A process was developed using an acidic system to hydrolyze fish protein, followed by a membrane-based separation process to purify and separate the desired liquid portion from the remaining solid prior to deodorization using activated bamboo charcoal and Zero Valet Iron. 85.97 percent of initial protein was recovered into the final product. Acidic hydrolysis and innovative deodorization method alleviated unpleasant odor of the fish hydolyzate (FH) by reducing the most important volatile compounds of the FH. Employing buffer system elongated the shelf life of final product compared to solely acid system. The results of field trials of the deodorized hydrolyzed fish fertilizer showed the product led to more yield and less sprouts and stalk in the fertilized plants compared to three other commercially available fertilizers. This is promising especially for organic farming due to more yields and less waste in the plants.
A NOVEL APPROACH TO PRODUCE ORGANIC FERTILIZER FROM FISH SCRAPS

By

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Chapter 1: Introduction

The environmental implications of traditional disposal methods of the waste of seafood processing companies, besides the strengthening of environmental regulations in many countries, have created an interest in alternative methods of disposal/utilization of this type of waste. One way for making a value added product out of proteinaceous fish waste is to convert it to hydrolysate. Such hydrolysates have multiple potential applications, for instance as ingredients in food (Adler 1986; Lahl and others 1994; Frøkjaer 1994; Mahmoud 1994), or animal feed (Faid and others 1997; Ouellet and others 1997), as the peptone ingredient in microbial growth media (Gildberg and others 1989; Vecht-Lifshitz and others 1990; Frøkjaer 1994), or as fertilizer (Kurbanoglu and others 2002). The term “peptone” is used for protein hydrolysates that are soluble in water and not heat coagulable (Green and others 1977). Because of the higher price of peptones compared to the common by-products from fish such as fish silage and fish meal, the production of peptones can be considered as an expensive method for the fish and seafood industry.

Traditional methods for the development of fish hydrolysates such as fish silage (Raa and others 1983; Arason 1994) or fish sauce (Saisithi 1994; Gildberg 2001) employ the endogenous enzymes of the fish intestines and are still the most commonly used methods for adding value to fish by-products. These autolytic hydrolysates are produced at low temperatures. Microbial growth is hindered by lowering the pH in silage by short chain organic acids (Woolford 1975), or in the case of fish sauce, via using high salt content. The activity of endogenous enzymes in a by-product is associated with both the physiological condition of the fish and the processing method of the by-product. This matter can restrict the efficiency and controllability of the process (Kristinsson and others 2000).
Chapter 2: Literature Review

2.1 Fish scraps and their recovery

Currently there are immense amounts of protein-rich by-product materials from seafood processing plants discarded with no attempt of recovery. Numerous processors are no longer allowed to discard their offal directly into open waters. This leads to a very high cost of refining the waste before it is discarded (Kristinsson and others 2000). For seafood processing industry to avoid this loss, an alternative to discarding these byproducts needs to be developed. Recovery and alteration of fish muscle proteins present in the byproduct material and use these as functional ingredients in food systems is a very promising and inviting alternative. However, for the industry to develop processes for byproduct recovery and exploitation, it should be more economically feasible than discarding the byproducts. With a drastically increasing world population and a world catch of fish, there is an increased need to utilize aquatics more intelligently (Kristinsson and others 2000).

Food protein hydrolysis has a long history, particularly for vegetable and milk proteins; these sorts of proteins are widely used in the food industry. Most studies on the hydrolysis of fish proteins were conducted in the 1960s. At that time, some cases of fish protein hydrolysate (FPH) production led to a relative success (Hoyle and others 1994). During the 1960s, research was conducted to the production of economically feasible nutritious protein sources for swiftly growing developing countries, or toward animal feed production, primarily through production of fish protein concentrates (FPC) (Kristinsson and others 2000).

2.2 Fish muscle protein and its biochemical properties

A protein in foods is traditionally categorized as a fibrous or globular protein based on its tertiary structure. Each protein in food has a particular molecular conformation determining its
functional properties, rather than a verity of environmental conditions over which it manifests these attributes (Demetriades and others 1997).

Due to the structural and functional complexity of food proteins, it is difficult to completely understand the process of protein hydrolysis without a deep understanding of the nature of the protein substrate and the hydrolyzing agent. Within protein hydrolysate development, the protein substrate is hydrolyzed by either a proteolytic enzyme or a chemical (Kristinsson and others 2000). The daily diet contains a vast range of proteins from different sources. In general, it is agreed that the relative quantities of dietary essential amino acids is the most important criterion which determines the nutritional value of food protein (Sikorski and others 1994).

Animal based proteins are recognized to be nutritionally more valuable to those from plant sources since they contain a preferable balance of the dietary essential amino acids. Among these, egg and milk proteins (casein) are mostly used as proteins of reference for assaying protein quality. Proteins sourced from meat and poultry muscle are also of very high quality. Fish muscle proteins are equivalent to both from a nutrient point of view (Friedman and others 1996).

Fish muscle possesses an excellent amino acid composition and is a valuable source of nutritive and proteins with high rate of digestion (Yanez and others 1976; Venugopal and others 1996a,b). However, since fish is greatly susceptible to spoilage, and also due to the possibility of chemical composition variation, the utilization of fish as a primary raw food material indicates unprecedented food processing problems (Spinelli and others 1982).

The fundamental of various animals’ muscle is very similar; they include similar protein and similar amino acid profiles. There are trivial differences between fish muscle and the muscle of terrestrial animals. These differences are mainly attributed to the variation in muscle structure
needed for swimming and buoyancy. Fish are surrounded by a mass of water; therefore the muscle fibers require less structural support than those in land animals. Due to this fact, fish muscle tends to have less connective tissue than muscles from land animals, which results in more tender texture. In addition, due to the unparalleled movement of fish, the structural conformation of muscle fibers is quite different from land animals (Kristinsson and others 2000). Most of commercially important fish species are cold adapted or poikilothermic, and as a result their muscle proteins have different biochemical characteristics compared with endothermic animals (Fennema and others 1985). The poikilothermic nature of fish proteins causes them to be more heat sensitive than mammalian muscle proteins, with a greater rate of denaturation at high temperatures. Muscle proteins of cold water fish have even greater tendency for denaturation than warm water ones (Venugopal and others 1994; Sikorski and others 1994).

The T-50 value is the required temperature for 50% denaturation. The T-50 values of fish muscle proteins are also prone to change by pH and were reported to be 29 to 35°C at pH 7.0 and 11 to 27°C at pH 5.5 (Suzuki 1981). Protein composition in muscles varies by muscle type. The three types of muscle are: striated, smooth, and cardiac muscle. Among these three types of muscles, fish mostly possess the striated type of muscle. Striated muscle tissue is arranged into muscle fibers that are bound together by a connective tissue to make a fiber bundle. Fish muscle includes “white” and “dark” meat (Suzuki 1981). The white meat is generally more predominant, contains fewer lipids than the dark meat, and is the most preferable type of muscle tissue for consumption. It is composed of about 18 to 23% of protein, based upon the species and harvesting time (Kristinsson and others 2000).

In a separate categorization, fish proteins are divided into various groups based on their solubility. Almost 70 to 80% of fish muscle is made from structural proteins. These structural
proteins are soluble in cold neutral salt solutions of relatively significant ionic strength. The rest (20 to 30%) contain sarcoplasmic proteins which are soluble in water and dilute buffers, and the last part of the structural proteins (2 to 3%) is insoluble connective tissue proteins (Spinelli and others 1982). However, some studies defy these generally recognized solubility data; they offer that the muscle protein components can be highly soluble at low ionic strengths (Stefansson and others 1994; Feng and others 1997). The primary food proteins of fish are myofibrillar proteins, encompassing 66 to 77% of the total protein in fish meat (Kristinsson and others 2000).

The myofibril protein complexes consist of myosin and actin. These are the most important components of the thick filament, and thin filament, respectively. Myosin contains 50 to 60% of the myofibrillar contractile proteins, and actin only 15 to 30% (Suzuki 1981; Fennema 1985). Myosin is the most predominant of the single muscle proteins, constructing near 38% of the total, and is a large molecule consisting of two similar large chains (223 kDa) and two small chains (22 and 18 kDa). The molecule has two similar globular head regions that accommodate the small chains and a great fraction of the large chains. The tails of the large chains form very long α-helices that wrap around each other (Zubay 1993) (see Figure 2.1).

Myosin can be cut by proteases at two spots on the molecule, one known by both trypsin and chymotrypsin and the other by papain. Papain cut the area close to head, cause the head to separate from the tail. Trypsin and chymotrypsin cut away from the head which split the molecule into two components called the heavy meromyosin (with the head region) and the light meromyosin, both with different functionalities. Myosin molecules are linked through their head region to the polymerized actin molecules in the thin filaments because of the ATPase activity of the head molecules. This complex is called actomyosin and its responsibility is contraction and relaxation of the muscle. Actomyosin is important in determining the quality of fish meat since it
is fairly unstable and readily affected during processing and storage. For instance, frizzing the solubility of actomyosin progressively decreases and the flesh becomes increasingly stiffer (DeMan 1990). The thin filament is a complex of actin molecules making a double helix. Tropomyosin places within the grooves of the thin filaments and two troponin molecules bind the actin filament at each helical repeat. Actin is the most abundant protein of the three proteins in the thin filaments which forms about 13% of the whole muscle proteins. The two forms of actin are G-actin, a spherical monomer, and F-actin, a large polymer that links to myosin. The thin filaments have a leading role by adjusting muscle contraction. From the viewpoint of muscle biochemistry, thin filaments are of great importance, in spite of their trivial content in meat.

The other important contractile proteins are C-protein, α-, and β actinin, connectin and paramyosin. However, their importance is less than food proteins. With regard to enzymatic protein hydrolysis, the myofibrillar protein myosin, actin, or actomyosin are the substrate of enzymatic cleavage (Kristinsson and others 2000).

![Fig. 2.1 Myosine molecule in fish muscle](Adopted from Kristinsson and others 2000)

### 2.3 Hydrolysis of protein

Modifying proteolytically the proteins of food protein for improving palatability and storage stability of the available protein resources has a long history in food technology (Adler-Nissen 1986). In one definition, hydrolysates are proteins that are chemically or enzymatically broken down into peptides of different sizes (Skanderby 1994). Protein hydrolysates are produced for a wide variety of applications in the food industry, including protein supplements, milk replacers, flavor enhancers in confectionery products, and stabilizers in beverages.
(Kristinsson and others 2000). The advantages of hydrolyzed food proteins to make functional protein ingredients and nutritional supplements are a more recent technology. The first commercialized protein hydrolysates were produced during the late 1940s. Although there is a considerable production worldwide, the proper control of the process and the exact mechanism of protein hydrolysis in a number of instances is not completely comprehended. Recent advances have granted researchers insight into the relation between the process and extent of hydrolysis, and the physicochemical mechanisms responsible for particular functionalities of the hydrolyzed protein. Recent research on enzyme catalysis has also assisted with the proper selection of enzyme catalysts and processing conditions to obtain more control over the reaction and attributes of the final product. The most widely employed methods are chemical and biological methods which protein hydrolysis with chemical hydrolysis used more regularly in industry due to being more economically feasible. Biological processes with the aid of added enzymes are used more frequently, and enzyme hydrolysis is promising in some applications since it results in products of superior nutritive value and functional properties (Kristinsson and others 2000).

There are also many other potential techniques for extracting protein from animal tissue. These include the application of aqueous and organic solvents; the conventional processes of cooking, pressing, drying, and hot oil extraction (Pigott 1982). The extraction of protein by means of solvent is also worth mentioning due to its industrial and historical importance for fish protein recovery (Kristinsson and others 2000).

2.3.1 Production of fish protein concentrate (FPC) using chemical extractions

Protein hydrolysis does not occur in the extraction methods. Chemical extraction methods are employed basically used to concentrate intact protein by the removal of water and oil from the initial materials. Solvent extraction methods have been frequently used in production of fish
protein concentrate (FPC) (Kristinsson and others 2000). The production of FPC was one of the first methods to recover fish protein from processing waste and to make a protein ingredient from underutilized species. FPC was the initial material of enzymatic hydrolysis of fish proteins. An extensive research program on the scaled-up production of FPC by the Bureau of Commercial Fisheries, currently the National Marine Fisheries Service (NMFS) of the Department of the Commerce, started in 1961 (Kristinsson and others 2000). The overall goal of the program was studying the development and application of FPC as a solution to global protein malnutrition and as a potential economic motive to the American fisheries industry (Snyder 1967). Solvent-extracted FPC or type A FPC is made by using primarily isopropanol or azeotropic extraction with ethylene dichloride, although other solvents like ethanol also have been used successfully (Kristinsson and others 2000). A standard process presented by Sikorski and Naczk (Sikorski and others 1981) which illustrated in Figure 2.2, is to grind a whole or eviscerated fish, extract it with isopropanol at a low temperature (20 to 30°C) for 50 min, followed by collecting the supernatant and two time extractions, first at 75°C for 90 min in isopropanol and then at 75°C for 70 min with azeotropic isopropanol. The final supernatant layer is collected, dried, milled, and screened to separate out bone particles. The final product is biologically of superior significant and is colorless and odorless, with less than 1% lipids. The disadvantage of solvent-extracted FPC is that it is not readily soluble or dispersible in foods and has poor emulsification characteristics (Cheftel and others 1971; Mackie 1974). Dubrow and others (Dubrow and others 1973; Venugopal and others 1996) reported that FPC made at higher temperatures (50°C) compared with lower temperatures (20°C) had lower emulsifying properties, but both had very inferior solubility. Overall not satisfying functional properties, potent fishy smell, expensive production, and staying of the trivial amount of solvent
in the final product did not let solvent extracted FPC to become commercially promising despite all the work (Finch 1977; Mackie 1982). Although FPC suffered from lack of solubility, based on the studies it has good foaming properties in variety of pH (pH 2 to 11), making stable and strong foams (Sheustone 1953; Hermansson and others 1971; Kinsella 1976). In spite of problems with protein functional properties, solvent extraction is the method of preference for numerous fatty pelagic fish species such as capelin, herring, and sardine since the protein is to large extent separated from the lipids, while reducing stability problems normally related with residual oxidizable lipid. For fatty fish, isopropanol to some extent was more efficient solvent than ethanol due to the residual amounts of lipids, but absolute ethanol produced FPC of lighter color and a neutral flavor (Moorjani and others 1968). Different research projects with FPC have also been conducted with solvent-extracted FPC as a substrate for enzyme hydrolysis for two reasons: to defat the substrate and to make it available to enzymatic hydrolysis. The resultant has great functional and nutritional properties (Cheftel and others 1971; Hale 1972; Spinelli and others 1972; Quaglia and others 1987a,b Hoyle and others 1994). Enzymatic hydrolysis, however, using FPC as an initial material led to loss of number of functionalities due to excessive protein breakdown but increased nitrogen solubility (Hermansson and others 1971). Odor and taste issues are generally decreased with a FPC initial material (Hale 1972). Some studies showed with solvent-extracted FPC produced FPC with better protein functionality. For instance, Vareltzis and others (Vareltzis and others 1990) studied the addition of ethanol-extracted FPC prepared from sardine to hamburger patties and explored that the overall functionality (water binding and cooking yield) and the penetration depth and shear force value of the hamburger raised by the addition of FPC. However, the hamburgers had a traceable unpleasant fishy flavor. Hoyle and Merritt (Hoyle and others 1994) reported that herring protein
extracted with ethanol in a same way and then hydrolyzed with either alcalase or papain produced a hydrolysate with a significantly decreased bitterness and unpleasant fishy odor.

2.3.2 Fish protein hydrolysis using biochemical methods

Biochemical hydrolysis to produce fish protein hydrolysates is performed by employing enzymes to hydrolyze peptide bonds. This can be conducted via proteolytic enzymes presenting in the fish viscera and muscle (endogenous proteases), or via addition of enzymes from other sources (Kristinsson and others 2000).
By employing enzyme technology for protein recovery in fish processing, it may be possible to produce a broad spectrum of food ingredients or industrial products for a wide range of applications. However, production cost would be considerably higher than the other methods like chemical hydrolysis. This would utilize either fishery byproducts or secondary raw materials. This is in addition to the underutilized species that would otherwise be discarded (Kristinsson and others 2000). Enzymatic modification of proteins using particular proteolytic enzyme preparations to cleave specific peptide bonds is extensively used in the food industry (Mullally and others 1994).

2.3.3 Chemical hydrolysis

Chemical hydrolysis of proteins is accomplished by cleaving peptide bonds by using of acids or bases. Different processes have been offered for the acid or alkaline hydrolysis of fish (Hale 1972). This has been the selected method in some cases for the industry primarily because it is relatively inexpensive and not complicated to conduct. However, there are multiple limitations to food ingredients using this method. Chemical hydrolysis is not easily controlled, and in most of cases invariably leads to products with variable chemical composition and functionalities (Pigott 1982; Blenford, 1994). Protein hydrolysis with strong chemicals and solvents is usually conducted at extreme temperatures and pH and generally yield products with less than ideal nutritional qualities, inferior functionality, and in some cases limited to be applied as flavor enhancers (Webster and others 1982; Loffler 1986).

2.3.3.1 Alkaline hydrolysis

The application of alkaline reactants, usually sodium hydroxide, for protein hydrolysis often results in inferior functional properties and also can have adverse effect on the nutritive value of the hydrolysate (Kristinsson and others 2000). In spite of this, in the food industry
restricted alkaline treatment is employed to recover and solubilize a variety of proteins. For instance, mechanically deboned turkey residue (MDTR) contains a considerable proportion of alkali-soluble proteins that can be recovered by alkaline treatment and applied in food stuff. Fonkwe and Singh (Fonkwe and others 1996) studied the application of alkali extraction for the recovery of MDTR with an alkaline sodium chloride solution, but they resulted that the method was not suitable because of low recovery. Alkaline hydrolysis of fish proteins has basically employed FPC as the initial material. Within alkaline hydrolysis of fish protein, quick cleavage to large water-soluble polypeptides occurs, followed by further degradation at a slower rate.

Alkali treatment can be helpful with modifying the properties of insoluble FPC (Sikorski, and others 1981). Tannenbaum and others (Tannenbaum and others 1970a,b) have discussed the alkaline process for hydrolyzing insoluble FPC and its applications. They explored a small-scale batch process that utilizes high pH (12.5) and 95°C for 20 min. The product contained large peptides, some relatively insoluble at the isoelectric point, but with a general progress in functional properties with respect to the original FPC. Application of the solubilized FPC as a milk substitute resulted in a product much better than the one obtained with FPC initial substrate, which had inferior solubility and dispersibility. Several disadvantageous reactions take place in alkaline solutions within hydrolysis. These are triggered by hydrogen abstraction from the alpha carbon of an amino acid and contain racemization of L amino acids, which leads to D-amino acids that are not digestible by humans. Disulfide bonds are split with loss of cysteine, serine, and threonine as well, via beta elimination reactions and formations of lysinoalanine, ornithinoalanine, lanthionine, and beta amino alanine is possible to occur as well (Kinsella 1976). Part of these elimination and addition reactions may cause formation of toxic compounds (e.g., lysinoalanine) that are greatly unsuitable in foods (Linder and others 1995). Alkaline
hydrolysis reaction products have an inhibiting effect on proteolytic enzymes, which reduces the rate of hydrolysis (Krause and others 1974). Some of the possible reaction products that can form within alkali hydrolysis are shown in Figure 2.3 (Sikorski and others 1981). Alkaline treatment can also lead to high collagen solubility (Ledward and others 1984).

![Diagram of possible chemical compounds that may form during the alkaline treatment of proteins.](image)

Fig. 2.3 Different possible chemical compounds that may form during the alkaline treatment of proteins.
(Adopted from Sikorski and others 1981)

### 2.3.3.2 Acidic hydrolysis

Acidic hydrolysis of proteins is used more regularly than alkaline hydrolysis. A large fraction of hydrolyzed proteins consumed in the U.S. are prepared by acidic hydrolysis which most of them are from not very expensive vegetable protein sources that otherwise would have inferior nutritive and low functional value in foods. Although this is a tough process which is hard to control, it is still the method of choice for hydrolyzed vegetable proteins. Hydrolyzed vegetable protein, which is commonly utilized for flavor and taste enhancement properties, needs extensive acid hydrolysis (Blenford 1994). Applications of hydrolyzed vegetable proteins are primarily as flavoring agents in processed meat, soup mixes, and crackers. Acidic hydrolysis of fish protein has involved reacting fish proteins with hydrochloric acid in the most of applications, or in the other cases sulfuric acid, and the proteins are completely hydrolyzed at high temperature, and often high pressure. In some cases the hydrolysate is neutralized to pH 6.0
to 7.0 after the completion of hydrolysis, and becomes concentrated to either a paste or further dried (Thakar and others 1991).

Since the final product is hydrolyzed extensively, its most important functional attribute is high solubility. Total hydrolysis of fish protein raw material can be accomplished in 18 h at 118°C in 6N hydrochloric acid (Thomas and others 1994). Following the neutralization of the digest, the hydrolysate contains considerable amount of salt (NaCl), which can make the product unpalatable and interferes with functionality in food systems. Another pertained issue of acidic hydrolysis is the destruction of tryptophan, which is an essential amino acid. Orlova et al. (Orlova and others 1979) reported an acid hydrolysis process of whole fish, where steam distillation is used to remove volatile odorant compounds followed by filtration and concentration. The concentrate was used in dehydrated soup cubes and as a microbial media (Orlova and others 1979). The acidic hydrolysis is also extensively used to convert useless and secondary raw material from fish into fertilizer due to the low production cost and resulting extensive hydrolysis (Kristinsson and others 2000).

2.4 Conventional production of fertilizers

Conventionally, soil additives and plant fertilizers have been made synthetically from petroleum byproducts and mineral salts. However, increasing public awareness of the environmental drawbacks of utilizing chemical-based fertilizers has created a demand for safe, natural, and more environmentally-benign fertilizers. In recent decades organic fertilizers have been considered more economically feasible to produce and environmentally friendly, with a potential to improve the health of soil because they contain micronutrients and macronutrients that are not found in chemical fertilizers (Sharma 1991; Qin and others 2008).
The most important macronutrients are nitrogen, phosphorus, and potassium. Secondary macronutrients include calcium, magnesium, and sulfur. Micronutrients are elements essential for plant growth and development that are required in trivial quantities, including boron, copper, iron, chloride, manganese, molybdenum, and zinc. In addition to these essential elements, organic fertilizer which the instances are not petroleum-based or chemical fertilizers, contain amino acids that replenish the nutrient level of the soil and feed important soil organisms, such as nematodes, earthworms, and microorganisms that are essential for overall plant and soil health.

The industry has been relying solely on in-house formulations which are limited in quality due to the incomplete existing knowledge on the development of organic fertilizers. That was mainly because of the short turnaround time during development, while new products often fall “under or near the tree” (as evidenced by the limited product varieties) due to the pressure of profits outweighs risk-taking, leaving considerable unknowns of the science involved. Consequently, the applicability of new ingredients from specific sources has been greatly hindered, resulting in limited number of choices for farmers.

2.5 Technologies for fish-based fertilizer production

Technologies that are currently used to produce fish-based fertilizer as a type of organic fertilizers mainly include the formation of a fish emulsion (Abbasi and others 2009), and the hydrolysis of whole fish or fish parts to produce hydrolyzed fish fertilizer. To produce a fish emulsion, the fish parts are heated to extract oil and the solid material is pressed into a cake and dried to make fish meal, which can be used for livestock feed. The liquid residue that has been pressed out of the fish cake is the fish emulsion used for fertilizer.
There are two main drawbacks associated with fish emulsion fertilizer. First, fish emulsion has a very strong, unpleasant odor due to the presence of decomposing proteins resulted from the high temperatures used during the extraction process. Secondly, fish emulsion, which is composed of the liquid pressed out of the fish cake, is primarily composed of water soluble nutrients and contains a relatively low concentration of oil-soluble nutrients. Thus, fish emulsion is lacking in a number of macro and micronutrients, including oils, proteins, and vitamins that are beneficial for optimal plant and soil health.

The second method is the hydrolysis of fish or fish parts to produce hydrolyzed fish fertilizer. In this method, the starting material is ground into meal and then digested or hydrolyzed. There are numerous methods of hydrolyzing fish protein to break down solid fish into a liquid form, including enzymatic hydrolysis (Aspmo and others 2005) and chemical digestion (Bueno-Solano and others 2008).

Fertilizers produced by hydrolysis have a number of advantages over fertilizer produced by the emulsion process. The hydrolysis process retains much more of the nutrients than the emulsion process, as the hydrolyzed fertilizer utilize all of the initial material. In particular, hydrolyzed fish fertilizer retains the oil soluble nutrients that are excluded from fish emulsion. Another advantage of the hydrolysis process is that it commonly does not involve high temperature. Consequently, the hydrolysates has very low odor as it does not contain decomposing proteins.

Recently enzymatic hydrolysis of fish has become more commercially available (Kristinsson and others 2000) and usually is carried out using enzymes such as papain, which break down proteins into smaller peptides and individual amino acids. However, enzymes are sensitive to pH and temperature, as well as inhibitors and denaturants (Bhaskar and others 2008)
Also, enzymes only catalyze reactions within a specific pH and temperature range. Thus, the hydrolysis reaction must be meticulously monitored and controlled in this method, in addition to its high manufacturing expenses.

Chemical hydrolysis, on the other hand, usually involves stringent conditions, for instance concentrated acid or base and heat to thoroughly hydrolyze the fish protein, due to the strength of peptide bond. Consequently, the application of heat generally results in a reduction in micronutrient concentration, while leaving some unpleasant odors in the product. Table 2.1 shows the comparison between the main fish protein recovery methods and the drawbacks associated to each of them.

Table 2.1 Comparison of different methods of fish protein recovery; fish emulsion formation, enzymatic hydrolysis, and chemical hydrolysis.

<table>
<thead>
<tr>
<th>Approach</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Fish emulsion formed from liquid residue from fish oil & fish meal processing | 1) Very unpleasant odor due to decomposed protein resulted from high temperature  
2) Lack of oil soluble nutrients         | Abbasi et al., 2009                                                      |
| Enzymatic hydrolysis                          | 1) Enzymes are sensitive to pH and temperature variations  
2) High manufacturing costs                   | Aspmo et al., 2005                                                      |
| Chemical hydrolysis                           | 1) Stringent conditions (concentrated acid, base, and heat) needed to achieve complete hydrolysis  
2) Reduction in micronutrient concentration and unpleasant odor resulted from heat | Bueno-Solano et al., 2008 |
2.6 Susceptibility of fish products to spoilage

Currently, control of food quality is of vital importance for food industry because of the constantly increasing demand of good quality and hygienic food products by consumers. In particular, fish products that belong to vulnerable types of products are readily infected by microorganisms at ambient conditions and result in the loosing of freshness shortly after being processed (Serena and others 2006). Hence, extensive efforts of preserving fish products such as vacuum-packed and carbon dioxide enriched cold storage (De la Holz and others 2000) after harvesting in fisheries and being processed in food industry are done for maintaining safety, nutritional, and commercial values.

2.7 Microbiological deterioration of aquatics

Microbiological spoilage of foods may occur in various forms, but all of them are a consequence of microbial growth and/or activity, which manifests itself as alteration in the sensory attributes (Gram and others 1996).

Raw foods are initially contaminated with a wide variety of microorganisms, but only a limited number of these contaminants are able to colonize the food and grow to a high population. The term ‘spoilage association’ has been used for such a specific microbial community. The precise mechanism by which one group of bacteria predominates over another, closely related group is not always completely understood. For a particular product, spoilage may develop differently, depending on geographical origin and other unknown factors dealing with the microbial development (Gram and others 1996).
2.7.1 Susceptibility of aquatics for bacterial spoilage

All food commodities have their own unique microbiology. Specific and non-specific contamination of the live animal from the environment and of products while being processed, growth conditions for microorganisms due to particular intrinsic, and extrinsic factors (temperature, \(a_w\), pH, Eh, microbial interactions etc.) are important factors which contribute to the complicated microbiology of seafood (Gram and others 1996).

The wide range of environmental habitats (freshwater to saltwater, cold waters to warm waters, pelagic swimmers to bottom habitants and degree of pollution) and the variety of processing practices (iced fish products to (sterile) canned products) are all important factors in determining the initial contamination of fish and fish products. The part of the micro flora which will ultimately grow on the products will be determined by the intrinsic and extrinsic factors. There are numerous important specific intrinsic parameters in fish which to a great extent influence the microbiology and spoilage, for instance the poikilothermic nature of the fish and its aquatic environment, a high post mortem pH in the flesh (usually > 6.0), the presence of large quantities of Non-protein-nitrogen (NPN), or the presence of trimethylamine oxide (TMAO) as part of the NPN fraction (Gram and others 1996).

Bacteria establish themselves on the outer and inner surfaces of the live fish (gills, skin, and gastro-intestinal tract). The poikilothermic nature of fish allows bacteria with a broad temperature range to grow. Therefore, the micro flora of cold water fish is dominated by psychrotrothpic Gram-negative, rod-shaped bacteria belonging to the genera \textit{Pseudomonos}, \textit{Moraxellu}, \textit{Acinetobacter}, \textit{Shewanellu}, \textit{Fluvobucterium}, \textit{Vibrionuceue} and \textit{Aeroemonaduceue}, but Gram-positive organisms such as \textit{Bacillus}, \textit{Micrococcus}, \textit{Clostridium}, \textit{Luctobacillu} and \textit{Corynebacterium} can also be found in various proportions. The flora on warm water fish often
carries a slightly higher load of Gram-positive and enteric bacteria, but is otherwise similar to the flora on cold water fish (Liston 1980).

An important intrinsic factor related to fish flesh is the very high post-mortem pH (> 6.0). Most fish contain only trivial amount of carbohydrate (< 0.5%) in the muscle tissue and only small quantities of lactic acid are produced post mortem. This has important consequences for the microbiology of fish as among other factors it allows the pH sensitive spoilage bacteria \textit{Shewanella putrefaciens} to grow. The non-protein-nitrogen (NPN) fraction of the fish flesh consists of low-molecular-weight water-soluble nitrogen which contains compounds such as free amino acids and nucleotides and is a readily available bacterial growth substrate. The decomposition of the sulfur containing amino acids cysteine and methionine is of particular importance in spoilage, as it causes undesirable odors and flavors due to formation of hydrogen sulfides and methylmercaptane respectively (Herbert 1975; Herbert and others 1976).

Trimethylamineoxide (TMAO) is part of the NPN fraction and its presence in all marine (Hebard and others 1982) and some fresh water fish (Gram and others 1989; Anthoni and others 1990) species is well known. TMAO is known to cause a high (positive) redox potential (Eh) in the fish flesh (Huss and others 1979, 1980); however, the significance of this is not clear. The spoilage of fresh fish is certainly influenced by the presence of TMAO, particularly under conditions where oxygen is excluded. A number of well-recognized spoilage bacteria \textit{(Shewunella putrefaciens, Photobacterium phosphoreum, Vibrionaceae)} are able to utilize TMAO as the terminal electron acceptor in an anaerobic respiration resulting in off-odors and -flavors due to formation of trimethylamine (TMA) (Gram and others 1987, 1990; Dalgaard and others 1993). In sugar-salted herring, the presence of TMAO and the high Eh was recognized as the protective mechanism against the most common type of spoilage (sweet-sour, rotten putrid)
as the organism which causes this type spoilage highly require a low Eh for growth (Knechel and others 1984a,b).

### 2.8 The volatile compounds causing fishy odor

There is almost 3% of different salts by weight in the water of the oceans, but the optimal level of dissolved minerals inside an animal cell is less than 1%. For maintaining the fluid balance, ocean creatures must fill their cells with amino acids and amines to counter the saltiness of seawater. Ocean fish mostly rely on trimethylamine oxide (TMAO) for this purpose. When fish are caught, bacteria and fish enzymes convert TMAO into trimethylamine (TMA), which cause the characteristic of “fishy” odor (McGee and others 2004).

Alcohols, esters, carbonyls, and sulfur compounds which can be formed by Pseudomonads typically cause sweet, malty, fruity, and onion like odors in fish (Miller and others 1973a,b). High levels of sulfur compounds (Herbert and others 1975) and fishy odors because of the reduction of TMAO to trimethylamine (TMA) forming by *Shewanella putrefaciens* can lead to more potent odors in fish (Shewan 1962); also dimethylamine (DMA) has been well known to be released from TMAO through intrinsic enzymatic activity during cold storage (Sotelo and others 1995). Trimethylamine-\(n\)-oxide (TMAO) is a nitrogenous osmolyte widely distributed in marine organisms (Spinelli and others 1981; Bechmann and others 1998), which can reach high concentration in some species (e.g. squid and cod). Numerous studies have reported that dimethylamine (DMA), formaldehyde (FA) and trimethylamine (TMA) in aquatic products are derived from the breakdown of TMAO (Fu and others 2007; Rey-Mansilla and others 1999). TMA is of particular attention because it is used as an indicator of spoilage in fish due to its typical fishy odor. TMAO can be converted to equimolar quantities of DMA and FA (Bechmann and others 1998).
Trimethylamine which is formed by bacterial reduction of trimethylamine oxide (TMAO) is one of the unique chemicals indicating the spoilage process in fish. The changes in TMA values were recognized to have good relationship with sensory tests, storage temperature, storage time and viable bacteria count (Dondero and others 2004). Additionally, methylamine (MA) was reported in spoilage crustaceans (Kruse and others 1989) and ham (Jones and others 1998); and dimethylamine (DMA) was mentioned to be released from TMAO through intrinsic enzymatic activity during cold storage (Sotelo and others 1995). These three alkylamines are also recognized to be the precursors of carcinogenic nitrosodimethylamine upon consumption (Zeisel and others 1988). Therefore, particular studies of MA, DMA and TMA could provide extra information for evaluation of the deterioration or freshness status of fish during storage and safety assessment regime (Serena and others 2006).

Treating fish with acidic ingredients can cause TMA to bind to water and become less volatile. Freshwater fish in most cases do not accumulate TMAO because their environment is less salty than their cells. As a result, their flesh tends to be milder, and they do not become as “fishy” as saltwater fish. However, freshwater fish sometimes suffer from an unpleasant “muddy” aroma. This often takes place in bottom-feeders such as catfish, and is caused by two compounds produced by blue-green algae (geosmin and methylisoborneol). These chemicals concentrate in the skin and dark muscle tissue of the fish. Acidic conditions will cause these compounds to break down (McGee and others 2004).

Determination of alkylamines in fish was used to be routinely performed using colorimetry by coupling with chemical agents like dimethyldithiocarbamate for DMA and picric acid for TMA, to form colored complex. These methods are straightforward, but procedures involved are not recognized applicable for high throughput operations. Data obtained are also
inherently subject to interference from co-existing amines in food matrices. Other chemical methods, with better turnaround time and improved selectivity such as gas chromatography (Veciana-Nogues and others 1996), liquid chromatography (Hwang and others 1997), flow injection analysis using potentiometric detection (Adhoum and others 2003) and capillary electrophoresis (Lista and others 2001) have later been established. Headspace solid-phase microextraction (HS-SPME) that was developed for analysis of volatile compounds in the early nineties (Arthur and others 1990) has proved unique capability of incorporating extraction and concentration in one single step. The technique also suggested advantages of solvent-free, simple manipulation, simplicity of automation and compatibility with GC-MS or LC-MS (Snow and others 2002; Kataoka 2002). These comprehensive and versatile characteristics enable HS-SPME being one of the most popular methodologies for quantitative analysis of volatile components in a wide variety of food samples. Recent studies on cheese (Verzera and others 2004) and whiting (Duflos and others 2005) samples were the examples amongst its diversified applications. A study using HS-SPME-GC-MS (Mills and others 1999) for determination of TMA as a diagnostic tool for trimethylaminuria in biological matrices explained the potential usefulness of HS-SPME for volatile amines.

2.9 Activated carbon as a deodorant substance

Adsorption processes using activated carbons are employed for verity of purposes. The examples are removing pollutants from wastewaters (Hameed and others 2007), removal of odorous compounds in wastewater (Hwang and others 1994), decontamination of fish oil (Oterhals and others 2007), removal of dioxins and polychlorinated biphenyl (PCB) from fish oil (Maes and others 2005), adsorption of phenolic compounds (Dabrowski and others 2005), adsorption of methylene blue (Hameed and others 2007). However, commercially available
activated carbon is relatively expensive. In the recent years, special attention on the preparation of activated carbons from several agricultural by-products has been directed respecting the growing interest in low cost activated carbons from renewable, abundant, especially for various applications (Hameed and others 2007). The advantage of using agricultural by-products as initial materials for manufacturing activated carbon is that these raw materials are renewable and potentially more economically feasible to manufacture. Plant biomass is a natural renewable resource that can be converted into useful materials and energy (Klass 1998).

2.9.1 Bamboo charcoal

Bamboo is the most diverse group of plants in the grass family. It belongs to the subfamily Bambusoidae of the family Poaceae (Graminae). Approximately 1500 commercial applications of bamboo have been identified, mostly in Asia (Scurlock and others 2000). It is a tenacious, versatile, and highly renewable material that people and communities have known and utilized for several years. Bamboo has been used as the structural material for steps at construction sites in China, India, Malaysia and other countries since it is a strong, stiff and low-cost material (Hameed and others 2007).

Bamboo charcoal is made of bamboo culms and waste material from bamboo processing. On one hand, bamboo charcoal not only provides a new path to utilize bamboo, but also benefits to environment protection by decreasing the residues pollution. On the other hand, bamboo charcoal is a kind of environmentally functional material which has supreme absorption and has benign properties from the environmental point of view. The production of bamboo charcoal will promote the sustainable development of economy and ecology (Mingjie 2004). Bamboo charcoal as a type of carbon materials has the adsorption function. This characteristic of bamboo charcoal grants it various functionalities, for instance as a purifying agent for water and air in
dwelling environment (Mingjie 2004). Bamboo charcoal also can be processed into bamboo active carbon after activation process. Activated bamboo carbon is extensively used in many fields of industry as absorbent, additive, as well as the other applications (Mingjie 2004).

2.10 ZVI (Zero Valent Iron) for deodorization

In general, zero valent iron (ZVI) or Fe(0) is a granular cast iron product obtained as a by-product from the automotive industry (Morrison and others 2002). ZVI has recognized to be an effective material for removing multiple contaminants including halogenated organic solvents (O'Hannesin and others 1998; Puls and others 1999; Vogan and others 1999; Wilkin and others 2003), radionuclides (Morrison and others 2002), arsenic (Lackovic 2000; Farrell and others 2001; Melitas 2002; Nikolaidis 2003; Su and others 2004), and heavy metals (Shokes and others 1999).

Passive methods employing permeable reactive barriers (PRBs) have been employed in order to reduce the cost of groundwater remediation. A PRB is an engineered zone of reactive material placed in an aquifer that allows passage of groundwater while retaining or degrading the contaminants (Morrison and others 2002). Many PRBs contain Fe(0) that has been used to treat organic contaminants (Gillham and others 1994; Gavaskar and others 1998) has also been employed in PRBs to treat U and associated inorganic contaminants at Durango, CO; Monticello, UT (Morrison and others 1995); Fry Canyon, UT (Naftz and others 1999); and Oak Ridge, TN (Gu and others 1999).

Metallic iron provides an alternative method to degrade chlorinated organics abiotically. Under anaerobic conditions, zero valent iron is able to reduce common chlorinated hydrocarbon (CHC) via intermediates, to non-toxic hydrocarbons. The reaction does not need high temperature. The reaction is thermodynamically feasible and is limited kinetically. The lower the
degree of chlorination causes the rate of dechlorination reaction to become slower (Matheson and others 1994; Johnson and others 1998).

Zero valent iron serves as a donor of electrons; this indicates that ZVI is a reducing agent. The reaction occurs in multiple stages. However, intermediate compounds like vinyl chloride are never present in water in high concentrations. This fact is of great importance since these compounds exhibit higher toxicity than the predecessor compounds. The final products of the reaction are simple hydrocarbons like ethylene, ethane and acetylene (Schreierer and others 1994; Orth and others 1996; Roberts and others 1996; Allen-King and others 1997; Campbell and others 1997; Su and others 1999; Farrell and others 2000).

Zero valent iron also reacts with water producing hydrogen gas and hydroxide ions which causes the pH of the water to increase. Hydrogen gas can also react with tetrachloroethylene (PCE) and trichloroethylene (TCE). Decontamination of waters using zero valent iron is not restricted to volatile halogenated hydrocarbons. It can also be utilized for the removal of DDT (Sayles and others 1997) and multiple other pesticides (Ghauch and others 2001) from water.

2.11 Environmental and agricultural significance of the project

Demand for organically produced food has increased in the US, as many consumers have expressed concern over pesticide residues on foods (Govindasamy and others 1998; Thompson and others 1998). Food and environmental safety are often-cited reasons for the use of alternative soil amendments, but increasingly, economic considerations are becoming important with a rise in popularity of organically produced foods (Govindasamy and others 1998; Klonsky and others 1998; Thompson and others 1998). A premium of 12–60% is often obtained from organic produce (Lohr 1998). Since this premium exists, organic agriculture has become more attractive to farmers (Langley and others 1983; Klonsky and others 1998; Thompson 1998).
Nutrients in surface and groundwater can affect human and aquatic organisms that rely on water for consumption and habitat (Easton and others 2004). The application of organic soil amendments has been attributed to desirable soil properties including higher plant available water holding capacity and cation exchange capacity (CEC) and lower bulk density, and can foster beneficial microorganisms (Doran 1995); (Drinkwater and others 1995). Soil chemical characteristics are influenced by soil amendment and production system. For example, at the Rodale Institute, long-term legume-based and organic production systems have resulted in an increase in soil organic matter and reduced nitrate runoff (Drinkwater and others 1995). Soils in organic production systems lost less nitrogen into nearby water systems than did conventional production systems (Liebhardt and others 1989). The amount of soil nitrogen in fields under conventional production systems has been negatively correlated with soil microbial components, while soil nitrogen in fields under organic production was positively correlated with soil microbial components (Gunapala and others 1998). Yields of crops grown in organic and conventional production systems can be equivalent. Vegetable fields under organic production in California produced yields similar to those under conventional production (Drinkwater and others 1995; Stamatiadis and others 1999). Long-run study in Pennsylvania has also indicated insignificant difference in yields between conventional and organic production systems (Drinkwater and others 1995).

It has been shown that microbial activity and biomass is higher in fields with organic amendments than fields with conventional fertilizers (Drinkwater and others 1995). Many studies on soil microbial communities, as affected by organic amendments, have examined functional groups, or classes of organisms, while a limited number of studies have examined the impact on community composition and genera within these groups. The other similar research in organic
tomato fields in California revealed that suppression of corky root disease was related to increased actinomycete activity (Workneh and others 1993, 1994).

The product developed in this project is intended to be sold to various farmers throughout the Mid-Atlantic region. Several unique environmental benefits are anticipated, including the reduction of damaging fertilizer run-off into the Chesapeake Bay, reduction of waste taken into landfills, and a beneficial application of an otherwise worthless product produced from a key component in our food chain. A complete understanding of the mechanisms by which chemical constituents interact and volatile compounds form is crucial in the value-added bioconversion of fish products and represents a phenomenon of worldwide importance with vast economic impact. Additionally, the novelty of combining acidic hydrolysis with membrane-based separation for liquid organic fertilizer production, which will be discussed further, is of scientific merit and should generate new intellectual properties.

In this project a process was developed using an acidic system to hydrolyze fish protein, followed by a membrane-based separation process to purify and separate the desired liquid portion from the remaining solid and deodorization of the final product using zero valent iron (ZVI) and activated bamboo charcoal (ABC).
Chapter 3: Hypothesis & Objectives

3.1 Hypothesis

It was hypothesized that the scrapes from fish processing plants can be converted into a liquid fertilizer which can be certified as organic fertilizer. The formulation of the fish hydrolysate can be engineered to bear upon the inherent nutritional value in all-natural, preservative-free organic fertilizer with optimal composition and performance for farming.

3.2 Research Objectives

To achieve the goal, three specific objectives were defined for the research project:

1. Recovery of fish hydrolysates (FH) from fish waste using a combination of isoelectric purification and membrane separation (Stage 1) to recover high yield of fish protein from fish waste. The specific objectives of this step were:
   - Explore the feasibility of using acidic systems to produce high yield of FH considering the organic product criteria.
   - To identify an appropriate sieving size to produce FH to be compatible with common agricultural tools.

2. Removal of undesired volatile compounds to produce odor free FH that meets the standard quality indicators of organic fertilizers (Stage 2). The particular objectives of this critical stage were:
   - Employing innovative deodorization methods to produce odor-free FH that meets the standard quality indicators of organic fertilizers.
   - To make a value-added product that in addition to carrying high nutritional value, is much more appealing, marketable, and economically feasible than the other commercially available products.
(3) Optimization of organic fertilizer formulations based on the functional constituents of FH against critical criteria and application conditions (Stage 3). Physically and chemically optimized product which meets the requirements of a standard organic liquid fertilizer will be of a great value for food and agricultural industries. During this stage the following properties were tested from optimizations standpoint to ensure the high quality of the final product:

- Functional nutrients of FH to have reasonable absorption ratio by plants.
- Rheological characteristics of FH to be conveniently applicable by common agricultural equipment.
- Physical, chemical, and sensory characteristics of FH to produce a high quality product.
- Shelf stability of FH to project the shelf life of the optimized product.
Chapter 4: Materials and Methods

During this project the process and experiments were arranged to produce the optimized product in an effective and efficient manner. Figure 4.1 illustrates the process flowchart and the association of the stages of the project for sample preparation. These samples were used for further experiments and analysis of the product optimization stage.

Fig. 4.1 Odor-free fish-based organic fertilizer process flowchart

4.1 Materials

The initial materials were freshly ground fish scraps, which were provided by Profish Ltd. (Washington, DC) fish processing plant, phosphoric acid 85% provided by Fisher Scientific Inc. (Pittsburg, PA), potassium phosphate monobasic, anhydrous Dimethylamine with 99+% purity as GC/MS standard, anhydrous trimethylamine with >=99% purity as GC/MS standard, propylamine with 99+% purity as internal GC/MS standard which all were provided by Sigma-Aldrich (St. Louis, MO), activated bamboo charcoal particles which provided by Aquatop
Aquatic Supplies (St. Brea, CA), zero valent iron by Connelly-GPM, Inc. (Chicago, IL), and
potable water.

4.2 Experimental design and Sample Preparation

In the stage one, the fish scraps from the fish processing plant, were ground up on site and delivered to the Food Bioprocess Engineering laboratory at the University of Maryland where the remainder of the sample preparation and main part of experiments were conducted. The samples were stored at -30°C until processed. Upon the process time, pre-ground fish scraps were defrosted in Emerson microwave oven, MW8117 model (Emerson Radio Corp, Hackensack, NJ). The ground fish scrap was subjected to further grinding for 20 minutes using Kitchen Aid food processor, model KFPM770NK1 (KitchenAid, St. Joseph, MI), to have a more efficient hydrolysis process. The well-ground material was diluted with potable water with the ratio of 1.5 water to 1 sample (w/w) before screen separation. Moisture adjustment can aid the screen separation process, as well as hydrolysis process. A portion of phosphoric acid was added to the ground fish scraps to hydrolyze fish protein under continuous mixing for further reduction in the mean particle size of the parts. The pH of samples adjusted to 3.5 which is minimum allowed pH for organic liquid fish products. This was followed by aerating the samples using Rena Air 400 aquarium aerator (PlanetRena, Charlotte, NC) for 24 hours in room temperature. A membrane-based pressure-driven process was used to reduce the mean particle size in the resultant mixture to simultaneously purify, separate, and concentrate the liquefied FH. The samples were sieved with USA standard (ASTM E-11 standard) sieves No. 16, No. 25, No. 50, No. 70 and No. 100. The sieves were purchased from Hogentogler Co. (Columbia, MD). Prepared samples and retentates from sieving process were kept in different batches for further experiments and analysis.
The National List of Synthetic substances allowed for use in organic crop production (refer to 7 CFR Part 205.601(j)) published by USDA’s National Organic Program (NOP) clearly indicates that “Liquid fish products—can be pH adjusted with sulfuric, citric or phosphoric acid. The amount of acid used shall not exceed the minimum needed to lower the pH to 3.5.” In acidic hydrolysis of fish protein, three types of acids known to work well with proteins, individually or in combination, namely citric acid, phosphoric acid, and sulfuric acid, at various volumetric ratios to ground fish samples (Lo and others 2005). Phosphoric acid was the selected acid for hydrolysis in this project because it increases the phosphorus content of the fertilizer which is an important macro-nutrient in fertilizers and soil additives (Beckley and others 2006). As for the sample which hydrolysis was conducted using buffer system, the buffer was made by phosphoric acid and its salt, potassium phosphate monobasic and using Henderson-Hasselbalch equation (Tallarida and others 1986) with regard to the desired pH and the employed acid and salt. The National List of Nonagricultural (nonorganic) substances allowed as ingredients in or on processed products labeled as “organic” or “made with organic (specified ingredients or food group(s))” (refer to 7 CFR Part 205.605(b)) published by USDA’s National Organic Program (NOP) states that “Potassium phosphate—for use only in agricultural products labeled “made with organic (specific ingredients or food group(s)),” prohibited in agricultural products labeled “organic”.” This indicates that potassium phosphate can be used in the products labeled “made with organic (specific ingredients or food group(s))”.

At the stage two granulated activated bamboo charcoal (hereinafter ABC) particles, provided by Aquatop Aquatic Supplies (Aquatop, St. Brea, CA) and zero valent iron (hereinafter ZVI), provided by Connelly-GPM, Inc. (Connelly-GPM, Inc, Chicago, IL) were employed as synthetic bio-filter media for removing odors of FH. ABC particles are known to maintain
structural integrity at low pH (Chan and others 2004). Filter material resistance to low pH of FH was an extremely important trait for bio filters.

The National List of Synthetic substances allowed for use in organic crop production (refer to 7 CFR Part 205.601(j)) published by USDA’s National Organic Program (NOP) clearly states that “Micronutrients—not to be used as a defoliant, herbicide, or desiccant. Those made from nitrates or chlorides are not allowed. Soil deficiency must be documented by testing.

(i) Soluble boron products.
(ii) Sulfates, carbonates, oxides, or silicates of zinc, copper, iron, manganese, molybdenum, selenium, and cobalt.” This indicates that zero valent iron (ZVI) can be used in organic crop production considering the aforementioned circumstances.

After the completion of aeration, the samples were divided into two groups for deodorization. The first group was control with no deodorization treatment and the second group was deodorized with the combination of ZVI and ABC. The control samples went through the sieving process which consisted of series of the US standard number 16, 25, 50, 70, and 100 sieves. The deodorized group after aeration went through the series of the US standard number 16, 25, 50, 70 sieves. It was followed by placing 100 grams of ZVI No. CC 1339 on the sieve No. 100 and passing one liter of the production of the last stage along this sieve for deodorization purpose. In the next stage, 30 grams of activated bamboo charcoal was placed per one liter of the ZVI treated samples for 24 hours and after that period ABC was removed and samples were prepared for further experiments and analysis.

To find the optimum quantity of ZVI for deodorization of FH, in separate sets of experiments different values of ZVI were examined. Based on the preliminary sensory attributed of each ZVI treated FH the decision about the best treatment was made. Table 4.1 shows the
changes of odor in each treatment while six different quantities of ZVI were examined. The starting point was 25 grams per each liter of sample with 25 grams increment.

Table 4.1 Changes in the odor potency of FH treated by different quantities of ZVI

<table>
<thead>
<tr>
<th>Scrap (Gram/Liter)</th>
<th>Fishy odor</th>
<th>Rusted iron odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>+++*</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>125</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>150</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>175</td>
<td>+</td>
<td>+++*</td>
</tr>
</tbody>
</table>

* Level of fishy odor which the more “+” shows the more potent fishy odor
** Level of rusted iron odor which the more “+” shows the more potent rusted iron odor

Also to find the optimum amount of ABC for the purpose of deodorization, in separate sets of experiments different quantities of ABC were examined on FH. Based on the sensory attributed of each ABC treated FH the decision about the best treatment was made. Table 4.2 shows the changes of odor in each treatment while five different quantities of ABC were tested. The starting point was 10 grams per each liter of sample with 10 grams increment.

Table 4.2 Changes in the odor potency of FH treated by different quantities of ABC

<table>
<thead>
<tr>
<th>ABC amount (Gram/Liter)</th>
<th>Fishy odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>+++*</td>
</tr>
<tr>
<td>20</td>
<td>++</td>
</tr>
<tr>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td>50</td>
<td>+</td>
</tr>
</tbody>
</table>

* Level of fishy odor which the more “+” shows the more potent fishy odor

From this point the formula was subjected to a step-wise optimization sequence and modification until a quality product was developed (stage three). The formula was verified to meet the organic requirements so that it could become an effective fertilizer ready for field testing.
Samples which prepared from Step two were stored at 4°C and used in the optimization study at step three. The cold storage period varied based on the pertained experiment. To find the optimum product and process, the physiochemical, odor, and shelf life of the deodorized and non-deodorized samples were analyzed. On one hand, in deodorized group, ZVI and ABC were applied for the purpose of deodorization. On the other hand, in non-deodorized group there was no deodorization treatment. Samples were labeled as following for the experiments which hereinafter they will be addressed by their label:

FH : fish hydrolyzate- with no treatment
FH-ZB : fish hydrolyzate- ABC and ZVI treated
RET : retentate of sieving

Phase separation, pH, color, odor, density, moisture and solid content, total nitrogen content, mineral content, apparent viscosity, rheological properties (product consistency), volatile compounds profile of the samples were analyzed to establish the base of product quality. Shelf-life study was conducted to project the expiration date of the product. The effects of different treatments were evaluated, and were taken into consideration to make future study recommendations. The field trial of the optimized product was performed by Harry Swartz, the retired professor of the Plant Science Department at the University of Maryland.

4.3 Total nitrogen and protein analysis

Total nitrogen analysis was conducted using Perkin-Elmer nitrogen analyzer (Perkin-Elmer Corporation, Waltham, MA); model 2410, on freshly produced FH and RET groups. Percent of protein was calculated using the following formula:

\[ \text{Protein Percent} = \text{Protein conversion Factor} \times \text{Percent nitrogen} \]
In the above formula the protein conversion factor which was used (for meat and meat products) was 6.25. Protein yield percentage (the protein recovered in FH) and the percentage of the protein in RET were calculated using the following formulas.

\[
Protein\ yield(\%) = \frac{FH\ protein\ weight}{Raw\ material\ protein\ weight} \times 100
\]

\[
RET\ protein(\%) = \frac{RET\ protein\ weight}{Raw\ material\ protein\ weight} \times 100
\]

4.4 Physiochemical Analysis

The phase separation of the FH and FH-ZB groups was observed and measured one month after the cold storage (4°C). The density of freshly produced FH and FH-ZB groups was measured at room temperature once each type of product was produced. The moisture of freshly produced FH, FH-ZB, and RET groups was determined by drying the samples in a hot air oven at 100°C until constant weight was obtained (952.45; AOAC, 1990), and the solid content was calculated accordingly. The pH of the FH and FH-ZB groups was measured on the freshly produced samples using Fisher Scientific (Fisher Scientific Inc., Pittsburg, PA) pH meter; model AB15. The pH measurement was also repeated at the end of each month of storage on different groups for shelf life analysis. The apparent viscosity of the freshly produced FH and FH-ZB groups was measured at room temperature by BROOLFIELD viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA). The color of the freshly produced FH and FH-ZB groups was measured by ColorFlex; Model No. 54/0 color-meter (Hunter Associates Laboratory, Inc., Reston, VA). The color measurement was also repeated at the end of each month of storage on each groups for shelf life analysis.
4.5 Preliminary odor evaluation

The odor of FH and FH-ZB groups was measured by four panelists at freshly produced samples. The odor evaluation was also repeated at the end of each month of storage on different groups for shelf life analysis. The odor analysis protocol was based on the method proposed by Lawless and others (Lawless and others 2010) for descriptive sensory analysis. The odor scale which was used to rate the amplitude of odor in each sample is shown in Table 4.3:

Table 4.3 Odor amplitude scale which was used for rating the odor of samples

<table>
<thead>
<tr>
<th>Rating</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low</td>
</tr>
<tr>
<td>2</td>
<td>Medium</td>
</tr>
<tr>
<td>3</td>
<td>High</td>
</tr>
<tr>
<td>4</td>
<td>Very low</td>
</tr>
</tbody>
</table>

4.6 Mineral profile analysis

Using ICP technique, mineral content of the freshly produced FH, FH-ZB, and RET groups was analyzed in dried weight basis and wet weight basis at the Soil Testing Laboratory at the University of Delaware (College of Agriculture & Natural Resources, Newark, DE) under the supervision of Ms. Karen L. Gartley. The protocol employed for mineral profile analysis was based on the method offered by Nuray and others (Nuray and others 2007).

4.7 Fertilizer consistency analysis (rheological studies)

Rheological properties of the freshly produced FH and FH-ZB groups were analyzed based on the method proposed by Rudrarajua and others (Rudrarajua and others 2005). The TA instruments AR2000 controlled stress rheometer (TA Instruments, New Castle, DE) was used for the study. Each sample was gently loaded onto the peltier plate using a tablespoon. Care was
taken to minimize shearing during sample removal and sample loading. All rheology studies were done at 20 °C. Once the system was calibrated, viscosity-shear rate studies were conducted. The flow behavior of the samples was characterized in a period of time. This experiment was done over a range of shear stresses (0.1 to 10 or 25 Pa), and the resulting deformation was obtained (Rudrarajua and others 2005).

4.8 Volatile compounds gas chromatography analysis

Trimethylamine (TMA) and dimethylamine (DMA) as the most important fishy odor indicators (Bechmann and others 1998) were the focus of volatile compounds analysis. The volatile compound analysis was conducted at the Food Science Department of the Louisiana State University under the supervision of Dr. Zhimin Xu. Volatile compounds of the freshly produced FH and FH-ZB groups were analyzed using GC-MS with solid-phase micro extraction (SPME) using the method presented by Serena and others (Serena and others 2006), to assess the effectiveness of the deodorization operation.

4.9 Shelf stability evaluation

Shelf stability evaluation was conducted on the FH and FH-ZB groups which were hydrolyzed through acid and buffer systems. The samples were stored at 4°C for 9 months and were analyzed in forty five day intervals. Each sample was evaluated for its visual mold growth, color, pH, and odor properties.

4.10 Field trials of FH-ZB

The field trials of FH-ZB were conducted at Five Aces Breeding farms (Oakland, MD) under the supervision of Dr. Harry Swarts. The FH-ZB samples were compared to three other commercially available fertilizers, namely Shafer’s SF Organics (SF Organics, Thomson, IL),
Neptune Harvest Organic (Neptune's Harvest, Gloucester, MA), and Miller’s Nutrient Express (Miller Chemical & Fertilizer Corp, Hanover, PA). All fertilizers were applied on raspberry (*Rubus idaeus*) (Five Aces Breeding LLC, Oakland MD) plants. Randomized complete block statistical design was used for the field trial experiments, which were performed during the growth season of raspberry from April to September 2013. FH-ZB and conventional Miller’s Nutrient Express fertilizers were applied in three dosage levels (low, medium, and high) defined based on the typical nutrient needs of raspberry and compared together. The low, medium, and high dosage levels of application were established at 40, 80, and 120 lbs/Acre of actual N, respectively. Medium level of FH-ZB and Miller’s Nutrient Express was the base of comparison with Shafer’s SF Organics and Neptune Harvest Organic. The plants were measured and analyzed twice during their growth, one at month 4 (July) and another at month 6 (September). Individual plant yield, fruit size, sprouts per plant, and average height were measured for each group at the end of the treatment (September 2013), and the plant’s leaves were sampled in July and September for minerals and nutrients analysis.

### 4.11 Statistical Analysis

Statistical differences in the results were determined by applying one-way analysis of variance (ANOVA) methods to the data using SPSS software (IBM SPSS Statistics version 22) (IBM Corporation, Armonk, NY). Each sample was analyzed three times and each experiment was conducted in triplicate (n= 3). The results were expressed as means ± SE (standard error). Differences were considered to be significant when the p-values were under 0.05.
Chapter 5: Results and Discussions

5.1 Process yield

While the protein content appears low in FH due to the significant amount of water used to dilute the ground fish waste prior to screen separation, it is important to note that the majority of protein went into the FH as evidenced by the protein weight (see protein distribution from raw material into the FH and RET in Table 5.1). The protein yield and the percent of protein in the RET were calculated based on the protein weight in each group (see the protein yield and RET protein content in Table 5.1).

Table 5.1 Protein distribution from initial material into the FH and RET; 85% of protein yielded in the FH and 14.03% rejected into the RET.

<table>
<thead>
<tr>
<th>Protein content (%)</th>
<th>Weight (g)</th>
<th>Protein weight (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material</td>
<td>22.2 ± 1.15</td>
<td>1192</td>
<td>264.62</td>
</tr>
<tr>
<td>FH</td>
<td>7 ± 0.28</td>
<td>3250</td>
<td>227.5</td>
</tr>
<tr>
<td>RET</td>
<td>11.4 ± 0.57</td>
<td>326</td>
<td>37.16</td>
</tr>
</tbody>
</table>

All values are means ± SE, n=3

The yields of recovered protein in FH and the RET protein reflect the distribution ratio of initial protein into the product and by-product of the process. This is an important measurement for two reasons: first, the importance of protein as a source of nitrogen for plant growth and development, and secondly because a maximum recovery is desired in the production of hydrolyzed fish fertilizer.

Nitrogen is biologically combined with carbon, hydrogen, oxygen, and sulfur to create amino acids, which are the building blocks of proteins. Amino acids are used in forming protoplasms, the site for cell division and therefore for plant growth and development. Because all plant enzymes are made of proteins, nitrogen is necessary for all of the enzymatic reactions in a plant. Nitrogen is a major part of the chlorophyll molecule and is therefore necessary for

In the study conducted by Undeland and others on the recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process (Undeland and others 2002) the proteins from herring light muscle were extracted using acidic or alkaline solubilization; 92 and 89 percent of the initial muscle proteins were solubilized at pH 2.7 and 10.8, respectively, of which 96 and 94 percent were recovered during precipitation at pH 5.5. Therefore, their ultimate process yield varied from 88.3 to 88.38 percent.

Kristinsson and others (Kristinsson and others 2000) reported that the fish hydrolysates made from Atlantic salmon (*Salmo salar*) produced by Flavourzyme, which is an alkaline protease, contained close to 80 percent of the initial protein/nitrogen. In their study nitrogen recovery was calculated as the amount of protein (%N 6.25) present in the hydrolysates relative to the initial amount of protein presented in the reaction mixture. Rebeca and others (Rebeca and others 2006) reported yields of 13 to 15 percent in terms of whole fish which were obtained at pilot plant using bacterial proteases. Their products contained 83 to 86 percent protein of which 70 to 80 percent was soluble. They produced concentrated fish protein hydrolyzate with high protein content, and the difference between the yields of their process and the process yield of this project associated to the nature of the final products of these research projects, which the final product of either one contained different amounts of protein.

Due to the relatively high process yield of the innovative hydrolysis method of this project and its simplicity and more economic feasibility compared to the other hydrolysis methods, it can be an effective method for the production of an affordable, high quality organic
fish fertilizer. However, more detailed economic studies should be conducted before the project industrial scale-up.

5.2 Physiochemical properties

The phase separation value of FH-ZB was significantly lower (p< 0.05) than FH (see the comparison in Table 5.2). Less syneresis leads to higher stability of the product. Syneresis is spontaneous contraction of a gel, accompanied by expulsion of liquid from the pores. This occurs even if evaporation is prevented and the gel is immersed in liquid (Scherer 1989). From this point of view FH-ZB showed superior stability over FH due to less syneresis.

As can be seen in Figure 5.1, the color of the FH-ZB group was significantly darker (p < 0.05) than the FH group; as for a and b values (Table 5.2), there was significantly difference (p< 0.05) between FH-ZB and FH samples (see the color comparison in Table 5.2).

![Fig. 5.1 Visual comparison of the appearance of a) FH-ZB; and b) FH.](image)

In Hunter color system the L, a, and b color space is a 3-dimentional rectangular color space based on the opponent-color theory. L (lightness) axis which 0 is black and 100 is white; a (red-green) axis which positive values are red, and negative values are green and 0 is neutral; b (blue-yellow) axis which positive values are yellow, and negative values are blue and 0 is neutral.

As the FH goes through the deodorization process by ABC and ZVI the L value decreases; it means that the product grew darker (see the color comparison in Figure 5.1).
Moreover, FH-ZB turned reddish according to the $a$ measurements, and it turned bluish according to the $b$ measurements. The $L$ difference ($\Delta L$) between FH and FH-ZB is -17.94, the $a$ difference ($\Delta a$) is 2.59, the $b$ difference ($\Delta b$) is -2.27, and the total color difference or $\Delta E$ is 18.27. The changes in the color of FH-ZB could be due to the addition of ZVI and ABC and the nature of their dark color, also the oxidation of ZVI can be the other reason of the color changes.

As it is shown in the Table 5.2 the density of the FH-ZB sample was significantly higher (p< 0.05) than the FH sample. This fact can be related to the fine ZVI particles which entered the FH-ZB sample during deodorization process. Density is an important parameter for product quality. The specific gravity (the ratio of the density of a substance to the density of water) of FH-ZB is 1.052. If a fertilizer density is higher than 1 g/cm³ (approximate density of water), it sinks into the soil, so when it is applied on the field, rainfalls cause it to sink into the soil. If otherwise, it will float on the surface, which will make it unavailable to the plant root in the soil. From this viewpoint FH-ZB can easily sink into the soil and become accessible for plant root.

The moisture content of FH was significantly higher (p< 0.05) than FH-ZB, and also the solids content of FH was significantly lower (p< 0.05) than FH-ZB. The solids and moisture content of RET were 36.08 and 63.92 percent, respectively. The solid content in RET was significantly higher (p< 0.05) than FH and there was no statistically significant difference (p< 0.05) between the solid content of RET and FH-ZB (see the comparisons in Table 5.2). The high moisture content in a product leads to high water activity, which causes concerns over mold and other microorganisms growth. From this point of view FH-ZB is less susceptible for microorganism activities. On the other hand, high solid content means the product packs in more nutrients, which is expected to deliver more per unit application. Respecting this fact, the higher solid of FH-ZB compared to FH can be associated to the higher nutrients of FH-ZB.
There was no statistically significant difference (p< 0.05) between viscosity values of FH-ZB and FH (see the comparisons in Table 5.2). Viscosity is an important factor affecting the fertilizer flow behavior. The viscosity of water at 20 °C is 1.0020 cP and the viscosity of regular mayonnaise at 20 °C is approximately 20000 cP. The viscosity of FH-ZB at room temperature is 17 cP (see Table 5.2); it is more viscous than water, but it is not as viscose as mayonnaise. This indicates that the product can be easily bottled by industrial bottling equipment, while it is also easy for application by common farming equipment due to its low viscosity. However, as the viscosity of FH-ZB is higher than water, the flow rates at the same pressure as water will be less. Therefore, for spraying by common agricultural or home sprayers, to get the same volume of liquid out of the same sprayer the pressure will need to be higher. Dilution of the liquid fertilizer with water upon application is another approach for convenient use of this product.
Table 5.2 Comparison between physiological properties of FH and FH-ZB; the innovative deodorization method improved these properties in FH-ZB sample.

<table>
<thead>
<tr>
<th>Physiochemical properties</th>
<th>FH</th>
<th>FH-ZB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase separation (%)</td>
<td>5 ± 0.57(^1)</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Color</td>
<td>L 72.85 ± 0.57</td>
<td>L 54.87 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>a -1.09 ± 0.11</td>
<td>a 2.48 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>b 11.88 ± 0.88</td>
<td>b 9.11 ± 0.11</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>0.993 ± 0.001</td>
<td>1.052 ± 0.003</td>
</tr>
<tr>
<td>Solids content (%)</td>
<td>24.13 ± 1.16</td>
<td>34.96 ± 1.15</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>75.87 ± 1.15</td>
<td>65.04 ± 1.15</td>
</tr>
<tr>
<td>Apparent viscosity (cP)</td>
<td>24.3 ± 2.02</td>
<td>17 ± 0.57</td>
</tr>
</tbody>
</table>

\(^1\) All values are means ± SE, n=3
5.3 Mineral profile

Tables 5.3 and 5.4 show the minerals content of FH, FH-ZB, and RET in wet weight basis and dried weight basis, respectively. In the wet weight basis measurement, the Al, Ca, Cu, Fe, K, Mg, Mn, S, and Zn contents of FH-ZB were significantly higher (p< 0.05) than FH, and also Na, and P contents of FH-ZB were statistically lower (p< 0.05) than FH. There was no significant difference (p< 0.05) in B content between FH-ZB and FH (see the comparisons in Table 5.3).

In the dried weight basis measurement, the Al, B, Ca, Cu, Fe, Mg, Mn, S, and Zn contents of FH-ZB were statistically higher (p< 0.05) than FH, and also P content of FH-ZB was statistically lower (p< 0.05) than FH. There was no statistically significant difference (p< 0.05) in K, and Na values between FH-ZB and FH (see the comparisons in Table 5.4).

Plants, similar to all other living organisms, need food for their growth and survival. Plants require 16 essential elements. Carbon, hydrogen, and oxygen are derived from the atmosphere, soil, and water. The remaining 13 essential elements (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine) are supplied either from soil minerals and soil organic materials or by organic or inorganic fertilizers (Uchida 2000).

Phosphorus is extremely important in photosynthesis and respiration. Phosphorus is required in large quantities in young cells, and it aids in root development, flower initiation, and seed and fruit development. Phosphorus has been shown to reduce disease incidence in some plants. Potassium is known to be an enzyme activator that promotes metabolism. Potassium assists in regulating the plant’s use of water. It has a key role in photosynthesis and has been shown to improve disease resistance in plants, improve the size of grains and seeds, and improve
the quality of fruits and vegetables. Calcium has a major role in the formation of the cell wall membrane and its plasticity. Calcium is an activator of several enzyme systems in protein synthesis and carbohydrate transfer. Calcium indirectly assists in improving crop yields by reducing soil acidity when soils are limed. Magnesium is a major constituent of the chlorophyll molecule, and it is therefore actively involved in photosynthesis. Magnesium also assists the movement of sugars within a plant. Sulfur is essential in forming plant proteins, and it aids in seed production, chlorophyll formation, and stabilizing protein structure. Boron has been shown to promote root growth. Boron is essential for pollen germination and growth of the pollen tube; it has been associated with activities of certain enzymes, seed and cell wall formation, and sugar transport. Copper is essential in several plant enzyme systems involved in photosynthesis. Copper may have a role in the synthesis and/or stability of chlorophyll and other plant pigments. Iron is essential in the heme enzyme system in plant metabolism (photosynthesis and respiration). Iron is essential in the synthesis and maintenance of chlorophyll in plants; it has been strongly associated with protein metabolism. Manganese is involved in the oxidation-reduction process in photosynthesis. Zinc is required in the synthesis of tryptophan and necessary for several different functions in plant metabolism (Uchida 2000).

As for the other minerals, aluminum in low concentrations can sometimes increase plant growth or induce other desirable effects. Aluminum toxicity is an important growth-limiting factor for plants in acid soils (Rout and others 2001). Sodium has a very specific function in the concentration of carbon dioxide in a limited number of plants (Subbarao and others 2003).

In general, the content of the most minerals in FH-ZB was higher than FH (see the comparisons in Table 5.3 and 5.4) which is expected to promote plant growth. The result of minerals content analysis of RET also shows that the retentate of sieving which is the by-product
of the process contains considerable amount of minerals. It shows the potential of the by-product of this process for further study to turn it to another value-added product.
Table 5.3 Minerals content (Wet weight basis) of FH, FH-ZB, and RET

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>B</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>P</th>
<th>S</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH</td>
<td>57.31</td>
<td>0.90</td>
<td>7524.81</td>
<td>7.72</td>
<td>15.22</td>
<td>3796.66</td>
<td>179.63</td>
<td>1.72</td>
<td>714.33</td>
<td>55419.53</td>
<td>539.21</td>
<td>9.80</td>
</tr>
<tr>
<td></td>
<td>±1.1</td>
<td>±0.1</td>
<td>±115</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±115</td>
<td>±1.1</td>
<td>±0.2</td>
<td>±11</td>
<td>±115</td>
<td>±11</td>
<td>±0.2</td>
</tr>
<tr>
<td>FH-ZB</td>
<td>76.87</td>
<td>1.25</td>
<td>8750.34</td>
<td>11.76</td>
<td>1894.07</td>
<td>5047.34</td>
<td>222.64</td>
<td>14.04</td>
<td>591.28</td>
<td>28644.41</td>
<td>648.50</td>
<td>14.34</td>
</tr>
<tr>
<td></td>
<td>±1.1</td>
<td>±0.1</td>
<td>±115</td>
<td>±0.1</td>
<td>±11</td>
<td>±115</td>
<td>±1.1</td>
<td>±0.2</td>
<td>±11</td>
<td>±115</td>
<td>±11</td>
<td>±0.2</td>
</tr>
<tr>
<td>RET</td>
<td>85.54</td>
<td>0.31</td>
<td>13574.29</td>
<td>3.75</td>
<td>34.90</td>
<td>4602.27</td>
<td>201.27</td>
<td>2.400</td>
<td>531.16</td>
<td>33566.47</td>
<td>1393.92</td>
<td>12.93</td>
</tr>
<tr>
<td></td>
<td>±1.1</td>
<td>±0.1</td>
<td>±115</td>
<td>±0.1</td>
<td>±115</td>
<td>±115</td>
<td>±1.1</td>
<td>±0.2</td>
<td>±11</td>
<td>±115</td>
<td>±11</td>
<td>±0.2</td>
</tr>
</tbody>
</table>

1 All values in mg/kg
2 All values are means ± SE, n=3

Table 5.4 Minerals content (Dried weight basis) of FH, FH-ZB, and RET

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>B</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>P</th>
<th>S</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH</td>
<td>163.93</td>
<td>2.56</td>
<td>21525.90</td>
<td>22.08</td>
<td>43.54</td>
<td>19852.34</td>
<td>513.86</td>
<td>4.91</td>
<td>2043.46</td>
<td>158536.21</td>
<td>1542.49</td>
<td>28.03</td>
</tr>
<tr>
<td></td>
<td>±11</td>
<td>±0.2</td>
<td>±115</td>
<td>±0.5</td>
<td>±17</td>
<td>±115</td>
<td>±57</td>
<td>±0.2</td>
<td>±115</td>
<td>±5773</td>
<td>±115</td>
<td>±1.1</td>
</tr>
<tr>
<td>FH-ZB</td>
<td>318.52</td>
<td>5.17</td>
<td>36256.85</td>
<td>48.75</td>
<td>7848.05</td>
<td>20913.56</td>
<td>922.49</td>
<td>58.18</td>
<td>2449.96</td>
<td>118687.51</td>
<td>2687.05</td>
<td>59.40</td>
</tr>
<tr>
<td></td>
<td>±11</td>
<td>±0.2</td>
<td>±115</td>
<td>±0.5</td>
<td>±115</td>
<td>±115</td>
<td>±57</td>
<td>±0.2</td>
<td>±115</td>
<td>±5773</td>
<td>±115</td>
<td>±1.1</td>
</tr>
<tr>
<td>RET</td>
<td>237.09</td>
<td>0.85</td>
<td>37622.34</td>
<td>10.38</td>
<td>96.73</td>
<td>12755.59</td>
<td>557.84</td>
<td>6.64</td>
<td>1472.14</td>
<td>93032.41</td>
<td>3863.36</td>
<td>35.83</td>
</tr>
<tr>
<td></td>
<td>±11</td>
<td>±0.2</td>
<td>±115</td>
<td>±0.5</td>
<td>±17</td>
<td>±115</td>
<td>±57</td>
<td>±0.2</td>
<td>±115</td>
<td>±5773</td>
<td>±115</td>
<td>±1.1</td>
</tr>
</tbody>
</table>

1 All values in mg/kg
2 Values are means ± SE, n=3
5.4 Rheological properties

Both FH and FH-ZB had the properties of shear-thinning (pseudoplastic) fluid (see the viscosity-shear rate curve in Figure 5.2). The shear-thinning non-Newtoninan liquids are the one that their viscosity was found to decrease with increase in shear rate, giving rise to what is now generally called “shear-thinning”, “temporary viscosity loss”, “pseudoplasticity”, or “thixotopic” behavior (Barnes and others 1989). An example of a shear-thinning liquid is ketchup. In contrast, it is possible that the very act of deforming a material can cause rearrangement of its microstructure such that the resistance to flow increases with shear rate. This type of flow behavior is called “shear-thickening. An example of a shear-thickening liquid is corn starch in water (Barnes and others 1989). The flow behavior of a liquid fertilizer is extremely important trait for its application, as well as its production. The shear-thinning flow behavior of FH and FH-ZB is promising for their application by common agricultural and home sprayers as liquid fertilizers, and also this indicates that the product can be easily bottled by industrial bottling equipment.

Fig. 5.2 Rheological properties; viscosity-shear rate curve shows that FH and FH-ZB had the properties of shear-thinning (pseudoplastic) fluid.
5.5 Preliminary odor evaluation

The odor amplitude in initial material, FH, and FH-ZB was shown in Table 5.5. The odor of each sample was stated according to the scaling method described in chapter 4. Treating fish with acidic ingredients causes trimethylamine (the most important volatile odorant compound in salt-water fish) to bind to water and become less volatile, and also acidic conditions causes geosmin and methylisoborneol (the important volatile odorant compounds in fresh-water fish) to break down (McGee and others 2004). With regard to the aforementioned information acidic hydrolysis in first stage of the project already decreased fishy odor in FH and FH-ZB. The descriptive odor evaluation did not reveal the odor differences between FH and FH-ZB, so volatile compounds analysis was the base for the judgment about the effectiveness of deodorization practice.

In the study conducted by Hoyle and others (Hoyle and others 1994), fish protein hydrolysates were prepared, using minced fillets and alcalase and papain, from raw herring (Clupea harengus) and from herring defatted by ethanol extraction, cooking and pressing. The researchers reported ethanol extraction reduced fishy odor to barely detectable levels.

Based on the study of Hale (Hale 1972) on making fish protein concentrate (FPC) by enzymatic hydrolysis, taste and odor problems can generally be minimized with a FPC starting material. It was also reported that the alewife (Alosa pseudoharengus) hydrolysate suffered from very fishy taste; however, the red hake (Urophycis chuss) hydrolysate had a less fishy taste and odor.

The fishy smell in the aquatic-based fertilizers causes detrimental effects on the quality of them if it is not removed. In this project the fishy smell was successfully removed to a large
extent during the acidic hydrolysis stage; removal of the volatile odorants was also supported by using the innovative method of deodorization during stage two of the process.

Table 5.5 Odor comparison: Initial material, FH, and FH-ZB

<table>
<thead>
<tr>
<th>Odor</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial material</td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>FH</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>FH-ZB</td>
<td></td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

5.6 Volatile compounds analysis

Trimethylamine (TMA) quantity of FH was statistically higher (p< 0.05) than FH-ZB, and also dimethylamine (DMA) quantity of FH was significantly higher (p< 0.05) than FH-ZB (see the comparison in Table 5.6). The results indicates that the deodorization practice using ZVI and ABC significantly lowered (p< 0.05) the quantities of TMA and DMA which are the most important volatile compounds causing fishy smell (Bechmann and others 1998), in the fish hydrolyzate.

During storage bacteria and fish enzymes convert trimethylamine oxide (TMAO) into TMA, which cause the characteristic of “fishy” odor (McGee and others 2004). A well-known bacterium which forms TMA from TMAO is Shewanella putrefaciens. Fishy odors because of the reduction of TMAO to TMA can produce potent odors in fish (Shewan 1962); also DMA, as the other important cause of fishy smell, was noted to be released from TMAO through intrinsic enzymatic activity during cold storage (Sotelo and others 1995).

The reduction in the volatile compound quantities might be related to the absorptive properties of ABC (Mingjie 2004). It also can be associated to the organic compound-removing
characteristics of ZVI (O'Hannesin and others 1998; Puls and others 1999; Vogan and others 1999; Wilkin and others 2003).

Table 5.6 Respective quantities of residual TMA and DMA in FH and FH-ZB

<table>
<thead>
<tr>
<th></th>
<th>TMA</th>
<th>DMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH</td>
<td>4.210 ± 0.123</td>
<td>0.869 ± 0.061</td>
</tr>
<tr>
<td>FH-ZB</td>
<td>0.558 ± 0.052</td>
<td>0.134 ± 0.001</td>
</tr>
</tbody>
</table>

1 All values in mg/100 mL
2 All values are means ± SE, n=3

5.7 Shelf stability evaluation

Shelf-life of FH and FH-ZB hydrolyzed in the acid system was analyzed and compared to the FH-ZB and FH samples hydrolyzed in a buffer system which was formed using phosphoric acid and potassium phosphate monobasic. Visual mold growth, pH, and color of the samples were monitored, and a preliminary odor monitoring was conducted on different groups in a course of 270 days; the samples were analyzed in 45 day intervals.

5.7.1 pH

Figure 5.3 illustrates the pH changes of FH and FH-ZB samples hydrolyzed in acid and buffer systems during the 270 day cold storage. The pH values of FH hydrolyzed in acid system statistically increased (p< 0.05) during cold storage period, pH values of FH-ZB hydrolyzed in acid system also statistically elevated (p< 0.05) during the cold storage period, there was no significant change (p< 0.05) in pH values of FH hydrolyzed in buffer system, and also there was no statistically significant change (p< 0.05) in the pH values of FH-ZB hydrolyzed in buffer system. The results indicate that buffer system maintained a relatively constant pH of 3.5 in FH and FH-ZB groups (see the trend in Figure 5.3). That means the buffer system functioned to resist changes in the pH of the solution in these groups.
5.7.2 Visual mold growth observation

The results of visual mold growth observation during cold storage for FH and FH-ZB samples hydrolyzed in acid and buffer systems are shown in Table 5.7. Mold growth in FH group (see the molded FH sample in Figure 5.4) after day 45 (see Table 5.7) can be related to pH increase in this group to neutral range that provides better environment for mold growth.

Table 5.7 Visual mold growth observation during 270 day cold (4°C) storage

<table>
<thead>
<tr>
<th>Day</th>
<th>FH (acid)</th>
<th>FH-ZB (acid)</th>
<th>FH (buffer)</th>
<th>FH-ZB (buffer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>135</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>180</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>225</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>270</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* “-” shows no presence of mold in the samples and “+” shows the mold growth in the samples which the more “+” shows the more mold growth.

Mold growth is highly unacceptable for the consumers. pH control is known to be an effective hurdle technology to control mold growth, especially with the moisture content in FH and FH-ZB are both higher than 65%, which makes the samples more susceptible to mold contamination. Therefore, tight control of pH is needed to extend the product shelf life.
5.7.3 Color analysis

Figure 5.5 shows the changes in \( L, a, \) and \( b \) during cold storage for FH and FH-ZB samples hydrolyzed in acid and buffer systems. The \( L \) values of FH and FH-ZB hydrolyzed in acid system significantly decreased (\( p < 0.05 \)) during the cold storage period, and the \( L \) values of FH hydrolyzed in buffer system also statistically decreased (\( p < 0.05 \)) during the cold storage period. There was no statistically significant change (\( p < 0.05 \)) in \( L \) values of FH-ZB hydrolyzed in buffer system during the cold storage (see the trend in Figure 5.5, a). Based on the results FH and FH-ZB hydrolyzed in acid system and also FH hydrolyzed in buffer system turned statistically (\( p < 0.05 \)) darker during the cold storage time, but no significant (\( p < 0.05 \)) darkness was observed in FH-ZB hydrolyzed in buffer system during the cold storage time.

There was no statistically significant change (\( p < 0.05 \)) in \( a \) values of FH hydrolyzed in acid system; \( a \) values of FH-ZB hydrolyzed in acid system statistically decreased (\( p < 0.05 \)) from day 1 to day 270; \( a \) values of FH hydrolyzed in buffer system statistically increased (\( p < 0.05 \)) from day 1 to day 270; and \( a \) values of FH-ZB hydrolyzed in buffer system statistically decreased (\( p < 0.05 \)) from day 45 to day 270 (see the trend in Figure 5.5, b).

The \( b \) values of FH and FH-ZB hydrolyzed in acid system, and FH-ZB hydrolyzed in buffer system statistically increased (\( p < 0.05 \)) from day 1 to day 270. The \( b \) values of FH hydrolyzed in buffer system statistically increased (\( p < 0.05 \)) from day 45 to day 270 (see the
trend in Figure 5.5, c). The results indicate that the samples turned more yellowish over the cold storage period.

![Graph showing color changes in samples during cold storage](image)

**Fig. 5.5** Color changes in samples during cold storage; a: L values, b: a values, and c: b values.

### 5.7.4 Preliminary odor monitoring

Table 5.8 shows the changes in odor amplitude during cold storage for FH and FH-ZB samples hydrolyzed in acid and buffer systems. The odor of each sample was stated according to the scaling method described in chapter 4. FH hydrolyzed in acid system showed pungent odor
after day 45 of cold storage and FH-ZB hydrolyzed in acid system showed pungent odor after
day 90 of cold storage. However, FH and FH-ZB hydrolyzed in buffer system did not show
pungent odor within the cold storage period (see the odor change in Table 5.8). This might be
related to the constant acidic pH of the FH and FH-ZB hydrolyzed in buffer system which
prevented pungent odor due to minimizing microorganism’s activities. Microorganisms and fish
enzymes cause the characteristic of “fishy” odor in aquaric products (McGee and others 2004)
and the accumulation of the volatile compounds causing by microorganism’s activities and
chemical reactions can lead to a pungent odor in these products. Controlling the microorganism’s
activities by maintaining an acidic condition in the product can lead to a better odor quality
during storage and consequently longer shelf life.

Table 5.8 Changes in the odor of samples during cold storage (4°C); the sign and numbers represent the amplitude of odor
which ), 1, 2, and 3 show very low, low, medium, and high amplitude, respectively.

<table>
<thead>
<tr>
<th>Day</th>
<th>Odor</th>
<th>FH (acid)</th>
<th>FH-ZB (acid)</th>
<th>FH (buffer)</th>
<th>FH-ZB (buffer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>45</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>90</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>135</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>180</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>225</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>270</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
5.8 Field trials of FH-ZB

The field trials of FH-ZB, which was the optimized product of the project, was of great importance since the trials revealed the functionality of this product on the growth and development of plants. FH-ZB underwent the innovative deodorization practice and it was tested for various physiochemical, rheological, odor, and shelf stability attributes to check the compliance of these properties with a high quality product. FH-ZB was compared to three other commercially available fertilizers, namely Shafer’s SF Organics, Neptune Harvest Organic, and Miller’s Nutrient Express. FH-ZB and conventional Miller’s Nutrient Express fertilizers were applied in three dosage levels (low, medium, and high). Medium level of FH-ZB and Miller’s Nutrient Express was the base of comparison with Shafer’s SF Organics and Neptune Harvest Organic. Individual plant yield, fruit size, sprouts per plant, and average height were measured for each group at the end of the treatment, and the plant’s leaves were sampled for minerals and nutrients analysis.

5.8.1 Nutrients comparison

Table 5.9 illustrates the nutrient comparison of FH-ZB to its competitors (Shafer’s SF Organics, Neptune Harvest Organic, and Miller’s Nutrient Express). FH-ZB contained higher quantity of Al, Ca, Cu, Fe, K, Mn, Zn compared to Neptune Harvest Organic, higher amount of Fe, K, P compared to Shafer’s SF Organics, and higher volume of Fe compared to conventional Miller’s Nutrient Express. A high quantity of essential nutrients is an important trait for a fertilizer which is necessary for the growth and survival of plants. As can be seen from Table 5.9 FH-ZB contains greater quantities of various nutrients in comparison to its competitors.
Table 5.9 Nutrients comparison of FH-ZB to its competitors (Shafer’s SF Organics, Neptune Harvest Organic, and Miller’s Nutrient Express).

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>B</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>P</th>
<th>S</th>
<th>Zn</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH-ZB</td>
<td>76.87(^1)</td>
<td>1.25</td>
<td>8750.34</td>
<td>11.76</td>
<td>1894.07</td>
<td>5047.34</td>
<td>222.64</td>
<td>14.04</td>
<td>591.28</td>
<td>28644.41</td>
<td>648.50</td>
<td>14.34</td>
<td>11000</td>
</tr>
<tr>
<td>Shafer</td>
<td>-</td>
<td>-</td>
<td>14200</td>
<td>-</td>
<td>1000</td>
<td>2600*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20000</td>
<td>10000</td>
<td>500</td>
<td>26000</td>
</tr>
<tr>
<td>Neptune</td>
<td>8</td>
<td>2.5</td>
<td>7500</td>
<td>&lt;0.1</td>
<td>26</td>
<td>3000</td>
<td>400</td>
<td>3</td>
<td>1600</td>
<td>43500</td>
<td>1700</td>
<td>9</td>
<td>22300</td>
</tr>
<tr>
<td>Miller</td>
<td>-</td>
<td>200</td>
<td>-</td>
<td>500</td>
<td>1000</td>
<td>180000*</td>
<td>5000</td>
<td>500</td>
<td>-</td>
<td>180000**</td>
<td>-</td>
<td>500</td>
<td>180000</td>
</tr>
</tbody>
</table>

\(^1\) All values in mg/kg
*K_2O
**P_2O_5
5.8.2 Field application comparison

FH-ZB showed field performance superior to its competitors (Shafer’s SF Organics, Neptune Harvest Organic, and Miller’s Nutrient Express) on raspberry fertilization (see the comparison in Figure 5.6).

Fig. 5.6 Raspberry fertilized with FH-ZB (A) showed superior fruit sizes and yields to competitors (B: conventional Miller’s Nutrient Express; C: Neptune’s Harvest Organic; D: Schafer’s SF Organic).

Figure 5.7 to 5.10 illustrate the comparison between FH-ZB field trials and its competitors (Shafer’s SF Organics, Neptune Harvest Organic, and Miller’s Nutrient Express). FH-ZB and conventional Miller’s Nutrient Express were applied in three different dosage levels (low, medium, and high). Individual plant yield, fruit size, sprouts per plant, and average height of the fertilized plants were the criteria of the comparison. The results are shown for each criterion respectively.
The individual yields of the plants fertilized with medium and high dosage levels of FH-ZB were significantly higher (p< 0.05) than the plants fertilized by the other competitors (see the comparison in Figure 5.7). The results of this part indicate the superior performance of FH-ZB for raspberry fertilization. Higher yield means more fruitful farming which is a desirable for every agronomist. In addition to the increase in the yield of the plants fertilized with FH-ZB, the fruits from these plants can be considered as organic fruits. A premium of 12–60% is often obtained from organic produce (Lohr 1998). Both high yield and organic production will lead to a lucrative farming for agronomists.
The fruit size of the plants fertilized with high dose of FH-ZB was statistically higher (p<0.05) than the plants fertilized by Shafer’s SF Organics, as well as the plants fertilized with medium and high doses of conventional Miller’s Nutrient Express (see the comparison in Figure 5.8). Larger fruit size usually is an appealing property for fruits from marketing point of view and from this viewpoint, FH-ZB showed a satisfying performance compared to the other competitors.
The quantities of sprouts of the plants fertilized with low, medium, and high dosage levels of FH-ZB were statistically lower (p < 0.05) than the plants fertilized with high dose of conventional Miller’s Nutrient Express (see the comparison in Figure 5.9). Less sprouts and higher yield of larger fruits mean that nutrients of FH-ZB derived the raspberry plants to produce higher amount of larger fruits instead of making more sprouts which can be considered as the by-product of farming.
There was no statistically significant difference (p< 0.05) between the average height of the plants fertilized with low, medium, and high dosage levels of FH-ZB and the other competitor fertilizers (see the comparison in Figure 5.10). In general, shorter plant means less waste of energy in the plant’s stalk. From this viewpoint all fertilizers performed almost equally.

### 5.8.3 Nutrients analysis of leaves

Two sets of comparison between nutrients of raspberry leaves fertilized by FH-ZB and its competitors were performed. In the first comparison the effect of medium dose of FH-ZB application on plant leaves was compared to Shafer’s SF Organics, Neptune Harvest Organic, and medium dose of conventional Miller’s Nutrient Express fertilization (Table 5.10). In the second comparison the effect of low, medium, and high dosage levels of FH-ZB application on raspberry leaves was compared to low, medium, and high dosage levels of conventional Miller’s Nutrient Express fertilization (Table 5.11). The leaves were selected from the plants at month 4 (July) and at month 6 (September) for these experiments.
Table 5.10 Leaf nutrients comparison between Neptune, Shafer, Miller (medium dose), and FH-ZB (medium dose) fertilizers

<table>
<thead>
<tr>
<th></th>
<th>Calcium</th>
<th>Phosphorous</th>
<th>Potassium</th>
<th>Nitrogen</th>
<th>Magnesium</th>
<th>Boron</th>
<th>Iron</th>
<th>Zinc</th>
<th>Manganese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neptune</td>
<td>1.89</td>
<td>2.02</td>
<td>0.32</td>
<td>0.38</td>
<td>1.04</td>
<td>0.76</td>
<td>2.98</td>
<td>2.97</td>
<td>0.68</td>
</tr>
<tr>
<td>Shafer</td>
<td>2.12</td>
<td>1.86</td>
<td>0.35</td>
<td>0.35</td>
<td>0.98</td>
<td>0.62</td>
<td>3.10</td>
<td>3.03</td>
<td>0.76</td>
</tr>
<tr>
<td>Miller(med)</td>
<td>1.61</td>
<td>1.49</td>
<td>0.33</td>
<td>0.28</td>
<td>1.68</td>
<td>1.40</td>
<td>3.42</td>
<td>2.85</td>
<td>0.57</td>
</tr>
<tr>
<td>FH-ZB(med)</td>
<td>2.31</td>
<td>2.31</td>
<td>0.37</td>
<td>0.48</td>
<td>1.24</td>
<td>0.38</td>
<td>2.38</td>
<td>2.47</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Table 5.11 Leaf nutrients comparison between Miller and FH-ZB fertilizers in low, medium, and high dosage levels

<table>
<thead>
<tr>
<th></th>
<th>Calcium</th>
<th>Phosphorous</th>
<th>Potassium</th>
<th>Nitrogen</th>
<th>Magnesium</th>
<th>Boron</th>
<th>Iron</th>
<th>Zinc</th>
<th>Manganese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller(low)</td>
<td>1.76</td>
<td>1.89</td>
<td>0.28</td>
<td>0.22</td>
<td>1.44</td>
<td>1.16</td>
<td>2.81</td>
<td>2.03</td>
<td>0.55</td>
</tr>
<tr>
<td>Miller(med)</td>
<td>1.61</td>
<td>1.49</td>
<td>0.33</td>
<td>0.28</td>
<td>1.68</td>
<td>1.40</td>
<td>3.42</td>
<td>2.85</td>
<td>0.57</td>
</tr>
<tr>
<td>Miller(high)</td>
<td>1.60</td>
<td>1.29</td>
<td>0.28</td>
<td>0.29</td>
<td>1.61</td>
<td>1.65</td>
<td>3.15</td>
<td>3.11</td>
<td>0.57</td>
</tr>
<tr>
<td>FH-ZB(low)</td>
<td>2.09</td>
<td>2.09</td>
<td>0.31</td>
<td>0.53</td>
<td>1.23</td>
<td>0.57</td>
<td>2.33</td>
<td>1.91</td>
<td>0.58</td>
</tr>
<tr>
<td>FH-ZB(med)</td>
<td>2.31</td>
<td>2.31</td>
<td>0.37</td>
<td>0.48</td>
<td>1.24</td>
<td>0.38</td>
<td>2.38</td>
<td>2.47</td>
<td>0.67</td>
</tr>
<tr>
<td>FH-ZB(high)</td>
<td>2.23</td>
<td>2.26</td>
<td>0.39</td>
<td>0.46</td>
<td>1.01</td>
<td>0.42</td>
<td>3.12</td>
<td>2.64</td>
<td>0.72</td>
</tr>
</tbody>
</table>
The level of calcium in the leaves of the plants fertilized with FH-ZB was statistically higher (p< 0.05) than this level in the leaves of the plants fertilized with the other competitor fertilizers (see the comparison in Table 5.10). The levels of magnesium and manganese in the leaves of the plants fertilized with FH-ZB were statistically higher (p< 0.05) than these levels in the leaves of the plants fertilized with medium dose of conventional Miller’s Nutrient Express (see the comparison in Table 5.10). The levels of potassium and zinc in the leaves of the plants fertilized with FH-ZB were statistically lower (p< 0.05) than these levels in the leaves of the plants fertilized at least with one of the other competitor fertilizers (see the comparison in Table 5.10). There was no statistically significant difference (p< 0.05) between the levels of phosphate, nitrogen, boron, and iron in the leaves of the plants fertilized with FH-ZB and these levels in the leaves of the plants fertilized with the other competitor fertilizers (see the comparison in Table 5.10).

The levels of calcium, phosphate, magnesium, and manganese in the leaves of the plants fertilized with at least one dose of FH-ZB were statistically higher (p< 0.05) than these levels in the leaves of the plants fertilized with at least one dose of conventional Miller’s Nutrient Express (see the comparison in Table 5.11). The levels of potassium and nitrogen in the leaves of the plants fertilized with at least one dose of FH-ZB were statistically lower (p< 0.05) than these levels in the leaves of the plants fertilized with at least one dose of conventional Miller’s Nutrient Express (see the comparison in Table 5.11). There was no statistically significant difference (p< 0.05) between the levels of boron, iron, and zinc in the leaves of the plants fertilized with any doses of FH-ZB and these levels in the leaves of the plants fertilized with any of the three dosage levels of conventional Miller’s Nutrient Express (see the comparison in Table 5.11).
The data shows that the nutrients which were delivered to the plant’s leaves from FH-ZB caused sustainable growth and survival for those plants. Also, it shows that the nutrients in FH-ZB are available in soil, and plants can readily uptake these nutrients. This is another indicator of the supreme quality of FH-ZB.
Chapter 6: Conclusion and Recommendations

6.1 Conclusion

High rate of protein recovery into the final product (85.97%) via the hydrolysis process shows the efficiency of the applied method. Superior physiochemical properties, significant decrease in the quantities of volatile odorant compounds and better odor properties of FH-ZB compared to FH are the indications of effectiveness of the deodorization method. The employed deodorization method using ZVI and ABC reduced the most important volatile odorant compounds in the final product without any adverse effects on the nutrients of the fertilizer. Hence, it can be considered as an effective deodorization method for the production of an odor-free, high quality organic liquid fertilizer. Employing buffer system for the purpose of hydrolysis led to a stable pH of 3.5 and consequently elongated the shelf life of the product, so hydrolysis using buffer system is the selected method for fish-based organic fertilizer production.

Based on the rheological properties of the final product it is readily applicable by common farming equipment and also indicates that the product can be easily bottled by industrial bottling equipment. The results of the field trial of FH-ZB reflected the superior quality of this product compared to the other commercially available fish-based fertilizers (Shafer’s SF Organics, Neptune Harvest Organic) and a conventional fertilizer (Miller’s Nutrient Express). FH-ZB field application showed that the plants fertilized with this product yielded more fruits and made less stalk and sprout. This fact shows that the final product of this project can lead to more fruitful organic farming. In addition, the fruits from the plants fertilized with this product are organic fruits which will lead to extra benefits for agronomists.
It is worth it to mention that during this project the majority of the fish waste was converted to highly value-added product and the by-product of the process which is about 13% (the portion rejected by the screens) can be further processed with more potent acidic systems or employing enzymatic hydrolysis for a non-organic product (fertilizer). The other innovative methods can be designed to add value to the by-product of this research project.

6.2 Research recommendations

It is recommended for the prospective research projects the following points to be considered as a supplement of this work:

- Study the effect of ZVI and ABC on lipid oxidation of fish hydrolyzate.
- Study the solubility of nitrogen in final product.
- Study the degree of hydrolysis in the final product.
- Study the recovery of the retentate of sieving into a value-added product.
- Conduct shelf-life study for the commercialized product.
- Conduct packaging studies for the final product.
- Conduct marketing studies for the final product.
- Conduct economic feasibility studies for the scaled-up project.
Works Cited


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