Waste Management and Byproducts Recovery for the Blue Crab (*Callinectes sapidus*) Industry

Part II: Flavor and Pigment Extraction from Blue Crab Processing Byproducts

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INTRODUCTION

1. General Introduction

Crab production in the Chesapeake Bay is approximately 77 million pounds annually (NMFS, 1989). Approximately 85% or 65 million pounds of the product is solid waste (crab scrap) which has a limited economic value. Presently, the byproducts or crab scrap is dehydrated, ground and sold as a feed ingredient. The price varies according to the soy and corn commodity markets and it is subject to other variable cost, such as energy. Many times, the product is sold lower than its production cost. The meal must be produced irrespective of profitability, since alternative disposal methods are unavailable. Some landfills will not accept the product due to the relative large volume of the waste, its rapid deterioration, characteristic odor, and potential as a pest attractant.

A recently formed corporation, Ducon, which is a cooperative effort between the Dupont and ConAgra firms, is interested in the commercial production of chitin and chitosan from shellfish waste but has stated that their production facility would not be located on the East coast. Consequently, this option of disposal would not be available to Virginia crab processors.

It is possible, however, to increase the economic value of the crab scrap by identifying commercial uses for components of the waste. The crab scrap could be used as a raw material in the production of a natural crab flavor and as a source of natural pigments.

Changing lifestyles in the United States during the last thirty years have made convenience foods an increasingly popular food item. Additionally, consumers have demonstrated a decided preference for "natural foods" which contain no artificial additives or ingredients which have a negative health connotation. These changes in consumption attitudes have had a significant impact on the food industry. One of which has been the increased demand for natural, rather than synthetic, food flavors. While the cost of natural flavors is relatively expensive, averaging 12-70 dollars/pound, availability, rather than cost, is usually the limiting factor (Heath, 1981).

Previous research at Virginia Polytechnic Institute and State University by Sea Grant investigators has indicated the possibility of producing a natural blue crab flavor that would be commercially acceptable (Flick, 1990) for engineered seafoods (surimi) and further processed seafood products. An acceptable (cost vs supply) natural blue crab flavor is unavailable at present although some blue crab bases are currently produced. Blue crab flavor uses would include seafood analogs and further processed products as crab cakes, stuffed crabs, crab mixes and soups. The production of a surimi based blue crab analog could result in substantial market approaching that of the king or snow crab products. A blue crab product would have substantial consumer appeal to both East and Gulf coast consumers were the blue crab is considered a shellfish favorite.

Consumers tend to avoid unfamiliar compounds that include food additives, such as antioxidants, aromas or colors. For this reason, the food industry requires a wide array of acceptable, safe food colorants to satisfy consumer preferences.

Colors that have flourished in the plant and animal kingdoms for centuries still serve in many instances as coloring substances in the food supply today. Carotenoids are widely represented in crustaceans as crabs, lobsters, shrimp, and barnacles. Crustaceans generally contain the same major carotenoids which are: B-carotene, astaxanthin, echinone, canthaxanthin, cryptoxanthin, zeaxanthine and lutein.

Investigations about the carotenoids of the carapace of the crabs (Portunus trituberculatum and Callinectes sapidus) have confirmed the presence of canthaxanthin, 4-hydroxiequinone, 3-ketocanthaxanthin, and astaxanthin. These pigments can be extracted from the carapace and the extremities of the blue crab, which are a component of processing byproducts.

2. Objectives

The objectives of this project are to produce a natural blue crab (Callinectes sapidus) flavor or flavorings, and to extract the astaxanthin pigment from the processing byproducts. Specific strategies include:

A. Examining various crab byproducts for their potential as a natural flavor source.

B. Examining various extraction processes for potential commercial application based on: resulting product quality and consistency, cost, hygiene, and safety.

C. Recovery efficiency and production efficiency of astaxanthin pigment from the crab byproducts.

D. Cooperating with seafood processing and flavor production firms to facilitate commercialization of the technology.

II. MATERIALS AND METHODS

The crab byproducts used in this project were obtained at the RCV Seafood Corporation's blue crab plant in Morattico, Virginia. The crabs were captured by commercial methods from May to September because crabs harvested during winter months are covered by sand and grit which imparts a muddy or earthy flavor to the extracts. Once the crabs were washed and cooked, the meat was picked from the body by professional pickers, who hygienically deposited the scrap (with still some meat attached) in special containers. The meat was separated from the claws using a Harris Claw Machine. The shells were also collected for use in this project. The blue crab scrap was composed of carapace with and without the mass of egg, legs, and tips (white scrap) with gills (Figures 1, 2, 3, and 4). The scrap was periodically removed from the line to prevent deterioration, deposited in bags, frozen at -10°F (-37.5°C) and transported to Virginia Polytechnic Institute and State University.

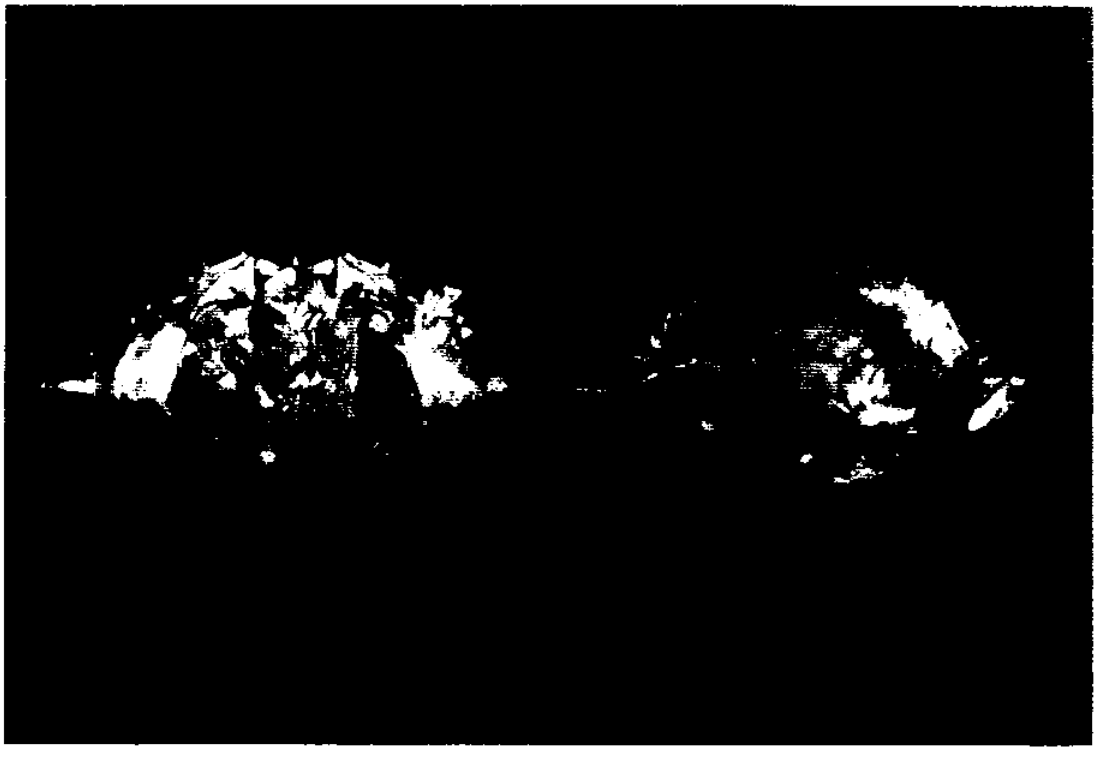

FIGURE 1: BLUE CRAB PROCESSING BYPRODUCTS: CARAPACE WITH EGGS

FIGURE 2: BLUE CRAB PROCESSING BYPRODUCTS: CARAPACE WITHOUT EGGS

FIGURE 3: BLUE CRAB PROCESSING BYPRODUCTS: (A) LEGS AND (B) CLAW SHELLS (BYPRODUCT OF THE HARRIS CLAW MACHINE)

FIGURE 4: BLUE CRAB PROCESSING BYPRODUCTS: TIPS (WHITE SCRAP)

1. Flavor Extractions

1.1. Extractions Performed in the Facilities of the Food Science and Technology Department at Virginia Tech

1.1.1. Determination of Boiling Time in Hot Water Extraction Institute and State University in an Open Vessel

1.1.1.1. Hot Water Extraction

A hot water flavor extraction method was utilized as suggested by Ochi (1980). Since the boiling time was not specified in his paper, several boiling-times (1,2,3, and 4 hr) were evaluated (Figure 5). The quality of the extracted flavors was evaluated by sensory analysis.

The blue crab scrap was stored in a freezer at -20°C after arrival at Virginia Tech. The frozen scrap was composed of: carapace, legs, viscera and tips with the gills attached (Figures 1, 2, 3, and 4). Five kg of scrap were ground in a blender with 10 kg of water. The mixture was placed in an open steamed jacketed kettle, Legion E., model TWP 60 (Legion Equipment Co., New York, NY) and boiled for 4 hr at a constant temperature (100°C). Two L of the mixture were taken from the kettle after 1, 2, 3, and 4 hr of boiling (Ochi, 1980). The samples were collected in Nalgene jars, and stored at 4°C. After filtration through cheese cloth followed by n_o 2 Whatman paper, the samples were subdivided into 3 fractions. One fraction was spray dried and a second was freeze dried. A third sample was stored in the freezer for use as a liquid flavoring.

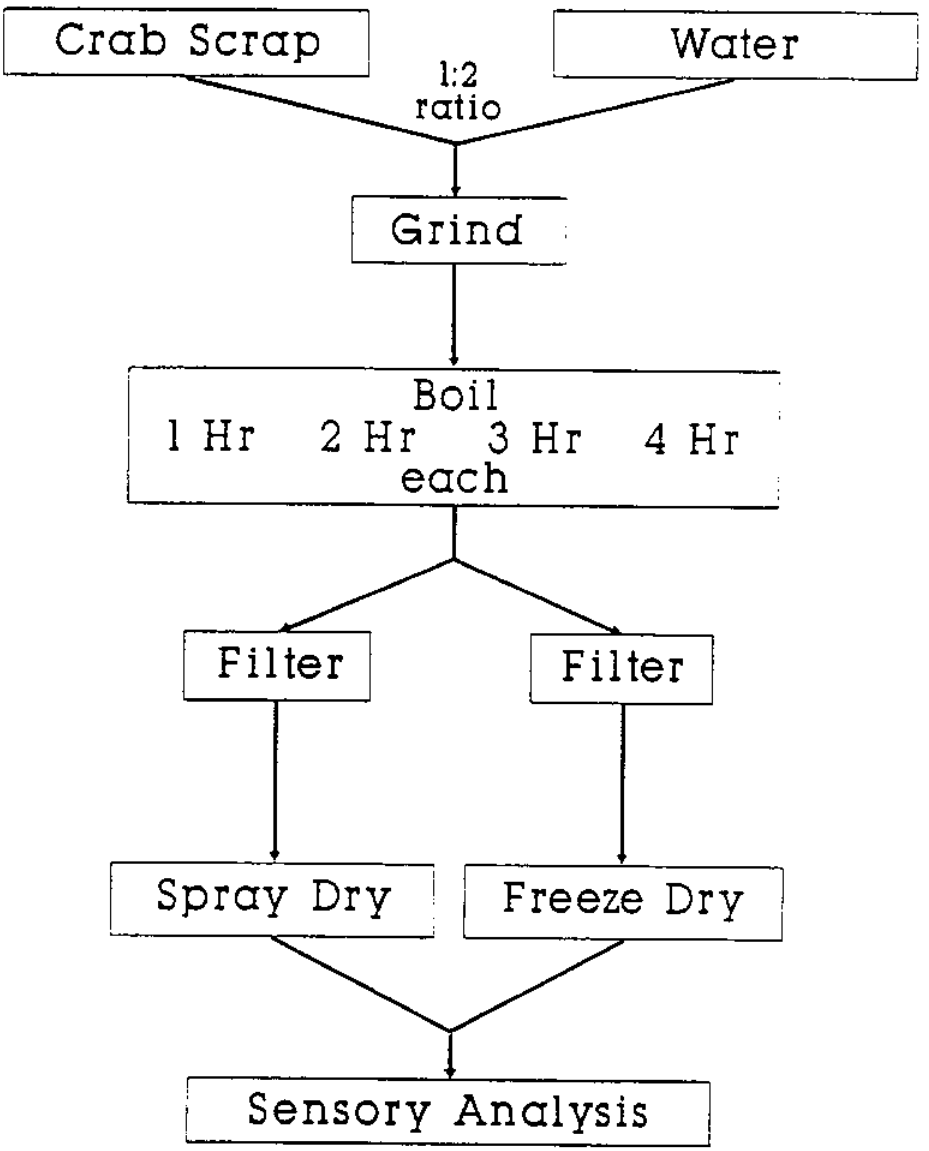

FIGURE 5: FLAVOR EXTRACTION PROCESS USED TO DETERMINE THE BOILING TIME IN AN OPEN VESSEL

1.1.1.2. Spray Drying Process

One L of each liquid sample was spray dried using a Buchi model 190 (Brickmann Inst. Co. Westbury, NY) spray drier. The inlet and outlet temperatures were set at 170°C and 80°C respectively. The powder obtained was placed in colored bottles, flushed with nitrogen, and stored in a desiccator at room temperature.

1.1.1.3. Freeze Drying Process

One L of each liquid sample was boiled in the open kettle and reduced to 5,000 ml. The reduced extract was freeze dried for 20 hours with a Virtis model 10-145 MR (Virtis Co, Gardiner, NY) freeze dryer (Brockman, 1974). The powder obtained was placed in colored bottles, flushed with nitrogen, and stored in a desiccator at room temperature.

1.1.2. The Contribution of Each Scrap Part to the Blue Crab Flavor

Blue crab scrap was composed of carapace, legs, viscera and tips with the gills attached (Figures 1, 2, 3, and 4) as previously stated. This experiment was designed to determine the contribution of each of these parts on the extracted flavor.

The scrap was separated into two groups: (i) carapace, tails, and appendages (ii) tips with gills. Extraction from each of the above groups was performed following the procedure mentioned above. . The boiling time was held constant at 1 hr.

In order to determine which scrap group contained the best crab flavor, all extracts were evaluated by sensory analysis.

1.1.3. Enzyme Digestion of the Crab Scrap Prior to Extraction

In this study, the crab scrap was enzymatically treated prior to extraction. Crab tips have a substantial quantity of attached meat which is high in protein. This protein can be broken down with various proteases liberating amino acids, like taurine and glycine, which are major contributors to crab flavor (Konosu and Yamaguchi, 1982). The enzyme treatment also increases the yield of extractable flavor materials. Three proteolytic enzymes were chosen for the pretreatment process. The enzymes were evaluated in three different experiments. Each experiment was designed to identify enzyme concentration and the reaction time that produced the best flavor (Figure 6). By comparing the flavors obtained in the three extractions (sensory analysis), the combination of enzyme and reaction time which produced the best flavor was identified.

Before the experiment was initiated, the percentage of meat present in the scrap was determined by carefully separating the meat from 500 g of scrap. The total percentage was calculated as follows:

$$\% \; Meat \in Scrap = \left(\frac{Weight \; of \; Meat \in 500 \; g \; of \; Scrap}{500 \; g \; of \; Scrap} \right) x \; 100.$$

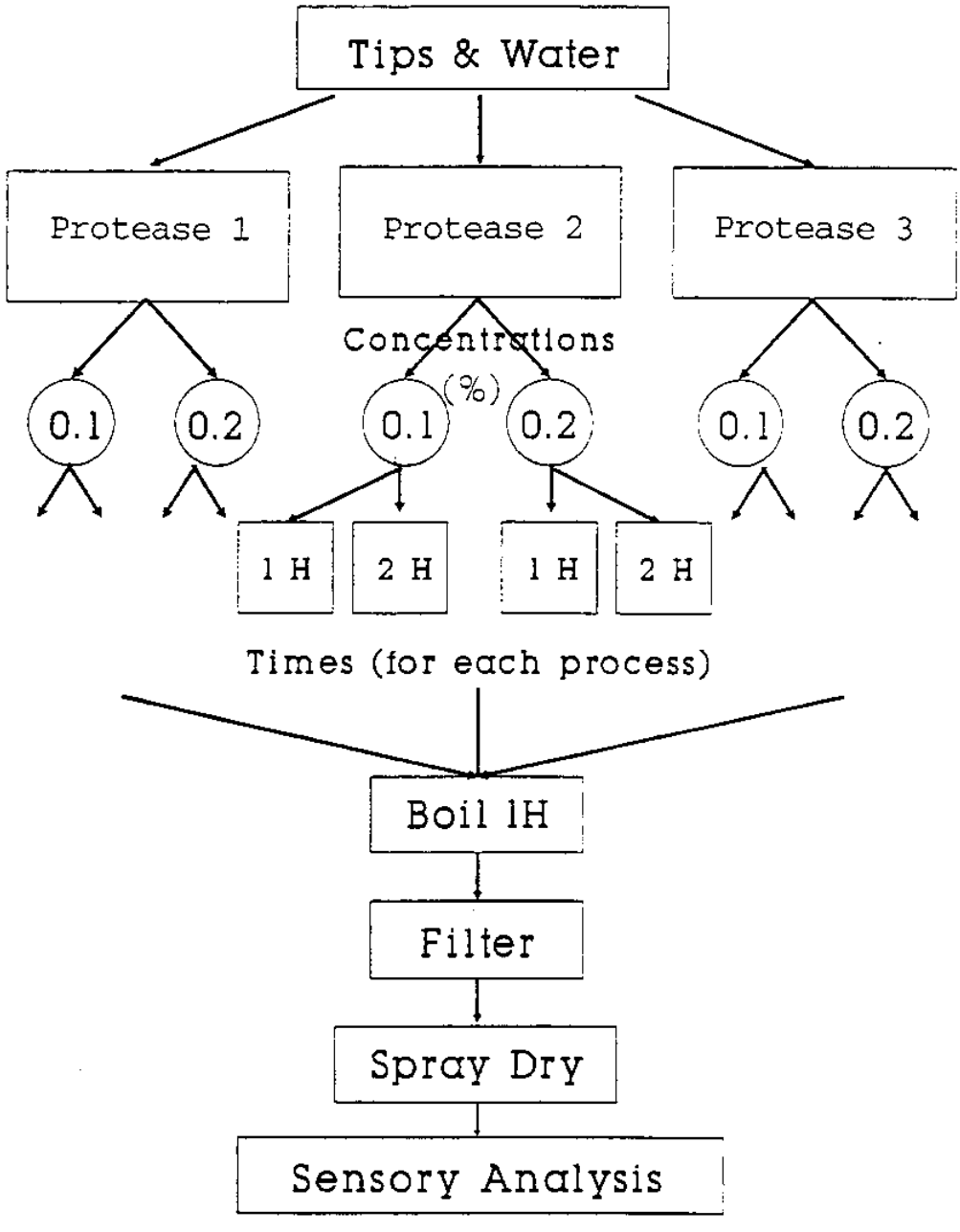

FIGURE 6: FLAVOR EXTRACTION PROCESSED FROM BYPRODUCTS PRE-TREATED WITH ENZYMES

Four samples (A, B, C, D) of crab scrap were prepared by grinding 2.5 kg of tips and appendages and adding 5 kg of water. The water and the scrap were well mixed in Nalgene containers which were placed in a walk-in incubator maintained at 55±1°C (optimum temperature of proteases). When the mixture reached 55°C, the enzyme was added according to the directions in Table 1.

The samples were stirred every 10 min to assure optimum reaction. When the reaction time of each sample was completed, the samples were introduced in the kettle and allowed to boil for 1 hr. Each sample was spray dried after filtering through a N_o 2 Whatman paper and evaluated for crab flavor by sensory analysis as previously described.

TABLE 1: REACTION TIME AND ENZYME CONCENTRATION USED IN THE ENZYMATIC TREATMENT OF RAW MATERIAL

SAMPLE	% ENZYME	REACTION TIME (hr)
Sample A	0.1	2.0
Sample B	0.1	4.0
Sample C	0.2	2.0
Sample D	0.2	4.0

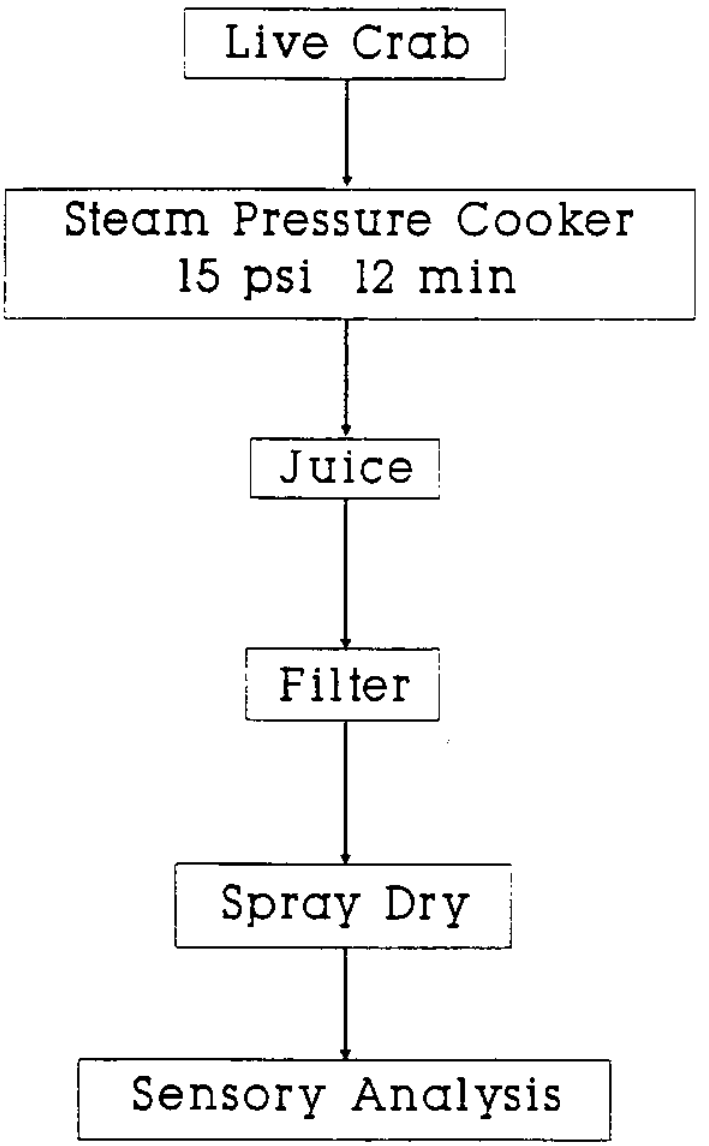

FIGURE 7: FLAVOR EXTRACTION FROM THE RETORT JUICE

1.1.4. Flavor Extraction From the Retort Juice

In this study, the retort juice was tested as a possible raw material for production of an acceptable crab flavor. The retort juice is the liquid that is exuded by the crab during the retorting (steaming) process.

This process (Figure 7) was simulated in the pilot plant by using a 3 gallon (11.4 L) pressure cooker, in which 30 pounds (13.50 kg) of live crabs were cooked for 12 min at 15 psig. Six L of crab juice were recovered, filtered through a N_o 2 Whatman filter paper and spray dried. The flavor quality was analyzed by sensory analysis.

1.2. Extractions Performed in the Facilities of a Commercial Flavor Firm

1.2.1. Flavor Extractions From the Tips With and Without Gills

All the previously discussed extractions were performed in an open vessel. Because industrial flavor extractions are usually conducted in a reactor (a closed vessel under pressure), the following extractions were performed in a pilot plant, using a 2 kg reactor (Figure 8). The effect of enzymes on the flavor extraction process from the crab scrap (only tips) was tested. The contribution of the attached gills to the final flavor was also tested by producing enzyme extracts from the scrap both with gills and without gills. The following enzyme reactions were performed: (1) tips with gills digested with Protease 1 for 9 hr, (2) tips without gills digested with Protease 2 for 6 hr, (3) tips without gills digested with Protease 2 for 9 hr, (4) tips with gills digested with Protease 2 for 6 hr, (5) tips with gills digested with Protease 3 for 9 hr, (6) tips without gills digested with Protease 3 for 9 hr, (7) tips with gills digested with Protease 4 for 9 hr, and (8) tips without gills digested with Protease 4 for 9 hr (Table 2).

1.2.1.1. Digestion

The samples were prepared by combining 1.5 kg of tips (with or without gills) with 3.0 kg of water in a metallic beaker, placed on a heating plate. The mixture was stirred at a constant rate with a Fisher stirrer model SL 2400 (Fisher Scientific, Springfield, NJ).

The temperature of digestion varied depending on the enzyme. The pH of the extract was adjusted to the optimum pH of each enzyme. The pH was decreased by the addition of 10 N hydrochloric acid. The enzyme was then added to the slurry.

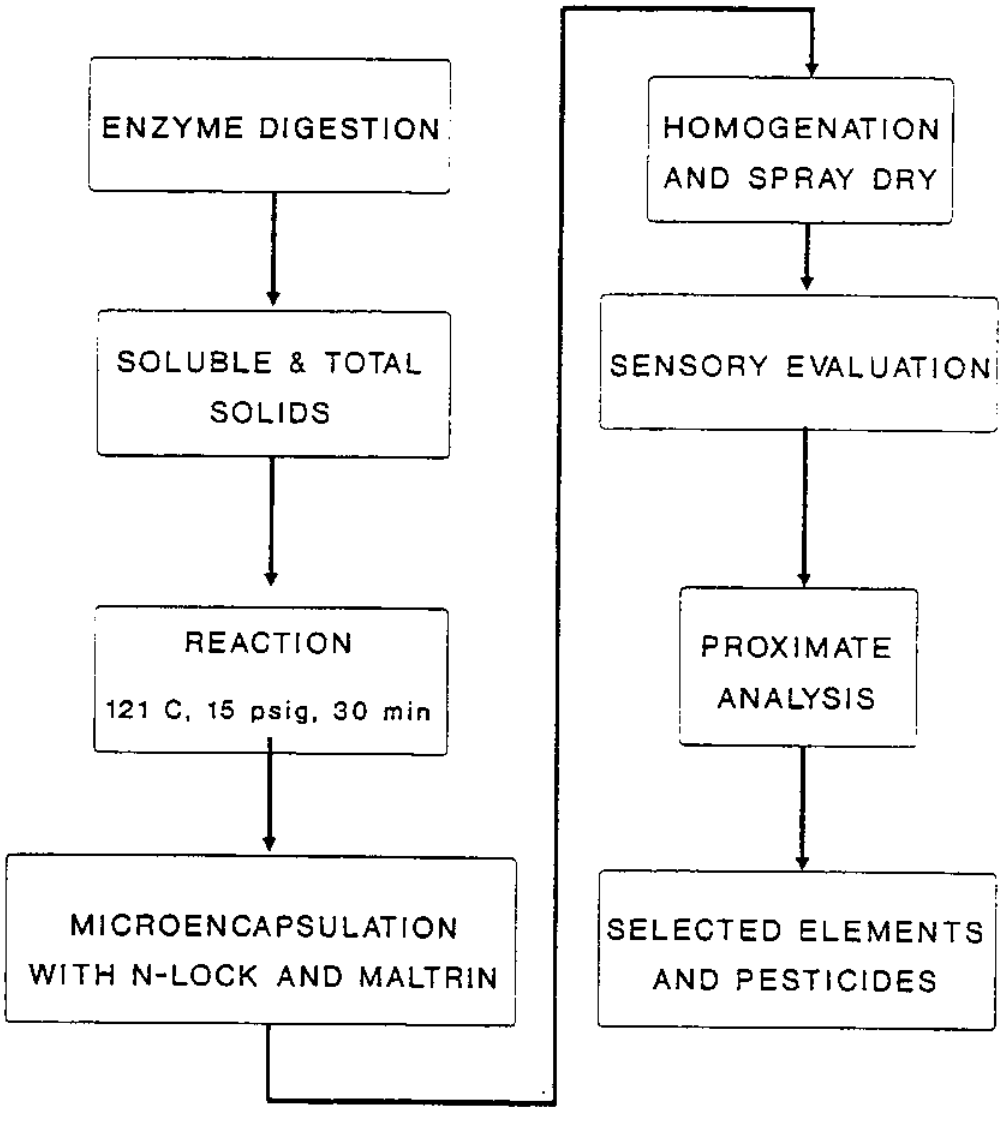

FIGURE 8: FLAVOR EXTRACTION PERFORMED AT A COMMERCIAL FLAVOR FIRM

TABLE 2: RAW MATERIALS, ENZYMES, AND TIME OF REACTION USED IN THE DIGESTIONS PERFORMED AT A COMMERCIAL FLAVOR FIRM

SAMPLE	RAW MATERIAL	ENZYME USED	TIME OF REACTION (hr)
1	Tips with gills	Protease 1	9
2	Tips without gills	Protease 2	6
3	Tips without gills	Protease 2	9
4	Tips with gills	Protease 2	6
5	Tips with gills	Protease 3	9
6	Tips without gills	Protease 2	9
7	Tips with gills	Protease 4	9
8	Tips without gills	Protease 4	9

The digestions were allowed to proceed for 9 hr. At the end of this time, the mixture was filtered through a 20 mesh sieve. The particles that did not pass through the sieve were collected for a proximate analysis (marc). The filtrates were collected in bottles and the soluble solids (SS) and total solids of the liquid (TS) were determined.

1.2.1.2. Soluble and Total Solids Measurements

The higher the SS of the digested mixture, the more crab meat that was dissolved in the water and the higher the percentage of the crab meat in the final product. The TS were measured as well because some crab meat was suspended as very small particles in the liquid. These solids became incorporated into the crab meat percentage of the final product. The SS were measured with a refractometer (Bauch-Lomb Optical Co, Rochester, NY). The TS were measured by evaporating all the water of a pre-weighed sample. Two analysis were performed for each treatment since the variation was small. Each sample was weighed in a drying pan and placed in a drying oven set at 110°C for 18 hr. The percentage of total solids was calculated as follows:

Percentage TS = 1 - [(initial weight of sample) - (weight of dry sample)] X 100 / initial weight of sample

1.2.1.3. Reaction and Microencapsulation

Two kg of the filtered sample were placed in the interior vessel of a 2 kg oil heated reactor for 30 min at elevated temperatures and pressures. Once the reaction was completed, a microencapsulation mixture was added to the extract in order to increase the total soluble solids.

1.2.1.4. Spray Drying Process

When the mixture was all dissolved, it was homogenized in Cherry Burrel homogenizer, model 200 (Cherry Burrel Co. Cedar Rock, IA) prior to being spray dried. The dry product was collected in a plastic bag and stored in a desiccator at room temperature for further analysis.

1.2.2. Flavor Extraction From the Claws

Blue crab claws are either picked by hand or in a Harris Claw Machine. The meat produced by the Harris Claw Machine is inferior in texture and flavor to hand picked meat (Hong, 1990) and is sold at lower prices. The machine picking operation consumes large volumes of brine and because of the high concentration of salt used in this operation, proper disposal of the brine becomes a problem.

The crab claws were evaluated as a possible raw flavor material to eliminate the problem of waste water disposal.

The claws were ground in order to expose the flesh to the enzyme. The ground claws were then mixed with water using the same quantities as the previous extractions (2 parts of water to 1 part of scrap). The identical process was followed as mentioned above: digestion (the claws were only digested with protease for 6 hr), soluble solids and total solids measurement, filtering, microencapsulation, homogenization, and spray drying. The flavor produced was evaluated for its quality by a trained panel.

1.3. Analysis Performed on Flavors

1.3.1. Sensory Analysis of the Flavors Obtained at Virginia Tech

The flavors obtained from the extractions produced at the Food Science and Technology Department of Virginia Tech were evaluated for crab flavor intensity by a sensory panel.

The sensory evaluations were conducted using a 14 member experienced panel selected from the Department of Food Science and Technology. The panel consisted of faculty, staff and graduate students of varying sex, ethnic background, and age. The panelists were non-smokers, in good health and with no allergies to seafood.

Two training sessions were held in the week prior to the tests. The training sessions consisted of a discussion on the objectives of the study and the methods used to evaluate the crab extracts. Fresh blue crab meat was presented to the panelists in the training sessions, thereby allowing them to familiarize themselves with its characteristic taste and odor.

The sensory evaluation tests were conducted during the mid-morning and mid-afternoon to prevent taste masking caused by previous meals. The panelists were seated in individual testing booths and all tests were performed under a fluorescent white light.

A preference test with a nine point hedonic scale was utilized. Panelists were asked to evaluate the quality of the crab flavor present in the sample and identify the answer that better described the flavor (Figure 9). The carrier in which the flavor was dispersed was Light Philadelphia^{R} Cream cheese (this cheese has a very light flavor that did not mask the blue crab flavor and provided the texture of a dip). The cheese was mixed with powdered flavor 1 hr prior to every session in order to allow the flavor to equilibrate and the mixture to reach room temperature. The amount of dry powder flavor to be added to the cream cheese was determined by the panelists in the first two sessions. The desired amount gave a distinct flavor to the cream cheese, without overwhelming the panelist's taste buds.

Each flavor was presented to the panel in triplicate. Twenty-five g of powder flavor were added to 250 g of cream cheese. The samples were presented on an unsalted cracker, with no

Name _____ Code_____ Date _____

Please circle the term that best describes your attitude about the product whose code matches the code on this score card.

XYA

Like extremely
Like very much
Like moderately
Like slightly
Neither like nor dislike
Dislike slightly
Dislike moderately
Dislike very much
Dislike extremely

FIGURE 9: AN EXAMPLE OF PREFERENCE TEST SCORE CARD USING A HEDONIC SCALE (Sidel, 1985)

specific order, only 6 samples were evaluated in each session to avoid fatigue. The panelists were instructed to eat the cheese only and not to bite into the cracker. Water and unsalted crackers were provided to the judges so they could rinse and refresh their mouths between samples.

The panelists were asked to select the statement that better defined their feelings toward the flavors and to write any comments on the preference test with the hedonic scale score card (Figure 9). Numbers from 1 to 9 were assigned to the statements of the hedonic scale in order to statistically analyze the results. Data was subjected to an analysis of variance and Duncans's multiple range test using the SAS computer program (SAS, 1985).

1.3.2. Sensory Analysis of the Flavors Obtained in at a Commercial Flavor Firm

The flavors obtained at a commercial flavor firm were evaluated for their quality by a highly trained panel. A simple preference test was used in which the best flavors were identified. The panelists were free to comment and give their opinion. The flavors (0.5%) were dispersed in 99% water and 0.5% of a mixture containing 70% salt, 20% lactose, 10% shortening.

1.3.3. Flavor Composition

The flavor selected by the expert sensory panel was analyzed at Virginia Polytechnic Institute and State University for amino acid composition, proximate analysis, pesticide residues, and selected elements.

1.3.3.1. Proximate Analysis

The flavors extracted at the commercial flavor firm were evaluated for their quality by an expert panel. Two samples from the most preferred two treatments were analyzed for their proximate composition according to the A.O.A.C. procedures (1984).

Protein was determined using a Kjeltec™ Auto 1030 Analyzer (Tecator), and fat by ether extract using a Soxtec™ System HT (Tecator). For ash determination, two 5 g samples were dry-ashed at 525°C (977°F) in oven dried crucibles using a furnace (Lindberg series 51000, Sola Basic Ind., Watertown, WI).

The carbohydrate composition was calculated by difference.

1.3.3.2. Amino Acid Composition

The flavors obtained form the tips without gills digested with Protease 2 for 6 and 9 hr and the flavor obtained from the claws digested with Protease 2 for 7 hr were sent to a private lab for analysis. The results will be included on a final report on the project that will be prepared by the Virginia Tech Sea Grant Program.

1.3.3.3. Selected Elements: Presence and Concentration

The flavors extracted in the commercial flavor firm that were selected as the most preferred by the trained sensory panel (the flavors obtained form the tips without gills digested with Protease 2 for 6 and 9 hr), were analyzed for selected elements at Virginia Tech using a ICP (inductively coupled plasma spectrometry) system. This system consists of a simultaneous spectrometer (Jarrel-Ash ICAP, model 9000) and a sequential scanning spectrometer (Jarrel-Ash Atomscan, model 2400, Thermojarrel Ash Corp. Franklin, MA). The simultaneous spectrometer records the concentration of the following elements simultaneously: Al, Ca, Fe, Li, Mn, P, Zn, B,

Cu, K, Mg, Na, and S. The sequential spectrometer scans the spectrum from 190 to 535 nm, measuring specified wavelengths corresponding to over 60 individual elements.

The samples were injected in the ICP system in a solution form. One g of each flavor was dissolved in 10 ml of deionized water.

1.3.3.4. Pesticide Residue Determination

The determination of pesticide residues in the flavors selected by the sensory panel that took place in the commercial flavor firm, was performed following the procedures described by Bertuzzy et al. (1967).

A gas chromatograph, Tracor model 540 GC (Tracor Instruments Austin, Inc., Austin, TX), equipped with an Electron Capture Ni63 and a Flame Photometric Detector was utilized. The EC Detector column packing was: 1.5/1.95% SP2250/SP2401, 100/120 mesh Supelcoport in a 6' glass column, and 1/4" inner diameter. The column temperature was 200°C, the inlet temperature 235°C and the detector temperature was 350°C. The FID Detector column packing was: 10% SP-2100, 80/100 mesh Supelcoport in a 6' glass column, 1/4" inner diameter. The column, inlet and detector temperature were identical.

1.3.4. Microbiological Analyses Performed on the Flavors

Because the raw material in flavor extractions is highly contaminated, it is important to determine the bacteriological condition of the final flavors.

Two flavors were analyzed for selected microbiological populations. These were the flavors obtained from the tips with gills digested with Protease 2 and reacted in a closed vessel, and the flavor obtained from the tips without gills digested with Protease 2 and reacted in a closed

vessel.

Microbiological analyses of the crab flavor consisted of the following counts: mesophilic aerobes, mesophilic anaerobes, total coliforms, fecal coliforms and Listeria.

A heat shock recovery test was performed as well in order to recover all thermophile microorganisms that could have survived the high temperatures used during extraction.

1.3.4.1. Sample Preparation

The crab flavor samples were aseptically taken from the containers where the flavors were collected after spry drying, and stored in sterile bags. Eleven g of the flavor were dissolved in 99 ml of sterile 0.1% (W/V) peptone broth (Difco, Detroit, MI). This 1:10 dilution was used for subsequent serial dilutions and pour plates.

1.3.4.2. Aerobic Plate Counts

Aerobic plate counts were conducted on duplicate pour plates made with Standard Methods Agar (SMA; Difco, Detroit, MI) and incubated at 35°C for 48 hr for enumeration of mesophiles. Aerobic bacterial numbers were calculated and expressed on a per gram of crab flavor basis.

1.3.4.3. Anaerobic Plate Counts

Duplicate pour plates with Standard Method Agar (SMA; Difco) were placed into GasPak™ Anaerobic Jars (BBL Microbiological Systems, Cockeysville, MD) with GasPak Plus™ Hydrogen + CO_2 Envelope (BBL) and an anaerobic indicator. The plates in the anaerobic

jar were incubated at 35°C for 48 hr for enumeration of mesophiles.

Anaerobic bacterial numbers were calculated and expressed on a per gram of crab flavor basis.

1.3.4.4. Total Coliforms

Total coliforms were estimated by a three tube MPN technique. Three consecutive dilutions of each flavor/peptone solution were inoculated in triplicate into Lauryl Sulfate Tryptone (LST broth; BBL Microbiological Systems) broth tubes and incubated at 32°C for 48 hr. Tubes showing gas formation were recorded as positive and used to calculate the Most Probable Number (MPN) of presumptive coliforms per gram of crab flavor (FDA, 1978).

1.3.4.5. Fecal Coliforms

Fecal coliforms were determined by inoculating separate tubes of EC Broth with a loopful of broth from each positive LST tube. EC tubes were incubated at 44.5°C for 48 hr. Tubes showing gas formation were used to calculate the Most Probable Number of fecal coliforms per gram of crab flavor.

1.3.4.6. Heat Shock Recovery of Thermophile Microorganisms

Thermophile microorganisms could have survived the high temperature and high pressure used in the extraction process due to the heat shock phenomenon. When bacteria are subjected to temperatures slightly higher than optimal conditions for growth, these "heat shocked" cells may increase heat resistance compared to "non-heat shocked cells" (Neidhart et al., 1984).

Enzymatic digestion at 55°C of the raw material prior to reaction at 120°C could have had this "heat shock" effect on the microorganisms present. These microorganisms would not be detected in mesophile plate counts, but would be observed in thermophile plate counts.

Aerobic plate counts were conducted on duplicate pour plates made with T-Soy Agar (Difco, Detroit, MI) and incubated at 55°C for 48 hr for enumeration of thermophiles. Aerobic bacterial numbers were calculated and expressed on a per gram of crab flavor basis.

2. Pigment Extraction from Blue Crab Processing Byproducts

The carotenoid pigments were extracted from the crab scrap and the yield was calculated. The crab scrap was separated into clean carapaces, carapaces containing the eggs, and legs. The carotenoid pigments were also extracted from the claws after the meat was separated using a Harris Claw Machine. The pigment was extracted from each part and the respective percent yield calculated.

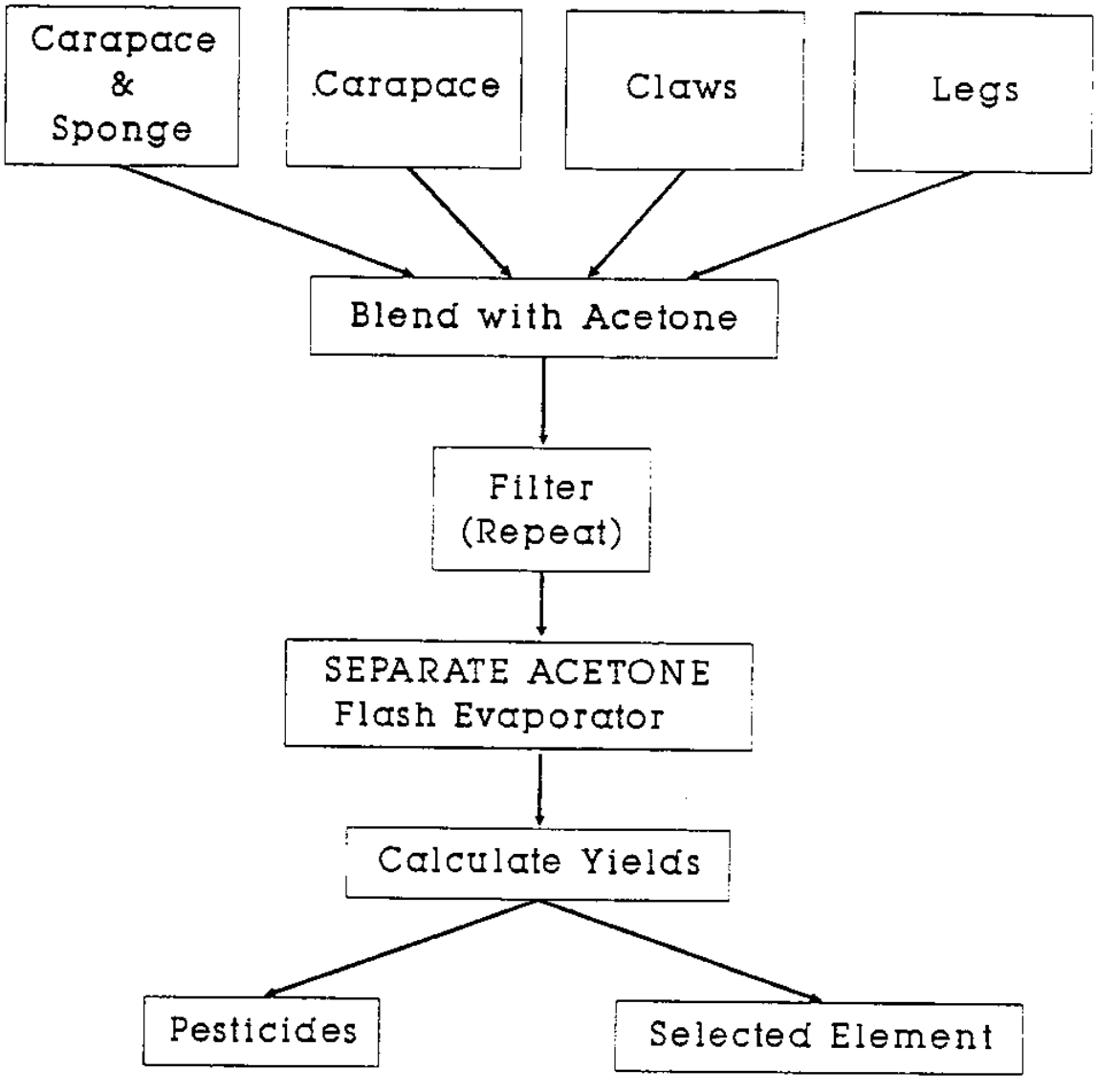

FIGURE 10: PIGMENT EXTRACTION OF BLUE CRAB BY-PRODUCTS

2.1. Pigment Extraction Process

The extraction method used was that suggested by Inoue (1987).

Two hundred g of the scrap were blended in 100 ml of acetone at 4°C in a blender for 1 min (Figure 10). The slurry was poured into a glass bottle and allowed to stand for 10 min. Then it was then filtered through 4 layers of cheese cloth. The marc was washed in acetone and filtered again until it acquired a gray color. The acetone which acquires an orange color, was filtered through a N_o 2 Whatman filter paper, and introduced into a pre-weighed 500 ml round bottom flask. The acetone was removed from the sample by using a Buckler Flash Evaporator under reduced pressure. The temperature of the water in the Flash-Evaporator bath was maintained at 56°C (boiling temperature of the acetone). Glycol (10°C) was used as the coolant in the condensation column. The flask containing the concentrated pigment was weighed and the yield calculated as follows:

Percent Yield = [(weight of flask + pigment) - (Weight of flask)] X 100 / initial scrap weight

2.2. Color Measurements

The pigment extracts obtained from the carapace with and without eggs were analyzed to determine color difference.

The samples were prepared for color measurements by drying the extracts in the oven.

Fifteen g of the dried samples (thick paste texture) were spread on a Petri dish.

Color measurements were taken with a Minolta Chroma Meter, model CR 200 (Minolta Corp., Osaka, Japan). The Chroma Meter was calibrated on the standard calibration surface (part of the Minolta Chroma Meter equipment) covered by a Petri dish. Six color measurements were taken per sample, and the results were statistically analyzed in order to compare both samples.

Color is expressed in the Minolta Chroma Meter system with the values "L*", "a*", and "b*". "L*" refers to the darkness-lightness scale with values of 0 to 100. The "a*" value refers to the red to green color scale; this scale goes from +a (redness) to -a (greenness). The "b*" value refers to the yellow to blue color scale; this scale goes from +b (yellowness) to -b (blueness).

2.3. Pigment Extract Composition

The extracted pigments were analyzed to determine their astaxanthin concentrations. A quick HPLC method for the separation of astaxanthin and its isomers on a H_3PO_4 coated silica column. Dichloromethane/2-propanol/n-hexane (10/2/88, v/v %) was used as eluent. The flow rate was 1.8 ml/min and the injection volume was 10ml.

2.4. Pesticide Residues Determination

The determination of pesticides in the color extracts was performed following the procedures described by Bertuzzy et al. (1967).

A gas chromatograph, Tracor model 540 GC (Tracor Instruments Austin, Inc., Austin, TX), equipped with an Electron Capture Ni63 and a Flame Photometric Detector was utilized. The EC Detector column packing and the FID Detector packing was similar to the packing used for detection of pesticides in flavors.

III. RESULTS AND DISCUSSION

1. Flavor Extraction Results

1.1. Flavor Extractions Performed at Virginia Tech

1.1.1. Boiling Time Determination for Extractions Performed in an Open Vessel

The spray dried and freeze dried extracts from the raw crab scrap boiled for 1, 2, 3, and 4 hr were subjected to sensory analysis. The results are summarized in Table 3.

In order avoid the possibility that the panelists' responses could be influenced by their likes or dislikes towards the carrier (cream cheese), samples containing only cream cheese (without crab flavor) were presented to the panelists with samples containing a crab flavor. All panelists detected the lack of crab flavor in the sample and these samples were rejected by the panelists.

Significant differences ($p < 0.05$) were found among the flavors boiled for 1, 2, 3, and 4 hr. The flavor obtained from the crab scrap boiled for 1 hr was found to be significantly different from the flavors extracted from the samples boiled for 2, 3, and 4 hr; but no significant difference ($p < 0.05$) was found between the flavors extracted from samples boiled for 2 and 3 hr. From these results it can be concluded that the best flavor was obtained from the sample boiled for 1 hr and the least preferred flavor resulted from the sample boiled for 4 hr. Seven out of 13 panelists defined the flavor extracted from the sample boiled for 4 hr as burnt, pungent, and bitter. Samples that boiled for 4 hr acquired a burnt flavor which was found to be unacceptable.

TABLE 3: SENSORY PREFERENCE RATINGS[pq] OF FLAVORS EXTRACTED FROM CRAB SCRAP BOILED FOR 1, 2, 3, AND 4 HOURS

BOILING TIME (hr)	DRYING PROCESS	MEAN SCORE
1	Spray Drying	3.00 (a)
	Freeze Drying	3.88 (b)
2	Spray Drying	4.66 (c)
	Freeze Drying	4.73 (c)
3	Spray Drying	4.76 (c)
	Freeze Drying	5.69 (d)
4	Spray Drying	-
	Freeze Drying	7.73 (e)

p Rating scale: like extremely = 1, Like very much = 2, like moderately = 3, like slightly = 4, neutral = 5, dislike slightly = 6, dislike moderately = 7, dislike very much = 8, dislike extremely = 9.

q Means followed by same letter within rows are not significantly different ($p > 0.05$)

A significant difference ($p < o.05$) was found between flavors that were obtained by spray drying and freeze drying the extracts resulting from the samples boiled for 1 hr. The sprayed dried flavor obtained from the samples boiled for 1 hr was given a mean score of 3.0 while the freeze dried flavor from the same extract was given a mean score of 3.88. This difference was found between the spray dried and freeze dried flavors obtained from the extract boiled for 3 hr but not between the flavors obtained from the extract boiled for 2 hr. From this experiment it can not be concluded that the spray dried flavors were preferred over the freeze dried flavors.

1.1.2. Contribution of the Crab Scrap Parts to the Flavor

The scrap was separated into two groups: tips (white scrap) and carapace with legs. These parts were boiled in an open vessel for 1 hr (the best flavor was obtained by boiling the scrap for 1 hr as indicated by the results in Table 3). The extracts were sprayed dried and freeze dried and were subjected to sensory analysis. The results are summarized in Table 4. A significant difference ($p < 0.05$) was found between the flavors extracted from the tips with gills and the carapaces with legs. The flavors extracted from the tips were preferred to the flavors extracted from the carapaces and legs. One reason for this difference is the presence the eggs attached to the carapace from the female crabs which imparted a bitter taste to the extract. Another major reason for this difference is that the tips contain up to 50% crab meat. This meat is the major contributor to blue crab flavor.

Spray dried and freeze dried flavors from the same extract were compared as well. A significant difference ($p < 0.05$) was found between the sprayed dried flavor and freeze dried flavor obtained from the tips. The spray dried flavor was given a mean score of 2.30 while the freeze dried flavor was given a mean score of 5.42. The same significant difference was found between spray dried and freeze dried flavors obtained from the carapace with legs (mean scores of 5.72 and 7.85 respectively). These significant differences allow the conclusion that the sprayed dried flavors were preferred to the freeze dried flavors.

TABLE 4: SENSORY PREFERENCE RATINGS[pq] OF CRAB FLAVORS EXTRACTED FROM DIFFERENT CRAB PROCESSING BY-PRODUCTS

SCRAP PARTS	DRYING PROCESS	MEAN SCORE
Carapace and Legs	Spray Drying	5.72 (a)
	Freeze Drying	7.85 (b)
Tips with Gills	Spray Drying	2.30 (c)
	Freeze Drying	5.42 (a)

p Rating scale: like extremely = 1, Like very much = 2, like moderately = 3, like slightly = 4, neutral = 5, dislike slightly = 6, dislike moderately = 7, dislike very much = 8, dislike extremely = 9

q Means followed by same letter within rows are not significantly different ($p > 0.05$)

This preference can be explain by examining the method employed for preparing the samples to be freeze dried. The samples to be freeze dried were concentrated by evaporating water from the extract. This process could have affected some components dissolved in the extract giving the characteristic burnt and pungent taste.

Since the flavor industry does not utilize freeze drying for flavor production, this method was not used again. The rest of the flavors produced in this research were spray dried.

1.1.3. Flavor Extractions from the Crab Tips Pre-Treated with Enzymes

The flavors obtained from the tips treated with protease at different concentrations and times of reaction were analyzed for their quality in the same manner as the flavors obtained in the previous extractions. The sensory preference rating are summarized in Table 5.

No significant differences ($p > 0.05$) were found among the flavors extracted from the samples treated with 0.1% or 0.2% Protease 2, or Protease 1 but a significant difference ($p < 0.05$) was found between these flavors and tips. The flavor extracted from the sample treated with 0.2% Protease 2 for 2 hr. This flavor was found to have greater acceptability (mean score was 1.73). From these results it can not be concluded that treating the samples with Protease 2 imparts a better flavor than treating the samples with Protease 2 or Protease 1 or that the concentration to be used should be 0.2% and the time of reaction 2 hr. But when an analysis of variance test was performed on the preference rating scores of these flavors and the scores given to the flavors extracted previously (without enzyme digestion) from the tips, it can be concluded that the flavors extracted from the samples pre-treated with enzymes were preferred to those extracted from the tips without enzymatic treatment.

TABLE 5: SENSORY PREFERENCE RATINGS[pq] OF CRAB FLAVORS EXTRACTED FROM ENZYMATICALLY PRE-TREATED TIPS

ENZYME USED	ENZYME CONCENT. (%)	REACTION TIME (HR)	MEAN SCORE
Protease 1	0.1	1	1.97 (a)
		2	2.36 (a)
	0.2	1	2.39 (a)
		2	2.38 (a)
Protease 2	0.1	1	1.88 (a)
		2	2.06 (a)
	0.2	1	1.85 (a)
		2	2.03 (a)
Protease 3	0.1	1	2.21 (a)
		2	1.85 (a)
	0.2	1	1.86 (a)
		2	1.73 (b)

p Rating scale: like extremely = 1, Like very much = 2, like moderately = 3, like slightly = 4, neutral = 5, dislike slightly = 6, dislike moderately = 7, dislike very much =8, dislike extremely = 9

q Means followed by same letter within rows are not significantly different ($p > 0.05$)

1.1.4. Flavor Extraction from Retort Juice

The flavor extracted from the retort juice was analyzed for its quality and the sensory preference rating of this flavor was compared (Anova and Duncan tests) to the rating of spray dried flavors extracted from the carapace and the tips. The flavor extracted from the retort juice was given a mean score of 8.60 which is significantly different ($p < 0.05$) from the scores given to the spray dried flavors extracted from the tips and carapace. These results are summarized in Table 6. The spray dried flavor extracted from the retort juice was given a mean score of 8.60. There is significant difference between this flavor and the spray dried flavors extracted from the tips and the carapace (mean score of 2.30 and 5.72 respectively). This flavor was extremely disliked by the panelist. All thirteen panelists wrote comments like very bitter and uneatable on the score cards.

From these results it can be concluded that the retort juice produced during the blue crab cooking operation, with no further treatment, is not an acceptable raw material for flavor extraction. The bitter taste is produced by peptide chains present in the juice. These chains can be broken into free amino acids by treating the retort juice with proteolytic enzymes, improving the extracted flavor.

TABLE 6: SENSORY PREFERENCE RATINGS[pq] OF SPRAY DRIED CRAB FLAVORS EXTRACTED FROM TIPS, CARAPACE AND THE RETORT JUICE

CRAB BY-PRODUCTS	MEAN SCORE
Carapace and Legs	5.72 (a)
Tips	2.30 (b)
Retort Juice	8.60 (c)

p Rating scale: like extremely = 1, Like very much = 2, like moderately = 3, like slightly = 4, neutral = 5, dislike slightly = 6, dislike moderately = 7, dislike very much = 8, dislike extremely = 9.

q Means followed by same letter within rows are not significantly different ($p > 0.05$)

1.2. Flavor Extractions Performed at a Commercial Flavor Firm

1.2.1. Soluble and Total Solids Present in the Extracts and the Sensory Analysis Results

Blue crab tips with and without gills and claws were utilized as raw materials for flavor extraction. The tips and claws were digested with Proteases 1,2, and 3. The results are summarized in Table 7.

TABLE 7: SOLUBLE AND TOTAL SOLIDS (SS AND TS) PRESENT IN THE BLUE CRAB EXTRACTS FROM TIPS WITH AND WITHOUT GILLS AND CLAWS AFTER DIGESTION WITH ENZYMES

RAW MATERIAL	ENZYME USED	REACTION TIME(hr)	SS (%)	TS (%)
Tips with gills	Protease 1	9	10	11.0
Tips w/o gills	Protease 2	6	8	9.3
Tips w/o gills	Protease 2	9	10	11.9
Tips with gills	Protease 2	6	6	s
Tips with gills	Protease 3	9	11	12.8
Tips w/o gills	Protease 3	9	10	11.8
Tips with gills	Protease 4	9	14	15.2
Tips w/o gills	Protease 4	9	14.5	15.0
Claws	Protease 4	6	7.0	7.8

s Analysis not performed

Tips with and without gills digested with Protease 2 for 9 hr had the higher percentage of soluble and total solids. The extract obtained from tips with gills digested with Protease 2 had 14% soluble solids and 15.2% total solids. The remainder of the extracts had total solids values between 11.0% and 11.8%. Protease 4 was more efficient in hydrolyzing the crab meat proteins than the other enzymes. The total soluble solids percentage obtained with each enzyme was one of the factors used to decide which enzyme to use in the industrial production of a crab flavor. A high percentage of total solids present in the flavor is preferred.

The variable used to identify the optimum process was the results of the sensory analysis. The flavor obtained from tips without gills digested with Protease 2 (15% TS) was described as more fish-like than crab-like. The blue crab flavor which was preferred by the panel was the flavor obtained from the tips without gills digested with Protease 2 for 6 hr. This process resulted in a lower amount of total solids (9.3%), but the flavor was more preferred. The flavor obtained from the tips digested with Protease 2 for 9 hr was found to be the second best (with a slightly fishy taste) although it had a higher percentage of total solids.

If the digestion process is too long, the resulting reduced compounds will develop a bitter taste or flavor. Six or seven hr appeared to be the digestion time which provided the best results. The flavors obtained from the tips without gills were preferred to the flavors obtained from the tips with gills attached. The gills imparted a bitter taste to the extract.

The extract obtained from the claws digested with Protease 2 for 6 hr had a low TS percentage (7.8%). This flavor was analyzed for its quality by a trained panel. This flavor was found to be the best of all flavors extracted. The low percentage of total solids present in the claw extract problem can be avoided if the claws were processed with the tips (with the gills), avoiding at the same time the high cost of separating the gills from the tips. The flavor of the gills could be improved by the flavor generated by the claws.

1.2.2. Flavor Composition

1.2.2.1. Proximate Analysis

The proximate composition of the flavors selected by the sensory panel (the flavor obtained from the tips without gills digested with Protease 2 for 6 hr, and the flavor obtained from the tips without gills digested with Protease 2 for 9 hr) was determined as well as the proximate composition of the marc that was recovered from both extraction processes after filtering the digested raw materials through a 20 mesh sieve. The results are summarized in Table 8.

F1 (flavor obtained from the tips without the gills digested with Protease 2 for 6 hr) had a protein percentage similar to the total solids percentage present in the extract prior to drying (9.3% total solids compared to 9.28% protein). F2 (flavor obtained from the tips without the gills digested with Protease 2 for 9 hr) had a protein percentage lower than the total solids present prior to drying (11.9% total solids compared to 10.18% protein). From these results, it can be concluded that the total solids present in the extract are primarily composed of protein. The higher the total solids, the higher the protein content of the flavor. Lipids were present in the flavors but only in trace amounts. Most of the carbohydrates present in the flavors were added to the extract as starch (microencapsulation).

The proximate composition of the marcs recovered from the extracts from which the best flavors were extracted indicated that the by-product of flavor extraction is very high in protein and ash. The marc from the extract obtained from the digestion of tips without gills with Protease 2 for 6 hr (M1) was 53.89% protein and 42.94% ash. The marc from the extract obtained from the digestion of tips without gills for 9 hr (M2) was 52.92% protein and 45.95% ash. The ash content includes the percentage of chitin present in the marc. These results indicate that the flavor extraction by-product can be utilized as a feed ingredient and a potential raw material for chitin and chitosan production.

TABLE 8: PROXIMATE COMPOSITION OF THE SELECTED FLAVORS AND PROXIMATE COMPOSITION OF THE RECOVERED "MARC"

SAMPLE	PROTEIN %	ETHER EXTRACT %	ASH %	CARBO-HYDRATE %
	dry weight basis			
F1	9.28	0.00	0.09	90.71
F2	10.19	0.03	0.01	89.78
M1	53.89	1.087	45.94	0.03
M2	52.92	1.092	45.95	0.05

F1 = Flavor obtained from the tips without gills digested with protease for 6 hours.
F2 = Flavor obtained from the tips without gills digested with protease for 9 hours.
M1 = Marc recovered after filtering the extract obtained from the tips without gills digested with protease for 6 hours.
M2 = Marc recovered after filtering the extract obtained from the tips without gills digested with protease for 9 hours.

1.2.2.2. Amino Acid Composition

The amino acid composition of the selected flavors will be included in a final report on the project that will be prepared by the Virginia Tech Sea Grant Program.

1.2.2.3. Selected Elements Determination

The flavor extracted from the tips without gills digested with Protease 2 for 6 hr was analyzed for selected elements. The detected elements and concentrations are shown in Table 9. These concentrations do not present any health hazard as indicated by the tolerance limits of each element (Dell'Aria, 1992).

No mercury or lead was detected in the sample.

TABLE 9: ELEMENTS DETECTED IN THE FLAVOR EXTRACTED FROM THE TIPS WITHOUT THE GILLS DIGESTED WITH PROTEASE FOR 6 HOURS

ELEMENTS	CONCENTRATION (ppm)
Mn	0.345
Cu	0.493
Fe	2.118
Al	3.285
B	0.071
Cd	0.006
P	78.850
K	87.44
Ca	196.1
Mg	26.34
Na	186.8
S	57.46
Zn	0.924

1.2.2.4. Pesticide Residues

The flavor obtained from the tips without gills digested with Protease 2 for 6 hours was analyzed for pesticide residues.

No organophosphorus (OP) pesticides or organochloride (OC) pesticides were detected in the flavor.

1.2.3. Microbiological Analyses Performed on the Flavors

The microbiological quality of the flavors extracted from the tips without gills digested with Protease 2 for 6 and 9 hr was investigated. The aerobic plate counts, anaerobic plate counts, and total coliforms were all negative. The heat shock recovery test also had negative results. The °extract was sterilized prior to drying by the high temperature (121°C) applied during the reaction procedure. Also, post processing contamination did not occur due to the quality control procedures employed during the drying and packaging operations.

2. Pigment Extraction

The pigment was extracted from all the crab processing byproducts: carapace with adhering eggs, carapace without eggs, legs and shells from the claws (shells were separated from the claw by the Harris Claw process). A pigment extract is shown in the photograph in Figure 11.

2.1. Pigment Extraction Yields

The pigment extract yields of each crab by-product were calculated. The carapace with eggs produced the highest yield, 7.5%, followed by the clean carapace 6.0%, claws with 3.5% and legs 1.0% yield. Prior to extraction, the mass of eggs had a bright orange color. This pigment was extracted with the pigment present in the shells, increasing the final yield of extraction from the carapace. Based on the results, it can be concluded that the claws and the legs were not suitable raw materials for industrial pigment extraction.

2.2. Color Measurements

Color measurements were taken from the color extracts recovered from the carapace with and without eggs.

"a*", "b*", and "L*" values of each sample were subjected to T tests in order to identify significant differences between samples (Table 11). The color extracted from the carapace with eggs had a "L*" value of 26.15, a "a*" value of +6.38 and a "b*" value of +7.92. The color extract from the carapace without eggs had a "L*" value of 27.2, a "a*" value of +6.10 and a "b*" value of +7.92. No significant difference ($p > 0.05$) was detected between color values determined for each sample. The pigment extraction process not only the pigment, but also the lipids present in the eggs. This pigment did not increase the color intensity, only the total yield.

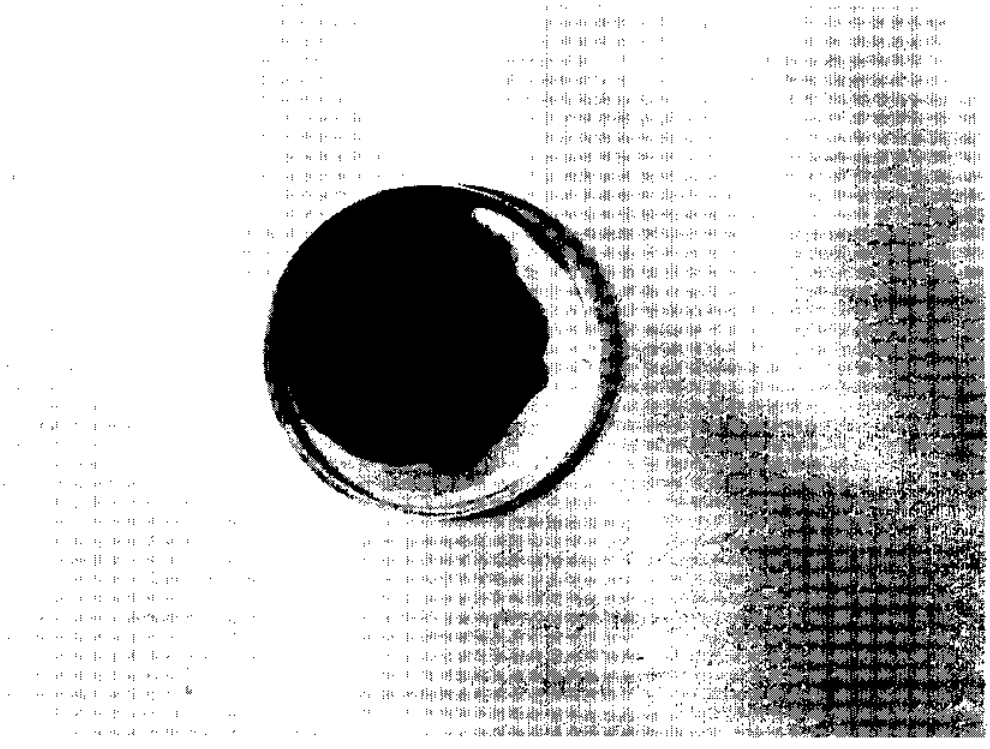

FIGURE 11: COLOR EXTRACT FROM CARAPACE WITH EGGS

TABLE 10: COLOR MEASUREMENT[1] USING MINOLTA CHROMA METER.

SAMPLE	CIE L*	CIE a*	CIE b*
Extract from carapace with eggs	26.15	+6.36	+7.38
Extract from carapace w/o eggs	27.2	+6.10	+7.92

[1] Means of 6 observations on each sample.

2.3. Astaxanthin Determination in the Color Extract

The color extracts contained 20-40 ppm of astaxanthin in the palmitate form. A final concentration of 1,200-3,000 ppm is preferred for industrial extraction of the pigment (Bowen, 1992). Chen and Meyers (1982) utilized a soy oil extraction procedure on crayfish and on blue crab waste. The crayfish waste had an astaxanthin concentration of 1,800 ppm but only 90 ppm of astaxanthin were recovered from the blue crab waste. Based on these results it can be concluded that the blue crab waste is a good raw material for canthaxanthin extraction but not for astaxanthin.

2.4. Pesticide Residues

The extract recovered from the carapace with adhering eggs was analyzed for possible pesticide residues.

The color extraction process also extracted all the fat present in the tissues attached to the carapace, concentrating any possible pesticide residue.

The following pesticides were detected in the color extract: Heptachlor, 0.036 ppm; Heptachlor Epoxide, 0.07 ppm; ortho-ortho DDE, 0.0031 ppm; para-para DDE 0.0183 ppm; para-para DDT, 0.043 ppm; Endrin, 0.033 ppm; Dieldrin 0.030 ppm; and Atrazine, 1.851 ppm. The tolerance limit concentration of each pesticide to be found in animal feed (Dell'Aria, 1992) and the actual concentrations found in the color extract are summarized in Table 11. The concentration of Heptachlor and Heptachlor epoxide (0.036 ppm and 0.07 ppm respectively) exceeded the tolerance limits (0.01 ppm) for a feed. The other pesticides present are within the tolerance limits. It is important to remark that these limits are for feed and not a feed ingredient. The incorporation of the color extract into a commercial animal feed would occur at minimal concentration (less than 1%). Therefore any pesticide residues would not occur in a concentration that would exceed the established tolerance limits.

TABLE 11: PESTICIDE AND HERBICIDE RESIDUES FOUND IN THE COLOR EXTRACT AND THE TOLERANCE LIMITS OF THESE RESIDUES IN ANIMAL FEED*

PESTICIDE HERBICIDES	CONCENTRATIONS IN EXTRACT (ppm)	TOLERANCE* LIMITS (ppm)
Heptachlor	0.036	0.01
Heptachlor Epoxide	0.07	0.01
Ortho-Ortho DDE	0.031	0.5
Para-Para DDE	0.018	0.5
Para-Para DDT	0.043	0.5
Endrin	0.03	0.03
Dieldrin	0.03	0.03
Atracine	1.851	15.0

* Tolerance Limits dictated by the Virginia Department of Agriculture and Consumer Services.

IV. SUMMARY AND CONCLUSIONS

A crab or seafood flavor was obtained from all blue crab processing byproducts utilizing two methods of extraction. Using an open vessel, the method and raw material that produced the best flavor was hot water extraction for 1 hr from the tips pretreated with proteolytic enzymes. Using a closed vessel, the by-product which produced the best flavor was the tips (white scrap) without the gills due to its high percentage of crab meat (40%). The gills attached to the tips imparted a bitter taste to the flavor which was found to be unacceptable. The flavor extracted from the claws was found to be the best of all flavors extracted at the facilities of a commercial flavor firm. An improved flavor may be obtained by increasing the total solids in the claw extract prior to reaction. In order to avoid the high cost of separating the gills from the tips, the claws should be processed together with the tips (gills attached). The resulting flavor should be of better quality than the flavor obtained form the tips with the gills attached. This extract could be used as a blue crab flavor or a seafood flavor. The extract obtained from the tips could be marketed as a seafood flavor while the extract obtained from the claws could be marketed as a blue crab flavor. The flavors extracted from the tips digested with proteolytic enzymes were preferred to the flavors extracted from the tips without previous enzyme treatment.

The digestion time that produced the best flavor was between 6 and 8 hr. If the digestion was allowed to continue, the crab taste was substituted by a fishy taste which was undesirable. The flavors were found to be commercially sterile and contained no pesticide or herbicide residues.

The blue crab white scrap was found to be a good source for a natural blue crab flavor. Not only was the flavor found to be acceptable, but sufficient amounts of the blue crab scrap are available on a regular basis for commercial use. Two companies control over 50% of the total blue crab processing in the Chesapeake Bay area and both are situated close to the flavor

manufacturer. The available raw material can be supplied on the basis of uniform composition, and can meet specification criteria consistently. The blue crab scrap is safe for use and is available at competitive prizes.

The flavor extraction process is economical (it does not require high technology) and will not produce any waste disposal problem, because the resulting byproducts have a high concentration of protein and chitin/chitosan which can be recovered to produce an ingredient for animal feeds and/or chitin and chitosan production.

The pigment extracted does not have a preferred astaxanthin concentration (1,200-3,000 ppm). Only 20-40 ppm were recovered from the carapace which contained which contained the highest concentration of the pigment. Other extraction methods, like the soy-oil extraction procedure should be considered, since it was possible to obtain 90 ppm of astaxanthin from Louisiana blue crabs (Meyers, 1992). Blue crab scrap, however is an excellent source of the canthaxanthin pigment. Astaxanthin has not been approved as a feed ingredient by the FDA. The possibility of extracting canthaxanthin industrially from the blue crab waste instead of astaxanthin should be considered not only because of the higher concentration of this pigment in the blue crab carapace, but the fact that these pigments contain similar tinctorial properties.

Future research should consider: (i) the treatment of the retort juice with proteolytic enzymes in order to break down the peptide chains that contribute to the bitter flavor; (ii) if the extraction process to be utilized includes the use of an open vessel, other boiling times (less than 1 hr) should be considered; (iii) flavors should be extracted from a combination of claws and tips with attached gills. These flavors should be compared to the flavor obtained from the tips without gills in order to determine any differences; (iv) the marc recovered from the extraction processes should be investigated as a possible ingredient for animal feed or raw material for chitin/chitosan production; and (v) studies with a feed containing canthaxanthin should be performed on fish in order to determine if canthaxanthin could replace all or a portion of the astaxanthin.

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