UTILIZATION OF FISH PROCESSING BY-PRODUCTS FOR NUTRITIONAL FORMULATION OF FISH FEED

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Abstract

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Small-scale fish farms market roughly 50 percent of the farm production. Processing of fish to produce fillets yields an immense quantity of underutilized by-products. Depending on the species, 30 to 80 percent by weight of the fish is not utilized for direct human consumption and is discarded as by-products or waste. For example, in a typical trout processing operation the finished trout fillet yield is approximately 50 percent of live weight. By-products consisting of trimmings, heads, frames, fins, skin, and viscera are as high in protein as the fillet and are disposed of as waste. Such disposal creates environmental problems and is a loss of valuable nutrients. This study was an attempt to develop a low-cost farm technology for production of fish feed pellets utilizing trout processing by-products. The process consisted of five unit operations: thermal processing, grinding, mixing, extrusion, and drying. Pretreatment requirements (heating time and temperature) to produce fish slurry with no microbial load were determined.

Cooked fish by-products were ground to reduce the particle size of the softened bones and to create a smooth slurry. Nutrient amendment requirements were established by proximate analysis (moisture, fat, protein, and minerals) of the fish slurry to meet the dietary requirements of trout. The by-products and supplementary ingredients were mixed and then extruded through a specially designed die using a Hobart meat grinding attachment. The pellets were dried using a forced-convection drier.

The response variables evaluated during process development were aerobic and anaerobic plate counts, pathogenic bacteria, apparent density, floating time, and sinking velocity. The raw by-products had a high aerobic ($6.7x10^5$-$5.7x10^6$ CFU/g) and anaerobic ($3.3x10^4$- $6.5x10^5$ CFU/g) load with no pathogens. Thermal processing at 121°C and $131x10^3$ Pa for 15 minutes was sufficient to destroy microbial populations and soften the bones of the raw by-products. A subsequent grinding was needed for production of a smooth slurry; grinding time ranged from 12 to 17 minutes. Proximate analysis conducted on the by-products indicated that the fish slurry needed supplementation with protein, lipid, minerals, and vitamins to meet the dietary requirements of trout. The by-products and supplementary dry ingredients were mixed using a Hobart mixer at a low speed for 15 minutes into an extrudable dough. A 50 mm-long multi-channel die provided enough pressure for pelletizing. The die had 10 openings (4.5 mm each) distributed around the circumference. The fish feed pellets were dried to approximately 5 percent moisture using a conventional oven for 45 to 49 minutes to impart structural integrity, shelf-life, and water stability to the pellets. The apparent density of the fish pellets ($1.1x10^2$ kg/m^3) was higher than that of water, which resulted in fish, feed pellets that sank. More research is needed to optimize the technology and scale up the process.

The process developed can be applied to small scale processing of by-products from other fish species leading to full utilization of cultured fish. In addition, this resource recovery system eliminates solid waste disposal problems. Such a technology can potentially benefit fish farmers everywhere in the country and the world.

To my teacher...

Gour Choudhury

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Chapter One

The annual world catch of fish is about 100 million metric tons (Ruiter, 1995) and one-third of this catch is not utilized for human consumption and considered as fishery by-products (Barlow & Widsor, 1984). The processing of fish for filleting, canning, and surimi production results in an immense quantity of by-products which include trimmings, belly flaps, heads, frames, fins, skins, and viscera (Choudhury & Bublitz, 1996). Every year, thousands of tons of fish by-products of high nutrient content are dumped or discarded by fish processing plants throughout the world. Discarding these by-products creates two major problems. First, is the underutilization of a huge amount of nutrients such as protein, minerals, and oil. Second, disposal of such huge quantities of highly polluting organic matter contributes to major environmental and economic problems. The fish processing industry is faced with the need to develop efficient by-product recovery and utilization methods to comply with the federal pollution control regulations (Choudhury & Bublitz, 1996).

In large-scale fish processing operations, the by-products are combined and converted to fishmeal and oil (Choudhury & Bublitz, 1996). Fishmeal is an important and expensive component of commercial fish feed pellets. It is a good source of essential amino acids and is rich in energy, minerals and essential fatty acids (Li, 1998). Production of fishmeal is by far the most successful and efficient method to recover the nutrients lost as a result of discarding the fish processing by-products. Fishmeal production is increasing consistently and was estimated at 6.4 metric tons in 1991 (Ruiter, 1995). However, in small-scale fish farms, fishmeal production seems neither feasible nor economically viable. For example, a typical small trout farm produces

roughly 10,000 kg of live weight every year. Processing of trout reduces this harvest to 5,000 kg of marketable products, with concurrent production of 5,000 kg of by-products (Choudhury & Bublitz, 1996). Non-availability of a suitable by-product utilization/disposal system will pose an impediment to any attempt for small trout farm expansion, because any expansion will result in increasing production, which leads to oversupply of underutilized by-products.

Feed, containing fishmeal, is the most expensive input in small-scale fish farms. Utilization of by-products to produce fish feed in the farm would significantly reduce the feed cost and improve the economic performance of the operation. Fish feed produced directly from by-products would be less expensive because this approach avoids numerous unit operations involved in fishmeal production. In addition, the feed produced from fresh by-products, instead of fishmeal, would be of higher quality. Overall, farm production of a better quality fish feed from fresh by-products would be lower in cost, thus improving profitability.

Objectives

The overall objective of the project was to develop a low-cost farm technology for the production of fish feed pellets utilizing trout processing by-products. The specific objectives were to:

1. Determine by-products handling and pretreatment requirements;
2. Develop a formulation that would maximize by-product utilization;
3. Determine process conditions to extrude fish feed pellets using a low-cost pelletizer; and
4. Evaluate the characteristics of feed pellets.

Chapter Two

Trout Production

In the 1870s, the business of raising trout and marketing began in the northern United States. The main species grown at the commercial farms were brook trout. Since 1870, trout farms have increased steadily in number and size by constructing raceways. Rainbow trout were officially introduced into the eastern United States in the 1880s. At that time they were raised as game fish by private fishing clubs until after 1900 when their commercial farming began (Brown, 1983).

The trout industry started to grow remarkably after the end of the World War II. The total production reached about 12 million kg in 1973 (Klontz and King, 1975). Most of this production came from the prime trout producing states such as Idaho, California, Wisconsin, Michigan, Colorado, and Pennsylvania (Brown, 1983).

Trout production in the United Sates reached 54 million fish in 2001, with concurrent sales of $57 million in the same year (Rainbow Trout Production in Western North Carolina, 2002). The state of Idaho is leading the trout production in the U.S. (41 million pounds sold in 1998), followed by North Carolina, which leads trout production (1.6 million kg sold in 1998) among the southeastern states (Trout, 2002).

Trout Processing

Trout can be processed to produce various forms of products depending on demand. For example, fish can be sold as totally whole, gutted, gutted and gilled or headed and gutted. However, the most dominant and desirable form of processed fish is the fillet (Regenstein & Regenstein 1991). Fish fillet production is one of the major operations in the fish processing industry. The unit operations comprising the filleting

process are shown in Figure 1. The harvested fish are headed, gutted, filleted, trimmed, and packed in ice (Choudhury & Bublitz, 1996).

Processing of fish to produce fillets yields a large quantity of underutilized by-products. Depending on the species, 30 to 80 percent by weight of the fish caught is not utilized for direct human consumption and is discarded as by-products or waste (Choudhury & Gogoi, 1995). In a typical trout processing operation the finished product (trout fillet) yield is approximately 50 percent of live weight (Figure 2). Fish processing by-products are of high nutrient content which, if not properly utilized for human or animal nutrition, are likely to be dumped in nearby water creating environmental pollution problems. These by-products consisting of trimmings, heads, frames, fins, skin, and viscera are as high in protein as the fillet (Choudhury & Bublitz, 1996).

In the United States, trout production in 2001 amounted to 25.5 million kg (Rainbow Trout Production in Western North Carolina, 2002). Processing of trout reduces this harvest to million pounds of marketable products, with concurrent kg production of 12.7 million kg by-products. The protein, ash, and oil of the composite by-products are 14.9%, 3.3%, and 4.5%, respectively. Accordingly, underutilization of the processing by-products, results in discarding about 1896 tons kg of high quality protein, 420 tons of minerals, and 1845 tons of fish oil. The seafood industry is faced with the need to develop efficient by-product recovery and utilization methods to comply with the federal pollution control regulations (Choudhury, 2001).

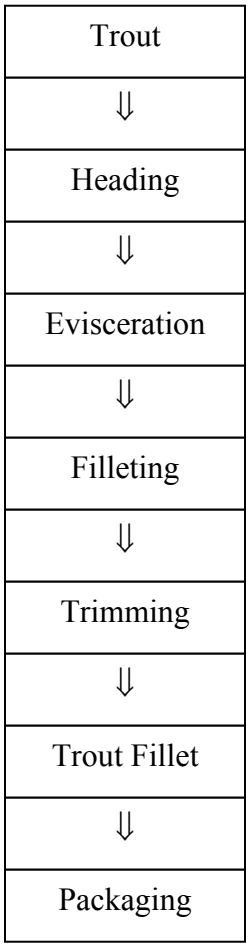

Figure 1. The fish filleting process (Modified from Choudhury & Bublitz, 1996).

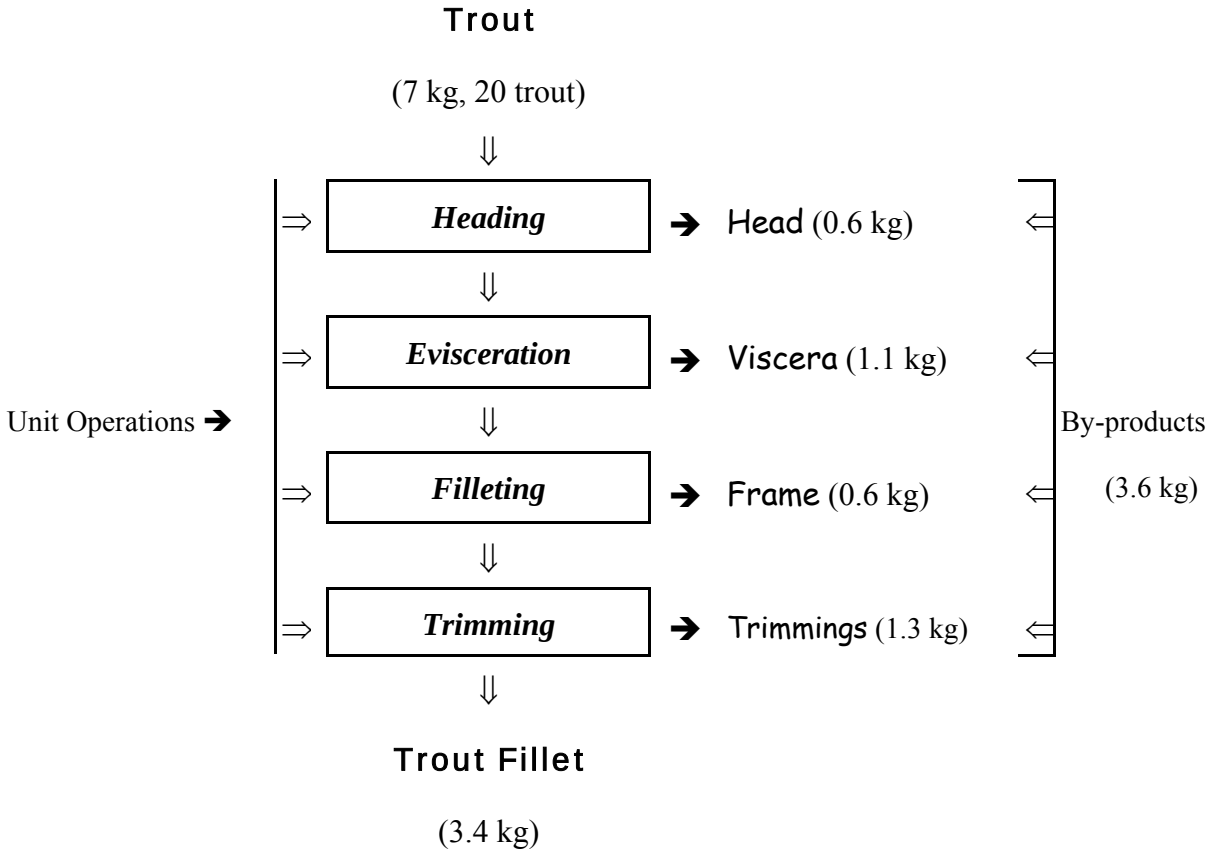

Figure 2. By-product generation at different points during trout filleting. (Choudhury, 2001).

Therefore, any successful development of a by-product utilization technology will result in recovering these discarded valuable nutrients and elimination of the environmental pollution caused by the improper disposal of the processing by-products (Choudhury, 2001).

By-Products Utilization

The world catch of fish is about 100 million metric tons annually (Ruiter, 1995). Large portion of this catch is not directed for human consumption, rather to make non-edible products (Barlow &Windsor, 1984). The production of fishmeal and oil is the most common and valuable utilization method for the non-edible fish and fish by-products resulting from filleting operations.

Fish Meal: Production, Composition and Use

Fishmeal is made from a variety of whole fish, which are caught exclusively for the purpose of producing meal and oil, and from the fish processing by-products of species of fish caught mainly for human consumption (Hardy, 1992). The world fishmeal production is around six to eight million metric tons (Hardy, 1992). Presently, fishmeal is produced throughout the world and is used practically in every country. The major fishmeal producing countries include Peru, Chile, South Africa, Norway, Iceland, Denmark, the United States, and Japan (Windsor & Barlow, 1981).

Fresh fish is very susceptible to spoilage and processing into fish meal results in a stable high protein product with longer shelf life ranging from a few months to years. The process of manufacturing fish meal involves cooking, pressing, centrifuging, drying, and grinding fish and fish by-products in machinery designed for this purpose

(Choudhury & Bublitz, 1995). The objective of the process is to separate three major components of the raw material: solids, oil and water. The water content must be lowered from 70 to 80 percent to about 10 percent to stop any kind of decomposition. The oil in the finished meal must be less than 15 percent to inhibit lipid oxidation and reduce the likelihood of fishy taint being developed in animals being fed the meal (Windsor & Barlow, 1981).

The raw material is ground after passing through a metal detector to remove pieces of metal and other undesirable metallic contaminants (Hardy, 1992). The fish are then cooked at temperatures of approximately100°C resulting in coagulation of protein and rupturing the fat depots thus liberating oil and bound water (Windsor & Barlow, 1981). The cooked material is then compressed, squeezing out a mixture known as press liquor made up of water, soluble protein, and oil. The remaining solid is known as press cake (Hardy, 1992). Pressing not only separates the oil and water from the raw material but also reduces the moisture content of the presscake (Ruiter, 1995). The press liquor (78% water, 6% solids and 16% oil) is screened to remove coarse pieces of solid material. It then passes to a desuldger, which separates the press liquors into two major components: water solids, and oil water solids (Windsor & Barlow, 1981). The water solids can be returned to the process and dried along with the press cake. The oil water solid mixture is then separated by centrifugation into oil and stickwater (Choudhury & Bublitz, 1996). The final oil-refining step is called polishing, which is washing the oil with hot water to remove impurities (Ruiter, 1995). The oil is now ready to be stored in clean dry tanks. The stickwater contains about 20 percent of the solids in the final meal,

therefore it is concentrated in evaporators to recover solids, which are added back to the press cake and dried along with it to produce whole meal (Windsor & Barlow, 1981). Press cake, along with the stickwater, are dried to a moisture content of around 10 percent to inhibit the growth of bacteria and any enzymatic reactions leading to fish meal deterioration (Windsor & Barlow, 1981). The final operations are grinding and screening to the correct particle size to produce a homogeneous powder free of foreign matter. During or before drying antioxidants are added to stabilize the final product and suppress lipid oxidation (Ruiter, 1995).

Fishmeal is considered to be a high source of quality protein (62-72% protein, depending on the species) (Babbit, 1990). The protein of fishmeal has a high biological value and is rich in the essential amino acids (Barlow & Windsor, 1984). Fishmeal is the richest natural source of two essential amino acids: lysine and methionine, which are the limiting amino acids in many animal diets.

The fat content of fishmeal ranges between 8 to10 percent (Babbitt, 1990). The fat from fishmeal differs remarkably from fat derived from plant in as much as it contains high level of long-chain (C20 and greater), polyunsaturated fatty acids. Moreover, fishmeal fat is high in the essential fatty acid linolenic acid (18:2ω3), which is important to chick growth, reproduction, and egg production (Barlow & Windsor, 1984). For many farmed fish and animals long-chain ω-3 polyunsaturated fatty acids must be supplied in the diet. As a result, diets containing fish meal meet this condition as it contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), C20:5ω-3 and C22:6ω-3, respectively (Ruiter, 1995). Although fat is needed as a good source of energy, its level in

most diets for animals is kept low so that no fishy flavor can be developed in animals being fed the fishmeal.

Fishmeal is a rich source of vitamins and minerals, which play an important role in the nutrition of animals. Ash content in fishmeal varies between 12 to 20 percent depending on species (Babbitt, 1990). Fishmeal contains pantothenic acid, riboflavin, niacin, B12, calcium, phosphorus, sodium, selenium and magnesium (Barlow & Windsor, 1984).

Fishmeal is regularly used to supplement feeds containing plant proteins (Li, 1998). Of the six to eight million metric tons of the world fishmeal production, approximately 60 percent of it is used for poultry feeds, 20 percent for the swine industry, 10 percent for aquaculture, and the remaining 10 percent for the pet food industry (Hardy, 1992).

Fishmeal is used in manufacturing fish pellets for salmon, trout, and catfish. All these species need the fish protein to maintain adequate health and growth. The amount of fishmeal used is around 10 percent of the final product. Hardy (1992) stated that around 60 percent of the fish meal produced is used in poultry feeds, while aquaculture uses 10 percent. The remaining 30 percent of the fishmeal produced is used mainly by the swine industry (20%) and the pet food industry.

Recent research revealed that fishmeal contains what is called unknown growth factors. As a result, chickens fed on fish meal-based diets showed significant increases in growth, egg production, and improved reproduction concurrent with better feed utilization (Barlow &Windsor, 1984). The use of fishmeal in feeding calves and pigs has been shown to be advantageous. Researchers have been attempting to replace milk protein with animal or vegetable protein. Calves and young pigs fed on a low fat content

fishmeal showed no allergy to the fishmeal (Barlow & Windsor, 1984)

Fish Oil: Production, Composition and Use

Fish oil is the second major product of rendering the inedible fish and fish by-products. Fish oils contain mainly triglycerides of fatty acids with variable amounts of phospholipids, glycerol ethers and wax esters (Ruiter, 1995). Moreover, fish oils contain a wide range of long-chain fatty acids (14-22 C) with high degrees of unsaturation. Due to their functional properties, fish oils have been utilized to manufacture food and pharmaceutical products. Hydrogenated fish oils are used to manufacture edible products such as margarine, shortenings, and salad oils (Ruiter, 1995). Since fish oils have a widely varied chain length (14-22 C), margarines prepared from them have an excellent plastic consistency (Barlow & Windsor, 1984). The highly unsaturated properties of unhydrogented fish oils make them very beneficial to human health. Medical and nutritional researchers have found that the long-chain polyunsaturated ω-3 fatty acids found in DHA and EPA are essential to the fetus and young child to have normal brain and nervous tissue development (Ruiter, 1995). Barlow et al. (1990) pointed out that fish oils containing EPA and DHA have positive effects on cardiovascular diseases as they help to reduce blood cholesterol levels. Finally, oil made up from fish livers possesses potential health benefits due to high contents of vitamins A and D.

Fish Silage: Production, Composition and Use

In some regions, the production of fishmeal from fish and fish by-products is not economically viable, due to an inadequate variable supply, remote locations, and high energy and labor costs (Hardy, 1992). Liquefied fish products (fish silage) offer an economical alternative that converts by-products into a stable product for further

processing or transportation to another location (Barlow & Windsor, 1981). Fish silage production involves mincing the by-products, adding sufficient acids, usually formic acid, to lower the pH to below 4 to prevent any microbial growth, and enabling the endogenous enzymes to digest the material under the favorable conditions provided by the acids (Ruiter, 1995). The product is then stored for further use. Fish silage is a major component in the feed of swine, fur animals, and fish (Hardy, 1992).

Other Uses of By-Products

Fish processing by-products have a number of uses other as raw material for fishmeal, oil and silage production. Fish heads are used as bait in lobster and crab pots and in other fish traps. Pet foods provide a relatively large market for fish processing by-products. Willard (1990) stated that 6 million metric tons of pet foods were sold in 1990. By-products are used in both canned and pelleted pet food (Hardy, 1992). The use of fish as fertilizer for crops was well known to Native Americans, and its use continues today. The use of fish by-products is an area of increasing interest in organic farming. Production of fish fertilizer involves hydrolysis of by-products, followed by fine grinding and preservation by acidification (Hardy, 1992). Fish fertilizers are used on turf, lawns, and row crops. Some recent studies indicate that the nitrogen from fish fertilizer remains in the soil longer than nitrogen from inorganic fertilizer (Hardy, 1992). Fish leather is used to manufacture belts, wallets, purses, and boots (Tressler & Lemon, 1951).

Although most of the methods described above focus on mass-scale utilization of by-products at a centralized facility, these methods do not provide a feasible choice for small-scale fish farms. This study was undertaken to develop a technology for on-farm utilization of by-products by small farmers.

Chapter Three

Materials and Methods

Materials

Trout Processing By-Products

Rainbow trout (*Oncorhynchus mykiss*) by-products used in this study were heads, frames, viscera, and trimmings (Figure 3). These by-products were provided by a local fish farm (Bullfrog Fish Farm, Menomonie, Wisconsin). By-products were collected during filleting operations and immediately transported to the laboratory in an icebox, and stored at 4 ±1°C until used.

Fish Feed Ingredients

Feed ingredients used to produce fish feed pellets are listed in Table 1. The proximate composition of these ingredients is listed in Table 2.

Materials Used in the Study

The materials used in the study are listed in Table 3.

Equipment

The equipment used in this study is listed in Table 4.

Figure 3. Trout processing by-products

Table 1

Summary of fish feed ingredients used in production of fish feed pellets

Ingredient	Source	Address	Purpose
Blood Meal	Griffin Industries	Cold Spring, KY	Protein source
Soy Flour	Cenex Harvest States, Inc.	Mankato, MN	Protein and starch source
Wheat Flour	Lammers Foods	Menomonie,WI	Binding agent
Fish Oil	Omega Protein, Inc.	Reedville, VA	Flavor and fat source
Vitamin premix	Bio-Oregon, Inc.	Warrenton, OR	Vitamin source
Mineral premix	Bio-Oregon, Inc.	Warrenton, OR	Minerals source
Lecithin	Lucas Meyer, Inc.	Decature, IL	Emulsifier

Table 2

Proximate composition of feed ingredients

Ingredient	Proximate Composition (%)			
	Moisture	Fat	Carbohydrate	Protein
Blood Meal	9	0.5	1	88
Soy Flour	6	0.54	43	51
Wheat Flour	9	0	73	10
Fish Oil	0	100	0	0

Table 3

Summary of materials

Item	Source	Address	Purpose
Stomacher bag	Seward Medical	London,UK	Mixing the sample in the stomacher
Petrifilm	3M	St.Paul, MN	Media for microbial growth
Anaerobic agar	Difco laboratories	Detroit, MI	Media for microbial growth
Baird Packer agar	Difco laboratories	Detroit, MI	Media for microbial growth
Violet red Bile	Difco laboratories	Detroit, MI	Media for microbial growth
Fraser broth	Difco laboratories	Detroit, MI	Media for microbial growth
Enterotube	Becton Dickinson & Co.	Cockeysville, MD	*Enterics* Bacteria identification
Hexane	VWR Scientific	Minneapolis, MN	Fat extraction
Thimble	VWR Scientific	Minneapolis, MN	Fat extraction
Sulfuric acid	VWR Scientific	Minneapolis, MN	Protein determination
Selenized hengar granules	VWR Scientific	Minneapolis, MN	Protein determination
Potassium sulfate	VWR Scientific	Minneapolis, MN	Protein determination
Sodium hydroxide	VWR Scientific	Minneapolis, MN	Protein determination
Boric acid	VWR Scientific	Minneapolis, MN	Protein determination
Hydrochloric acid	VWR Scientific	Minneapolis, MN	Protein determination

Table 4

Summary of equipment

Equipment	Source	Address	Purpose
Stomacher	Tekmark Seward Medical	London,UK	Mixing & homogenizing samples
Blender	Waring Products	New Hartfort, CT	Grinding fish by-products
Incubator	VWR Scientific	Minneapolis, MN	Provided optimal temp. for microbial growth
Hot Plate Stirrer	VWR Scientific	Minneapolis, MN	For creating turbulence
Steam Sterilizer (Figure 4)	American Sterilizers	Manitowoc, WI	Thermal processing
Sterilizer pans (Figure 4)	An old set was modified at UW-Stout, Menomonie		Separate sterilization of by-products
Mechanical Oven	Linder/Blue	Asheville, NC	Moisture determination
Muffle Furnace	Barnstead/Thermolyne	Bubuque, IA	Ash estimation
Soxhlet Extraction Apparatus	VWR Scientific	Minneapolis, MN	Lipid extraction
Digestor	Labconco Inc.	Kansas City, MO	Protein determination
Rapid Distillation Unit	Labconco Inc.	Kansas City, MO	Protein determination
Hobart Mixer (Figure 5)	Hobart Corporation	Troy, OH	Mixing and extrusion
Extrusion Die (Figure 6)	Tainter Shop	Tainter,WI	Pellet Production

Figure 4. Pans and Steam sterilizer.

Figure 5. Hobart mixer

Figure 6. Die used for extrusion of the fish pellets

Methodology

Sample Collection for Microbiological Analysis

Trout processing by-products, fish feed, inlet and outlet water samples (to and from fish tanks) were provided by the Bullfrog Fish Farm (Menomonie, WI). Fish by-products, feed, and water samples were taken twice a week. On each sampling day, fish were removed from the tanks and processed. Aseptically, fish by-products (heads, frames, viscera, and trimming) were then individually transferred to sterile plastic bags, and immediately packed in ice. Water samples were collected in sterile plastic flasks (100 mL) from the inlet and outlet of fish tanks and immediately placed in ice. Commercial feed samples were taken from the current supply using sterile scoops and held in sterile bags on ice until examined. Examination of samples began within 2 h of collection. Samples were separately ground into smaller pieces using a blender that had been disinfected.

Microbiological Analysis

A sample from each by-product type (1 g) was taken and placed in a sterile standard stomacher bag (Seward Medical, London) containing enough distilled water to make a 1:100 dilution, and were blended and homogenized for 60 seconds in a stomacher. Fish and water samples were then serially diluted (1:10), and spread plated onto Petrifilm aerobic count plates (3M Inc., St. Paul, MN), and anaerobic agar (Difco Laboratories, Detroit, MI) for an anaerobic plate counts. The same procedure was used for fish feed and water samples.

Isolation and Identification of Microorganisms

All samples from fish by-products, feed, and water were also plated on selective media designed for recovery of *Staphylococcus, Micrococcus, Listeria, and Escherichia*

coli. One milileter from each sample was transferred to a sterile stomacher bag containing enough distilled water to make 1:100 dilution, and was homogenized for 60 seconds in the stomacher. All samples were then serially diluted, spread plated onto various medium depending on the type of microorganism type to be tested as shown in Table 5. The same procedure was followed in all isolation and identification tests.

Table 5.

Microbiological Isolation and Identification

Microorganism	Media	Incubator		Indicator
		Temperature (°C)	Time (Days)	
Staphylococcus and Micrococcus	Baird -Packer agar	35	2	Development of round white colonies
Listeria	Fraser broth	35	2	Production of dark broth color
Coliforms	Violet Red Bile agar	35	2	Development of blue to red-blue colonies associated with entrapped gas
E.coli	*E.coli* Petrifilms	35	2	Development of blue to red-blue colonies associated with entrapped gas

Identification of Enterobacteriales Using the Enterotube Test

Members of the order Enterobacteriales (enterics) are facultatively anaerobic, gram-negative rods that inhabit the intestinal tracts of humans and other animals (Tortora et al., 2001). Since members of this family such as *Escherichia, Salmonella, Shigella, Klebsiella, and Enterobacter* are potentially pathogenic, colonies grown on Violet Red Bile agar were streaked and inoculated into an Enterotube for rapid identification of enteric family members.

Chemical Analysis

In order to formulate a fish feed that met the nutritional requirements of trout fish, the proximate composition (moisture, ash, fat, protein) of the trout processing by-products was determined according to the Association of Official Analytical Chemists (AOAC, 1984) methods to estimate the quantity of supplementary ingredients needed to produce the desired fish feed.

Moisture Determination

A known amount of sample (10g) was weighed and placed into a mechanical oven set at 105°C for 24 h. After drying, the sample was removed and placed in a desiccator. The sample was then reweighed. Sample weight was calculated by subtracting the weight of the empty dish from the weight of the dish plus its contents. The dry weight was divided by original sample weight and expressed in percent.

Ash Determination

A sample weighing approximately 3 g was weighed and incinerated at 525 °C for 24 h in a muffle furnace. After this, it was placed in a desiccator for cooling and then re-weighed. The ash weight was divided by original sample weight and expressed in percent.

Fat Extraction

A known amount (150 mL) of hexane was poured into a preweighed 225 mL round bottom flask. Approximately 3 g sample was weighed and placed in a thimble and extracted with hexane for five hours. The flask, containing a mixture of hexane and extracted fat, was placed in a boiling water bath to remove hexane. The flask was dried in a mechanical oven, cooled in a desiccator, and re-weighed. The fat content was divided by the original sample weight and expressed in percent.

Protein Determination

Protein determination was carried out in two stages, as follows:

Digestion: Approximately 1 g sample was weighed and placed into a flask followed by the addition of 25.0 mL of H_2SO_4, 3 selenized Hengar granules, and a half tablet of Kjeldahl digestion mixture (K_2SO_4 and Se). The sample mixture was heated until it became colorless, and was allowed to cool. The sample was diluted to a 100 mL volume and mixed thoroughly. The mixture was then allowed to cool before distillation.

Distillation: A known aliquot (20 mL) was transferred to the sample addition funnel of the Rapid Distillation Apparatus and then introduced to the sample chamber. Approximately 25-30 mL of 40 percent concentrated NaOH was added to the sample addition funnel and released to the sample chamber at a slow rate. The ammonia was entrapped in a receiving solution containing boric acid with a purple indicator. The distillation lasted for 20 minutes and the boric acid color turned to green. The solution was then titrated with 0.1N HCl acid.

Feed Formulation

Fish feed manufacturing involved extruding a mixture of trout processing by-products and feed additives into a usable form. Many nutritional and non-nutritional considerations were taken into account during feed formulation. Nutritional considerations such as protein, lipid, vitamin, mineral, and energy requirements were met for normal growth and other physiological functions. Fish feed ingredients were also selected on the basis of availability, low cost, and simplicity of handling during processing. These characteristics were the primary reasons for choosing blood meal, soy flour, wheat flour, vitamin and mineral premixes. In the formulation process, the proximate composition of

trout processing by-products was determined in order to calculate the required amounts of the complementary ingredients. A Microsoft Excel spreadsheet (Redmond, Washington) was used to calculate the amount of ingredients needed to meet the nutritional requirements of trout fish (Table 6).

Process Development

The process for production of fish feed from trout processing by-products involved four unit operations (Figure 7)**.**

Thermal Processing/Grinding of Trout Processing By-Products

In order for the finished product (fish feed) to be microbiologically safe, the trout processing by-products were subjected to thermal processing to destroy the bacterial load. In addition, thermal treatment of by-products softened the fishbone making it easier to grind. Pressure and temperature were maintained constant throughout the experiment at 131 $x10^3$ Pa, and 124°C, respectively, whereas sterilization time varied from 10 to 20 minutes (Table 7). The response variable was the bacterial count of the thermal processed by-products. Three experiments were conducted to estimate the optimal time for a bacteria-free product. To do so, each by-product was assigned to a single pan (total of four pans). The pans were then stacked on the top of each other and placed in the sterilizer. By-products were cooked for 10, 15, and 20 minutes and examined for bacterial count after each treatment. Each experiment was conducted three times to ensure accurate and consistent results. A one-gram sample from each pan was aseptically transferred into a sterile standard stomacher bag containing enough distilled water to make 1:100 dilution, and blended and homogenized for 60 seconds in a stomacher.

Table 6

Standard fish feed composition

Feed components	Amount (%)
Protein	≥52
Fat	≥14
Moisture	10
Vitamins	2
Minerals	≤12
Carbohydrates	≤20

Mixing of By-product Slurry with Dry Ingredients

Appropriate quantities of dry ingredients other than by-products were weighed, ground, and mixed utilizing a food processor. The dry ingredients were ground an average of 15 minutes to reduce the particle size. Likewise, the processed by-products were ground to soften the fish bones. After this, both dry ingredients and by-products were transferred into the Hobart mixer. The ingredients involved in the fish feed production were trout by-products, blood meal, soybean meal, wheat flour, fish oil, vitamin and mineral premix. The mixture was mixed to produce dry dough that could be extruded.

Extrusion of Dough to Form Pellets

A multi-channel die was designed and fabricated locally. The die was then attached to the Hobart extrusion attachment to produce pellets 4.5 mm in diameter. The extruded threads were cut manually using a knife in a regular manner as they came out the die to ensure similar pellet size.

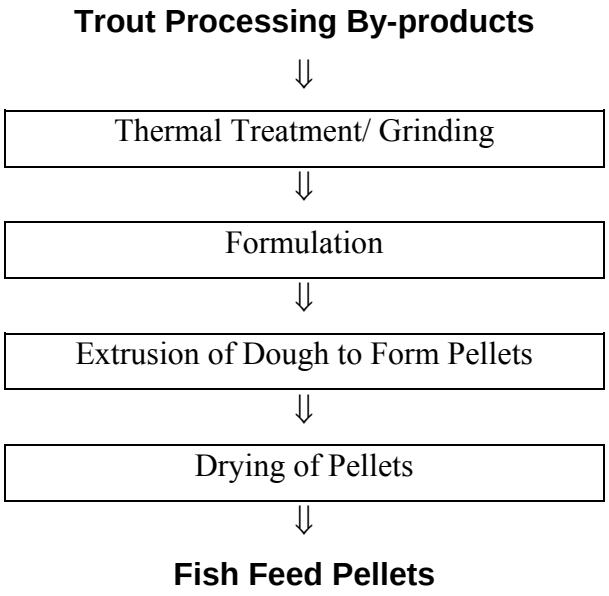

Figure 7. Process for production of fish feed from trout processing by-products

Drying of Pellets

A mechanical oven was used to dry the pellets. Which were spread over the oven shelf to a thickness of about 4.5 mm. Drying time at 105°C was determined to residual moisture content about 5 percent.

Table 7.

Fixed, independent, and response variables used during thermal processing

Fixed Variables	Independent Variable Heat Treatment Time (min)	Response Variable
1. Temperature at 124°C	10	Bacterial Count (CFU)
	15	
2. Pressure at 131 x10^3 Pa	20	

Chemical and Physical Evaluation of the Finished Product

Chemical Evaluation of Fish Feed Pellets

The proximate composition of the fish feed pellets was estimated following the same procedures mentioned earlier. The chemical analysis was conducted to ensure that the

final product contained all the nutritional and energy requirements needed for optimal fish growth and physiological functions.

Physical Evaluation of Fish Feed Pellets

The following response variables were evaluated to determine the effects of the composition and process parameters on feed pellet characteristics:

1. Apparent Density: The apparent density was estimated by determining the mass and apparent volume of individual dry, cylindrical extruded rods (Choudhury & Gautam, 1999). Apparent volume was calculated as the product of the length and cross-section area of the extruded rods. An average of ten measurements were used.

2. Floating Time: The floating time is an important parameter for feed consumption. The longer the floating time, the greater the opportunity for the fish to consume the pellets. Floating time was determined by recording the time taken by a pellet to go just below the surface of water. An average of ten measurements were used.

3. Sinking Velocity in Still Water: Sinking velocity, expressed as m/s, was determined in a measuring beaker (volume 2000 ml, length 21 cm, and diameter 16.5 cm) by recording the time required by a pellet to sink in water from the surface to a fixed depth (Das et al., 1993). An average of ten measurements were used.

4. Sinking Velocity in Turbulent Water: Turbulent conditions were created using a magnetic stirrer in a transparent beaker (same specifications as above). The sinking velocity (m/s) was measured the same way above. An average of ten measurements were used.

Chapter Four

Results and Discussion

Microbiological Evaluation of Trout By-Products

Aerobic Microflora

Initial numbers of microflora associated with cultured freshwater trout by-products were determined. The environment can influence numbers and types of microorganisms of fish (Nedohula & Westhoff, 1997). Therefore, the load and identity of bacteria in the water and feed were determined. Bacterial levels for trout by-products (heads, viscera, trimmings, and frames) and those for water (from which the fish were caught) and the feed appear in Tables 8 and 9, respectively. The aerobic counts among the by-products were fairly similar with trimmings and viscera displaying the highest and lowest bacterial load, respectively. In a similar study on bacterial load of cultured rainbow trout it was found that heads yielded the highest aerobic count of 7.08×10^3 CFU/g whereas trimmings had the lowest microbial load of 8.32×10^2 CFU/g (Gonzalez et al., 1999). These differences in findings are in agreement with the generally accepted concept that the environment can influence the number and types of bacteria associated with the skin, gills, and guts (Nedohula & Westhoff, 1995). In this study, aerobic counts for the heads, trimmings, viscera, and frames were lower than the maximum values (5×10^7 CFU/cm^2) recommended by the International Commission on Microbiological Specifications for Foods (ICMSF) for freshwater fish (ICMSF, 1986).

To study the effect of the environment on the bacterial load and flora of fish, the aerobic counts of growing water and fish feed were examined (Table 9). The microbial count of the incoming water was lower than that of the outlet water, which indicated a

relatively lower level in the incoming water. The bacterial loads of both the incoming and outlet water were lower than the bacterial count of freshwater (1.74 $x10^3$CFU/g) containing rainbow trout reported by (González et al., 1999).

Table 8

Aerobic counts (CFU/g) from the by-products of rainbow trout processing

By-product type	Microbial load (mean ± standard error)	Coefficient of variation (%)
Head	$1.4x10^6 \pm 2.3x10^4$	2.9
Viscera	$6.7x10^5 \pm 1.9x10^4$	4.8
Trimmings	$5.7x10^6 \pm 1.5x10^5$	4.4
Frames	$1.4x10^6 \pm 4.3x10^4$	5.45

Table 9

Aerobic counts (CFU/g) for water and fish feed

Variable	Microbial load (mean ± standard error)	Coefficient of variation (%)
Water in	$5 \pm 6x10^{-1}$	20
Water out	$2.7 x10^2 \pm 1.5x10^1$	9.2
Commercial fish feed	$4.5x10^3 \pm 2.3x10^2$	8.9

Anaerobic Microflora

Unlike the aerobic counts, the highest anaerobic microbial load was found in viscera whereas the lowest count was observed in heads (Table 10). The high bacterial load found in the viscera agreed with data reported by others, which indicated that fish intestines provide a favorable ecological environment for bacteria (Huss, 1995, Westerdahl et al., 1991).

Table 10

Anaerobic counts from (CFU/g) from the by-products of rainbow trout processing

By-product type	Microbial load (mean ± standard error)	Coefficient of variation (%)
Head	$3.3 \times 10^4 \pm 1.8 \times 10^3$	9.4
Viscera	$6.5 \times 10^5 \pm 1.5 \times 10^4$	3.9
Trimmings	$8.4 \times 10^4 \pm 1.8 \times 10^3$	3.6
Frames	$6.2 \times 10^4 \pm 1.7 \times 10^3$	4.8

In this study, the range of the anaerobic counts (10^3 to 10^4 CFU/g) was higher than (10^2 to 10^4 CFU/g) found in a similar study on farmed rainbow trout (González et al., 1999) but was in agreement with the anaerobic counts of striped bass raised in flow-through tanks (Nedohula et al., 1995). For the heads, viscera, trimmings, and frames, the aerobic counts were consistently higher than the anaerobic counts in each round.

The anaerobic bacterial count of the incoming freshwater was lower than that of outlet water (Table 11) and both of them were lower than that found by González et al. (1999).

Table 11

Anaerobic counts (CFU/g) for water and fish feed

Material Examined	Microbial load (CFU/g) (mean ± standard error)	Coefficient of variation (%)
Water in	$6.7 \times 10^1 \pm 3.3 \times 10^0$	8.7
Water out	$1.6 \times 10^2 \pm 1.2 \times 10^1$	13
Commercial fish feed	$5.3 \times 10^2 \pm 8.8 \times 10^1$	29

Water Quality and Environment Effect

Nedoluha and Westhoff. (1993) reported water bacterial counts collected from commercial freshwater ponds varied between 10^2 to 10^5 CFU/g. The fact that the microbial load of water samples in this study was remarkably lower than that found in other studies indicated that water tanks were not heavily contaminated and in a good sanitary condition. In another study, bacterial counts of water in a recirculating system were very high and averaged 6.3x 10^6 CFU/g (Nedoluha and Westhoff, 1997). Since the water is reused in a recirculating system rather than replaced, bacteria from the diet and the intestines accumulated and established themselves as a resident microbiota, which resulted in elevated bacterial counts.

The findings of this study supported the generally accepted thesis regarding the influence of the environment on the bacterial load. This was clearly demonstrated by observing higher bacterial counts in the by-products than the growing water. In addition, the substantial difference in bacterial counts existing between inlet and outlet water indicated that water became contaminated after being mixed with fish. Sugita et al. (1985) found that fish excreta influenced the bacterial load of the tanks containing fish and concluded that fish can be a source of bacteria for the surrounding water. In this study, the aerobic and anaerobic counts for fish feed were slightly higher than those found in similar study on striped bass feed (Nedoluha and Westhoff, 1995). Some studies have suggested that fish feed contributes to the bacterial load and species identity of fish. In addition, they suggested that bacterial flora of the gut depends primarily on the food source (Margolis, 1953; Seki, 1969).

Pathogenic Bacteria

Since fish are considered to be an excellent host of nonpathogenic and pathogenic bacteria, it was important to test for the presence of some pathogenic bacteria that are primarily found in fish. Gram-negative bacteria compose most of the freshwater fish microbiota (Frazier & Westhoff, 1988). In a study on the microbiota of farmed rainbow trout González et al. (1999) found a wide variety of bacterial species with the predominant species being members of the genera *Enterobacter, Aeromonas, Acinetobacter, Alcaligenes,*and *Micrococcus.* In another study on the bacterial flora in the alimentary tract of freshwater salmonid fishes, the most prevalent bacterial species were members of the genera *Enterobacter, Aeromonas*, and *Acinetobacter* (Trust & Sparrow, 1974). Several studies found that *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Aeromonas hydrophila, Enterobacter cloacae,* and *Clostridium botulinum* were predominant pathogenic bacteria of freshwater fish (González et al., 1999, Trust et al. 1974, & Nedoluha andWesthoff, 1993). In this study, selective media were employed to detect the presence of coliforms*, E. coli, Staphylococcus, Listeria, and Micrococcus bacterial* genera*.* As shown in Table 12, the by-products were free of any of the species mentioned above.

Coliforms

Margolis (1953) reported that bacteria on fish might reflect the bacteriological conditions of the water in which they were grown and that microbial populations can be used as a good indicator of pollution. The test for water purity in this study was carried out

using coliforms as indicator organisms of water pollution. Coliform population in the fish by-products, fish feed, and growing water are shown in Tables 12 and 13.

Table 12

Coliform count (CFU/g) and pathogenic bacteria of the by-products

By-product type	Coliforms		*E.coli*	*Listeria*	*Staphylococcus*	*Micrococcus*
	(mean ± standard error)	CV (%)				
Head	$1.7x10^4 \pm 1.2x10^3$	12	ND*	ND	ND	ND
Viscera	$1.6x10^5 \pm 1.2x10^4$	13	ND	ND	ND	ND
Trimmings	$3.2x10^4 \pm 1.5x10^3$	7.8	ND	ND	ND	ND
Frames	$9.4x10^4 \pm 1.5x10^2$	2.7	ND	ND	ND	ND

* ND = Not detected in this study.

Table 13

Coliform counts (CFU/g) for water and fish feed

Material Examined	Coliform load (mean ± standard error)	Coefficient of variation (%)
Water in	ND	0.0
Water out	$1.9 \times 10^2 \pm 1.2x10^2$	11
Commercial fish feed	$3.8 \times 10^4 \pm 1.5x10^3$	6.7

ND= Not Detected

The coliform counts for fish by-products were in conformity with Huang et al. (1993) with viscera being the highest and heads the lowest (Table 12). However, the coliform population of outlet water (1.9×10^2 CFU/g) (Table 13) was higher than that reported by Huang et al. (7.2×10^1 CFU/g), whereas the incoming water contained no coliforms. The fact that the coliform load of water was less than the by-products signified that fish and fish feed could be the source of coliforms to the growing water and supported

the finding mentioned above regarding the interrelationship between the environment and fish.

The coliform colonies, which grew on violet-red bile agar, were streaked on a prepared multimedia tube (Enterotube II) for the identification of members of the family Enterobacteriaceae (Table 14). None of the isolated identified species were pathogenic. This finding is similar to results of studies from freshwater hybrid striped bass and salmon fish (Nedoluha Westhoffl., 1993, Trust and Sparrow., 1974). Since the microorganisms recovered from by-products were absent or not detected in the growing water but existed in the fish feed, the findings supported the generally accepted idea of the influence of environment on the bacterial flora of fish and agrees with the findings of Gonzáles et al. (1999).

Table 14

Species isolated from coliform plates and grown on violet-red bile agar

Species	Head	Viscera	Trimming	Frame	Fish Feed	Water out
Acinetobacter anitratus	P	P	P	P	P	A
Serratia liquifaciens	P	A	P	P	P	A
Enterobacter aerogenes	P	P	P	A	P	A
Enterobacter agglomerans	P	P	A	P	P	A
Enterobacter ammigenus	A	A	A	A	A	P
Serratia plymuthica	A	A	A	A	A	P
Enterobacter hafniae	A	A	A	A	A	P

P= Present A= Absent

Chemical Composition of Trout Processing By-Products

The chemical compositions of by-products were determined to estimate the additional ingredients needed to formulate fish feed similar in composition to the standard fish feed to ensure an acceptable consistent growth rate of fish. Using the official AOAC methods (1984), the proximate composition (moisture, protein, fat, and ash) of the fish by-products was determined. Table 15 compares the proximate composition of trout by-products with that of pollock (Babbitt, 1990). Overall, the values seem to be similar with respect to protein and ash contents, however, the differences in relation to moisture and fat contents are attributed to the species and feeding method.

Table 15

Proximate composition of trout and pollock by-products in percentage

	Composition (%) [a] (mean ± standard error)	CV (%)	Composition (%) Pollock by-products[b]
Moisture	$67.4 \pm 8.7x10^{!2}$	0.22	74
Protein	$14.9 \pm 4.0x10^{!2}$	0.47	14
Fat	$14.5 \pm 3.5x10^{!2}$	0.45	9
Ash	$3.34 \pm 1.7x10^{!2}$	0.75	3

[a] Trout by-products

[b] Source: Babbitt, J. K., (1990). Intrinsic quality and species of north pacific fish. *Proceedings of the International Conference on Fish By-Product*. Fairbanks, AK. (pp.39-43).

The available amounts of protein, fat, ash, and moisture coming from the by-products were used as the baseline for formulating a fish feed similar to those that are commercially available. Knowing the final composition of the finished product, the by-products were supplemented with proteinaceous materials (blood meal and soy flour),

energy supplement (fish oil), binder (wheat flour), and vitamin and mineral premixes, to bring up the levels of protein, fat, minerals, and vitamin, to a level to meet the nutritional requirements of trout (Table 16). However, addition of dry ingredients dropped the water content but not to the needed level, therefore, drying the finished feed was essential.

Table 16

The available amounts from by-product, required in the finished feed, and the needed or to be removed amounts in percentage

	Available (%) Trout by-products	Required (%) Finished Product	Addition/Removal Needed
Moisture	67.4	5	Removal
Protein	14.9	55	Addition
Fat	14.5	18	Addition
Ash	3.34	10	Addition
Carbohydrate	0.0	10	Addition

Process Development

Thermal Processing/Grinding of Trout Processing By-Products

The by-products were subjected to thermal processing to:

1. Destroy the microbial load so that the finished product is safe.
2. Soften bones in heads and frames prior to grinding.

The by-products were sterilized utilizing a pressure cooker with pressure and temperature being held constant at 131x 10^3 Pa and 124 °C. The time at which there was no detection of bacterial growth was considered the optimal time. The time was varied to estimate the lowest time required to optimize energy, time and cost. The by-products were cooked for 10, 15, and 20 minutes; the optimum cooking time was estimated to be 15 minutes

(Tables 17 and 18). As can be seen from both tables the aerobic and anaerobic bacterial count after the ten minute cooking time was found to be the highest in trimmings and viscera, respectively. These findings are consistent with the initial bacterial load where trimmings had the highest aerobic load, and viscera had the highest anaerobic load (Tables 8 and 10).

Table 17

Aerobic bacterial count (CFU/g) of the trout by-products heat-treated for various times

Fixed Variables: Temperature (T) and Pressure (P)	Independent Variable: Thermal Process Time (min)	Response Variable: Bacterial Count (CFU/g)			
		Head	Viscera	Trimming	Frame
T = 124°C P = $131x10^3$ pa	10	66.7 ± 8.7	86.7 ± 8.7	303 ± 14.5	30 ± 11.5
	15	No Growth	No Growth	No Growth	No Growth
	20	No Growth	No Growth	No Growth	No Growth

Table 18

Anaerobic bacterial count (CFU/g) of the trout by-products heat-treated for various times

Fixed Variables: Temperature (T) and Pressure (P)	Independent Variable: Thermal Process Time (min)	Response Variable: Bacterial Count (CFU/g)			
		Head	Viscera	Trimming	Frame
T = 124°C P = $131x10^3$ pa	10	43.3 ± 8.7	517 ± 17.8	63.3 ± 8.7	147 ± 12.1
	15	No Growth	No Growth	No Growth	No Growth
	20	No Growth	No Growth	No Growth	No Growth

Cooking the by-products softened the bones and facilitated grinding. The grinding time was experimentally determined and ranged from 12-17 minutes depending on the load. Grinding for less than 12 minutes did not pulverize the bones, which resulted in coarse particle size. However, grinding for any time within the range was sufficient to make very smooth slurry with no large pieces of bones.

Feed Formulation

Nutritional and non-nutritional considerations were taken into account during fish feed formulation (Li, 1998). Since farm-raised fish have no access to natural food, the commercially prepared fish feed must be the primary source of nutrients. Therefore, a nutritive feed is necessary for fish raised in raceways to provide nutrients and energy required for optimal growth and other physiological functions. For commercial feed manufacturing, feed ingredients must be available all the time, easy to handle, withstand manufacturing conditions, and inexpensive (Li, 1998). Since the objective of this study was to develop a farm technology for production of fish feed pellets, the principal goal was to increase profits of fish production by maximizing the nutritional value of the manufactured feed at minimum cost. The above mentioned nutritional and non-nutritional characteristics were the main reasons why blood meal, soy flour, wheat flour, and fish oil (Table 2) were selected as additional ingredients for the prototype feed.

Nutrient requirements for fish are similar to those for terrestrial animals but with lower energy requirements (Lovell, 1998). Unlike warm-blooded animals, fish poorly utilize carbohydrates as a source of energy. Fish are born and raised in aquatic environments where carbohydrate sources are limited, which explains why their digestive systems became adapted to better utilize protein and lipids for energy than

carbohydrates (Lovell, 1998). Several studies have shown that fish can grow satisfactorily on a carbohydrate-free diet if lipids are provided in the diets to supply glycerol for carbohydrate synthesis (Brambila & Hill, 1996). Fish use protein efficiently as a source of energy. This is attributed to the efficient way fish excrete nitrogen (Lovell, 1998). The energy cost of synthesis for urea and uric acid is 3.1 and 2.4 kcal/g of nitrogen, respectively (Martin & Blaxter, 1965). Fish do not synthesize uric acid or urea to get rid of the nitrogenous waste (ammonia), rather they readily release ammonia into the water through gills, thus energy is saved by not synthesizing uric acid or urea (Cowey, 1975). Fish best utilize protein because they don't expend energy regulating body temperature, resulting in lower maintenance energy requirements (Lovell, 1998). Therefore, the top priority was to meet the requirements for protein, fat, minerals, and vitamins. Carbohydrate sources such as wheat flour and soy flour where used to provide binding properties so that the final dough would be extrudable.

Knowing the proximate composition of fish by-products and additives, the required quantities of each ingredient were calculated to formulate a complete fish feed that meets the energy and nutritional requirements of fish (Table 19). A Microsoft Excel spreadsheet was used to solve the mass balance equations to determine the feed composition shown in Table 20.

Table 19

Standard fish feed composition

Feed components	Amount (%)
Protein	≥52
Fat	≥14
Moisture	≤10
Minerals	≤12
Remainder	≤10

Table 20

Composition of the formulated mix feed on wet weight basis

Ingredient	Amount (g)	Protein	Fat	Minerals	Vitamins	Carbohydrate
Fish by-product	55.0	8.2	8.2	1.87		0.0
Soy flour	10.0	5.2	0.05			4.3
Blood meal	46	40	0.23	0.69		0.45
Wheat flour	5.9	0.59		0.04		4.4
Fish oil	9.7	0.0	9.72			0.0
Mineral premix	18.3	1.0		7.50		
Vitamin Premix	2.0	0.0			2.0	
Total	146.5	55.0	18.2	10.0	2.0	9.1
Removed Moisture	46.5					
Final Product	100	55.0	18.2	10.0	2.0	9.1

Mixing/Extrusion of Dough to Form Pellets

Cooked ground fish-products and dry ingredients were weighed following the formula and mixed together using a Hobart mixer. It was experimentally determined that the fish by-products must comprise 10-15 percent of the dry matter of the total finished product in order to formulate a dough that was neither sticky nor dry and was extrudable.

The mixture was mixed at a low speed for 15 minutes. It was important to pause mixing and manually stir top and bottom ingredients**,** so that the added fish oil and other ingredients would mix together to form a homogeneous mixture with consistent composition.

Die Design

A multi-channel die was designed and fabricated locally for extrusion of the dough. The die length was 50 mm, which was enough to provide the pressure needed during extrusion to form the pellets. The diameter of the die block containing 10 openings (4.5 mm in diameter each) was 60 mm (Figure 8). The first die design consisted of two rows of openings in the center of the die each row contained five openings**.** The design was not satisfactory due on inconsistent flow rate, which resulted in having pellets of various sizes**.** After this, the die design was modified and the openings were then distributed around the circumference of the die and were 10 mm apart as shown in Figures 8 and 9. This design ensured a consistent and equal flow rate resulting in feed pellets with nearly similar sizes. The cutting rate was adjusted to obtain pellets about 4.5 mm long (Figure 10).

Drying Fish Feed Pellets

Fish feed pellets were dried to a 5 percent residual moisture content using a mechanical oven. The pellets were spread over the shelf with layer thickness of about 4.5 mm. The drying temperature was kept fixed at 105 °C. The drying time needed to result in 5 percent moisture content ranged from 45-49 minutes.

Figure 8. Die used for extrusion of the of the fish pellets

Figure 9. Die attached to the Hobart extrusion attachment

Figure 10. Flow through the die and fish pellets

Chemical and Physical Evaluation of the Fish Feed Pellets

Chemical Evaluation of Fish Feed Pellets

To verify that the manufactured fish feed contained the correct requirements needed by fish for optimal growth, a chemical analysis was conducted on the final product. Table 21 lists the proximate composition of the manufactured fish feed. The actual yield was similar to the predicted values (Table 20). Carbohydrates and vitamins were not determined. However, the remaining amount (17%) was composed of carbohydrates, vitamins, and miscellaneous found in mineral premix.

Table 21

Proximate composition of the manufactured fish feed pellets

Component	Actual % (mean±standard error)	CV%
Protein	53.6 ± 0.25	0.82
Fat	17.8 ± 0.14	1.4
Ash	9.37 ± 0.08	1.6
Carbohydrates	NE[a]	NE
Vitamins	NE	NE
Moisture	2.00 ± 0.06	5.00

[a] NE= Not estimated in this study

Physical Evaluation of Fish Feed Pellets

Table 22 lists the physical attributes measured in the study to determine the effect of the composition and process parameters on feed pellet characteristics. The apparent density of the feed was higher than that of water, which indicated the sinking nature of the pellets. Numerous attempts to produce floating pellets by changing feed composition

did not generate the desirable results**.** The sinking velocity was used as an indicator of the rate at which the pellet would sink in still and turbulent water in order to provide the fish with a greater opportunity to consume the pellets before it reaches the bottom.

Table 22

Physical attributes of fish feed pellets

Attribute	Mean	CV (%)
Apparent density (kg/m^3)	$1.1x10^2 \pm 17.4$	0.05
Sinking velocity in still water (m/s)	$7.6x10^{-2} \pm 8.2x10^{-4}$	1.0
Sinking velocity in turbulent water (m/s)	$9.1x10^{-2} \pm 2.8x10^{-3}$	3.0
Floating time (s)	0.0	0.0

Chapter Five

Conclusion

A low-cost farm technology for the production of fish feed pellets utilizing trout processing by-products was developed. The process consisted of five unit operations: thermal processing, grinding, mixing, extrusion, and drying. A 15-minute heat treatment of the by-products was required to destroy the microbial load and soften the bones. A subsequent grinding was needed for production of a smooth slurry with grinding time ranging from 12 to 17 minutes. The by-products and supplementary dry ingredients were mixed using a Hobart mixer at a low speed for 15 minutes into extrudable dough. A 50 mm long multi-channel die provided enough pressure for pelletizing. The die had 10 openings (4.5 mm each) distributed around the circumference. The fish feed pellets were dried to approximately 5 percent moisture using a conventional oven for 45 to 49 minutes to impart structural integrity, shelf life, and water stability to the pellets.

The fish feed pellets produced were of sinking type. Numerous attempts to produce floating pellets by changing feed composition did not generate the desirable results. A change in process will be needed to produce floating feed. Use of a cooking extruder is common in the feed industry and introduction of a cooking extrusion step after mixing may produce a floating feed.

More research is needed to optimize the technology and scale up the process. The technology works at a pilot-scale. The process parameters need to be adjusted and optimized for farm production of fish feeds. The nutritional quality of the developed fish feed pellets needs be determined through a feeding experiment by comparing the growth rate of fish using the commercial and the farm feeds in two aquariums. An economic

analysis including labor cost, capital investment cost, and direct and fixed manufacturing cost for small scale on-farm production of fish feed, needs to be done before adaptation of the technology for on-farm feed production.

Further research is needed to harness the full benefit of this thesis project. Successful implementation of the technology will result in recovering the valuable nutrients from trout processing by-products and eliminating/reducing the environmental pollution created by improper disposal of the same. In addition, this technology will enable the fish farmers to manufacture their own requirement of fish feed pellets on site without the additional cost of the packaging, distribution and marketing steps. This resource recovery system will improve profitability by reducing feed cost and alleviating by-product disposal problems. Such a technology will benefit fish farmers everywhere in the country and the world.

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