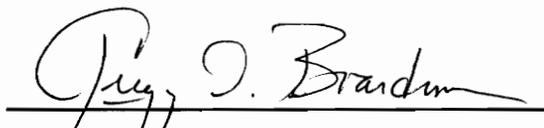


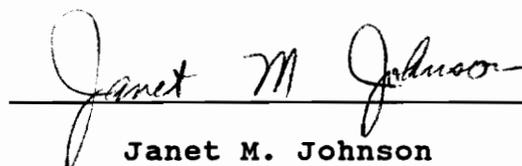
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PROCESSING BY-PRODUCTS
by
Eva Moral

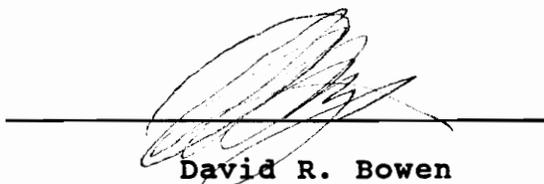
Thesis submitted to the Faculty of the
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in
Food Science and Technology

APPROVED:


George J. Flick, Jr., Chairman


Gregory D. Boardman


Janet M. Johnson


David R. Bowen

March, 1992
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(ABSTRACT)

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Extracts were obtained from the following blue crab processing by-products: carapace, legs, claws, tips (white

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DEDICATION

This manuscript is dedicated to my father and mother, Mr. and Mrs. Moral, for their constant support and encouragement throughout my undergraduate and graduate studies. Their love, sacrifices, and council were instrumental in my pursue of higher education.

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I. 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 by-products 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: β -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-hydroxyequinone, 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 by-products.

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 by-products 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 by-products.

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

II. REVIEW OF LITERATURE

1. Blue crab (Callinectes sapidus)

1.1. Biological Characteristics

Crabs are broad bodied, flattened crustaceans. They have hard shells or exoskeletons, four pairs of jointed legs, and claws. The last pair of legs are paddle shaped for the purpose of swimming (Warner, 1976). Most species are found in the sea. They can extend offshore to at least 120 feet deep, however, they are predominately a shallow water species, especially common in bays and estuaries and sometimes range into freshwater rivers. There are more than 4,500 species of crabs in the world.

The blue crab (Callinectes sapidus) is found on the Atlantic coast from the East coast of Canada to Northern South America; and have been reported from France, Holland, Denmark, and the East coast of the Mediterranean (Van Engel, 1958). When fully grown, they average 13 to 18 cm across the back of the shell. The shell color of these crabs ranges from brownish green to dark green while the underside of the body and the legs are white and the claws show varying amounts of blue. Sex is easily distinguished because the tips of the female's claws are bright red.

Other way to distinguish sex is by observing the crabs underside; the females have a round shaped tail while the males have an arrow shaped tail.

Blue crab mate while the female is in the soft stage. In the Chesapeake Bay, mating begins in early May and continues through October. Spawning is delayed at least two months after mating and occurs from early May through September (Van Engel, 1958).

The female carries her eggs in a mass called "sponge", on her underside. In two weeks the eggs hatch into free swimming larvae called zoea. These zoea, after several moltings, become adults. During development, crabs molt between 18 and 23 times. In early development, crabs molt every 3-5 days, but when they reach a marketable size of approximately 5 inches, molting occurs less frequently at about 30 to 50 days intervals. Crabs attain market size, approximately 5 inches, in about 12 to 18 months. They live about 4 years, with the largest crabs reaching 7 inches from point to point on the shell (Hong, 1990).

When the waters cool in October, the males and immature males migrate from the shallow waters to the deep holes and river channels where they burrow in the mud and remain in a state of semi-hibernation until the waters warm to above 60° F (16°C) generally in mid-April (Dressel et al., 1983).

The male blue crab, sometimes called a "jimmy" crab, has

a higher meat yield than the female, which is called a "sook" (Dressel et al., 1983).

1.2. Harvesting

The blue crab supports the largest crab fishery in the United States, representing about 50% of the total weight of all species of crabs harvested (Hill, 1989). Annual commercial landings in the United States averaged 86,000 tons (190 million pounds) in 1980-1985. Harvests from the mid-Atlantic region during 1977-1985 composed about half of the total U.S. commercial blue crab harvest. Almost 90% of the commercial blue crab landings in the mid-Atlantic are from the Chesapeake Bay region in Maryland and Virginia. Much of the harvest is processed for commercial packaging and represents a 60 million dollar industry (Hill, 1989).

Commercial harvests of blue crabs fluctuate widely. For example, the annual harvest in Chesapeake Bay fluctuated between 45 and 94 million pounds from 1966 to 1980. Rees (1963) found no direct relationship between commercial catches and recruitment of harvestable crabs in subsequent years.

The blue crab is the most important commercial species from Delaware to Florida, especially in the Chesapeake Bay

area which accounts for 40 percent of the catch. The South Atlantic accounts for 29 percent of the catch; the Gulf, 28 percent and the remaining 3 percent of the total United States catch comes from the mid-Atlantic region (Dressel et al., 1983).

Traditionally, blue crab harvesting is a summer fishery in the Atlantic and Chesapeake regions, where most of the landings occur from May to October.

On the Atlantic coast, different methods of capturing crabs are used as: crab dip nets, push nets, scrapes, fyke nets, haul seines, pots, dredges, and trot lines. The galvanized chicken wire crab pot introduced in 1938 is the dominant gear to fish hard crabs. Deep running dredges are used in the winter fishery to harvest hibernating crabs from the deeper waters of the lower Chesapeake Bay. Harvesting seasons, size requirement and catch limits are totally within the jurisdiction of state conservation laws (Dressel et al., 1983).

2. Blue Crab Industry

2.1. Blue Crab Processing

After harvesting, the unrefrigerated crabs are delivered by boat or truck to processing plants. The crabs are

weighed and dumped into stainless steel baskets. If the crabs are harvested during the winter (dredging season), they are run through a tumble spray washer prior being dumped in retort baskets in order to remove the sand and the grit that covers their surface (Hong, 1990).

The crabs are then placed into horizontal or vertical retorts, and cooked by steaming at 250°F (121°C) for 8 to 12 minutes at 15 psig (Hong, 1990). Maryland, Florida and North Carolina have regulations that stipulate that "crabs shall be cooked only under steam pressure"; however, on the Gulf coast, crabs are placed in boiling water and cooked for 20 to 30 minutes. The main purposes of the precooked processes are to facilitate removal of meat from the shell, give the product the characteristic crab meat odor and flavor and reduce microbial populations (Hong, 1990).

When the cooking process is completed, the crabs are moved to cooling areas and allowed to cool to ambient temperatures before being moved to the coolers.

Crabs are prepared prior to removing the meat (picking). Two different processes are utilized: a wet process and a dry process. In the wet process, the crabs are backed (carapace is removed), declawed, and washed by hand or machine and the meat is removed immediately, or the bodies may be refrigerated over night. The dry process, commonly used in the Chesapeake Bay area, does not include the

washing step. Each picker backs, declaws, and removes all the meat from each crab (Phillips and Peeler, 1972).

Picking meat from the crab is labor intensive. The picker uses a crab knife to remove the back and legs and then to open the body cavities. As the meat is picked, it is separated into three categories which are backfin or lump, regular or flake, and claw meat (Hong, 1990).

In a few large plants, hand picking is supplemented by mechanized picking. One process uses a machine that combines a hammer mill with a brine flotation tank but most processors use these machines only for picking claws because the crab meat produced by this process is inferior in texture and flavor (Hong, 1990). These machines are called Harris Claw Machines, which are based on a process where claws are put through a hammer mill type machine that breaks the claws in many small pieces. The crab meat is separated from the shells by a brine flotation process with the meat floating to the top and the shells sinking to the bottom where they are conveyed to a large container for disposal. The crab meat is picked up by a stainless steel belt that takes the meat to fresh water sprays to wash any adhering brine (Lopez, 1987). The meat is then collected from the conveyer belt and the excess water absorbed by the meat is removed with a metal squeezer.

The Quick-Pick Machine, which uses high speed vibration

and pressure, is utilized in few processing plants to remove body meat from crab cores. This process only produces flake meat since the lump and backfin is damaged during the extraction procedure.

After the picking process, the meat goes through a hand deboning step, followed by a packing process. The scrap which includes: carapace, tips, viscera and appendages, is deposited in containers. The scrap is ground and dried for animal feed within 24 hours after production.

2.2. Blue Crab Waste Management

The issue of seafood waste management is moving to the forefront of industry concerns for the 1990's. Waste disposal regulations and expenses are becoming major corporate concerns. The United States discards 130,000,000 tons of solid waste per year at a cost that has increased from 3 to 4 dollars per ton 10 years ago to 15 to 70 dollars per ton today (Gates, 1991).

Seafood operations generate substantial quantities of waste, much of it as edible protein. The blue crab processing industry faces serious solid and liquid waste disposal problems. For example, after blue crab hand picking operations, the waste accounts for 84% to 91% of the raw crab while the product obtained constitutes only 9% to

16%. Approximately 85% or 65 million pounds of the product is solid waste (NMFS, 1989). The liquid waste constitutes a problem as well. The liquid waste from the blue crab processing plants include: the cooker effluent (retort juice), crab washing water, and the effluent streams produced by the Harris Claw process. The first effluent stream produced in this process is the claw wash reel. The claws are washed prior to being picked, and 400 to 700 gallons of water per day are utilized in this process. The brine bath used in meat separation after the claws are broken in the hammer mill, constitutes the second effluent stream. The brine bath constitutes 200 to 400 gallons of water per day with a salt concentration of 130 g per liter. The claw meat conveyor wash effluent stream ranges from 2,000 to 9,800 gallons of water per day. This water is used to wash the brine solution adhered to the crab meat and contains a salt concentration of 3 g per liter. The disposal of this water is a major problem for the processing firms. The high salt concentration of this water is potentially toxic to biological treatment systems (Harrison et al., 1991). Retort juice is currently discharged directly overboard without further treatment. This liquid is high in BOD (Biochemical Oxygen Demand), SS (Suspended Solids), and TDS (Total Dissolved Solids).

In the 1970's, the United States Environmental Protection

Agency (EPA) developed documents which defined effluent limits for segments of the seafood processing industry but during the last decade, citizen and environmental groups have encouraged more stringent legislation and individual states have established waste disposal standards that exceed the current U.S. EPA requirements (Harrison et al., 1991). In Virginia, the State Water Control Board, has began an aggressive program to remove pollutants from the Chesapeake Bay. New standards for the disposal of liquid and solid wastes have been established. Consequently, processing firms will need to develop in-plant programs that will include: water conservation and recovery processes, improved by-product recovery systems, and the development of industrial products from wastes (including foods, feeds and biologics).

Recovery of edible by-products for human consumption or animal feed is hampered by the small scale and the seasonality of the blue crab fishery. Presently, the crab scrap is dehydrated, ground and sold as a feed ingredient. The price varies according to the soy and corn commodity markets and subject to other variable costs 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 and its rapid deterioration, characteristic odor and potential as a pest attractant.

Some proposed approaches to by-product utilization of the blue crab waste include: minced crab meat, chitin-chitosan recovery, carotenoid pigments recovery, and protein and flavor recovery (Gates, 1991).

Gates et al. (1988) recovered minced meat from blue crab picking by-products using a Baader deboner. He used the tips and all the by-products except claws and legs. Mince meats produced commercially in Georgia for human and pet food are sold for about \$1.00/pound. The trade-off for this by-product recovery is that the deboned picking scrap has less available protein making the scrap unsuitable for the production of animal feed.

Chitin and chitosan could be the most important recovered by-product from the estimated 900 million to 1.7 billion pounds of crustacean waste generated annually world wide. The Japanese produce 300 tons per year of chitin and chitosan while only pilot scale production occurs in the United States (Gates, 1991). For the last 10 years, the National Sea Grant Program, NOAA, Department of Commerce, has sponsored research activities on chitin and chitosan and has generated significant interest in the possibilities of these materials (Johnson et al., 1982). The growing demand

for these products and the pressing need for a practical solution to the waste disposal problems of the shellfish industry could result in a substantial production of chitin and chitosan. 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 the area where the greatest production of blue crab occurs.

Protein and flavor recovery from shellfish processing waste has been proposed by several investigators. Zall and Hood's (1981) surf clam processing proposal included the recovery of clam broth and dry clam flavor from wash water. Green and Kramer (1979) estimated additional yearly income of 200,000 dollars for one clam processor. Shiau and Chai (1988) used concentrated wash water to produce fermented oyster sauce. Perkins (1989) recovered 1.5% solids in the form of shrimp paste from shrimp canning operations. No and Meyer (1989) used crawfish chitosan to recover flavor compounds from crawfish and other seafood waste.

Limited 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). An acceptable (cost vs supply) natural blue crab flavor is unavailable at present although some blue crab bases are currently produced.

3. Flavor Industry

3.1. Flavor Industry Survey

Flavor in foods and food flavorings are terms which vary in definition. Flavor is created by aromatic chemicals which are bio-synthesized during normal metabolic process in plants and animals (Heath, 1988). Food flavorings are man made, compounded from natural or synthetic aromatic substances. They are used to impart a flavor of choice, to modify a flavor present or to mask an undesirable flavor so as to stimulate the palate to increase the pleasurable acceptability of the end product. The origin of the use of flavoring materials is lost in antiquity; but the first natural flavoring materials were probably spices whose use originated in the Orient to introduce variety into the rice dishes which were a large component of the diet. The ancient Greeks and Romans were well acquainted with flavoring materials and used flavors in their natural form and extracted oils. During the Renaissance period, with the

growth in the fields of chemistry, medicine and pharmacy, there was a significant expansion in the number of flavoring materials available (Schubert, 1977).

The flavor industry has developed only over the past 150 years beginning with companies specializing in marketing of vanilla, herbs, and vegetable drugs. The prime products of these companies were pharmaceuticals and fragrances; the development of flavorings occurred much later in history. Today, these companies have complex operations offering a whole range of flavorings as a service to the food and beverage related industries.

It is not until the latter half of the 19th century that chemists began to realize the flavoring possibilities of synthetic aromatic chemicals. By then, organic chemists were preparing a wide range of highly odorous aromatic chemicals which later had great value to the flavor industry (Bedoukian, 1967).

Today the flavor industry is dominated by several very large multinational flavor and fragrance companies specialized in natural or synthetic chemicals which are used in the compounding of almost limitless ranges of flavorings and fragrances.

Flavoring ingredients are certainly the most numerous single group of intentional food additives. The annual sales of food additives in the United States in 1973 was 804

million dollars of which about 40% were flavoring and seasonings. The flavor industry may be regarded as integral to the food processing industry. Currently, flavors are sold to such a variety of user industries that only major suppliers have been able to grow simultaneously with these industries simultaneously. Most companies have developed specialty product lines that are sold to selected end users. Regardless of the degree of specialization, three factors appear to stand out as being a key to growth within the flavor industry. These are: new product development; extensive technical interchange; and personalized service.

3.2. Flavors of the Future: Seafood Flavors

Society is demanding that the food industry provides the required quantity of good tasting, nourishing and low priced food as possible. The trend is to consume more processed and convenience foods which call for a wide spectrum of flavorings. The flavor industry, being bound to the food and beverage industries, is trying to meet this need. Consumers have demonstrated a decided preference for "natural foods" which contain no artificial additives or ingredients which have a negative health connotation. This change in attitude has had a significant impact on the food industry, one of which has been the increased demand for

natural, rather than synthetic, food flavorings. Flavors which were once quite acceptable are now considered to be "artificial", "synthetic", or "chemical" in character. There is an increasing demand for natural flavors produced from natural raw materials.

Schubert (1977), noted that the important criteria to the success of a food flavoring is the recognition and comparability of the new flavor with one which is already familiar to the consumer. Exotic or fantasy flavor compositions, not representing any known flavor profile, are generally not acceptable. But people are traveling more every year than before, they are gaining wider experiences and likings in foreign tastes and flavors, and the consumer is starting to demand more exotic flavors.

An important source of raw material is seafood, in particular shellfish. Seafood extracts appeal to the food industry because they have a taste appeal based on their identification with the natural products from which they were derived. Seafood products like shellfish are in high demand though the market price is one of the highest for animal protein. Consumers consider shellfish products a sophisticated food based on the limited availability of these raw materials.

Seafood extracts are appealing to the food industry because they allow flavor standardization of seafood

products. Seafood extracts allow the development of a range and variety of new processed foods with flavors that meet consumer's demand. It is possible to meet the consumer preference for seafood products but also comply with the consumer requirement for natural products (Ochi, 1980). Some popular seafood extracts include: mackerel, tuna, oyster, clam, short-necked calm, scallop, mussel, crab shrimp and lobster. A blue crab product would have a substantial consumer appeal to United States consumers since the crab is considered a shellfish favorite. Crab is not only a United States favorite but it is very popular in Europe and Asia where the price of the crab is relatively expensive. A crab extract or flavor could provide the taste and flavor of the more expensive raw material to an inexpensive seafood analog. This would provide an opportunity for both new product development and increased products sales.

3.3. Raw Material Resources

Ochi (1980) emphasized that any source of seafood flavors needs to meet the following criteria:

- i. Availability. Sufficient amounts of the material must be available on a regular basis to satisfy industrial extraction needs.

ii. Consistency. The available material should be supplied on the basis of uniform composition, and should be able to meet specification criteria consistently.

iii. Cost. Supplies must be available at competitive prices in the world market.

iv. Hygiene and safety. All materials that available must be safe for use, and be preserved in such a manner as to assure satisfactory quality.

Blue crab scrap meets all these specifications and could be considered a satisfactory raw material resource for flavor extraction.

3.4. Blue Crab Flavor Applications

Blue crab flavor uses would include seafood analogs and further processed products as crab cakes, stuffed crabs, crab stuffing and mixes, soups, bisques, and a condiment for potato chips and dips.

Much of the crab meat used in the further processed products mentioned is machine produced using the Harris Claw Machine process (Flick, 1990). In this procedure, the crab is passed through a hammer mill to free the shell from the meat. The meat is then separated from the shell with a differential brine flotation process. The resulting product has a very high salt content (3-5%) and a significant flavor

loss. If this crab meat could be supplemented with a natural crab flavor, the quality of further processed products would be improved and receive greater consumer acceptance.

An important application of a blue crab flavor is its use in surimi. Surimi is an engineered seafood that has become common in the United States and throughout the world, as a lower priced seafood substitute (Ryan, 1984). Recently a number of surimi analogs have appeared in the supermarket. These new products are marketed trade names as: Sea Stix, Sea Legs, Ocean Magic or Delicasea to name a few. Not only are these products available in the supermarket, but many restaurants have included them in menu items.

According to the U.S. Food and Drug Administration (FDA), "Surimi is an intermediate processed seafood product used in the fabrication of a variety of finished seafood products". Surimi is minced fish meat which has been washed to remove the fat and undesirable materials (such as blood, pigments, and odorous substances), and mixed with cryoprotectants such sugar and sorbitol to extend frozen shelf life (Pigot, 1986). When formulating finished seafood products, surimi is thawed and blended with other ingredients and additives such as natural shellfish meat, and shellfish flavorings, salt, water, and starch. These ingredients are processed by heat for making fibrous, flake, chunk, and composite-molded

consumer products.

The production of a surimi based blue crab analog would have a substantial market. The amount of natural crab meat added when producing a surimi analog varies with the manufacturer and can range from 0 to 35%. Snow crab is usually included in addition to crab extracts and flavorings. Because blue crab is the favorite shellfish in the U.S. market, blue crab meat could be added to surimi in substitution of snow crab meat in order to make this product more successful. Surimi-based products are not only an economical substitute, they also can be made without seasonal quality and quantity variations. Consumers can buy brand name products and know they are getting quality and consistency (Ryan, 1984).

4. Flavor Research

Flavor research is aimed at obtaining solutions to problems which are of direct application in the provision of flavorful foods (Heath, 1981). The aim of flavor researchers is to acquire knowledge of the chemicals responsible for the characteristic odors and flavors of foods. This knowledge enables them to specify the flavor attributes of food and flavoring materials and thereby exercise control in food products. Flavor research is a

very important part of the flavor industry and it is a preliminary step for the mass production of natural flavorings.

Flavor research includes the development of natural flavors from new raw materials. This type of research includes the selection of methods by which the chemical constituents responsible for odor and flavor in plant and animal raw material are isolated and characterized. The steps involved in flavor research have been described by Forss (1969) and Chang (1973). The stages and techniques used in flavor research are:

1. Selection of starting material:
 - Sensory assessment
2. Isolation or Extraction:
 - Aqueous distillation
 - Adsorption
 - Expression
 - Formation of derivatives
 - Headspace vapor collection
 - Solvent partition
 - Steam distillation
 - Vacuum sublimation
3. Concentration:
 - Adsorption/desorption displacement
 - solvent recovery
 - Freeze concentration
 - Molecular distillation
 - vacuum distillation
 - zone concentration
4. Separation:
 - Chemical fractioning
 - Chromatography

- column
- gas-liquid
- gas-solid
- thin-layer
- gel-permeation

5. Identification:

- Retention index determination
- Sensory assessment of GLC effluent
- Spectroscopy
 - infrared
 - mass
 - nuclear magnetic resonance
 - ultraviolet

The selection of method for flavor extraction depends on the nature of chemical constituents responsible for odor and flavor in plant and animal materials. These constituents could be volatile, non volatile, or a mixture of both. Aromatic components are generally present in aqueous solutions or in droplets in the cells, although some compounds may exist in oil sacs and glandular hairs. These latter components are not water soluble and can not be extracted with the same methods as the water soluble components.

In order to obtain a flavor comparable to the original raw material, it is necessary to isolate the odor/flavor complex as completely as possible from the mass of inert cellular matter with the minimum amount of chemical change. Sometimes, by changing extraction methodology, or by isolation of only some aromatic compounds, a flavor not comparable to the original raw material is obtained. These

flavors could be more desirable and could have specific applications in the food industry. These changes in methodology are studied by the flavor industry in order to produce greater and improved flavor varieties.

All the methods for isolation, concentration, and separation can not be applied in the flavor industry for the mass production of flavorings. Some methods are preferred in the industry to others for economical reasons. The methods employed by the industry for the production of flavors are discussed in Chapter 2.5 of Literature Review "Flavor Manufacturing Methods".

The isolation of aromatic components and their precursors in flavor research is achieved by several techniques depending on the nature of the starting materials. These techniques could be altered for the mass production of flavor in the industry. These include: expression, solvent extraction, steam distillation, high vacuum degassing, and head space vapor collection.

5. Flavor Manufacturing Methods

The production of consistently uniform and high quality flavorings has evolved during the years. Several of the present leading flavor manufacturers had their origins in the drug, pharmaceutical and chemical industries in which

the manufacturing techniques now in use were developed to the present high standards of efficiency and safety (Dorland and Rogers, 1977). In some cases, flavor manufacturers are capable of preparing the necessary natural or synthetic raw materials required in their formulations as well as having the facilities for further processing to produce a whole range of flavorings. Even when the further processing component is absent, manufacturers have a close contact with their raw materials suppliers and should be well informed of the methods used to produce these materials.

Whatever the nature of the flavoring, the manufacturing processes involved may be considered in terms of unit operations, each with its own problems and limitations and with an interdependency necessary to ensure reproducibility of the end product (Heath, 1981). The following unit operations will be discussed:

- i. Raw material handling:
 comminution, enzyme digestion, extraction with solvents, distillation
- ii. Liquid flavor production:
 blending and compounding, pasteurization
- iii. Dry flavor production:
 spray-drying, microencapsulation, freeze drying
- iv. Packaging

v. Storage

5.1. Raw Material Handling

5.1.1. Comminution

Most natural raw materials, as handled commercially, consist of relatively large pieces of tissue. Plant materials are handled in large pieces of hard dried plant tissue. Size reduction of these tissues is an essential first step in the extraction of the aromatic compounds of those materials. The reduction of particle size enables solvent or steam to penetrate and come into close contact with the cellular tissues containing the active constituents. Because the starting materials show very wide variability in chemical and physical properties, it is important to determine the degree of comminution necessary, and the equipment required to produce this product.

Plants show enormous variations in function, design and capacity to satisfy specific parameters. All this machinery falls broadly into one of the following classes (Heath, 1981):

i. Coarse cutters, crushers, or breakers capable of handling large and irregular pieces of materials and producing a uniform broken product suitable for further size

reduction.

ii. Slow-speed attrition mills which reduce fibrous materials to very fine powders (e.g., ball mills, end runner mills, and roller mills).

iii. High-speed impact machines capable of producing a wide range of particle size with good volume throughput (e.g., hammer and pin mills, disintegrators).

Enzyme biotechnology is applied in flavor manufacturing as a particle size reduction method. After size reduction has been achieved by one of the methods mentioned above, further particle reduction and compound disintegration can be pursued by enzyme digestion of the raw material components. Enzyme biotechnology will be discussed in the following chapter.

5.1.2. Extraction

Most natural products used as raw materials for flavor manufacturing, consist of small proportions of active constituents carried in a relatively large proportion of inert matter. This is achieved by the treatment of the raw material with a selected solvent in which the maximum percentage of desired extractive are soluble.

The solvent used may be either water from which the aromatic components may be recovered by vaporization, low-

boiling point non-polar solvents (e.g., ether, cyclohexene, methylene dichloride) or liquified gaseous solvents. The solvent of choice depends on the physical nature of the start material and its susceptibility to oxidative reactions (Heath, 1981). The selection of the solvent is very important. This step takes place in the preliminary flavor research of the raw materials to be extracted.

If the solvent selected is water, the extraction is accelerated with the aid of heat. This process is called digestion, and is not recommended for raw materials with high percentages of aromatic compounds of low boiling points and heat instabilities.

Other methods of exposing the materials to the solvent are used if the materials are of botanical origin. The methods are most utilized are maceration and percolation.

The production of sea food extracts requires different processes: acid hydrolysis, fermentation, and aqueous extraction as well as a combination of the three. The most common route for obtaining seafood extracts is through aqueous extraction using boiling water either under normal pressure or elevated pressure (Ochi, 1980). In order to reduce the volatile loss, the extraction is performed inside a reactor (closed vessel under pressure).

While this method of obtaining the extracts provides the lowest yields, it also provide the highest flavor and aroma

retention of the original materials.

The unit operations involved in the process of solvent extraction include (Heath, 1981):

- i. Preparation of the raw material by comminution.
- ii. Exposure of the materials to the solvent.
- iii. Separation of the solute from the extracted material or "marc".
- iv. Removal of the solvent from the "marc".
- v. Disposal of the waste material.

5.1.3. Steam Distillation

Steam distillation is a different type of extraction in which the separation is achieved by the use of steam. This method is based on differences in the vapor pressures of the compounds of the base material constituents. Simple distillation consists of converting a liquid into its vapor by direct heat in a still. The process may be performed at atmospheric pressure or under reduced pressure. On the commercial scale, the batch or pot still consists of a steam-jacketed vessel in which the material is heated and the vapor is led from the vessel to a water-cooled condenser.

5.2. Enzyme Digestion of Raw Materials

In the search for natural aromas, the term "natural" must be defined. The meaning of the term "natural" may vary in different social communities, e.g., the U.S. definition of "natural" incorporates not only a number of raw materials, but also products modified by living cells or their components including enzymes (Schreier, 1988). In the search for natural aromas, the flavor industry employs enzyme technology. Enzymes are used to digest the raw materials prior to extraction, catalyze a wide variety of organic reactions, and produce molecules of great diversity.

When the raw material to be extracted is a protein or protein derivative, it is digested with proteolytic enzymes, which catalyze the break down of the long peptidic chains into small chains and free amino acids. The hydrolysis of proteins leads to changes in solubility, functional properties and often flavor (Johnson, 1977). The free amino acids contribute greatly to the specific flavors to be extracted from the raw material (Konosu, 1987); for example, L-glutamic acid is a very well known flavor enhancer. Glutamic acid is a common constituent of animal and plant protein, and is effective as a flavor enhancer after suitable enzymatic hydrolysis (Gatfield, 1988). But the most important effect of enzyme hydrolysis of the protein

present in raw materials for the flavor industry is the change in solubility of the hydrolyzed proteins. Hydrolyzed proteins are soluble in water and are easily processed for flavor production (Jonhson, 1977).

The enzymes most currently used in the flavor industry are: bromelain, papain, and ficin. Some microbial proteinases are used but the strains from which the enzymes are extracted are kept confidential by the industry.

5.2.1. Bromelain

Bromelain is a proteolytic enzyme naturally present in a large number of varieties of pinapples (Ananas comosus), as well as in many other species of the family Bromeliaceae.

Like other plant protease, bromelain is a sulfhydryl enzyme requiring activation by cysteine for attainment of maximal activity. The specificity of bromelain has been examined in a number of substrates, and it is known that this enzyme shows great similarities in its specificity to that exhibited by papain and ficin, as well as some differences (Glacer and Smith, 1971). Bromelain cleaves the Arg-Ala and Ala-Glu bonds but leaves the Arg-Arg and Lys-Tyr bonds intact. The temperature, pH, and the concentration of bromelin influences the hydrolysis of animal protein by this enzyme. Weiner, (1958) verified that meat proteins were

best digested by bromelin at 60°C to 70°C. El-Sharbawi and Whitaker (1963) demonstrated that the action of bromelain on meat protein increases by increasing the temperature to 80°C. Bromelain's optimum pH is between 7 and 8 (Glazer and Smith, 1971). Asakura et al. (1982) demonstrated that with an increasing concentration of enzyme, hydrolysis increases.

5.2.2. Papain

Papain is a major protein constituent of the latex and the melonlike green fruit of the small softwood tree Carica papaya. The term papain is used to describe a proteolytic principle of papaya latex. The enzymatic principle of papain is a cysteine protease. Papain hydrolyses the amides of α -amino-substituted arginine, lysine, glutamine, histidine, glycine and tyrosine. The amides of α -N-benzoyl-L-arginine and lysine are the most susceptible. On prolonged peptide hydrolysis bonds involving not only the above-mentioned but also those of a number of others are split as well. The wide variety of peptide bonds cleaved by papain may be interpreted as indicating a low degree of enzyme specificity. This low degree of specificity has been exceedingly valuable for the hydrolysis of proteins in the food industry (Glazer and Smith, 1971).

The stability of papain is very high. It is stable at pH

values from 2.8 to 10.8, with an optimum pH between 4.0 and 7.5. The enzyme is very thermostable in the presence of substrate between the temperatures 5°C to 66°C, with the optimum temperature between 40°C and 55°C (Glancer and Smith, 1971). Asakura et al. (1982) reported that papain was more efficient in animal protein hydrolysis at 60°C.

5.2.3. Ficin

The name of ficin has been used to describe both the crude dried latex from different species of the genus Ficus and the crystalline cysteine protease prepared from the latex.

Studies of the kinetic behavior and specificity by Miller et al. (1988) show striking similarities between ficin and papain.

5.3. Liquid Flavor Production

5.3.1. Mixing and Filtering

Once the active or desirable compounds are extracted from the inert material, the solvent is removed totally to produce a dry flavoring or partially removed to produce a liquid flavoring. The production of a liquid flavoring

involves other steps. Liquid flavorings consist of a large variety of different constituents ranging in physical characteristics from very fine powders or crystalline solids, through viscous pastes to mobile liquids. These ingredients may be present in very small amounts (ppm) or in significant percentages and could be carried in a liquid or solid solvent. This wide spectrum of conditions makes mixing a vitally important step in flavor production.

Frequently in the production of liquid flavorings, a haze of oily globules separates after compounding is completed. This opalescence is due to traces of other constituents which are not soluble in the solvent. Sometimes, when the active compounds are extracted by boiling water extraction, other compounds are dissolved in the water making the extraction solution appear cloudy and after a period of standing, a precipitate will form in the bottom of the container. This opalescence is often difficult to clear by simple filtration through paper; and the use of filtering aids may be required. The most prevalent filtering aids used in the industry are: gravity filters, pressure filters, filter presses, pumps, clarification filters, vacuum filters and centrifugation.

5.3.2. Sterilization

In the absence of preservatives, flavoring products are liable to spoil and therefore require a thermal destruction or inactivation of microorganisms. The heat treatment necessary to achieve sterilization varies with the type of organism present. The methods of heat treatment used are: pasteurization, flash pasteurization and ultra-high temperatures sterilization (UHT). In pasteurization, the product is heated before or after filling into final containers, at 65-85°C for 10 minutes. In flash pasteurization, the product is passed through a tube or plate heat exchanger and raised to about 95°C for 1-3 minutes before filling into sterile containers. In ultra-high temperature sterilization, the liquid product is heated to temperatures of 135°C for periods of only a few seconds, rapidly cooled and filled aseptically into sterile containers.

5.4. Dry Flavor Production

In the production of flavoring powders, drying is an essential stage of processing involving various techniques based on direct, indirect or radiant heat dryers. All these methods involve the evaporation of the contained solvent by

heat. For optimum drying conditions, it is necessary to regulate three directly related conditions: (i) the movement of the solvent from within the material to the surface, (ii) the evaporation of the solvent from the product surface, and (iii) the removal of the solvent from the system. Drying equipment is designed and operated to achieve a balance between these stages and also takes in account the volume of air required, the relative humidity of the incoming air and the velocity of air flow through the drying chamber. These conditions dictate the amount of energy to be provided to achieve optimum drying (Heath, 1981).

The operation of dryers is dictated by the need to conserve heat energy, optimize working space, minimize labor, and limit air pollution. The latter is of particular concern in the production of spray-dried flavorings where losses must be reduced to a minimum.

Both direct and indirect dryers may be designed for batch or continuous operations. The temperature and time parameters and the heat transfer rate of the drying operation are dictated by the physicochemical characteristics of the wet start and the dry end products desired.

5.4.1. Direct Heat Dryers (Spray Dryers)

Direct dryers use heated air or gases which are in direct contact with the material to be dried. The following types are of particular interest to the flavor industry (Labuza, 1976): tray dryers, vacuum oven dryers, tunnel dryers, fluid-bed dryers, rotary dryers, and spray dryers.

Spray dryers are used in flavor research but mostly by the flavor industry for the mass production of dry flavors. Spray dryers are designed to handle wet materials in the form of a solution, slurry, or emulsion. The drying is achieved by spraying the feed liquor as minute droplets into a stream of hot air. This produces a very large surface of contact which results in a high rate of liquid evaporation. The dried material is recovered as a finely divided powder with uniform spherical particles.

The principal of spray drying comprises several stages: (i) preparation of the solution to be dried, (ii) the atomization of this liquid in the dryer chamber, and (iii) the regulation of temperature and velocity of the drying gases and the removal and recovery of the dried product.

Atomization is an important factor in the spray-drying operation. The aim is to produce a high ratio of surface area to mass in the liquid interface. Atomization is achieved by three different systems: high pressure

centrifugal nozzles, two fluid nozzles, and high speed centrifugal discs.

The drying chamber is designed in order to: (i) achieve an intimate mixing of the atomized spray and drying gases, (ii) remove the moist gases and dry product from the hot drying chamber, (iii) prevent the buildup of dried product within the drying chamber and (iv) enable ready cleaning and hygienic operation (Williams-Gardener, 1971).

The great advantage of spray drying is that evaporation is rapid and the drying rate remains constant so long as there is free moisture on the droplet surface, in consequence, heat sensitive materials are less likely to be degraded. Once the particles are dry, their temperature rises significantly, and it is essential that they be removed from the hot drying chamber as rapidly as possible. In most spray dryers, the dry powder is in the drying gases and moves in the main air stream into a cyclone separator set apart from the drying chamber. Any powder which settles in the drying chamber itself may be recovered by suction and added to that collected in the cyclone (Heath, 1981). Microencapsulation of the extract is recommended prior to spray drying. Microencapsulation is achieved by emulsifying the liquid material to be dried with gum Arabic or one of the modified starches (approximately 20% by weight). This emulsion is then spray dried to produce the encapsulated

flavor. These flavorings are dry, free running, and protected from oxidation, evaporation or polymerization, which makes them quite stable over very long storage periods. If the mucilage to make the emulsion is prepared using gum Arabic, the resulting product may have a high total microbiological count which is carried through the spray-drying process to the finished dry product. The initial mucilage must be thoroughly sterilized before making the emulsion. Modified starches do not pose the same hygienic problems (Bakan, 1973; Andres, 1977).

5.4.2. Indirect Heat Dryers (Freeze Dryers)

In the indirect dryers, the heating source is quite separate from the air used to remove the evaporating water or solvent. This provides an advantage as the air stream does not have to be heated. Drying is achieved in several different ways depending on whether the machine is for batch or continuous operation. For continuous processing, plant facilities include: cylinder dryers, drum dryers, steam-tube rotary dryers, and vibrating tray dryers. Batch indirect dryers include the following types: agitated pan dryers, agitator/rotary dryers, vacuum tray dryers, and freeze dryers. Freeze drying is a method of concentrating the

aromatic isolates by removing all the aqueous solvent used in the extraction step. This is a technique for removing water from wet materials by sublimation. It involves freezing the wet material at atmospheric pressure, followed by transforming the ice directly into vapor which is removed from the system. The system is under an applied vacuum of 4.6 mm Hg. Sufficient heat is applied to the frozen product to provide the latent heat of evaporation of the ice to effect sublimation (Brockmann, 1974). This method is not extensively used in flavor manufacturing although it has some advantages over other drying technologies, including spray drying, in that the driving force of dehydration is not heat but rather the difference between the vapor pressure of the porous material and the condenser plate. With this method, aqueous solutions with a high degree of heat sensitivity can be dried without damaging the aromatic compounds.

One of the major problems of freeze drying is the operational costs. This is an expensive method of concentrating aromatic compounds, especially in the mass production of flavors.

Jones Ainsworth (1988) described the freeze drying process as, "Freeze drying, is not the only way to prepare powder, so you have to have an expensive product to justify the cost".

5.5. Packaging and Storage of Liquid and Dry Flavorings

The prime purpose of packaging is to protect the product and ensure that it reaches the customer intact. The flavorings must remain in perfect condition right up to the time it is used in the final end product.

Several factors contribute to the deterioration of flavoring materials and these influence the way in which these materials are handled, packed and stored. These factors are: presence of water, action of light, ambient temperatures, atmospheric oxygen, trace metals and microbiological growth.

If the final product desired is a dry flavoring, microencapsulation of the flavor is recommended. Microencapsulation is achieved by emulsifying the liquid material to be dried with gum Arabic or one of the modified starches (approximately 20% by weight).

Flavoring materials should always be stored in a cool and dry place (mean ambient temperature of 15°C). The containers should be out direct sunlight. Ideally, the storage area should be dark or indirectly lighted areas (Heath, 1981). The following are points which require most attention when handling flavorings: (i) store in cool temperature, (ii) keep away from moisture, (iii) avoid heat and sunlight, and (iv) keep containers closed.

6. Sensory Analysis of Flavor

Sensory Evaluation has been defined as "a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing" (IFT, 1975). Sensory evaluation accomplishes the measurement of the sensory properties and the determination of the importance of these properties to consumer product acceptance. The response of the consumer to the different food products is very important for the industry. The methodology to measure these responses is established by sensory evaluation science though it the responsibility of the sensory scientist to select the proper testing methodologies by understanding the experiments objectives.

Sensory evaluation is an important tool in new product development. Some new products are unique but most products are imitations or variations of the same standards. Sensory evaluation of a new product involves the following test sequence (IFT, 1975):

- i. Characterization of the product prototype to determine uniqueness or a point of differentiation form related products.

- ii. Evaluation of the experimental prototype samples to

establish whether differences exist among them or between the prototypes and the standard.

iii. Determination of whether the samples meet the acceptability requirements established.

Different tests can be applied to each of the steps mentioned above. There are two major classifications of tests: Analytical and Affective tests. Analytical tests are used to determine differences or similarities and for identification and quantification of sensory characteristics. Affective tests are used to evaluate preferences and acceptance of products.

Difference testing (analytical test) is used to determine if the panelists can notice differences among the prototype samples. Once established that the samples are different, the best sample is selected by using an affective test: multiple paired comparison with hedonic scale, in which a preference for an specific flavor is determined. Another difference test is the paired-comparison test. This test is a two-sample test, and the panelist task is to indicate if the samples are similar or different (Stone and Sidel, 1985). The paired comparison test is relatively easy to organize. The two coded samples are served simultaneously. The panelists makes a decision after tasting both samples. This test has four orders of presentation: AB, BA, AA, BB (Stone and Sidel, 1985).

The scale for the preference test was developed and described by Jones et al. (1955) and by Peryam and Pilgrim (1957). These investigators studied a number of different scales of varying length and number of categories, as well as selection of most appropriate words used as the anchors for each category. This research resulted in the production of a scale with 9 points or categories and 9 statements.

This scale has been used extensively both as a laboratory and market research tools. It measures how much a person likes or dislikes a particular product (Schutz, 1965). The 9 point hedonic scale is probably the most useful method to measure preference (Figure 1). It has been used extensively with a wide variety of products and with considerable success (Stone and Sidel, 1985).

The results from this scale's use are most informative. The computations yield means, variances, and frequency distributions because the data can be converted to ranks. The responses are converted to numerical values.

Once the test strategy is determined, other important factors in sensory evaluation must be considered. These factors are: the selection of panelists, training of panelists, sample preparation and serving, and analysis of data.

The choice of a panelist has a direct influence on the

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

results. It is important to screen for eligible respondents. The most obvious people to be included in the study are those who would have the greatest interest in the product but inclusion of light, moderate, and heavy category users will provide broader feedback reflecting differing patterns of consumption (Moskowitz, 1988). The screening will include the completion of a product attitude survey. The information includes special product requirements such as food allergies. The screening also includes, discrimination testing, which would reveal those panelists that are good discriminators for that product. The selected panelists should attend orientation or training sessions. The testing should be discussed and the subjects would have the opportunity to ask questions. During these sessions, the subjects learn to use their senses and to follow specific instructions. They get familiarized with the product and with the test procedures.

Once the panelists are trained, they are ready to start testing. The testing should take place in a separate area from the preparation area (Stone and Sidel, 1985). The panelists should not have any contact with the investigator or with the other panelists. The tests should be performed in individual booths, which contain a small door at the counter surface which communicates with the preparation area. The adequate environment for testing should be free

from any element that may distort normal perception. Lighting should be well balanced and not impart a strange color on the food. The room should be ventilated without pollutants from adjoining areas. The procedure for presenting products for testing should be standardized between sessions. Each product should be subjected to the same type of evaluation under similar conditions. It is important not to overload the panelists with too many samples during the same session. Fatigue from multiple testings will decrease the respondent's ability to make accurate distinctions. Between samples, it is essential to remove all traces of flavor influences from the prior sample. This may be done with a mouth rinse of water, a bite of unsalted cracker or white bread (Moskowitz, 1988).

7. Chemical Composition of Blue Crab Flavor

Flavor chemistry is the identification of the chemical nature of components responsible for the odor and flavor of natural products of plant and animal origin, and the objective study of their biogenesis, biosynthesis, and deterioration (Heath, 1981).

The flavor of shellfish originates principally in the ingredients that are soluble in water or in saliva when they are chewed. Proteins, polysaccharides, pigments, and

vitamins are seldom involved in producing flavor. It is the water-soluble low-molecular-weight components (extractive components) that are regarded as the principal flavor producers. The extractive components may be divided into two broad groups: nitrogenous compounds, comprising free amino acids, low-molecular-weight peptide, nucleotide and related compounds; and non-nitrogenous compounds comprising organic acids, sugars, and inorganic constituents.

The free amino acid content in crustaceans is high when compared with that in fish. High levels of taurine, proline, glycine, alanine and arginine, are general characteristics found among crustaceans (Konosu and Yamaguchi, 1987). Konosu (1979) studied the contribution of each extractive component to the characteristic taste of crabs. He used five species of common edible crabs, and found, as a striking feature, the free amino acid composition of crab meat. He found high amounts of glycine and arginine and high but some what lower amounts of proline and taurine. These four amino acids accounted for 60-80% of the total free amino acids, which amounted to 2,000 to 3,000 mg/100 g of meat.

An analysis of blue crab Callinectes sapidus meat revealed 445 mg/100 g of glycine, 330 mg/100 g of arginine, 213 mg/100 g of taurine, and 250 mg/100 g of proline (Konosu, 1987).

Since various free amino acids increase after hydrolysis in extracts of shellfish, it is evident that different low-molecular weight peptides are present in the extracts, however, a limited number have been identified.

Adenosine monophosphate (56mg/100g) and cytosin monophosphate (49mg/100g) were found to be the major nucleotides in the tissue of the blue crabs. Small amounts of guanidil monophosphate (GMP), uridil monophosphate, and adenosil diphosphate were also detected. Hayashi et al. (1978) proved that the existence of GMP together with glutamic acid showed a distinct taste enhancing effect. Konosu (1987) demonstrated that very small concentrations of glutamic acid and inosine monophosphate (IMP) were necessary to enhance the blue crab flavor due to their synergism.

Several kinds of nucleoside and free bases were detected in blue crab meat: Ado, Ino, Hyp, Gua, and Cyt (Hayashi et al., 1978). Common quaternary ammonium bases are found in the muscles of all five species of crabs analyzed by Konosu (1987). The two that appeared in higher concentrations are trimethylamine oxide (TMAO) and glycine betaine. TMAO accounted for more than 100 mg in all samples.

Konosu's (1979) results showed that seven nitrogenous constituents, glycine, taurine, proline, glutamic acid, arginine, AMP, CMP, GMP, and four inorganic ions, Na^+ , K^+ , Cl^- , PO_4^{2-} , contributed in different degrees to produce the

taste of crab. If glycine was omitted from the crab extracts, sweetness decreased considerably. Glutamic acid contributes greatly to the characteristic crab taste and the sweet sensation. If arginine was eliminated, the overall taste as well as the crab-like taste became weak.

8. Microbiological quality of Blue Crab Scrap

Crab meat is a highly perishable product even under the best of storage conditions. Crab meat and processing by-products microbiological quality is further jeopardized by the human contact to which they are subjected during processing operations (Hong, 1990).

Harris (1932) reported a bacteriological investigation of the spoilage of Maryland and Virginia crabs and crab meat. He concluded that the Proteus group was the most important in spoilage. Other genera isolated were Streptococcus, Sarcina, and Achromobacter. Tobin and McClesky (1941) examined fresh and iced crabmeat to determine the bacterial condition of the meat and incidence of E. coli. In initial studies, E. coli was not detected but total counts ranged from 1.5×10^4 to 5×10^5 organisms/g in steamed crabs. Following picking, however, a bacteriological examination indicated E. coli was present in many samples.

9. Astaxanthin Pigment Extraction from Blue Crab Processing By-products

9.1. Carotenoids: Astaxanthin Pigment

Consumers tend to avoid unfamiliar compounds that include food additives, such as antioxidants, preservatives, and colors, but they easily adopt the concept that if the additive is in natural food it must be safe and good, since apparently it has been consumed for centuries and has withstood the test of time. There has always been and always will be a desire for attractively colored foods as long as the eye signals the selection of the daily ingestion of food products for the stomach via the brain. For this reason, the food industry will continue to require a wide array of acceptable, safe ways to produce foods with acceptable colors that would to satisfy the consumer.

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; for example, carotenoid pigments (Klaur and Bauerfeind, 1981).

Astaxanthin pigments belong to the carotenoid family. The carotenoids normally used in the food industry yield only tints of yellow to red hues. However, with the use of other carotenoids like astaxanthin and zeaxanthin, more

colors of the rainbow may be covered (Noonan, 1968).

Carotenoids possess high tinctorial properties. Although they are oil-soluble, emulsion and finely colloidal forms have been developed which disperse in water based foods. These pigments are stable in the pH range of foods, and are quite acceptable for food use under reducing conditions in minimal oxygen atmospheres. Applications are best in selective product use. Costs are comparable to those of other colorants, and some times they are more economical. Supplies are not limited, and wide international acceptance has been demonstrated.

The physical and chemical form of the carotenoid as they occur in nature is of particular importance to the food scientist in developing and utilizing food coloring forms. In some instances, carotenoids in natural foods are present in a free form dissolved in the oily or fatty phase of the food tissue. However, little is known of the nature of protein carotenoid linkages, like carotenoproteins which contain the astaxanthin pigments present in invertebrates like lobster and crab.

Carotenes produce yellow to orange colors in solution, and in the food industry, they are applied to a variety of products: shortenings, margarine, butter, egg yolk, baked goods, pasta products, salad dressings, spreads, cheese, ice cream, yogurt, gravies, meat and fish products, icings,

puddings, juices and beverages, and pop corn (Bauernfeind, 1981). Another important use of carotenoids is in commercial fish feeds.

9.2. Use of Carotenoid Pigments in Commercial Fish Feeds

In recent years there has been an upsurge of interest in the commercial culture of marine and freshwater animals. In some countries the need is for a good protein source, whereas in others the demand for luxury items, such as trout, salmon, and shrimp exceeds the supply. If the operation is to be efficient in terms of land use, feed must be supplied to the fish. The feed must provide all of the essential elements of the natural diet if the product is to compete with the wild harvest. Carotenoids must therefore be supplied to aquatic animals that deposit them. They must be supplied in a form that can be used by the particular organism; i.e., the right type of carotenoid, such as astaxanthin in a nondegraded form (Simpson et al., 1981).

Studies in feeding habits of shrimp, salmon and trout show that these animals need the ingestion of carotenoids in their diet in order to show the desirable pigmentation (Ramaswamy et al., 1991).

Consumer acceptance of market prawns are largely based on color. The red prawn is preferred, whereas the blue prawn,

generally artificially cultured, is not. Prawn fed crab waste are red rather than blue (Simpson et al., 1981). Intensive cultivation of crustaceans will mean that natural sources of carotenoid will not be available, and these pigments will have to be added to the feed.

Nilsson and Anderson (1967) studied food of the allopatric brown trout in the lakes of northern Sweden and concluded that in the lakes where crustaceans are abundant and are found in trout stomach contents, the trout are pink to red and are considered to be of the highest quality.

Several studies have shown that carotenoids can rapidly be absorbed by trout and salmon in flesh and skin. Lambertsen and Braekkan (1971), and Saito and Reiger (1971) reported good coloration of salmonoids by feeding canthaxanthin. The hue of the flesh of salmonoids is slightly different when astaxanthin or canthaxanthin is fed. Canthaxanthin gives a slightly more orange color than salmon color. Joseph and Williams (1975) extracted shrimp heads and impregnated a commercial feed with the shrimp oil. The augmented diet was fed to the Malasyam prawn. It was found that the prawn on this diet had a higher survival rate, mean weight, biomass, total pigment content, and better feed conversion efficiency. The total pigment levels in the experiment prawn were 15 times higher than in the control. The experimental animals were visibly more red than the

control animals.

Since the purpose of aquaculture is to prepare a product for food, the characteristics of food quality must be considered to be important. Aquaculture animals must be supplied with the necessary pigments of "natural origin". As Simpson et al. (1981) wrote, "An oil extract of crustacean waste could supply pigments as well as needed growth factors for crustaceans and salmonoids".

Canthaxanthin was approved for food and feed use in the U.S. in 1969. It is used in tomato based foods, meat based foods and fish feeds. Astaxanthin has not been approved yet, and the fish imported from Europe which were fed a diet containing astaxanthin are being detained by the FDA.

9.3. Astaxanthin Pigments in Crustaceans

Carotenoids are widely represented in crustaceans like crab, lobster, shrimp, and barnacles. Crustaceans generally contain the same major carotenoid, which are: β -carotene, astaxanthin, echinone, canthaxanthin, cryptoxanthin, zeaxanthin and lutein. There is no doubt that the relative proportions of carotenoid occurring in different organs and tissues, fluctuate according to the species (Castillo et al., 1981). The carotenoid levels reflect the availability of pigment in food and the appetite of the animal for it.

It appears that female crustaceans are found to contain more pigment than males. Generally, the carapace as well as the ecdysial exoskeleton contain mainly astaxanthin and lutein. Astaxanthin represents 90% of the total pigment in the carapace of shrimps. The investigations about the carotenoid of the carapace of the crabs (Portunus trituberculatum and Callinectes sapidus), have confirmed the presence of canthaxanthine, 4-hydroxyquinone, 3-ketocanthaxanthin, and astaxanthin. Chen and Meyers (1982) extracted astaxanthin from crayfish and found concentrations ranging from 1,800 to 2,000 ppm. Meyers (1992) only extracted 90 ppm astaxanthin from blue crab waste. Canthaxanthin is the most abundant pigment in the blue crab (Castillo et al., 1981).

III. MATERIALS AND METHODS

The crab by-products 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 2, 3, 4, and 5). 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.

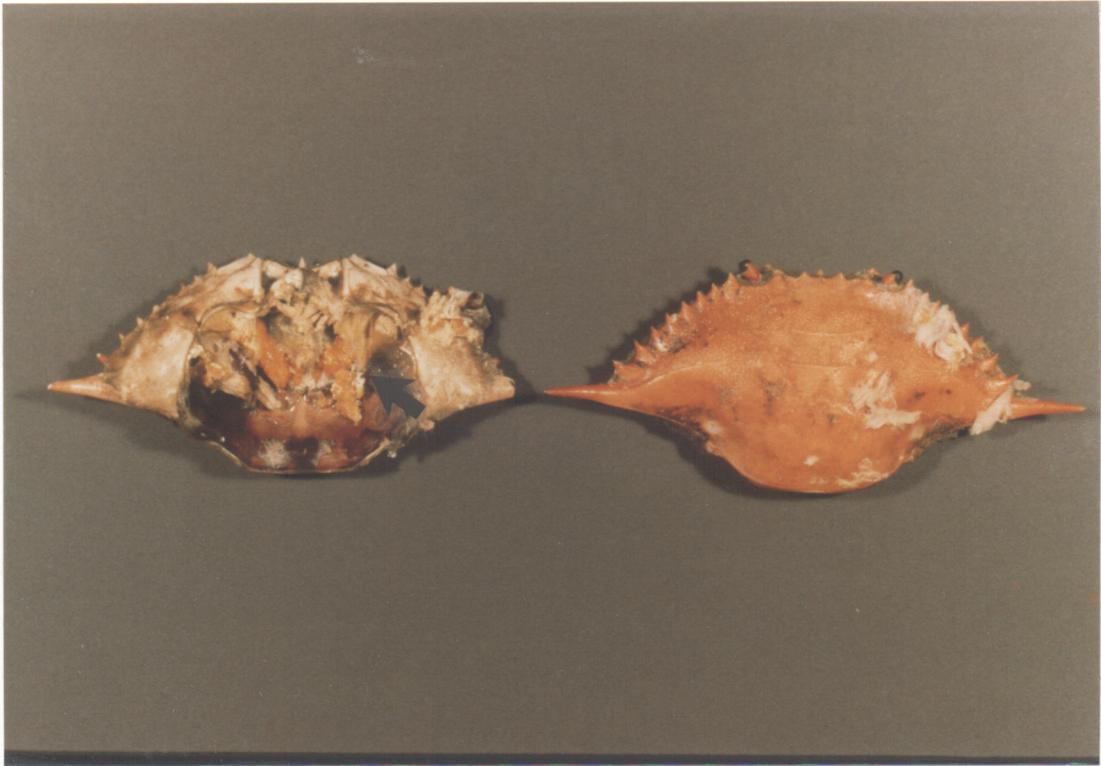


FIGURE 2: BLUE CRAB PROCESSING BY-PRODUCTS: CARAPACE WITH EGGS



FIGURE 3: BLUE CRAB PROCESSING BY-PRODUCTS: CARAPACE WITHOUT EGGS



FIGURE 4: BLUE CRAB PROCESSING BY-PRODUCTS: (A) LEGS AND (B) CLAW SHELLS (BYPRODUCT OF THE HARRIS CLAW MACHINE)



FIGURE 5: BLUE CRAB PROCESSING BY-PRODUCTS: 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 6). 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 2,3,4,and 5). 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

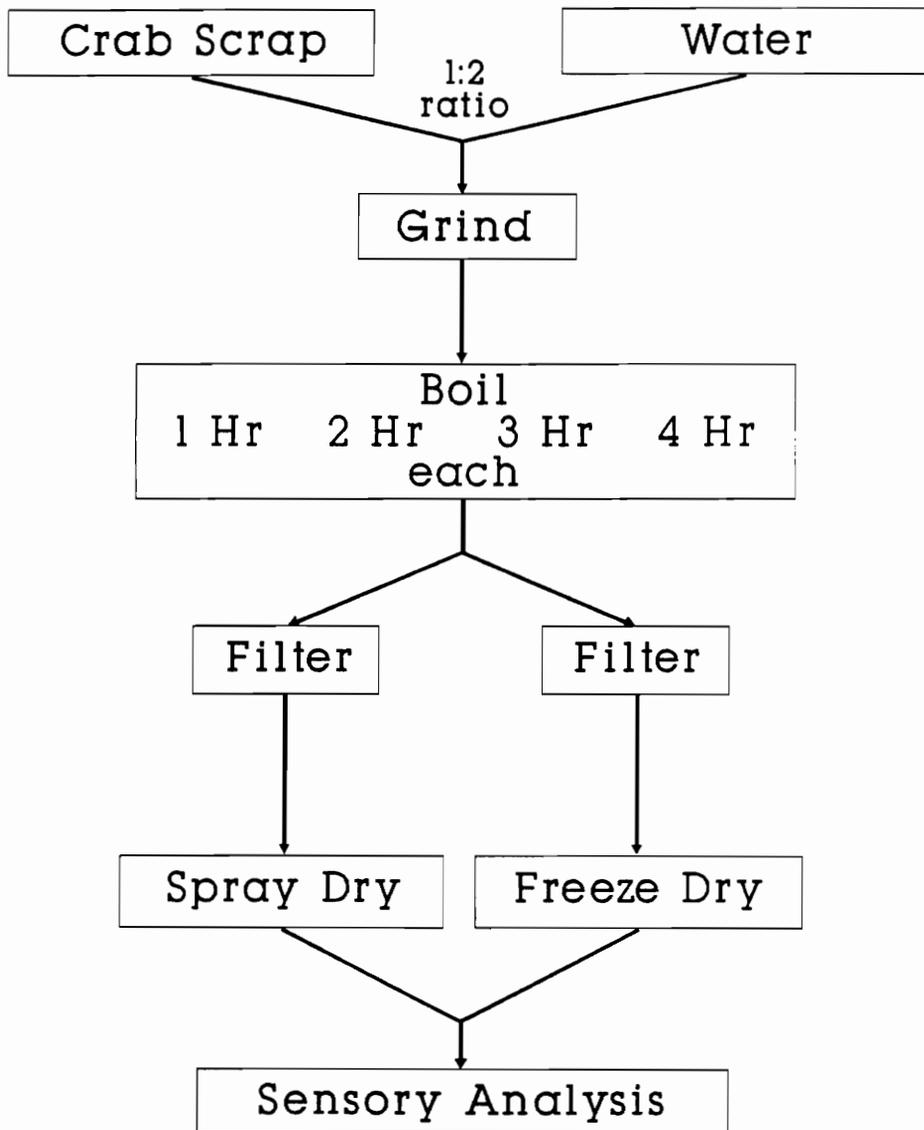


FIGURE 6: FLAVOR EXTRACTION PROCESS USED TO DETERMINE THE BOILING TIME IN AN OPEN VESSEL

jars, and stored at 4°C. After filtration through cheese cloth followed by no. 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.

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 2, 3, 4, and 5) 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 (Figure 7). 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

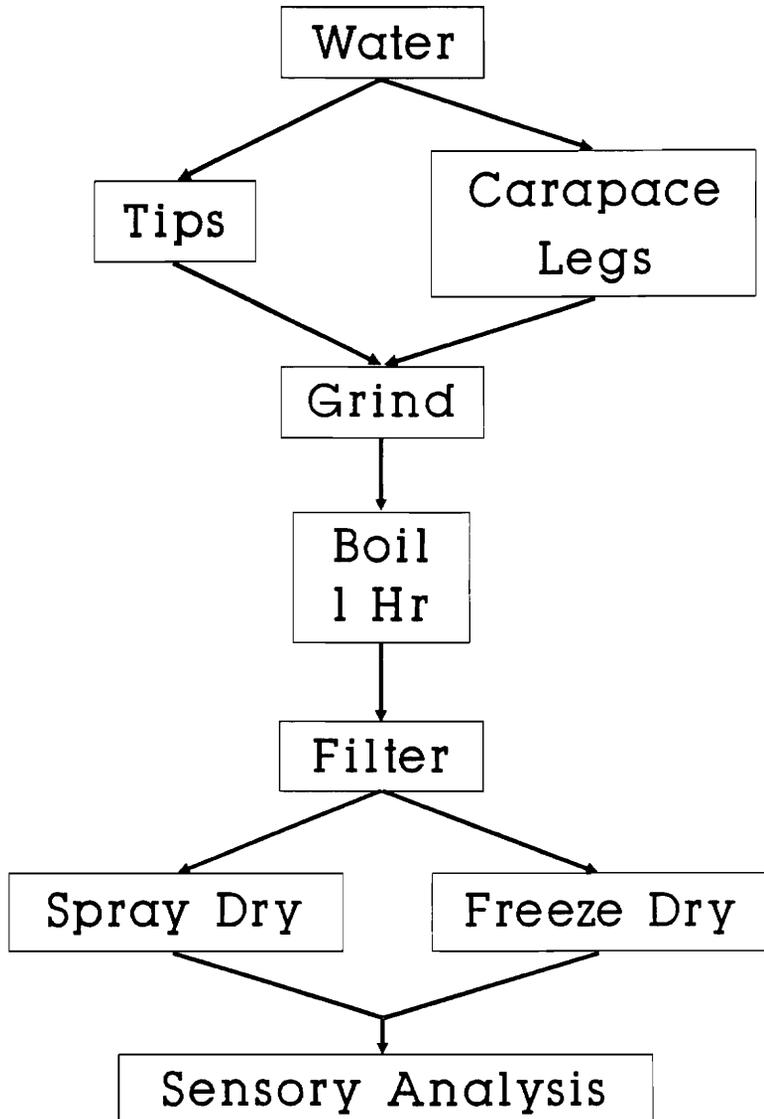


FIGURE 7: FLAVOR EXTRACTION PROCESS USED TO DETERMINE THE CONTRIBUTION OF EACH BY-PRODUCT TO THE FLAVOR

contributors to crab flavor (Konosu and Yamaguchi, 1982). The enzyme treatment also increases the yield of extractable flavor materials. Three enzymes were chosen for the pretreatment process: bromelain, papain, and neutral protease (proteolytic enzyme preparation produced by a selected strain of Bacillus subtilis). These 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 8). 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:

$$\% \text{ Meat } \epsilon \text{ Scrap} = \left(\frac{\text{Weight of Meat } \epsilon \text{ 500 g of Scrap}}{500 \text{ g of Scrap}} \right) \times 100.$$

Bromelain, supplied by Quest Bioproducts group (Sarasota, FL) with an activity of 375 GDU/mg (gel dissolving units) was the first enzyme tested. The concentrations of bromelain employed were: 0.1% and 0.2% (0.1 g and 0.2 g per

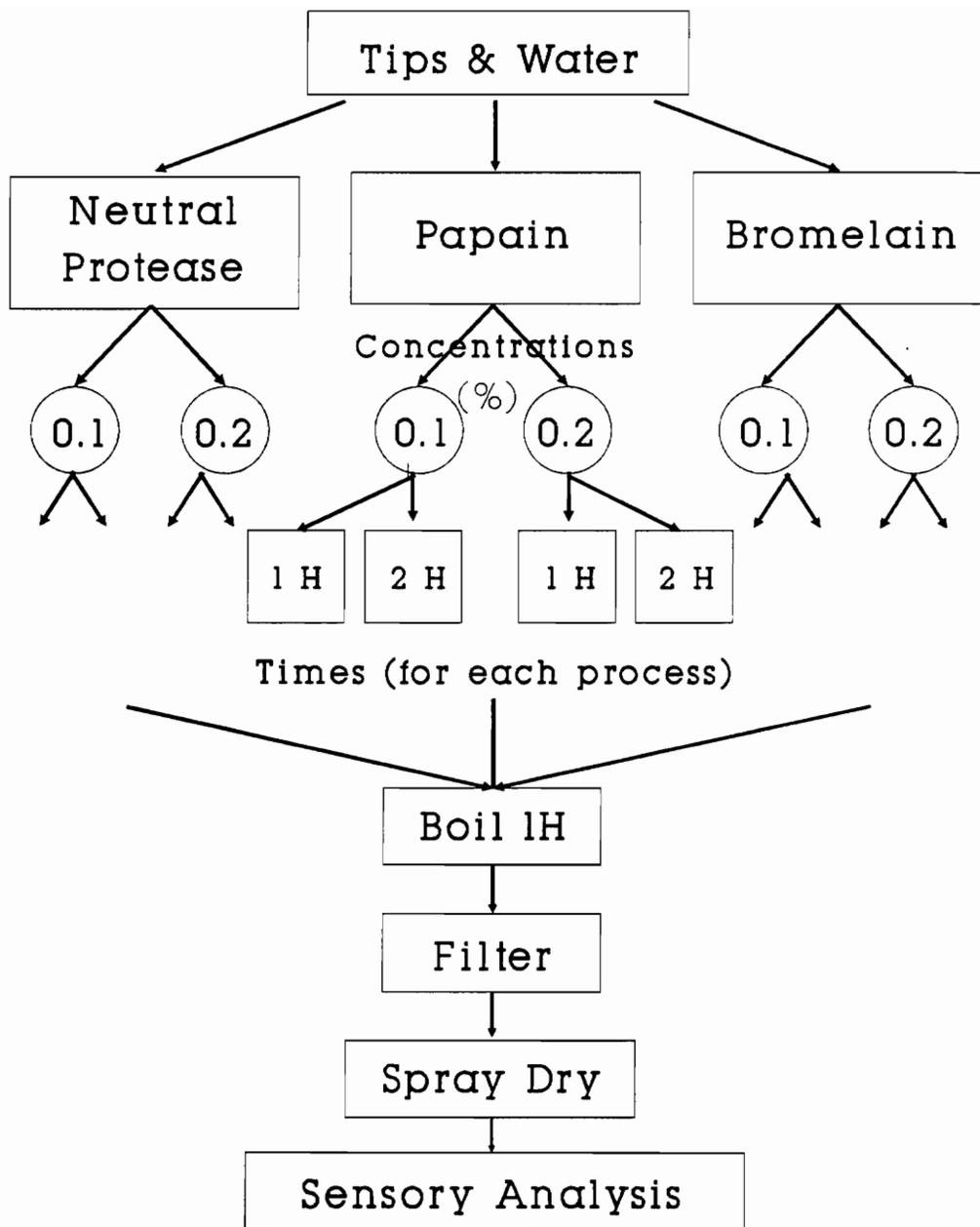


FIGURE 8: FLAVOR EXTRACTION PROCESSED FROM BY-PRODUCTS PRE-TREATED WITH ENZYMES

100 g of crab meat present in the scrap as calculated above). Both samples, 0.1% and 0.2%, were allowed to react for 2 and 4 hours.

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 $55\pm 1^{\circ}\text{C}$ (optimum temperature of bromelain, papain, and neutral protease). 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 No. 2 Whatman paper and evaluated for crab flavor by sensory analysis as previously described. The enzyme treatment was repeated using both papain, supplied by Quest Bioproducts Group with an activity of 200 MCU/mg (milk clotting units); and neutral protease, supplied by Quest Bioproducts Group with an activity of 48,000 units/mg. The activities selected for the enzymes are comparable (Quest Bioproducts group, 1992)

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

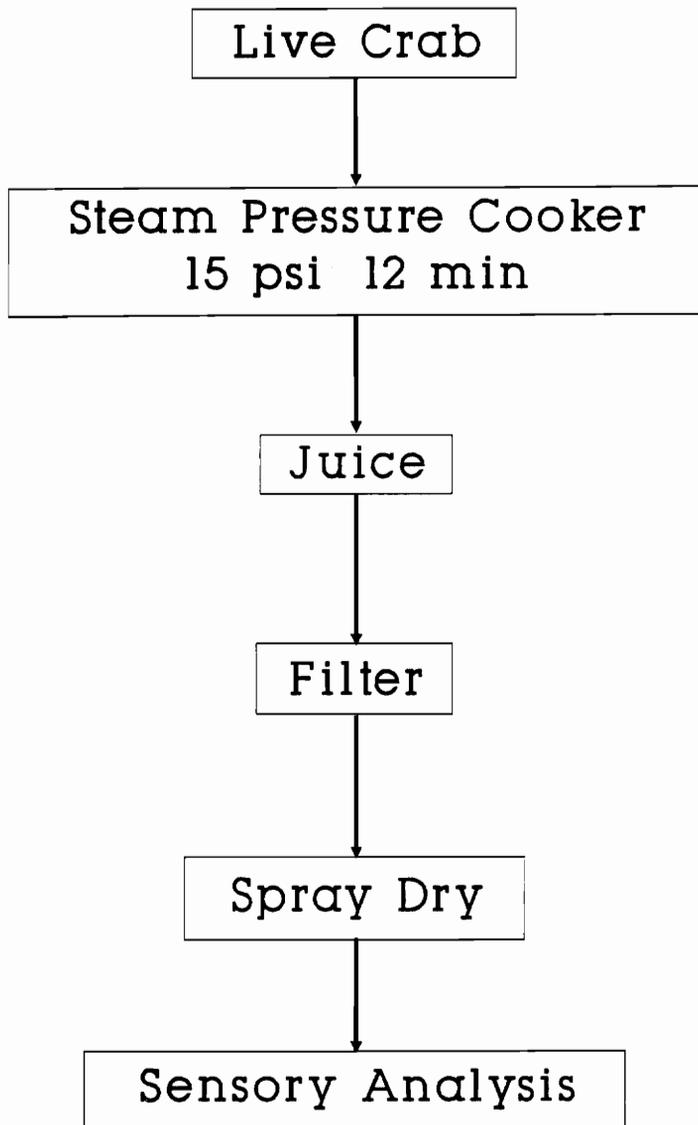


FIGURE 9: FLAVOR EXTRACTION FROM THE RETORT JUICE

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 9) 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 No. 2 Whatman filter paper and spray dried. The flavor quality was analyzed by sensory analysis.

1.2. Extractions Performed in the Facilities of Quest International, Using a Closed Vessel

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 the pilot plant of Quest International Flavors and Food Ingredients Co. (Owens Mills, MD), using a 2 kg reactor

(Figure 10). The effect of four enzymes: bromelain, papain, neutral protease and pepsin, 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 neutral protease for 9 hr, (2) tips without gills digested with papain for 6 hr, (3) tips without gills digested with papain for 9 hr, (4) tips with gills digested with papain for 6 hr, (5) tips with gills digested with bromelain for 9 hr, (6) tips without gills digested with bromelain for 9 hr, (7) tips with gills digested with pepsin for 9 hr, and (8) tips without gills digested with pepsin 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 optimum temperature for bromelain is 60°C, for papain and neutral protease 55°C, and for pepsin, 36°C. The

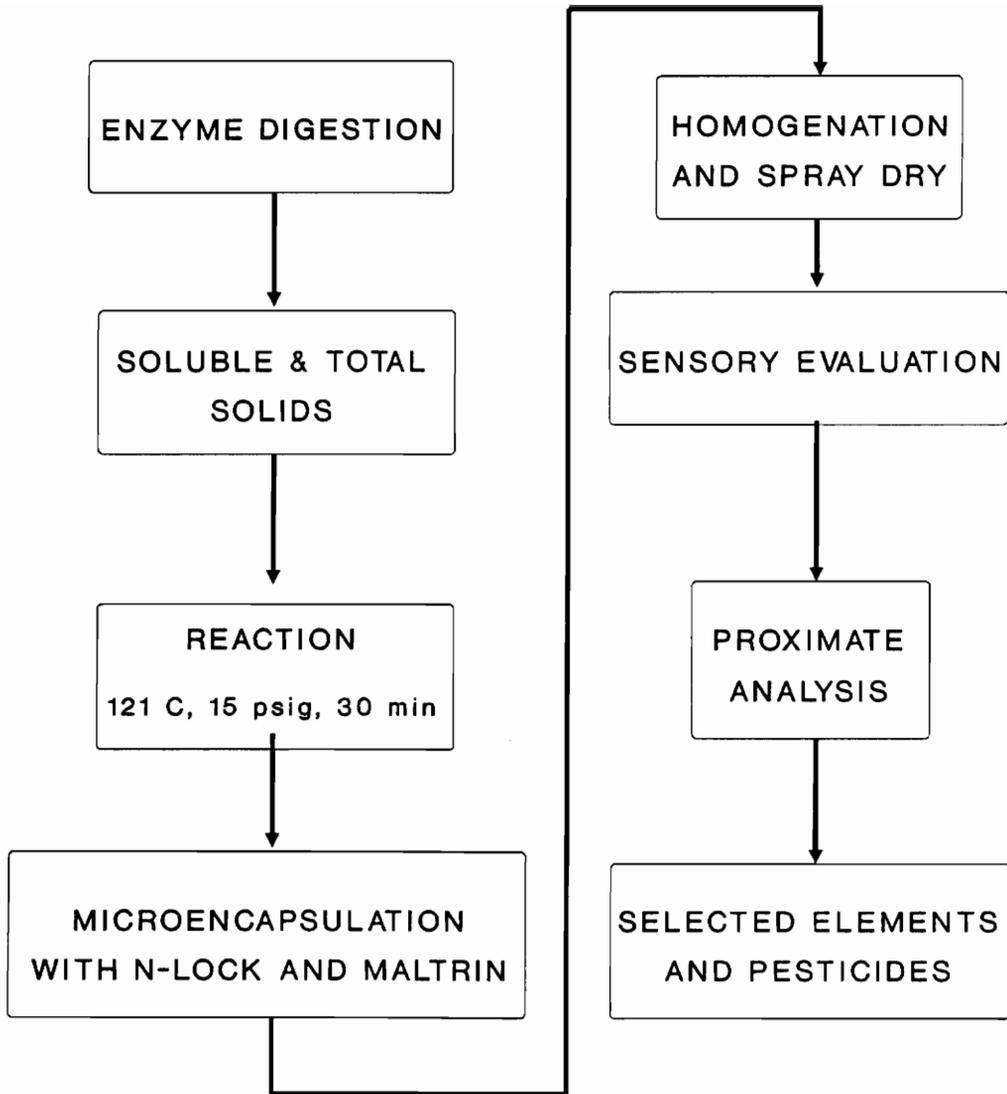


FIGURE 10: FLAVOR EXTRACTION PERFORMED AT QUEST INTERNATIONAL FLAVORS AND FOOD INGREDIENTS CO.

**TABLE 2: RAW MATERIALS, ENZYMES, AND TIME OF REACTION USED
IN THE DIGESTIONS PERFORMED AT QUEST INTERNATIONAL**

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

pH of the extract was adjusted to the optimum pH of each enzyme. The optimum pH of bromelin, papain, and neutral protease is 7.0-7.3; and the optimum pH of pepsin is 1.0-2.0. The pH was decreased by the addition of 10 N hydrochloric acid. The enzyme, 18 g, was then added to the slurry. The pepsin concentrate was supplied by Sigma Chemical Company (St. Luis, MO) with an activity of 510 Units/mg.

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:

$$\text{Percentage TS} = 1 - [(\text{initial weight of sample}) - (\text{weight of dry sample})] \times 100 / \text{initial weight of sample}$$

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, model JUVO D 25/2 (K. Kurt Juchheim, Berncastel, Germany) and reacted for 30 min at 121°C and 15 psig. Once the reaction was completed, the microencapsulation mixture (20% N-Lock starch and 80% Maltrin) was added to the extract in order to increase the total soluble solids to 40%. The amounts of the N-Lock and Maltrin added were calculated as follows:

$$\text{Percent solids to be added in form of Maltrin and N-Lock} = 40\% \text{ desired SS} - \text{SS\% measured after digestion}$$

$$\text{Grams of Maltrin and N-Lock to be added} = (\% \text{ solids to be added}/100) \times 2000 \text{ g of extract}$$

$$\text{Grams of Maltrin} = (40/100) \times \text{grams of Maltrin and N-Lock to be added}$$

$$\text{Grams of N-Lock} = \text{grams of Maltrin and N-Lock} - \text{grams of Maltrin.}$$

The N-Lock starch and the Maltrin were added to the extract while stirring constantly.

1.2.1.4. Spray Drying Process

When the starch was all dissolved, the mixture was homogenized in Cherry Burrel homogenizer, model 200 (Cherry Burrel Co. Cedar Rock, IA) prior to being spray dried. The spray dryer utilized was a Maxum Becker with a nozzle delephen 72. The inlet temperature was set at 340°F (171.1°C) and the outlet temperature was set at 190°F (87.7°C).

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 papain 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 at Quest International.

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 1). 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 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 1). 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 Quest International

The flavors obtained at Quest International 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 in Quest International 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 Quest International 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 KjeltacTM Auto 1030 Analyzer (Tecator), and fat by ether extract using a SoxtecTM 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 from the tips without gills digested with papain for 6 and 9 hr and the flavor obtained from the claws digested with papain 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 Seagrass Program.

1.3.3.3. Selected Elements: Presence and Concentration

The flavors extracted in Quest International that were selected as the most preferred by the trained sensory panel (the flavors obtained from the tips without gills digested with papain 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 Residues Identification

The determination of pesticide residues in the flavors selected by the sensory panel that took place in Quest International, was performed following the procedures described by Bertuzzi 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 pepsin and reacted in a closed vessel, and the flavor obtained from the tips without gills digested with pepsin 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 spray 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 + CO₂ 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 By-Products

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.

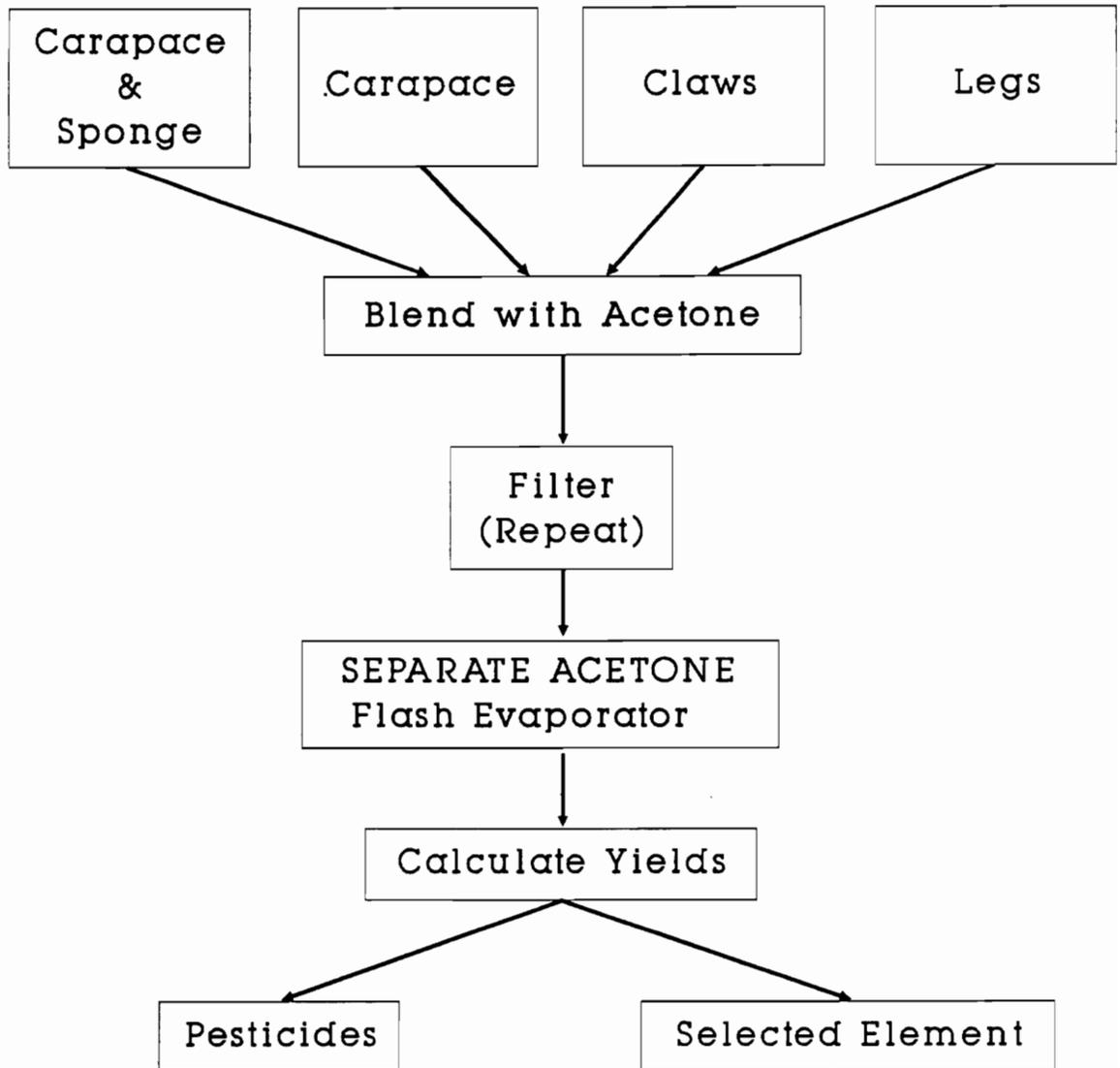


FIGURE 11: PIGMENT EXTRACTION OF BLUE CRAB BY-PRODUCTS

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 11). 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 No. 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:

$$\text{Percent Yield} = \frac{[(\text{weight of flask} + \text{pigment}) - (\text{Weight of flask})] \times 100}{\text{initial scrap weight}}$$

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 Meeter 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 10 L.

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.

IV. 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

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 ($p > 0.05$)

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.

A significant difference ($p < 0.05$) 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 Different Crab Processing By-products to 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

**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 ($p > 0.05$)

preferred to the freeze dried flavors. 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 papain, bromelain, and neutral 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% papain, or neutral protease but a significant

**TABLE 5: SENSORY PREFERENCE RATINGS^{pq} OF CRAB FLAVORS
EXTRACTED FROM ENZYMATICALLY PRE-TREATED TIPS**

ENZYME USED	ENZYME CONCENT. (%)	REACTION TIME (HR)	MEAN SCORE
Neutral Protease	0.1	1	1.97 (a)
		2	2.36 (a)
	0.2	1	2.39 (a)
		2	2.38 (a)
Papain	0.1	1	1.88 (a)
		2	2.06 (a)
	0.2	1	1.85 (a)
		2	2.03 (a)
Bromelain	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)

difference ($p < 0.05$) was found between these flavors and tips. The flavor extracted from the sample treated with 0.2% bromelain 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 bromelain imparts a better flavor than treating the samples with papain or neutral protease 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.

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.

1.2. Flavor Extractions Performed at Quest International

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 papain, bromelain, neutral protease. The results are summarized in Table 7.

Tips with and without gills digested with pepsin for 9 hr had the higher percentage of soluble and total solids. The extract obtained from tips with gills digested with pepsin had 14% soluble solids and 15.2% total solids. The remainder of the extracts had total solids values between 11.0% and 11.8%. Pepsin 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 pepsin (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 papain 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 papain 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

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	Neutral protease	9	10	11.0
Tips w/o gills	Papain	6	8	9.3
Tips w/o gills	Papain	9	10	11.9
Tips with gills	Papain	6	6	s
Tips with gills	Bromelin	9	11	12.8
Tips w/o gills	Bromelin	9	10	11.8
Tips with gills	Pepsin	9	14	15.2
Tips w/o gills	Pepsin	9	14.5	15.0
Claws	Papain	6	7.0	7.8

s Analysis not performed

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 papain for 6 hr had a low TS percentage (7.8%). This flavor was analyzed for its quality by a trained panel at Quest International. 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 papain for 6 hr, and the flavor obtained from the tips without gills digested with papain 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 papain 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 papain 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 papain for 6 hr (M1) was 53.89% protein and 42.94% ash. The marc from

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

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.

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 Seagrass Program.

1.2.2.3. Selected Elements Determination

The flavor extracted from the tips without gills digested with papain 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 (Dellaria, 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 PAPAIN 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. Pesticides Residue Determination

The flavor obtained from the tips without gills digested with papain 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 papain 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 by-products: 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 12.

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 10). 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

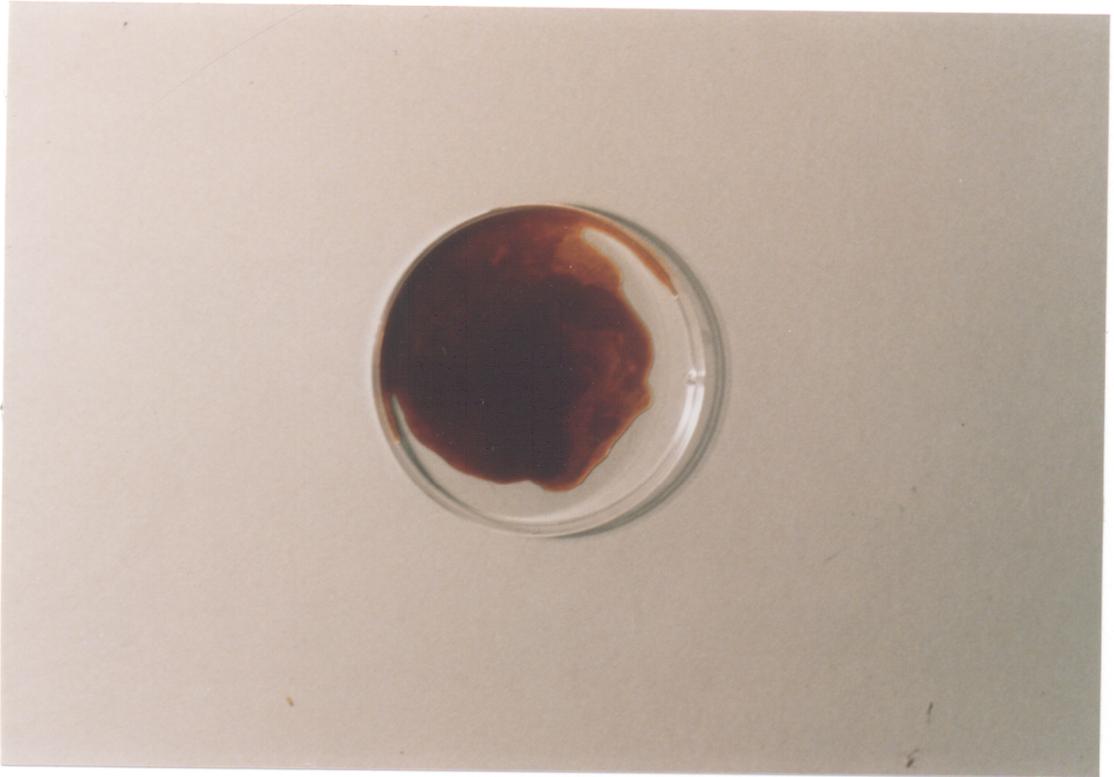


FIGURE 12: COLOR EXTRACT FROM CARAPACE WITH EGGS

TABLE 10: COLOR MEASUREMENT¹ 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.

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.

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. Pesticides 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 (Dellaria, 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.

V. SUMMARY AND CONCLUSIONS

A crab or seafood flavor was obtained from all blue crab processing by-products 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 Quest International. 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 from 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 by-products 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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VIII. VITAE

The author, Eva Moral, was born on May 20, 1964 in Valladolid, Spain. In May 1982, she received her high school diploma from La Compania de Maria high school, and in September of the same year, she was accepted in Valladolid medical school. She followed medical studies for the next three years, until she realized that a medical career was not her vocation. In september 1985, she entered the Center for International Studies in Madrid, Spain, where she studied the first two years of college in the American system. In 1987, the author entered Rollins college in Winter Park Florida, where she graduated in May 1989 with a Bachelor of Science in Biology.

In September 1989, the author was hired by Nestle AEPa, in Valladolid Spain, where she worked in the Quality Control Department until July 1990, when she began graduate study at Virginia Polytechnic Institute and State University for a Master of Science Degree in Food Science and Technology.

The author is a member of the Institute of Food Technologists, the Carolina Virginia Institute of Food Technologists, and Phi Sigma Honor Society for the Biological Sciences.