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# AN INVESTIGATION OF THE CHEMISTRY OF TEXTURE CHANGES OF FROZEN BLUE CRAB MEAT[1/]

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ABSTRACT

A STUDY OF THE CHANGES IN TEXTURE IN FROZEN STORED BLUE CRAB MEAT INDICATED THAT A LOW-GRADE NONENZYMATIC RESPIRATION OF THE TISSUE CONTINUES EVEN AT 1.4° F. THE RESPIRATORY QUOTIENT STUDIES INDICATED THAT THE WARBURG RESPIRATION IS DUE TO OXIDATION OF TISSUE CARBOHYDRATES. MORE WORK IS SUGGESTED.

## INTRODUCTION

The texture of seafood alters when it is frozen and held in cold storage. With crab meat, there is a progressive increase in toughness. This increase hitherto has been ascribed to protein denaturation or to desiccation. We decided to study, however, the possibility that the alteration in texture might be caused by certain enzymic changes. In this paper, the question as to whether enzymic changes occur at all in frozen stored crab meat is investigated.

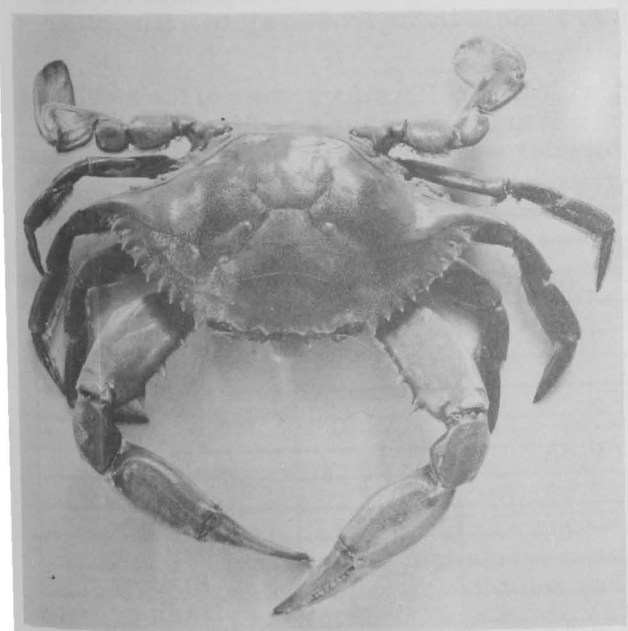

FIG. 1 - BLUE CRAB

The blue crab, Callinectes sapidus, was used as the source of the crab meat. For purposes of orientation it was decided to measure (1) changes in pH, and (2) respiration of the tissues. These measurements were chosen for the information they might yield regarding the type of chemical change occurring. Changes in pH, for example, might be the result of oxidative or nonoxidative decarboxylation or deamination, or simple hydrolytic cleavage of fats and/or proteins, providing that the natural buffering capacity did not hide these chemical changes. Measurement of changes in the respiration of the tissues in connection with any changes in pH would aid in the establishment of the oxidative or nonoxidative nature of reactions responsible for pH changes and also aid in determining the presence of decarboxylation or deamination reactions.

It was found that changes in the pH of frozen stored meat were so slight as to be of doubtful significance. Respiration studies revealed the presence of a constant low-grade tissue respiration. We attempted to stimulate the respiration by the addition of

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possible substrates such as glucose, l-leucine, oxalacetic acid, sodium pyruvate, dl-malic acid, l-malic acid, and dl-lactic acid. Adenosine triphosphate (A.T.P.) was added in all respiration studies to obviate the possibility that the concentration of it might become the limiting factor in any enzymic reaction.

Although claw meat, regular grade of white meat, and backfin lump meat (chunks of meat along the backfin) were picked and stored separately, most of the experimental work was done with only the backfin lump meat, since it was more uniform than were the other types of meat and formed better homogenates.

## EXPERIMENTAL

THE PREPARATION AND STORAGE OF CRAB MEAT SAMPLES: Three different lots of a dozen each of hard-shell blue crabs were cooked, and the meat was removed and placed in frozen storage. Several other lots also were prepared in about the same manner. In each case, the cooking procedure approximated the method used by commercial packers of crab meat. The live crabs were placed in wire-mesh cages and put in an autoclave. The steam was allowed to pass into the chamber for about 10 minutes during which time air was exhausted from the chamber. The crabs were autoclaved at 15 pounds for about 10 minutes, after which the steam was exhausted for 5 minutes before the door was opened. The crabs were removed, allowed to stand at room temperature until cool, and picked. The meat then was mixed thoroughly to give homogeneous samples. Lots I and II were comprised of 50-gram portions of meat packed in tight packages of moisture-vaporproof cellophane; and lot III was comprised of meat packed in 1-pound friction-top perforated-bottom cans used commercially. These samples were stored at -22° C. (-7.6° F.) for 2 days, after which time they were stored at -17° C. (1.4° F.). Just before being used for testing, each portion with the exception of those from lot III samples was removed to a 2° C. (35.6° F.) storage room for 18 hours and allowed to thaw. Since the portions of lot III were packed in much larger containers, the amount needed for testing was taken directly from one of the containers while in the frozen state. The other lots used were stored in small quantities.

DETERMINATION OF THE pH OF CRAB MEAT: Twenty grams of the meat to be tested were placed in an ice-cold glass cup of a Waring Blendor and 80 milliliters of ice-cold water was added gradually. The blendor was turned on between additions so

Table 1 - Data on pH and Texture of Backfin Crab Meat after Frozen Storage

| Period of Storage at -17° C. (1.4° F.) | pH Cooked Meat | | | pH Raw Meat | Texture as Determined Organoleptically |
|---|---|---|---|---|---|
| | Lot I | Lot II | Lot III | | |
| Days | | | | | |
| 0 | 7.59 | 8.00 | 7.87 | 6.49 1/ | Normal |
| 10 | 7.60 | | | | Normal |
| 12 | | 7.98 | | | Normal |
| 27 | 7.62 | | | | Normal |
| 40 | | | 7.90 | | 2/ |
| 56 | | | 7.84 | | 2/ |
| 61 | 7.74 | | | | Stringy |
| 86 | | 8.08 | | | Stringy |
| 111 | 7.89 | | | | Stringy |
| 160 | | 8.06 | | | Stringy |
| 298 | | | 8.04 | | Stringy |
| 305 | | 7.87 | | | Stringy |
| 345 | 7.69 | | | | Stringy |

1/ AVERAGE OF 5 DETERMINATIONS USING INDIVIDUAL FRESHLY-KILLED CRABS.
2/ SINCE SAMPLE WAS NOT THAWED BEFORE USING, IT WAS NOT POSSIBLE TO ESTIMATE TEXTURE.

that a smooth creamlike mixture was maintained during the process. This mixture, after being thoroughly blended, was transferred to a 250-milliliter beaker and tested, using the large electrodes of a Beckmann Model G pH meter. The sample was stirred occasionally, and readings were taken every 5 minutes until two successive readings

agreed within 0.01 pH unit. The texture of each sample was determined organoleptically at the time of each pH determination. The results of this procedure are summarized in table 1.

DETERMINATION OF ENDOGENOUS RESPIRATION IN CRAB MEAT: A homogenate of 20-percent backfin crab meat by weight was prepared in the same manner as in the determination of pH, except that an isotonic solution approximating the composition of sea water (Lange 1946) was used in place of the distilled water. The buffer solution thus obtained is much more concentrated than is the usual Krebs-Ringer solution, but was used because crab blood approximates the composition of sea water in salt content (Tessier 1938). The composition of this isotonic solution was as follows:

| | |
|---|---|
| 100 parts 0.6 M NaCl | 10 parts 0.6 M $MgSO_4$ |
| 2 parts 0.6 M KCl | 30 parts 0.6 M $CH_3COONa$ |
| 4 parts 0.4 M $CaCl_2$ | |

After the homogenate had been prepared by the use of this solution, the pH was adjusted to 7.00 by the dropwise addition of 1 molar acetic acid. Three-milliliter aloquots of this homogenate were placed in the main compartments of Warburg flasks, and the oxygen uptake was determined by the usual Warburg technique using KOH in the center wells. The temperature of the water bath was 37° C. (98.6° F.). Respiration values were calculated as microliters of oxygen consumed per hour for 3 milliliters of homogenate. Table 2 contains the results of these determinations.

Table 2 - Values for Endogenous Respiration of Backfin Crab Meat after Frozen Storage

| Period of Storage at -17° C. (1.4° F.) | Respiration as Microliters of Oxygen Consumed Per Hour Per 3 ml. of Homogenate Containing 20 Percent (by weight) Backfin Crab Meat | | | |
|---|---|---|---|---|
| | Cooked Meat | | | Raw Meat |
| | Lot I | Lot II | Lot III | |
| Days 0 | 2.9 | | | 14.9 1/ |
| 10 | 3.1 | | | |
| 12 | | 4.7 | | |
| 27 | 2.7 | | | |
| 40 | | | 8.0 | |
| 56 | | | 0.0 | |
| 61 | 6.8 | | | |
| 111 | 3.8 | | | |

1/ 10-PERCENT HOMOGENATE WAS USED HERE, AS THE 20-PERCENT HOMOGENATE WAS A GEL.

DETERMINATION OF THE ENZYMIC ACTIVITY OF UNFROZEN COOKED BACKFIN CRAB MEAT IN THE PRESENCE OF VARIOUS SUBSTRATES: The isotonic buffer solution used in the experiments on endogenous respiration was abandoned in favor of using a phosphate buffer, which was still approximately isotonic with sea water but which did not contain the same proportion of ions. This solution, having a pH of 7.0, was made as follows:

Solution A: 100 parts of 0.770 M NaCl
4 parts of 0.770 M KCl
3 parts of 0.154 M $CaCl_2$
1 part of 0.770 M $KH_2PO_4$
1 part of 0.770 M $MgSO_4$

Solution B: 0.1 M Phosphate buffer, pH 7.4.

Dilute 20 milliliters of solution A to 100 milliliters with solution B and filter if a slight precipitate appears.

This was done in order that the homogenate could be accurately buffered without having to adjust the pH before using, and also to provide phosphates.

To determine the activity in the presence of various substrates, we used two Warburg flasks for each substrate; one contained KOH in the center well and the other did not. In the main compartment were placed 2 milliliters of the substrate in a concentration of 1.5 times the desired working concentration, and 0.25 milliliters of 0.4-percent A.T.P. Three-quarters milliliter of the homogenate was placed in the side arm. Control flasks were prepared by using water in place of the substrate. The flasks and manometers were placed in the bath and allowed to equilibrate for 15 minutes after which time the stopcocks were closed and the initial readings were taken. The homogenate then was tipped in from the side arm, and readings were taken every 30 minutes for 2 hours. The results of this initial trial are summarized in table 3.

Table 3 - The Effect of Added Substrates on the Activity of Homogenates Containing 20-Percent Backfin Crab Meat

| Substrate Present | Total Oxygen Uptake Due To Substrate | Total Carbon Dioxide Production Due To Substrate |
|---|---|---|
| | Micromoles | |
| 0.0833 M glucose | 0 | 0 |
| 0.0133 M l-leucine | 0 | 0 |
| 0.0833 M dl-malic acid | 0 | 1.93 1/ |
| 0.0833 M sodium pyruvate | 0 | 0 |
| 0.0833 M oxalacetic acid | 0 | 2/ |

1/ THIS RESULT WAS NOT VERIFIED IN SUBSEQUENT WORK.
2/ REACTION WAS TOO FAST TO MEASURE. THIS RESULT CAN BE OBTAINED WITHOUT THE PRESENCE OF HOMOGENATE.

Since only dl-malic and oxalacetic acids produced carbon dioxide, the behavior of these acids were investigated further. Oxalacetic acid is known to be unstable, under these conditions, so the experiment with this substrate was repeated without the addition of the homogenate. The reaction proceeded at approximately the same rate as in the presence of the homogenate. In a similar experiment, however, dl-malic acid failed to produce carbon dioxide in the absence of homogenate. It was not possible to obtain the action of dl-malic acid in subsequent trials with the homogenate.

It should be noted here that the production of carbon dioxide shown in table 3 was virtually complete in 10 minutes after tipping in the homogenate. Under these same conditions 0.02 molar dl-lactic acid was also found to produce carbon dioxide This concentration of dl-lactic acid produced 1.09 micromoles (average of two determinations) of carbon dioxide.

The data in table 3 and from unpublished work with added dl-malic acid and l-malic acid as substrates indicated that l-malic acid stimulated the respiration of cooked and frozen crab meat homogenates more than did the dl-malic acid, indicating a stereospecific enzymatic reaction. Subsequent attempts to duplicate these results were unsuccessful. In fact, further experiments on the respiration of cooked and frozen crab meat at three different temperatures, both in the presence and absence of dl-malic acid, revealed that the addition of the dl-malic acid had no effect on respiration and that the 37° C. (98.6° F.) temperature gradient had only a small enhancing action. The data from these experiments are presented in table 4.

## DISCUSSION OF EXPERIMENTAL RESULTS

The data in table 1 show that the pH of the freshly-cooked meat varies widely and that no significance can be attached to the results obtained. This variation is

probably due to the comparatively great buffering capacity of crab meat. Data in tables 2 and 4 indicate that a low level of respiration occurs in the thawed meat even after being kept in frozen storage.

Table 4 - The Effect of Temperature on the Respiration of a 20-Percent Homogenate of Backfin Crab Meat in the Presence and Absence of Added dl-malic Acid

| Treatment of Sample of Crab Meat | Amount of dl-malic Acid Added as Substrate | Amount of 20-Percent Homogenate Added | Carbon Dioxide Produced at 1/: | | |
|---|---|---|---|---|---|
| | | | 0° C. | 20° C. | 37° C. |
| | Micromoles | Milliliters | Micromoles Per Hour | | |
| Cooked and then frozen for 1 week | 3 | 3 | 0.6 ± 0.1 | 0.55 ± 0.1 | 0.75 ± 0.04 |
| Cooked and then frozen for 1 week | 0 | 3 | 0.77 ± 0.03 | 0.64 ± 0.04 | 0.80 ± 0.04 |

1/ AVERAGE OF 3 TO 6 DETERMINATIONS.
± STANDARD ERROR: $\sqrt{\frac{\Sigma d^2}{n}}$

It was noted in the course of the experiments reported in table 4 that the oxygen consumption by these homogenates was equivalent to the carbon dioxide produced, giving a respiratory quotient of approximately 1.0. This quotient of 1.0 would indicate that the low-grade nonenzymatic respiration exhibited by this tissue is due to the oxidation of carbohydrate.

## CONCLUSIONS

1. Changes in the pH of stored frozen crab meat are variable and show no definite trend with length of storage. This variation probably is due to the natural buffering capacity of the crab meat.

2. Cooked and stored frozen crab meat exhibits after thawing a low-grade nonenzymatic respiration even after having been stored at -17° C. (1.4° F.). Further study along this line is suggested as a logical attack on the problem.

LITERATURE CITED

LANGE, N. A.
1946. HANDBOOK OF CHEMISTRY, P. 1237, HANDBOOK PUBLISHERS, INC., SANDUSKY, OHIO.

TESSIER, G.
1938. COMPTE RENDU SOCIETE BIOLOGIE, 129, P. 937.

HOW MANY DIFFERENT SPECIES OF FISHES IN THE WORLD?

According to the Curator of Fishes, Smithsonian Institution, there are 40,000 species and subspecies of fish in the entire world. The tropical Indo-Pacific region, which extends from the head of the Red Sea to Easter Island, is considered to be the richest in number of species of fish, containing over 9,000 species.