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To the Graduate Council:

I am submitting herewith a thesis written by Pathur Jagadeesh Reddy entitled "Enzymatic Peeling of Shrimp." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

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ENZYMATIC PEELING OF SHRIMP

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Pathur Jagadeesh Reddy

August 1971

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ABSTRACT

In general, shrimp are peeled by hand, but a limited amount of peeling is done by machine. Both operations are expensive. In addition, the inadequacy of available hand labor is becoming an acute problem. For these reasons, newer innovations of peeling are being sought. These methods include the use of chemicals and enzymes. Experimental results have been quite satisfactory. In studies using enzymes, 85 to 90 percent of shrimp can be peeled with the tail remaining. Yields of the peeled product do not differ from those resulting from hand peeling. The color of the product is an appealing light pink. The enzymatic treatment has a slight tenderizing effect and shrimp thus treated exhibited as much as a ten-fold reduction in the total plate count of bacteria.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	3
III.	MATERIALS AND METHODS	5
	Experiment I	5
	Selection of enzyme	5
	Activity of the selected enzyme	6
	Activation by cysteine	6
	Maintenance of pH with buffer	7
	Statistical analysis	7
	Experiment II	7
	Yield percentage	8
	Peel percentage	8
	Color measurement	9
	Texture measurement	10
	Microbial analysis	10
	Sensory panel evaluation	11
IV.	RESULTS AND DISCUSSION	13
	Experiment I	13
	Selection of enzyme	13
	Activity of the selected enzyme	13
	Activation by cysteine	18

CHAPTER PAGE

Maintenance of pH with buffer 19
Experiment II 20
Yield percentage 20
Peeling percentage 23
Color 26
Texture 35
Microbiology 35
Sensory panel evaluation 39
Comparison of enzyme-peeled shrimp with hand-peeled shrimp 39
V. SUMMARY 43
BIBLIOGRAPHY 44
VITA 48

LIST OF TABLES

TABLE		PAGE
I.	F-Table Showing Effect of Temperature, pH and Concentration of Enzyme on the Rate of Activity	14
II.	Effect of Temperature, pH and Concentration of Enzyme on Amount of Activity	16
III.	Interaction of Temperature and pH on the Rate of Enzyme Activity ($X10^{-2}$)	17
IV.	F-Table Showing Effect of Temperature, Time and pH on Percentage of Yield	21
V.	The Effect of Temperature, Time and pH on Percentage of Yield	22
VI.	F-Table Showing Effect of Temperature, Time and pH on Percentage of Peeling	24
VII.	The Effect of Temperature, Time and pH on Percentage Peeling	25
VIII.	F-Table Showing the Effect of Temperature, Time and pH on C.I.E. Coordinate "X"	27
IX.	Effect of Temperature, Time and pH of Enzyme Peeled Shrimp on C.I.E. Coordinate "X"	28
X.	Effect of the Interaction of Temperature, Time and pH on C.I.E. Coordinate "X"	
XI.	F-Table Showing Effect of Temperature, Time and pH of Enzyme Peeled Shrimp on C.I.E. Coordinate "Y"	30

TABLE PAGE

XII. Effect of Temperature, Time and pH of Enzyme Peeled Shrimp on C.I.E. Coordinate "Y" 31

XIII. Effect of the Interaction of Temperature, Time and pH on C.I.E. Coordinate "Y" 32

XIV. F-Table Showing Effect of Temperature, Time and pH of Enzyme Peeled Shrimp on Whiteness Index 33

XV. Effect of Temperature, Time and pH of Enzyme Peeled Shrimp on Whiteness Index 34

XVI. F-Table Showing Effect of Temperature, Time and pH on Texture of Shrimp Peeled by Enzymes 36

XVII. Effect of Temperature, Time and pH on Texture of Shrimp Peeled by Enzymes 37

XVIII. Effect of Temperature, Time and pH on Bacteria of the Enzyme-Peeled Shrimp ($X10^3$) 38

XIX. Comparison of Various Quality Factors of Shrimp Peeled by Enzymes (Treated) at 40°C for 40 Minutes at pH 8.5 With Hand-Peeled (Control) Shrimp 40

XX. Effect of Treatment Factors on Sensory Evaluation (Hedonic Scores) on Cooked Shrimp 41

CHAPTER I

INTRODUCTION

The United States is the largest producer and consumer of shrimp. Since 1950 shrimp consumption has increased nearly 6 percent annually. Per capita consumption increased more than 115 percent during the same period (8).[1]

Major determinants of shrimp consumption are (1) the price of shrimp relative to the general price level of consumer goods, (2) the price of competing foods, and (3) personal income levels. The income level is the most influential determinant, accounting for 90 percent of the change in per capita consumption since 1950. Two important observations have been made using disposable income and consumer price index (8):

1. Each one percent gain in per capita real income has been accompanied by a 1.77 percent increase in shrimp consumption.
2. Each one percent increase in the retail price of shrimp has been accompanied by a 0.46 percent decline in consumption.

Technologists should assist the industry in producing a quality product at a more economical price. Several authors (1, 4, 13) have recognized that the major deterrents for expanding the market for shrimp

---

[1]The number in parenthesis represents similarly numbered reference in the bibliography.

were the high cost of hand peeling operations, the high cost of shrimp peeling machinery, the loss of product quality during handling and the lack of ready markets. Thus, new methods of harvesting and processing shrimp are being sought.

The present study was conducted to determine the efficiency of proteolytic enzymes in the peeling of shrimp.

CHAPTER II

LITERATURE REVIEW

The peeling of shrimp with enzymes is a new approach. Little known research has been conducted on this phase of processing. The proteolytic enzyme Rhozyme PF (Rohm and Haas, Philadelphia, Pennsylvania) has been used for eviscerating fish (24). Recently, Fehmerling (12) used several classes of enzymes for separating the shell from the edible meat of numerous marine creatures. The classes include proteolytic, diastatic and cellulose enzymes. Rhozyme P-53 (Rohm and Haas) has been recommended for use on marine products (2). Miyada and Tappel (22) showed that ficin of all the proteolytic enzymes which they studied had the greatest activity on meat. Hale (15) reported that ficin has the most rapid activity on the hydrolysis of fish protein and is suitable for short term hydrolysis. Hale also showed that ficin supplied by various manufacturers had different proteolytic activity levels.

Ficin exhibits activity over a wide range of conditions. Robins (26) found a pH optimum of 5 on gelatin. Winnick et al. (29) found a pH optimum of 7 on casein. Krishnamurthi and Subramanyan (19) found a pH optimum ranging from 6.5 to 7.5 for edestin, egg albumin and hemoglobin hydrolysis. Whitaker (28) reported that ficin has two different peaks of optimum activity, one on casein near pH 6.7 and one near pH 9.5. Cohen (9) found one peak at pH 5.5 and another at pH 9.5 from ficin activity on arginine derivatives.

The action of all enzymes increases concurrently with the increase in temperature up to its optimum temperature. This temperature may vary according to the type of substrate, the pH and the concentration of the enzyme. Whitaker (28) showed that the activity of ficin ranged from 30°C to 63°C, the optimum being in the range of 40°-55°. Hale (15) found the maximum activity of ficin on fish protein at 40°C.

The concentration of commercial ficin preparations required to hydrolyze gelatin ranges from 2 to 8 percent (26). Miyada and Tappel (22) used 0.05 percent ficin for the hydrolysis of beef protein, whereas El-Garbawi and Whitaker (11) showed increased hydrolysis of beef protein at concentrations ranging from 1 to 5 percent. Hinrichs and Whitaker (16) showed that an 8 percent concentration of the enzyme was required to hydrolyze collagen completely. Normally, a 2 percent concentration of ficin is required to hydrolyze about 72 percent of collagen at pH 7 and 35°C temperature within one hour (26).

Ficin is activated by sulfhydryl compounds. Whitaker (28) determined that cysteine, thioglycolic acid, sodium cyanide and versene are the most effective activators. Hydrogen peroxide, iodine, iodoacetic acid and heavy metal salts are some of the inactivators. El-Garbawi and Whitaker (11) concluded that the presence of cysteine and sodium chloride increased the hydrolysis of collagen. Cohen (9) found cysteine to be the most effective activator even at 0.01 M concentration.

CHAPTER III

MATERIALS AND METHODS

I. EXPERIMENT I

Selection of Enzyme

Frozen, raw brown shrimp Penaeus aztecus (43-50 count, shell-on, headless) were bought from a local wholesaler. Before being utilized, they were thawed at room temperature for 6 hours.

The pH of the shrimp was determined on a slurry of 100 grams of shrimp in 200 ml. of distilled water.

One hundred grams (11-12 shrimp) of shrimp constituded a sample. Each shrimp was split dorsally by cutting through the exoskeleton and slightly through the meat just enough to expose the vein. A similar cut was made on the ventral side but only through the exoskeleton. The shrimp were transferred into a Dewar flask containing 300 ml. of distilled water maintained at 50°C. A freshly prepared solution of enzyme was added to the flask after the contents reached temperature equilibrium. The enzyme solutions studied were Rhozyme-PF and Rhozyme-53 (Rohm and Haas, Philadelphia, Pennsylvania) and Enzeco® Protease-J (Enzyme Development Corporation, New York, New York). The concentration of enzyme used was 0.5 percent (W/W) of the weight of the shrimp. The contents of the flask were stirred continuously. The pH was maintained at the natural pH of shrimp (pH 8.5) by titrating with 0.05 N sodium hydroxide, using the Beckman model K automatic titrator.

After one hour of hydrolysis the shrimp were peeled in a test tube basket and agitated for one minute under cold water. The shrimp were drained and the separated shells were removed. The extent of peeling was estimated by examining each shrimp (detailed method is described in Experiment II).

The enzyme preparation which exhibited the highest degree of peeling Enzeco® Protease-J was selected for further investigation.

Activity of the Selected Enzyme

One hundred grams of shrimp were blended for two minutes with 200 ml. of distilled water. Silicone antifoam was added. Fifty-gram samples of the slurry were transferred to 250 ml. beakers which were held in a water bath at 40°, 45°, 50°, and 55°C. The pH was adjusted to 7.5, 8.0, 8.5, and 9.0 by adding either 1N hydrochloric acid or 1N sodium hydroxide.

The enzyme activity was expressed in terms of acidity produced by hydrolysis of protein. The amount of standard sodium hydroxide solution required for titration was recorded. Twenty observations were recorded with one minute intervals between each. Milliequivalents were calculated from these observations.

Activation by Cysteine

The effect of cysteine as an activator was investigated. The hydrolysis of a shrimp slurry was carried out as described previously at pH 8.5 and 50°C, but 1 ml. of a 0.2M cysteine solution was added to

the slurry before adding the enzyme solution. Four samples of each treatment were evaluated.

Maintenance of pH with Buffer

The hydrolysis of a shrimp slurry was conducted at pH 8.5 and at 50°C. Borate buffer of pH 8.5 served as the medium instead of distilled water. The concentrations of buffer used were 0.05, 0.1, 0.2, and 0.3M. Four shrimp samples were hydrolyzed at each borate buffer concentration.

Statistical Analysis

Each factor was analyzed as a factorial arrangement of a completely randomized block design. The effect of time, temperature, and pH was tested for significance by analysis of variance. Significance among means was determined by the Duncan Multiple Range Test. All computations were carried out by IBM 7040 and IBM 360/65 computers, using Bone's (6) computer programs available at The University of Tennessee Computing Center.

II. EXPERIMENT II

One hundred grams of shrimp (11-12 shrimp) were split dorsally and ventrally as described in Experiment I. Then the shrimp were transferred into Dewar flasks containing 300 ml. of 0.2M borate buffer at pH 8.5 and pH 9.0. The incubation temperatures used were 40° and 50°C. A freshly prepared enzyme solution (Enzeco® Protease-J) was added to the flasks after the contents reached temperature equilibrium. The

concentration of the enzyme was 0.5 percent (W/W) of the shrimp. The solutions were stirred continuously.

The shrimp were held at the incubation temperature for 30, 40, 50, and 60 minutes. After each treatment the shrimp were removed and placed in a test tube basket in which they were agitated for one minute. The following observations were made after draining: yield and peel percentages, color, texture, microbial analysis and sensory evaluation (texture, color, and appearance).

Each factor measured was analyzed as a factorial arrangement of a completely randomized block design as in Experiment I.

Yield Percentage

The peeled shrimp were placed on waxed paper and the separated shells were removed. The yield percentage of shrimp was determined by the following equation:

$$\text{percent yield} = \frac{\text{weight after peeling}}{\text{weight before peeling}} \times 100.$$

Peel Percentage

The shrimp were graded by assigning a score to determine the efficiency of the enzyme in removal of the peel. The following scoring procedure was used:

1.00 completely peeled

0.75 three-fourths peeled

0.50 half peeled

0.25 one-fourth peeled

0.00 unpeeled.

The percentage of shrimp peeled was calculated by the following equation:

$$\text{percentage peeled} = \frac{\text{total points}}{\text{number of shrimp}} \times 100$$

Color Measurement

A Color-Eye Colorimeter (IDL, model D-1, Instrument Development Laboratories, Attleboro, Massachusetts) was used to measure the color by reflectance. The instrument was standardized with a barium sulfate plate.

A clear transparent slide was placed over the port to prevent the shrimp from falling inside the instrument. Two shrimp were placed closely together on the slide and the reflectance was measured. The instrument values $\bar{X}$, X_1, Y_1, and Z_1 were converted to the Commission Internationale de l'Eclarage (C.I.E.) system according to the following formulas:

C.I.E. Tristimulus values:

$$X = 0.783 \times X_1 + 0.197 \times \bar{X}$$

$$Y = Y_1$$

$$Z = 1.18 \times Z_1$$

Chromaticity coordinates:

$$x = \frac{X}{X + Y + Z}$$

$$y = \frac{Y}{X + Y + Z}$$

Whiteness index = $X + Y + Z$.

The chromaticity coordinates and the whiteness index were used for statistical computations. The dominant wavelength was obtained to facilitate determination of color, but this was not used in the statistical analysis.

Texture Measurement

Texture was measured in terms of the force required to shear a sample by use of an Allo-Kramer Shear Press (S-2HE). The shrimp were sheared at the second segment with a meat shear cell, using a 100 lb. proving ring and a 21 second stroke. The recorder (E-2EZ) was adjusted to receive the first 10 percent of the proving ring force over the full scale of the recorder. The 10 percent of the proving ring force was adjusted to record 0-10 lb. shear force. The area under the work force curve was measured with a Keuffel and Esser Compensating Polar Planimeter and area values were corrected to actual shear force by dividing by 10 (10 percent shear force expanded in the recorder). These values were used for statistical analysis.

The remainder of the samples not used in the previous determinations were frozen in sterile polyethylene bags for further analysis. The procedure was replicated four times and a control (sample peeled by hand) was run simultaneously with each replication.

Microbial Analysis

The total aerobic bacterial plate count was determined by the APHA (5) method using 11 grams of the sample.

Sensory Panel Evaluation

From the analysis of the previous factors, the following three samples were selected for sensory panel evaluation:

1. 40°C, 45 minutes at pH 8.5;
2. 50°C, 30 minutes at pH 8.5;
3. 40°C, 30 minutes at pH 9.0.

The hand peeled sample was considered the reference sample and given an arbitrary score of 0. The peeled shrimp were cooked in boiling water for three minutes and served to a panel of eight trained members. The panel was composed of students and the faculty members of the Department of Food Technology, The University of Tennessee. They scored the samples for general appearance, texture (mouth feel), and color. A 7-point hedonic score was used (Figure 1).

The panel results were analysed by analysis of variance as described by Kramer and Twigg (18).

SCORING CHART

Name_____ Date_____

Scoring System: Reference = 0 Give 0 score to any sample equivalent to the reference sample

+3 Very much better than reference
+2 Better than reference
+1 Slightly better than reference
0 Reference
-1 Slightly inferior to reference
-2 Inferior to reference
-3 Very inferior to reference

Sample Number	Ref		
Color			
Appearance			
Texture			

Rank according to preference including the reference sample. More than one sample may have the same rank.

First Choice_____

Second Choice_____

Third Choice_____

Fourth Choice_____

Which attribute influenced your choice?

Figure 1

Score Chart Used for Sensory Evaluation

CHAPTER IV

RESULTS AND DISCUSSION

I. EXPERIMENT I

Selection of Enzyme

Proteolytic enzyme Rhozyme PF and Rhozyme P-53 had low activity on shrimp protein. Exposure of shrimp to elevated temperatures for an extended length of time will cause deterioration and loss of quality of the product. Therefore, faster acting enzymes would be better suited for use with shrimp. Thus, Enzeco® Protease-J with a unique mixture of fast acting plant proteases was selected. This enzyme preparation for hydrolysis of the connective tissue present between the exoskeleton and the shrimp meat showed that the enzymes of the mixture exhibited satisfactory results.

Activity of the Selected Enzyme

Proteolytic enzymes hydrolyze protein by breaking the peptide bonds at specific linkages. The amino acids released can be measured by neutralizing with a standard sodium hydroxide solution.

The results of the analysis of Experiment I are presented in Table I. There were significant differences in the effect of temperature and pH, but no difference in the effect of concentration of enzyme on hydrolysis of protein. Probably the substrate was saturated with the

TABLE I

F-TABLE SHOWING EFFECT OF TEMPERATURE, pH AND CONCENTRATION OF ENZYME ON THE RATE OF ACTIVITY

Source	D.F.	Mean Square	F-ratio
A. Temperature	3	13.01	6.32**
B. pH	3	82.87	40.24
A x B	9	4.09	1.99*
C. Concentration	3	1.94	0.94
A x C	9	1.77	0.86
B x C	9	2.79	1.36
A x B x C	27	1.85	0.90
Residual error	1216	2.06	

**Significant at .01 level of probability.

*Significant at .05 level of probability.

enzyme, so that additional enzyme did not increase the rate of activity, indicating that lower enzyme concentrations should have been tried.

The temperature levels studied indicated both heat of activation and heat of inactivation. With a gradual increase in temperature, there was a gradual increase in enzymatic activity. The maximum activity occurred near 50°C, and declined abruptly above 50°C.

A change in the pH of the medium from pH 7.5 to 9.0 increased the enzymatic activity. However, there was no significant difference between pH 8.0 and 8.5 and between pH 8.5 and 9.0. The apparent increase in hydrolysis of protein at pH 9.0 over that at pH 8.5 was probably the result of the additional hydrolysis brought about by sodium hydroxide. The enzyme preparation (Enzeco® Protease-J) was mostly plant proteases (Table II).

An interaction between pH and temperature influenced the optimum levels of enzyme activity significantly (Table III). The combination of the higher pH and lower temperatures caused significantly greater activity than the lower pH and higher temperatures. The hydrolysis of the substrate with H^+ ions requires more activation energy than hydrolysis with OH^- ions (10). The activity of the enzyme was greater at pH 9.0 and 40°C than at pH 8.5 and 50°C. No difference was observed in the activity between the treatments of pH 9.0 at 45°C, and pH 8.5 at 50°C. It appears from the results that the enzyme had an appreciable heat of ionization which varies with the temperature. Whitaker (28) reported that ficin upon heating undergoes two reactions simultaneously, one of

TABLE II

EFFECT OF TEMPERATURE, pH AND CONCENTRATION OF ENZYME ON AMOUNT OF ACTIVITY

Temperature		pH		Concentration	
°C	Mean[1,2] X10^{-2}	Value	Mean[1,2] X10^{-2}	% (W/W)	Mean[1,2] X10^{-2}
40	4.6b[3]	7.5	1.8r	0.5	5.3
45	5.2b	8.0	5.1p	1.0	5.6
50	6.7a	8.5	6.4np	1.5	5.5
55	4.6b	9.0	7.8n	2.0	4.7

[1]Milliequivalents of sodium hydroxide required to neutralize.

[2]Means of 320 observations.

[3]Means followed by the same letter within a column are not significantly different at .01 level of probability.

TABLE III

INTERACTION OF TEMPERATURE AND pH ON THE RATE OF ENZYME ACTIVITY ($X10^{-2}$)[1,2,3]

Temperature °C	pH			
	7.5	8.0	8.5	9.0
40	0.0k	4.3efghj	6.2cde	9.0a
45	2.1jk	3.6fhgj	6.2bcde	8.6ab
50	2.8ghj	7.5abcd	7.9abc	5.7cdef
55	2.5hj	4.9fgh	5.2defg	0.0k

[1]Milliequivalents of sodium hydroxide required to neutralize.

[2]Means of 80 observations.

[3]Means followed by the same letter are not significantly different.

which is reversible. Letting N represent the native active ficin; I, irreversibly denatured ficin; and Nd, a form into which ficin is reversibly converted, the reactions may be written as:

$$I \longleftarrow N \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Nd \qquad \text{or} \qquad I \longleftarrow Nd \underset{k_1}{\overset{k_2}{\rightleftharpoons}} N$$

The equilibrium ratio between denatured and native protein at a given temperature is the same regardless of whether the equilibrium is reached with ficin kept previously at lower or higher temperatures. At temperatures above a certain point, the rate of k_2 becomes negligible in comparison to k_1. The amount of native protein at equiblibrium approaches zero; whereas, with temperatures below a certain point, the opposite is true and N approaches 100 percent.

Activation by Cysteine

Addition of cysteine did not increase the enzymatic hydrolysis of shrimp, even though ficin is a sulfhydryl enzyme. Liener (20) indicated that ficin contains at least two SH groups, only one of which is directly involved in the catalytic site of the enzyme, and at least one disulfide bond which appears to be unessential for the maintenance of activity. Ficin is active in the reduced form (29). The apparent function of the co-enzyme cysteine is to offer protection for the enzyme against inactivation by oxidation or by combination with heavy metals (29), or it may react preferentially with the inhibitor (14).

Several authors (9, 11, 20, 28) report that cysteine increases the activity of ficin. The failure of cysteine to increase activity

of the enzyme used in this study may have been the result of:

1. The shrimp protein may have possessed sufficient -SH groups for the activation of the enzyme.
2. The concentration of the cysteine may not have been sufficient at the pH under study.
3. The purified enzyme existed in the reduced (active) form and the shrimp protein may not have possessed ficin inhibitors.

Maintenance of pH with Buffer

The use of a buffer solution was substituted for titration primarily because the buffer solution would be more practical for industrial usage. Since the pH optima for the enzyme is near pH 8.5, a borate buffer was selected. A series of borate buffers (0.05 to 0.3M) was selected as the reaction media. Any change in the pH of the reaction medium during hydrolysis was recorded. The pH of the media of the lower molar concentrations did change. However, 0.2M and 0.3M concentrations did not change in pH.

Both the higher and lower concentrations of buffer affected the activity of the enzyme by changing the ionic strength. The stability of the enzyme increases with the increase in the molar concentration up to a certain point. Whitaker (28) determined the stability and activity of ficin at different concentrations of buffer. He concluded that ficin exhibits maximum stability above 0.2M and then it will level off. Based on Whitaker's conclusions and the results obtained in this study, it was decided that 0.2M buffer was practical for use.

II. EXPERIMENT II

Splitting the exoskeleton of the shrimp provided pathways for the enzyme to move easily between the exoskeleton and the muscle. The deeper cut on the dorsal side exposed the vein which was removed by the enzyme and thus eliminated the deveining step.

Yield Percentage

The temperature at which enzymatic hydrolysis was carried out influenced significantly the final yield of the product (Table IV). This occurred because the enzyme hydrolyzed more muscle protein at the higher temperature. It appears that Enzeco® Protease-J has at least two enzymes acting on protein: one which hydrolyzed the connective tissue between the exoskeleton and the edible meat, and one which hydrolyzed the edible meat or muscle protein. At higher temperatures, there was a more intense hydrolysis of muscle protein. The meat became mushy and fragmented. This reduced the yield because the small fragmented pieces were washed off during agitation.

The length of time for hydrolysis had a significant effect on the final yield of peeled shrimp (Table V). Treatments at 30 and 40 minutes gave significantly higher yields than those at 50 or 60 minutes. The longer the shrimp were exposed to the enzyme the greater the amount of hydrolysis of muscle protein. Increased hydrolysis causes a decrease in yield.

There was a significant difference between 40 and 50 minutes in percent of yield; at 40 minutes the efficiency of the enzyme acting on

TABLE IV

F-TABLE SHOWING EFFECT OF TEMPERATURE, TIME AND pH ON PERCENTAGE OF YIELD

Source	D.F.	Mean Square	F-ratio
A. Temperature	1	4104.00	105.44**
B. Time	3	390.95	10.04**
A x B	3	26.14	0.67
C. pH	1	58.03	1.49
A x C	1	61.04	1.57
B x C	3	14.54	0.37
A x B x C	3	6.02	0.15
Residual error	48	38.92	

**Significant at .01 level of probability.

TABLE V

THE EFFECT OF TEMPERATURE, TIME AND pH ON PERCENTAGE OF YIELD[1]

Temperature		Time		pH	
°C	Mean[2]	Minutes	Mean[3]	Value	Mean[2]
40	76.3a	30	73.6n	8.5	68.9x
50	60.3b	40	71.5n	9.0	67.7x
		50	64.5p		
		60	63.8p		

[1]Grams of peeled shrimp per 100 grams of shell-on shrimp.

[2]Means of 32 observations.

[3]Means of 16 observations.

Means followed by the same letter within a column are not significantly different at .01 level of probability.

the connective tissue in peeling the shrimp was greater than that at 50 minutes. The yield was dependent on the temperature and length of time instead of pH.

The results of Experiment I indicated that pH levels of 8.5 and 9.0 did not affect significantly the activity of the enzyme (at .01 level of probability). The slight increase in the hydrolysis of protein at pH 9.0 was probably due to hydrolysis brought about by OH^- concentration and may not have been caused by the enzyme. It is important to consider that pH may affect quality factors other than yield.

Peeling Percentage

None of the treatments or interactions affected significantly the percentage of peeling (Table VI) which ranged from 85 to 95 percent efficiency (Table VII). The lack of significant difference among the means is probably due to the fact that the enzyme was distributed equally over all of the shrimp. It appears that the enzyme which acted on the connective tissue did not act on the muscle protein. This assumption is based on the fact that the connective tissue was hydrolyzed at the lower temperature and shorter period of time to a similar degree as it was hydrolyzed at a higher temperature and longer time. But at lower temperature and shorter time, there were more half peeled shrimp than there were unpeeled shrimp, which will cause more shrimp to be taken back for hydrolysis.

The temperature had a greater influence on the enzyme that hydrolyzed the muscle protein than on the enzyme that hydrolyzed the

TABLE VI

F-TABLE SHOWING EFFECT OF TEMPERATURE, TIME AND pH ON PERCENTAGE OF PEELING

Source	D.F.	Mean Square	F-ratio
A. Temperature	1	23.10	0.21
B. Time	3	104.35	0.97
A x B	3	9.23	0.09
C. pH	1	18.13	0.17
A x C	1	156.91	1.46
B x C	3	25.17	0.23
A x B x C	3	35.70	0.33
Residual error	48	107.63	

TABLE VII

THE EFFECT OF TEMPERATURE, TIME AND pH ON PERCENTAGE PEELING[1]

Temperature		Time		pH	
°C	Mean[2]	Minutes	Mean[3]	Value	Mean[2]
40	86.5a[4]	30	85.0n	8.5	86.7x
50	87.7a	40	84.8n	9.0	87.5x
		50	89.6n		
		60	89.0n		

[1]Mean percentage values.

[2]Means of 32 observations.

[3]Means of 16 observations.

[4]Means followed by the same letter within a column are not significantly different at .01 level of probability.

connective tissue. Neither time nor pH had any influence on the peel percentage.

Color

The analysis of color data is presented in Tables VIII through XV. The effect of temperature caused a significant change in the color of the shrimp. The shrimp treated at 40°C were pink and orange-pink according to a plotting of the coordinates on a chromaticity chart, whereas at 50°C they were close to illuminant "C" of the C.I.E. system. These results are illustrated by calculating the whiteness index (Tables XIV and XV). The dominant wavelength was not used because there were no differences among them, even though there is a difference in purity.

The major constituent of shrimp pigment is Astaxanthin, a carotenoid pigment which is attached to the protein molecule of the muscle and is concentrated on the outer tissues. Under intense hydrolysis of the muscle protein, the carotenoids are released into the medium, thereby exposing the inner light colored meat. At relative low temperatures very little muscle protein was hydrolyzed; thus, the color was unchanged.

With a progressive lengthening of time that the shrimp were treated with the enzymes, there was an increase in the development of whiteness. This change caused a change in the C.I.E. coordinate x, not y, which shows a loss of pink color instead of orange. The longer the time of exposure of shrimp, the greater the hydrolysis of the muscle proteins resulting in loss of pink color.

TABLE VIII

F-TABLE SHOWING THE EFFECT OF TEMPERATURE, TIME AND pH ON C.I.E. COORDINATE "X"

Source	D.F.	Mean Square $X10^{-5}$	F-ratio
A. Temperature	1	0.03	23.47**
B. Time	3	0.60	4.28**
A x B	3	0.30	2.36
C. pH	1	0.20	1.12
A x C	1	0.40	2.89
B x C	3	15.00	0.20
A x B x C	3	0.70	5.25**
Residual error	48	7.00	

**Significant at .01 level of probability.

TABLE IX

EFFECT OF TEMPERATURE, TIME AND pH OF ENZYME PEELED SHRIMP ON C.I.E. COORDINATE "X"

Temperature		Time		pH	
°C	Mean[1]	Minutes	Mean[2]	Value	Mean[1]
40	0.348a[3]	30	0.347n	8.5	0.342x
50	0.334b	40	0.343p	9.0	0.339x
		50	0.341pr		
		60	0.332r		

[1]Means of 32 observations.

[2]Means of 16 observations.

[3]Means followed by the same letter within a column are not significantly different at .01 level of probability.

TABLE X

EFFECT OF THE INTERACTION OF TEMPERATURE, TIME AND pH ON C.I.E. COORDINATE "X"[1,2]

Time, Minutes	Temperature °C	
	40	50
	pH 8.5	
30	0.347abc	0.350abc
40	0.365a	0.323d
50	0.355ab	0.333bcd
60	0.340bcd	0.325d
	pH 9.0	
30	0.352abc	0.337bcd
40	0.340bcd	0.342bcd
50	0.351abc	0.325d
60	0.331cd	0.333bcd

[1]Means of four observations.

[2]Means followed by the same letter are not significantly different at .01 level of probability.

TABLE XI

F-TABLE SHOWING EFFECT OF TEMPERATURE, TIME AND pH OF ENZYME PEELED SHRIMP ON C.I.E. COORDINATE "Y"

Source	D.F.	Mean Square $X10^{-5}$	F-ratio
A. Temperature	1	0.37	7.54**
B. Time	3	0.15	3.06*
A x B	3	6.00	1.22
C. pH	1	0.43	0.86
A x C	1	0.20	3.93
B x C	3	17.00	0.03
A x B x C	3	0.39	7.75**
Residual error	48	5.00	

**Significant at .01 level of probability.

*Significant at .05 level of probability.

TABLE XII

EFFECT OF TEMPERATURE, TIME AND pH OF ENZYME PEELED SHRIMP ON C.I.E. COORDINATE "Y"

Temperature		Time		pH	
°C	Mean[1]	Minutes	Mean[2]	Value	Mean[1]
40	0.345a[4]	30	0.346n[3]	8.5	0.344x
50	0.340b	40	0.344n	9.0	0.342x
		50	0.343n		
		60	0.339p		

[1]Means of 32 observations.

[2]Means of 16 observations.

[3]Means followed by the same letter within a column are not significantly different at .01 level of probability.

[4]Significance test at .05 level of probability.

TABLE XIII

EFFECT OF THE INTERACTION OF TEMPERATURE, TIME AND pH ON C.I.E. COORDINATE "y"[1,2]

Time, Minutes	Temperature °C	
	40	50
	pH 8.5	
30	0.343abc	0.349ab
40	0.354a	0.334bc
50	0.348abc	0.341abc
60	0.345abc	0.333c
	pH 9.0	
30	0.349ab	0.342abc
40	0.339abc	0.347abc
50	0.348abc	0.336bc
60	0.335bc	0.341abc

[1]Means of four observations.

[2]Means followed by the same letter are not significantly different at .01 level of probability.

TABLE XIV

F-TABLE SHOWING EFFECT OF TEMPERATURE, TIME AND pH OF ENZYME PEELED SHRIMP ON WHITENESS INDEX

Source	D.F.	Mean Square	F-ratio
A. Temperature	1	34156	79.48**
B. Time	3	2060	4.79**
A x B	3	892	2.08
C. pH	1	170	0.40
A x C	1	470	1.09
B x C	3	170	0.39
A x B x C	3	379	0.88
Residual error	48	430	

**Significant at .01 level of probability.

TABLE XV

EFFECT OF TEMPERATURE, TIME AND pH OF ENZYME PEELED SHRIMP ON WHITENESS INDEX

Temperature		Time		pH	
°C	Mean[1,3]	Minutes	Mean[2,3]	Value	Mean[1,3]
40	131b	30	138p	8.5	155x
50	177a	40	154np	9.0	152x
		50	159n		
		60	164n		

[1]Means of 32 observations.

[2]Means of 16 observations.

[3]Means followed by the same letter within a column are not significantly different at .01 level of probability.

pH did not have a significant effect on the color of shrimp. However, an interaction between temperature, time, and pH did affect the color. The shrimp treated at 40°C, 40 minutes and at pH 8.5 were significantly more pink than the ones treated at 40°C, 40 minutes and at pH 9.0. This results from the fact that the enzyme acting on the connective tissue is more active at pH 8.5. Very little hydrolysis occurred by the enzyme acting on muscle tissue. The increase in hydrolysis of the muscle tissue is caused by the higher concentration of OH^- which hydrolyze the muscle and, in turn, the carotenoids.

Texture

Only temperature affected the texture of shrimp (Tables XVI and XVII). The shrimp treated at 40°C were significantly firmer than those treated at 50°C. It appears that the enzyme which hydrolyzed the muscle protein was more active at higher temperatures. The enzyme hydrolyzed more muscle protein and at the same time over-tenderized the muscle causing it to become very soft, mushy and fragmented, and at the same temperature the slow acting enzyme on muscle slightly tenderized the shrimp meat.

Microbiology

The bacterial count of enzyme-treated shrimp was related to the treatment given to the shrimp (Table XVIII). At the lower temperature the count was higher at 40 and 50 minutes than at 30 and 60 minutes. At 50°C and pH 8.5 there was a trend for a reduced count as the time was extended. At 50°C and pH 9.0 no important trend was observed.

TABLE XVI

F-TABLE SHOWING EFFECT OF TEMPERATURE, TIME AND pH ON TEXTURE OF SHRIMP PEELED BY ENZYMES

Source	D.F.	Mean Square	F-ratio
A. Temperature	1	16.13	14.34**
B. Time	3	0.79	0.70
A x B	3	0.91	0.81
C. pH	1	0.01	0.00
A x C	1	0.41	0.37
B x C	3	1.87	1.67
A x B x C	3	0.23	0.20
Residual error	48	1.12	

**Significant at .01 level of probability.

TABLE XVII

EFFECT OF TEMPERATURE, TIME AND pH ON TEXTURE OF SHRIMP PEELED BY ENZYMES[1]

Temperature		Time		pH	
°C	Mean[2] X10^{-1}	Minutes	Mean[3] X10^{-1}	Value	Mean[2] X10^{-1}
40	2.79a[4]	30	2.47n	8.5	2.30x
50	1.79ab	40	2.49n	9.0	2.84x
		50	2.17n		
		60	2.04n		

[1]Mean work-force area square inch.

[2]Means of 32 observations.

[3]Means of 16 observations.

[4]Means followed by the same letter within a column are not significantly different at .01 level of probability.

TABLE XVIII

EFFECT OF TEMPERATURE, TIME AND pH ON BACTERIA OF THE ENZYME-PEELED SHRIMP ($X10^3$)[1,2]

Time, Minutes	Temperature °C	
	40	50
	pH 8.5	
30	11.6	10.0
40	13.2	10.7
50	13.8	7.4
60	12.0	9.2
	pH 9.0	
30	12.3	5.9
40	16.5	5.4
50	14.0	5.5
60	9.0	6.5

[1]Total aerobic plate count per gram.

[2]Means of four observations.

However at this treatment the count was lower than the count for other temperature and pH treatments.

The bacteria are concentrated primarily just beneath the exoskeleton on the surface of the shrimp muscle and inside the vein. The enzyme acting on the muscle protein hydrolyzed a thin layer of muscle which caused the bacteria to be released and washed away during agitation under water. The vein was completely removed. Since bacteria are composed of protein, it is possible that some of the bacteria were hydrolyzed by the enzymes.

Sensory Panel Evaluation

The sensory panel did not give a mean positive score to any of the samples for appearance, texture and color, meaning that all the enzyme-treated samples were scored below the reference sample (Table XIX). However, some of the panelists preferred the sample treated at 40°C for 40 minutes at pH 8.5 to the reference because of its texture and flavor. The low scores assigned to the enzyme-peeled shrimp were probably the result of the cooking method. Higher scores might have been given to the shrimp if they had been prepared by another cooking method, such as breading and deep-frying.

Comparison of Enzyme-Peeled Shrimp with Hand-Peeled Shrimp

The differences between shrimp peeled with enzyme at 40°C, 40 minutes and pH 8.5 are presented in Table XX. There was no difference in yield, both being 78.6 percent. Enzyme treatment gave an average 90 percent peeling efficiency. Enzyme-treated shrimp had more pink

TABLE XIX

EFFECT OF TREATMENT FACTORS ON SENSORY EVALUATION (HEDONIC SCORES) ON COOKED SHRIMP

Treatments			Evaluation[1,2]		
Temperature °C	Time, Minutes	pH	Appearance	Texture	Color
Reference (hand peeled)			0.0a	0.0n	0.0x
40	30	8.5	-1.12a	-0.56n	-0.66x
50	30	8.5	-1.06a	-0.72n	-0.94x
40	30	9.0	-0.87a	-0.37n	-0.66x

[1]Means of 32 observations.

[2]Means followed by the same letter within a column are not significantly different at .05 level of probability.

TABLE XX

COMPARISON OF VARIOUS QUALITY FACTORS OF SHRIMP PEELED BY ENZYMES (TREATED) AT 40°C FOR 40 MINUTES AT pH 8.5 WITH HAND-PEELED (CONTROL) SHRIMP

Quality Factor	Expression	Method Peeling		Percent Change
		Hand Peeled (Control)	Enzyme (Treated)	
Yield	Percentage	78.62	78.62	0.00
Peeling	Percentage	100.00	85-95	-15-5
Color	Whiteness index	384.01	235.12	-38.77
Texture	Work force area $in.^2$	0.45	0.26	-41.68
Bacteria	Total plate count/g	180,000	13,225	-92.65
Sensory	Hedonic scores	0.00	-0.91	-0.91

color and were softer than hand-peeled shrimp. There was a 92 percent reduction in bacteria count in enzyme-treated shrimp. The sensory panel gave the enzyme-treated samples lower scores.

CHAPTER V

SUMMARY

Shrimp can be peeled satisfactorily by enzymatic hydrolysis using the enzyme Enzeco® Protease-J. The exoskeleton was split on the dorsal and ventral sides and the shrimp were treated in a borate buffer solution of the enzyme at pH 8.5 at 40°C. The length of time necessary to produce hydrolysis under the conditions of this study was 40 to 45 minutes.

The exoskeleton can be separated easily by agitating the shrimp under water. The peeled shrimp were light pink in color, slightly tenderized, low in bacterial count (see Table XVIII, page 38) and had the tail attached.

Treating shrimp with Enzeco® Protease-J could be used as a method for replacing the laborious hand peeling or the expensive machine peeling of shrimp. However, the peeled shrimp should probably be cooked by breading and deep-frying.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Allen, H. B. 1967. Technology's role in the seafood industry. Comm. Fisheries Rev. 29(2):59.

2. Anonymous. 1968. Enzyme products. Food Product Development 2(4):55.

3. Anonymous. 1970. Duncan's New Multiple Range Test. Bulletin SEQ 0141, G04. The University of Tennessee Computing Center, Knoxville, Tennessee.

4. Anonymous. 1970. Study on northern shrimp confirms best method to process product. Quick Frozen Foods 32(6):111.

5. A.P.H.A. 1970. "Recommended Methods for the Microbiological Examination of Foods." 2nd ed., p. 111. American Public Health Association, New York.

6. Bone, G. 1970. ANOVAR/360. IBM Corporation, Salt Lake City, Utah.

7. Carrol, B. J., Reese, G. B., and Ward, B. Q. 1968. Microbiological study of iced shrimp. Circular 284. U.S. Dept. Interior, Bureau of Comm. Fisheries, Washington, D.C.

8. Cleary, D. P. 1970. World demand for shrimp and prawns may outstrip supply during next decade. Comm. Fisheries Rev. 32:3, 19.

9. Cohen, W. 1958. Characterization of ficin. Nature 182:659.

10. Dixon, M., and Webb, E. C. 1964. "Enzymes." Pp. 81-181. Academic Press, New York.

11. El-Garbawi, M., and Whitaker, J. R. 1963. Factors affecting enzymatic solubilization of beef proteins. Food Research 28:168.

12. Fehmarling, G. B. 1970. Process and composition for loosening and removing edible tissue from shells of marine creatures. U.S. Patent 3,513,071.

13. Franklin, H. L., and Holm, P. 1970. Supply-demand factor necessitates finding frozen shrimp resources. Quick Frozen Foods 32:12, 31.

14. Gould, N. R., and Liener, I. E. 1965. Reaction of ficin with diisopropyphosphofluridate evidence for a contaminating inhibitor. Biochem. 4:90.

15. Hale, M. B. 1969. Relative activities of commercially available enzymes in the hydrolysis of fish protein. Food Technol. 23:107.

16. Hinrichs, J. R., and Whitaker, J. R. 1962. Enzymatic degradation of collagen. Food Research 27:250.

17. Karrer, P., and Jucker, E. 1950. Astacene and Astaxanthin. In "Carotenoids," p. 229. Elsevier Publishing Co., Inc., New York.

18. Kramer, A., and Twigg, B. A. 1966. "Fundamentals of Quality Control for the Food Industry." 2nd ed., p. 130. AVI Publishing Co., Westport, Conn.

19. Krishnamurthi, C. R., and Subramanyan, V. 1949. Studies on vegetable rennet. III Further observations on the milk clotting and protease components in the preparation from fig latex (Ficus Carica Linn.). Indian J. Dairy Sci. 2:19.

20. Liener, I. E. 1961. A study of the number and reactivity of the sulfhydryl groups of ficin. Biochem. Biophys. Acta. 53:332.

21. Love, T. D., and Thompson, Mary H. 1969. Report B.C.F. Technical Laboratory. Circular 327. U.S. Dept. Interior, Bureau of Comm. Fisheries, Washington, D.C.

22. Miyada, D. S., and Tappel, A. L. 1956. The hydrolysis of beef proteins by various proteolytic enzymes. Food Research 21:217.

23. Nakao, T. 1952. Ficin Specificity. Acta School Med. Univ. Kioto 29:167. Abstracted from Chem. Abstract, 1953, 45:3360.

24. Pully, J. E. 1969. Enzymes simplify processing. Food Eng. 41:2, 68.

25. Reed, G. 1966. "Enzymes in Food Processing." P. 126. Academic Press, New York.

26. Robins, B. H. 1930. Proteolytic enzymes in ficin. J. Biol. Chem. 87:251.

27. Whitaker, J. R. 1957. Assay and properties of commercial ficin. Food Research 22:468.

28. Whitaker, J. R. 1957. Properties of the proteolytic enzymes of commercial ficin. Food Research 22, 483.

29. Winnick, T., Cone, W. H., and Greenberg, D. M. 1944. Experiments on the activation of ficin. J. Biol. Chem. 153:465.

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