covering a few topics of interest to scientists working in the meat industry. For example, the pioneering study of Machlik and Draudt, J. Food Sci. *28*(6), 711, (1963), on collagen shrinkage and gelatin formation and their relationship to tenderness, was not included in an otherwise very complete review. An omission such as this one may not detract from the importance of this work to the basic researcher but diminishes the value of the paper with respect to its use as a teaching reference. Of course, the problem faced by all of the authors is one of attempting to distill down massive amounts of literature into a reasonably short article. The contribution on stress in meat animals by Lister, Gregory and Warriss exemplifies this problem. Perhaps no area in meat science has been a greater volume of scientific publication over the past 20 years. This problem may be sufficient in itself to

explain the omission of information concerning the genetics of the porcine stress syndrome and the effects of breeding programs on carcass musculature and quality. One could also argue that the halothane test is not particularly suitable for stress susceptibility diagnosis by individual farmers. Simpler methods, such as blood CPK, are easy to accomplish by the individual operator of a small farm.

In his article on the effects of postmortem changes in muscle on properties of comminuted meat, Hamm presents a very thorough review of work done by he and his coworkers and other investigators on the area of ATP depletion and pH decline postmortem and the influence of salting, freezing and freeze-drying. These are exciting areas in which the end result may be the development of new types of sausage materials which have extremely high water holding capacity and, in some cases, a very long shelf life (as in the case of freeze-dried, prerigor meat).

As stated earlier, all of the remaining articles are very useful and of very high quality. Some comments will be made about the article on the timely subject of meat and health by Pearson. This article, covering a very controversial subject, is done with a great deal of objectivity in my opinion. The author is to be complimented on being very up-to-date on some exciting new areas such as cholesterol oxidation and its potential role in atherosclerosis. Pearson thoroughly reviews and interprets the findings of Bruce Taylor that native cholesterol is not able to induce angiotoxic effects and the previously-ascribed blood vessel damage can now be attributed to the fact that cholesterol preparations contained oxidized forms of cholesterol and that these forms, not native cholesterol, were angiotoxic. This is welcome news for the meat industry. Nevertheless, research is needed to establish whether or not some cholesterol oxidation derivatives may be found in certain products. Pearson points this out, quoting Taylor's suggestion that some types of cured and smoked meats might contain cholesterol oxides. However, if it is considered that smoke is an antioxidant, that many of these products also contain phosphates and nitrite, two other substantial antioxidants, it is more probable that only those products which are partially dried and stored for a long period of time at elevated temperatures are likely to show oxides of cholesterol. Nevertheless, this area needs to be studied.

Pearson's discussion of meat and cancer is also very up-to-date and thorough, given constraints that he is under.

In conclusion, *Developments in Meat Science—2* edited by Ralston Lawrie, is a significant contribution to the meat science literature. Its

application in teaching would be mostly at the graduate level and its use in the meat industry would probably be more limited to those companies large enough to support some sort of basic research program. Nevertheless, it is full of up-to-date information.

PAUL B. ADDIS

MEETING

RECENT DEVELOPMENTS IN FOOD ANALYSIS
Focus on Food Science Symposium IV

Friday, September 24, 1982

Program

Morning Session: Big 8 Room—K-State Union

Presiding: Dr. F. E. Cunningham

8:30	Registration and coffee
9:00	Opening Remarks
9:20	Objective Measurement of Food Texture
10:00	Water Activity in Foods
10:30	Coffee Break
11:00	Use of Microprocessors in Food Analysis
11:30	Near Infared Reflectance Analysis
12:00	Luncheon—Flint Hills Room, K-State Union

Afternoon Session: Big 8 Room, K-State Union

Presiding: Dr. Richard Bassette

1:35	Computerized PER Values
2:00	Color: Reflections and Transmissions
2:30	Differential Scanning Calorimetry of Starch
3:00	New Rapid Methods for Moisture and Fat Analysis
3:30	Closing Remarks
3:45	Announcements and Adjournment

F
N
P

JOURNALS AND BOOKS IN FOOD SCIENCE AND NUTRITION

Journals

JOURNAL OF FOOD SERVICE SYSTEMS, G. E. Livingston and C. M. Chang

JOURNAL OF FOOD BIOCHEMISTRY, H. O. Hultin, N. F. Haard and J. R. Whitaker

JOURNAL OF FOOD PROCESS ENGINEERING, D. R. Heldman

JOURNAL OF FOOD PROCESSING AND PRESERVATION, T. P. Labuza

JOURNAL OF FOOD QUALITY, A. Kramer and M. P. DeFigueiredo

JOURNAL OF FOOD SAFETY, M. Solberg and J. D. Rosen

JOURNAL OF TEXTURE STUDIES, P. Sherman and M. C. Bourne

Books

ANTINUTRIENTS AND NATURAL TOXICANTS IN FOOD, R. L. Ory

UTILIZATION OF PROTEIN RESOURCES, D. W. Stanley, E. D. Murray and D. H. Lees

FOOD INDUSTRY ENERGY ALTERNATIVES, R. P. Ouellette, N. W. Lord and P. E. Cheremisinoff

VITAMIN B_6: METABOLISM AND ROLE IN GROWTH, G. P. TRYFIATES

HUMAN NUTRITION, 3RD ED., R. F. Mottram

DIETARY FIBER: CURRENT DEVELOPMENTS OF IMPORTANCE TO HEALTH, K. W. Heaton

RECENT ADVANCES IN OBESITY RESEARCH II, G. A. Bray

FOOD POISONING AND FOOD HYGIENE, 4TH ED., B. C. Hobbs and R. J. Gilbert

FOOD SCIENCE AND TECHNOLOGY, 3RD ED., M. Pyke

POSTHARVEST BIOLOGY AND BIOTECHNOLOGY, H. O. Hultin and M. Milner

THE SCIENCE OF MEAT AND MEAT PRODUCTS, 2ND ED., J. F. Price and B. S. Schweigert

GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title, which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom correspondence should be sent.

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

References: References should be given in the text by the surname of the authors and the year. *Et al.* should be used in the text when there are more than two authors. All authors should be given in the References section. In the Reference section the references should be listed alphabetically. See below for style to be used.

DEWALD, B., DULANEY, J. T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology*, Vol. xxxii, (S. Fleischer and L. Packer, eds.) pp. 82–91, Academic Press, New York.

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Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. The title of the table should appear as below:

Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids

Description of experimental work or explanation of symbols should go below the table proper.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) of author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

Acknowledgments: Acknowledgments should be listed on a separate page.

Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the scientific literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

EDITORIAL OFFICE: Prof. T. P. Labuza, Editor, Journal of Food Processing and Preservation, University of Minnesota, Department of Food Science and Nutrition, Saint Paul, Minnesota 55108 USA

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