Possibility of Rendering Koi Carp

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by

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ABSTRACT

Koi carp, one of the most invasive fresh water fish, is a pest in New Zealand waterways. Pest management plays a vital role in protecting the natural environment and efforts are being made to eradicate koi carp.

The aim of this study was to identify possible uses for koi carp that will help offset eradiation costs. An extensive literature search to identify feasible products that could be made from koi carp showed that producing fish meal and fish oil had potential. The commercial processes are well established and these processes were relatively simple and economic. The high protein meal and fish oil produced could be used as ingredient in formulated animal, pet and fish feeds. It is proposed that whole fish are processed, eliminating the need to clean, gut and fillet the fish.

A series of experiments were done out in the laboratory to simulate the two common commercial rendering systems – wet rendering and dry rendering. Minced whole koi carp caught in Lake Waikere were heat treated for various temperatures and processing times to cook the protein. The resultant meal was then dried. High temperature processing under pressure and solvent extraction (to remove the maximum amount of oil) were also simulated.

A dried meal could be obtained by continuously heating and stirring minced fish for 3 h to an endpoint temperature of 132°C. Free oil could be obtained by pressing or centrifuging the dried material. The final meal yield was 25-27% of the original wet fish weight. It had a composition of approximately 25% protein, 8-9% lipid and 2.2% minerals, based on a meal moisture content of 6% . The oil yield was only 1.5% (wet rendering) and 5.2% (dry rendering) of the original fish weight, indicating that koi carp are not a suitable source for oil.

Pressure processing produced similar yields as wet rendering process.

It is recommended that further work is done to determine the effect of seasonal variations in koi carp on meal yields and composition. Research should also be done to determine whether the acceptability of the meal in formulated animal of fish feeds. Lastly, an economic analysis on processing koi carp collected from eradication programmes should be done.

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1: OVERVIEW

1.1. New Zealand and the Environment

Millions of years ago, New Zealand separated from the massive supercontinent Gondwanaland and drifted into South Pacific. This created an environment where seventy five percentage of the native flora is unique (Walrond 2009). Maintaining the natural environment has become an important issue for the country.

In New Zealand, humans have been the invaders and a major cause of loss and even extinction of many flora and fauna. Some birds such as the moa and huia became extinct due to predation by humans. Large-scale hunting and destruction of forests has endangered populations of many species of birds (such as kakapo and takahe) and plants (such as Adams mistletoe, *Lepidum obtusatum* – a species of coastal cress).

As well as their impact on the natural environment, people have introduced many species of animals and plants, which increased the threat to native species by altering the natural habitat. These introduced species include animals such as possum, rabbit, rat, weasels, deer and carp and exotic plants such as grasses to create farmland, garden plants that have escaped, hedging and plantation trees. Their impacts are widespread.

1.2. Threats to the Aquatic Environment

The three main threats to the aquatic environment are pollution (normally caused by human activity), pesticides (used in large scale agriculture), and introduced species which have become pests. The primary sources of pollution are human sewage, urban storm runoff, industries, agriculture, agricultural processing, mining, forestry. This causes a decline in water quality and degradation of the aquatic ecosystem. Fertilizers on reaching the aquatic system alter the nutrient level of these systems. Run-off from farmland carries any excess fertilizer as well as pollution caused by animals. These increased nutrients cause eutrophication, with the following effects:

- Sedimentation rate increases, which shortens life of the waterway

- Turbidity increase
- Anoxic conditions are created
- Plant mass increases (often causing algal blooms) and animal biomass increases (due to the extra nutrient available)
- Species diversity decreases, resulting in change in the dominant biota:

Increased amount of pesticides in the aquatic ecosystem results in bioaccumulation of pesticides in organisms, which consequently enter the human food chain and affect human health. Bioaccumulation also causes heavy impacts on the aquatic life.

Introduced species are those that do not belong to the particular environment. They pose threats to the native flora and fauna and can hinder survival of the native species. Carp, rudd and gambusia are considered to be pest fish in New Zealand waterways.

1.2.1. Introduced Fish

Common carp (*Cyprinus carpio*) have been introduced to New Zealand waterways as a result of accidental release. Carp act as 'bioturbators' and change the nutrient level in the aquatic system. These changes can affect the activities of livestock as well as humans. These effects include:

- Decline in amenity value of the water
- Restraining water flow, and hence navigation, due to the increased vegetation
- Decline and even extinction of native fish and commercially-important fish population
- Difficulty in treating drinking water followed by the unacceptable change in taste and odour

The changes can make water from these waterways injurious to health and unsuitable for drinking and swimming, even for livestock.

1.3. Introduced Species – Koi Carp

Carp are oily, heavy-bodied, fresh water fish. Koi carp is an ornamental variety of common carp that resembles gold fish. Their accidental introduction into New Zealand occurred as a result of gold fish consignment in 1960 because of this resemblance (McDowall 1990). Koi carp have subsequently been released into waterways, along with some illegal introductions. They now have become a major aquatic pest in the Auckland and Waikato waterways.

The threat to the natural ecosystem demands the eradication of koi carp. The legal requirement is that once the koi is caught, it must be immediately killed. Generally these fish are wasted. Finding economic uses for this fish will act as an aid in the eradication process. However, major factor of consideration regarding the utilization of the koi is that that the raw material (caught koi) will no longer be available once their population in the natural environment has been reduced to low levels or completely eliminated.

1.4. Possible Options for Using Koi Carp

Fish can be used as whole or parts to obtain useful products with commercial value. These include fish burger, fish roe, collagen, bio-fertilizer, fish silage, dried fish, fish pickle, bio plastics, pet food, and fish oil. However, processing some of these products is not economically feasible. Regardless of the option(s) chosen, market viability and product acceptability must be taken into account. Fish meal (a bio-fertilizer), fish silage, ingredient in pet food, and fish oil appear to be potential feasible end-uses for carp caught during eradication programmes.

Rendering is a commercial process to separate water, fat and protein components of animal and fish into commercial products. The major aim of the rendering process is the production of stable, hygienic products of commercial value from raw material that is often unsuitable for human consumption. The rendering process involves separating fat from the raw material and drying the residue. Heat is the most common method for rupturing fat cells but the solvent extraction and enzymatic extraction methods can also be used.

1.5. Objectives

- Investigation of possibilities of feasible uses for koi carp (raw material)
- Primary processing which includes chopping and mincing followed
- Separation of components by dry rendering methods
- Separation of components by wet rendering methods
- Quantitative analysis of ash and moisture
- Quantitative analysis of oil yield
- Determination of the best method of rendering for carp

1.6. Outline of Thesis

The literature on koi carp and possible utilization will be presented in Chapter 2. This will finish with the specific aims of the thesis and the methodology that will be used. The methods, materials and trials are described in Chapter 3. Results are presented and discussed in Chapter 4. Finally, the conclusions and recommendations are presented in Chapter 5.

2: REVIEW OF LITERATURE

2.1. Koi Carp

The common carp is a durable fish suitable for aquaculture and hence the third most commonly introduced fish species in the world (Welcomme 1992). They belong to the Cyprinidae family and there are three main subspecies: East Asian common carp (*Cyprinus carpio haematopterus*) from Siberia and China; European common carp (*Cyprinus carpio carpio*) from Europe; and wild carp from central Asia (Balon 1995; Murakaeva *et al*. 2003).

Koi carp is an ornamental variety of East Asian common carp (Kohlmann and Kersten 1999; Gross *et al*. 2002; Zhou *et al*. 2003) that is believed to have been taken from China to Japan (Axelrod 1973), where they were then bred for their colouration. The time of the origin for koi carp is unknown. Assumptions range from 200 years ago in Japan to 2000 to 4000 years in China (Balon 1995). They exist in a variety of vivid colours (Balon 1995; Stuart and Jones 2002) and have an oily nature. Many studies link common carp to decrease in environmental quality and loss of species diversity (Crivelli 1981; Meijar *et al*. 1990; Lougheed *et al*. 1998; Sidorkewicj *et al*. 1998; Zambrano *et al*. 1999; Hickley *et al*. 2008).

Carp are usually found in lakes, water courses, estuarine habitats and wetlands. They can grow up to 700 m long and weigh up to 60 kg (Allen 1989, in Pinto *et al*. 2005). The common carp can be recognised by its small eyes, thick lips, large cycloid scales, a pair of barbels at each corner of the mouth, and strongly serrated spines in the anal and dorsal fins (NSW Department of Primary Industries 2005).

The description of carp is:

> "Dorsal spines (total): three to four; Dorsal soft rays (total): 17 to 23; Anal spines: two to three; Anal soft rays: five to six; Vertebrae : 36 to 37. Pharyngeal teeth 1, 1, 3:3, 1,1, robust, molar-like with crown flattened or somewhat furrowed. Scales large and thick. `Wild carp ' is generally distinguished by its less stocky build with height of body 1:3.2 to 4.8 in standard length. Very variable in form, proportions, squamation, development of fins, and colour. Caudal fin with three spines and 17 to 19 rays. Last simple anal ray bony and serrated posteriorly; four barbles; 17 to 20 branched dorsal rays; body grey to bronze." (FishBase 2003, in ISSG 2009).

Juvenile koi carp have a morphological resemblance to gold fish. Koi carp (Fig. 2.1) have the distinguishing factor of having two pair of barbels (whiskers) on sides of their mouth. Natural hybridisation can occur between gold fish (*Carassius auratus*) (Fig 2.2) and koi carp (Pullan and Smith 1987). The resulting koi-goldfish hybrids can be differentiated by the number of pair of barbels, as the hybrids only have a single pair of these. They can also be distinguished by using genetic markers and morphological analysis (Tempero 2004).

Figure 2.1: Koi carp caught from Lake Waikare

Figure 2.2: Gold fish from domestic aquarium

The composition of common carp collected from a brackish water pond in Pakistan was 65.6% moisture, 9.7% of ash (dry weight), 22.7% lipid (dry weight) and 66.2% protein (dry weight) (Ali *et al.* 2005).

Initial growth rate of koi carp in New Zealand is believed to be slow compared to growth rates in other countries (Tempero *et al.* 2006) and its lifespan in New Zealand is considered short compared to that in other countries. The oldest koi carp found in the New Zealand aquatic systems are females that could live up to 12 years and were up to 700 mm long (Tempero *et al.* 2006). Male carp could live up to 10 years. Male koi carp in New Zealand sexually mature at 1.1 years and females at 2.7 years (Tempero *et al*. 2006). However, the sex ratio of matured koi carp in New Zealand is balanced (Tempero *et al.* 2006).

Accidental importation of koi carp to New Zealand is believed to have occurred as a part of a gold fish assignment in 1960s (Section 1.3). Their naturalisation into wild is believed to have occurred during flooding but further intentional spreading may have been due to recreational angling. Koi were recognized as a possible threat to native aquatic system during late 1970s (Pullan and Little 1979). Koi carp were initially declared as ", noxious fish" by the 'Fresh Water Fisheries regulations' (1980). In 1993, Koi carp was designated as "unwanted organism" (MAF Biosecurity 2008) to prevent the further spreading of koi and for its eradication.

Feral common carp in Australia have dominated and degraded all accessible habitats (Kennard *et al*. 2005) and this possibility in New Zealand cannot be avoided. In early 1980s, self-sustaining koi carp populations were detected in Auckland and Waikato regions and soon ecological threats by koi became a concern. Today, koi carp are prominent in aquatic systems throughout the North Island of New Zealand (NIWA 2008) and dominate the fish biomass in Waikato River (Hicks *et al.* 2005). They are believed to be the major cause for the drastic decline in quality of the Waikato River and lakes and wetlands connected to the river (Hayes *et al*. 1992; Chapman 1996). These systems are of significant cultural and recreational values for New Zealanders. Funded research for management strategies for the Waikato River were undertaken in 2001 to identify feasible options for removing koi carp, and thus protect species diversity and improve water quality (Chadderton 2001).

Spawning and growth rate of koi carp

Fecundity of koi carp is very high. Female Koi carp in New Zealand lays about 300,000 to 1,000,000 of eggs in a single year. Out of these 90% are fertilized (Swee and McCrimmon 1996; Brown *et al*. 2005; Tempero *et al*. 2006). Such high fecundity along with durability makes common carp the major biomass in infested aquatic systems. Common carp spawns in dense submerged vegetation in shallow waters (Swee and McCrimmon 1996; Crivelli 1981; Koehn 2004) at water temperature between 15°C and 25°C (Swee and McCrimmon 1996; Crivelli 1981; Stuart and Jones 2002). Hardening and hatching of carp eggs depend on water temperature. Hardening can occur within 15 to 25 minutes and hatching occurs in 3 to 12 days (McCrimmon 1968). Koi can reach high densities in a short time period (Crivelli 1983; Hanchet 1990; Barton *et al*. 2000).

Koi carp are durable fish that can survive poor water quality. The filter feeding technique of common carp, which is often called as "mumbling", causes water quality to decline. During foraging, indigestible material is discharged and this re-suspends the sediments (bioturbation) (Cahn 1929; Roberts *et al*. 1995; Zambrano *et al*. 1999; Saikia and Das 2009). Mumbling softens the original sediment thereby re-suspending the trapped nutrients (Chumchal *et al*. 2005). This results in the loosening of the sediment layer, making it vulnerable to disturbance (Zambrano and Hinojosa 1999). Koi carp can physically cause the disturbance in the sediment layer during their search for food or burrowing activities.

At higher densities, carp increases aquatic turbidity (Cahoon 1953; Crivelli 1983; Drenner *et al*. 1997). Significant degradation to aquatic environment is believed to occur above a threshold density of 450kg/ha (Crivelli 1983, Zambrano 2001). The impact of benthivorous fish can be reversed. If much of the benthic fish biomass is removed from the aquatic system, submerged macrophytes can substitute the zoo planktons, followed by an improvement in water quality (Meijer *et al*. 1999; Jensen 2002). According to studies of Zambrano *et al.* (1999) 70% reduction of common carp biomass was essential for restoration of water clarity.

Macrophytes are important to aquatic system because they reduce turbidity by locking up nutrients and by stabilizing sediments (James and Barko 1994). The increase in turbidity due to the presence of carp (or other species) reduces the light availability for aquatic plants (Meijer *et al*. 1990), which subsequently reduces survival rate of macrophytes as they cannot photosynthesise. In severe situations, the submerged macrophytes may completely vanish from an area (Skubinna *et al*. 1995; Hootsmans 1999). Koi carp also physically uproots submerged macrophytes.

Koi carp could cause eutrophication (phytoplanktons becomes dominant vegetation replacing submerged macrophytes) by increasing the total amount of nutrients such as phosphorous by excretion (Richardson *et al*. 1990) or by suspending nutrients such as nitrogen by disturbing sediment layers (Roberts *et al*. 1995; Chumchal *et al*. 2005). The subsequent increase in phytoplankton reduces the available area and depth for the growth of macrophytes (Sidorkewicj *et al*. 1999). Habitat degradation and consumption of large amount of macro-invertebrates by common carp affects natural balance of aquatic system (Wilcox and Hornbach 1991; Hayes *et al.*1992).

Common carp has a wide range of feeding preferences including macroinvertebrates, zooplankptons, fish roe and seeds (Lammens and Hoogenboezm 1991; Khan 2003). They are opportunistic omnivores with varying dietary preferences between different seasons and locations according to the food availability (Lammens and Hoogenboezm 1991). A regime shift from clear water to turbid water could occur due to the direct and indirect effects of common carp (Scheffer *et al*. 2001; Zambrano *et al*. 2001). The changes can affect native aquatic species, reduce the number of other fish and reduce the recreational value of the aquatic system. The change may also cause navigational problems for boats.

Bioturbation and competition for food can also have a negative effect on waterfowl population (Eriksson 1979; Hanson and Butler 1994). Studies in gravel pits in South-West France showed that Coot (*Fulica atra*) and pochard (*Aythya farina*) populations were inversely proportional to carp numbers (Santoul and Mastrorillo 2003). Increases in carp biomass pose a threat of bioaccumulation. Pesticides from agricultural and industrial run-off may accumulate in carp and pass to higher levels in food chain

2.2. Management Techniques

Biomanipulation, barriers or screens, harvesting, biological control, bioacoustics, poisoning, immunocontraception and genetic manipulation can act as possible management techniques (Cahoon1953; Koehn *et al*. 2000; Stuart and Jones 2002; Sorensen and Stacey 2004; Hicks *et al.* 2006). Physical control methods involve barriers, water level manipulation, radio telemetry, traps and harvesting (Cahoon1953; Stuart and Jones 2002). Electro-fishing can be used for *s*elective fishing of koi carp (Hicks *et al*. 2006). This method uses an electric field to stun the fish, creating an effective koi carp fishing zone. The stunned fish can then be collected by using suitable nets. Fish other than carp are liberated and soon regain consciousness. Chemical management techniques involve the use of pesticides and pheromones (Sorensen and Stacey 2004). However large scale use of pesticides is not possible and also there are no available species-specific pesticide for carp. A non-selective natural chemical named rotenone considered to be relatively safe has been used with success in the USA (Koehn *et al*. 2000). Another management technique is biological control method that involves virus introduction (Matsui *et al.* 2008). However it is extremely difficult to eradicate koi carp from an aquatic system with a single technique. Therefore integrated pest management approach could be the key for successful eradication (Sorensen and Wyatt 2001, in Sorensen and Stacey 2004).

2.3. Possible Uses of Carp

Koi carp makes up the majority of fish biomass in the North Island (Section 2.1) and intensive research is being done to remove it from the aquatic system (Section 2.1). There are no New Zealand studies on the potential uses of koi carp, hence all the koi removed (killed) from the waterway in any eradication programme are wasted.

Fish is a good source of protein and a potential source of raw materials for various industries such as the pet, food, pharmaceutical, and cosmetic industries. It could also be used as the raw material for various energy processes. It is important to identify potential uses for wild koi carp collected as this may help offset the eradication costs. Finding a viable method to utilize the koi will also reduce any potential pollution costs (caused by improper disposal of fish) and thereby increase economic returns. A major factor when selecting an appropriate method is that it must support the aim of eradication. Once the koi population has been substantially reduced, the raw material (koi carp) to support the possible uses will no longer be available. Continued economic viability on any processes identified will be improved if alternative raw materials that can substitute for koi once the population has been reduced can be identified.

Possible products can be categorized as being for human or animal food applications, processed non-food applications and miscellaneous applications. (Figure 2.3)

2.3.1. Food Applications

- ***Fresh fish***

Both marine and freshwater fish are widely acceptable in the human diet and some species are regarded as delicacies. Fish constitutes the majority of vertebrates, with around 20,000 known species. The muscle mass on both sides of the fish makes up the fillet, which is the main part consumed by humans. Proper handling of the captured fish plays a vital role in its acceptance; rough handling may discolour the fillet (Valdimarsson *et al.* 1984; Botta *et al.* 1986). Majority of the body mass of fish is moisture. For example herring (whole fish) constitutes approximately 71% moisture, 18% protein, 8% lipid and 3% ash (Murray and Burt 1979).

Carp is farmed and used as a food source in many countries. However, acceptance of carp in many Western and European countries has recently decreased due to its environmental impacts and bony nature (Kirkagac and Demir 2004; Mutsuro *et al*. 2005).

- ***Dried Fish***

Dried fish is obtained by removing water by physically (drying) or by immobilising the water using humectants such as sugar, common salt and glycerol. The humectants lower the water activity, so free water is not available for microbial and chemical reaction. The process involves a combination of vapour, molecular and liquid diffusion, which causes water within the fish to move to the surface (Doe 1998). Simultaneously transfer of solutes can occur from outside to the inside of fish (Jason and Peters 1973), resulting in sugar and salt being deposited on the surface of some dried fish products.

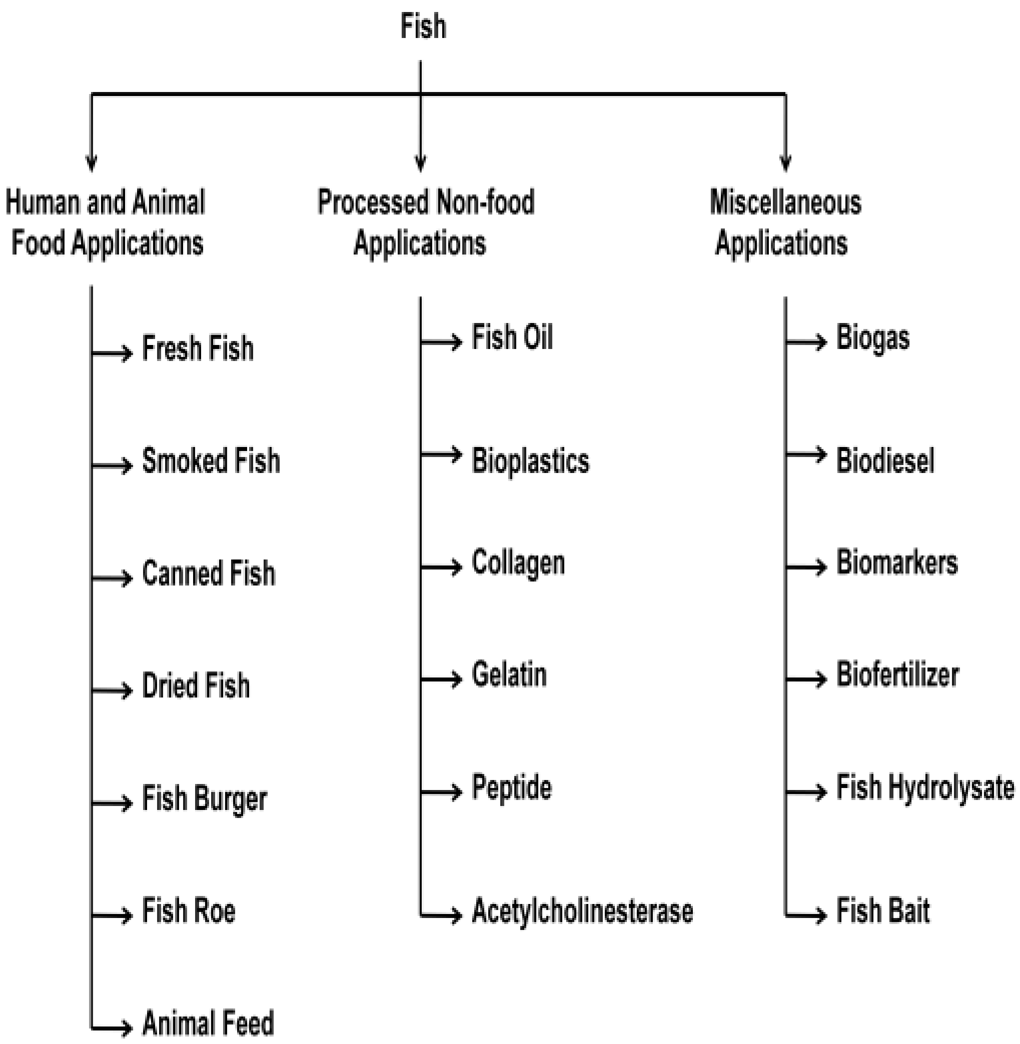

Figure 2.3: Possible uses of fish

Drying fish can be achieved using sun drying or kiln drying. Fish products can be dried, salted and dried, fermented or dried with sauce. During drying, heat and mass transfer occurs. The main factors influencing drying are humidity, air temperature and air speed (Doe 1998). Whole fish, fish powder, dried fillets are some of the products that can be produced by drying fish. These products are widely recognized to be highly nutritious and a source of good-quality animal protein, essential fatty acids, micro-nutrients, vitamins and minerals.

The possibility of microbial contamination is one of the main disadvantages of dried fish. Water activity of the product is one of the main determinants in microbial spoilage. Technologists and food scientists use sorption isotherms, which show the relationship between moisture content and water activity in a hygroscopic substance, to predict shelf life and texture (Doe 1998). Dried bonito sticks from Japan are an example of a well-known dried fish product. They have extremely low moisture content, are very hard and have a long shelf life (Sakakibara *et al*. 1990).

Although dried fish is highly nutritious, it is not as viable as other options for koi carp captured in New Zealand. These fish may contain toxic trace elements (Metcheva *et al*. 2010) and koi carp often have a muddy taste due to their feeding technique and habits. Developing the process probably requires high management, marketing and financial costs. Lastly, dried fish is not a common product in New Zealand and market studies on its acceptability in New Zealand would need to be done. This would increase the lead-in time for marketing a dried fish product.

- ***Canned Fish***

Canning food is a common method for extending shelf life and also it increases acceptance of fish. Fish used for canning must be fresh or well preserved. It should have an acceptable general appearance, firm flesh, no off-odours, not damaged and have no parasitic infestations (Footitt and Lewis 1995). If the starting material is frozen fish, it must be thawed carefully. Canning involves removing the head, skin and scales (if needed), pre-cooking or smoking, quality checks and then packing before high heat treatment (Footitt and Lewis 1995). Studies have shown that canned silver carp was more acceptable than fresh silver carp because many hard bones had been softened by the processing (Woodruff 1978).

Canned bighead carp has potential as a freshwater fish product. The product had less than 1% crude lipids and was a light in colour (Freeman 1999). Canning carp can reduce the muddy taste and calcium from bones is retained.

Certain bacteria can decarboxylate the histidine present in fish, leading to histamine poisoning if canned food has not been appropriately processed and stored (Taylor *et al*. 1989; Morrow *et al*. 1991; Kim *et al*. 2003).

Cooking protein foods usually leads to protein and water loss and increase in fat. This has been reported for steamed and canned Albacore tuna (*Thunnus alalunga*)

(Castrillon *et al*. 2006) and may also occur for canned carp. This gives overall product loss. The market acceptability of canned wild koi carp has not yet been studied in New Zealand, making production and viability of this product in New Zealand risky.

- ***Smoked Carp***

Smoking food is an ancient preservation method. Smoked product has a longer shelf-life than sun dried, and it also tastes better. Curing before smoking can increase both the flavour and shelf-life of the food. The curing process involves rubbing dry salt to meat or fish, immersing the meat or fish in brine solution, or injecting salt solutions into the flesh. Recently, herbs and spices are also added during the curing (Doe 1998).

There are three smoking methods: cold smoking, hot smoking and hot smoke / drying (Doe 1998). Cold smoking is usually done below 30°C whilst the temperature for hot smoking and hot smoke /drying is about 80°C (Doe 1998). The process can be done using a slow burning hard wood fire, a charcoal fire topped with hard wood, a pan with hardwood heated by using butane or natural gas, or an electric heating element with hard wood. The carboxylic acids, ketones, phenols and aldehydes in wood smoke play a vital role in the smoking process (Doe 1998) because they infuse into the food and provide the smoke flavour.

Smoking reduces the water content of the food because hot air from wood smoke partially dries the food. The characteristic colour of smoked food comes mainly from constituents in the wood smoke sticking to the food surface (Doe 1998). The main advantages of smoking include increased flavour, better preservation by reducing moisture content and number of microorganisms, and concentrating the nutrients. Process factors such as temperature, time, characteristics of the raw material and type of wood used affect final product quality.

A satisfactory smoked carp fish can be obtained by curing the fish in 15% NaCl at 4°C for two days, desalting the surface for 30 min and partially drying at room temperature before cold smoking at 36°C to 41°C for 6 hours (Hassan 1988). The product made by using a 24% brine solution was not as acceptable, indicating that process steps need to be controlled to obtain a good smoked carp. Carp could be smoked as whole fish or as fillets. However the possibility that carcinogens in smoked products may accumulate in the product (Ferguson 2002) has reduced its acceptability.

Factors decreasing the potential for making a smoked product from wild koi carp included: the likelihood that it will have a muddy flavour; it may contain toxic elements from the environment, and the perceived carcinogenic potential from the smoking process. Smoked fish can be vehicle for salmonellosis (Olitzky *et al.* 1956). Also, thiaminase present in some smoked fish products can destroy the thiamine in humans and hence present a factor for malnutrition (Melnick *et al*. 1945). In New Zealand, several cases of scromboid poisoning (a food-born chemical intoxication) have been associated with eating smoked fish (Fletcher *et al*. 1998). Lastly, canned fish is not a popular item in New Zealand diets, where there is plenty of fresh fish. Thus, the disadvantages of a smoked carp product seem too great to make this a potential product from wild koi carp.

- ***Fish Burgers***

Foods that can be prepared and served quickly are known as fast foods. Many people in countries such as the United States consume a lot of fast foods like burgers, food wraps and French fries. Patties, which are the vital part of burgers, can be made from vegetables, chicken, pork, beef, turkey or combinations of these. Recently, fish patties have been introduced.

Research has shown that flavour, colour, appearance and overall acceptability of fish patties are not affected by the size (weight) of the fish used. However patties prepared from fish in the 250-500 g and 501-750 g range had better texture than those in the 750-1000 g groups (Sehgal *et al.* 2008). Another research studied the shelf life of patties made from minced fish with modified tapioca starch, sodium sorbate or sodium chloride, structured vegetables and antioxidants (Bello and Pigott 1978). The fish patties were stable over four months at 25^{o}C and the product had a good amino acid profile and protein efficiency ratio.

The nutritional quality of fish patties made from Indian major carp was affected by the effect of type of extenders and fish weight (Sehgal *et al.* 2008). Cooking decreased the crude protein and increased total lipids and total soluble sugar content. Cooking yields were higher if larger fish were used and if the extender was corn flour. Recent studies on shelf life of fish patties (Sehgal *et al.* 2008) supported the observations made by Bello and Piggot (1978).

Preparing fillets from carp is a tedious process due to its bony structure. There is no information on market viability and acceptance of koi carp as fish patties. Much tastier fish such as hoki and pollock are already being used for fish patties and carp do not seem to be a better replacement for these fish. Hence, the potential of using wild koi carp for patties does not show much promise.

- ***Fish roe***

Fish roe (or eggs) is a delicacy. The best known, caviar, is obtained from sturgeon (Logan *et al* 1995). Making caviars from fish roe involves grading, sorting, singling-out, salting and curing (Bledsoe *et al.* 2003). Fish roe can also be sold as a frozen product. Fish roe contains omega-3 and omega-6 fatty acids, sodium, protein, and small amounts of fat and cholesterol (Gussoni *et al*. 2006). The lipid content is affected by the species (Body 1989). Common carp contains the omega-3 fatty acids eicosapentaenoic acids (EPA) and docosahexaenoic acid (DHA) (Yang *et al.* 1994). This makes fish roe a nutritious food. Many delicacies are also made from fish roe.

Koi carp in New Zealand are very fecund (Section 2.1) and this high fecundity proves it to be an excellent source of fish roe.

Presence of several unfavourable factors makes using fish roe from koi carp non-viable. The high fat content of fish roe means preservation methods must limit fat oxidation and rancidity. Toxic trace elements have been detected in fish roe from six commercial species of fish in New Zealand (Bekhit *et al.* 2008). Even though studies have not been conducted on fish roe of koi carp in New Zealand, there is a possibility that they may also contain toxic trace elements. These factors indicate that fish roe is not a suitable option. Also, as roe are only a small part of the fish, other processes will have to be identified to use the waste.

- ***Animal feed***

Pets play a major part in modern family life. Seventy percentage of American homes have at least one pet and the 2005 pet populations were estimated to be 81.4 million cats and 63 million dogs (Euromonitor 2005). Specialty pets such as rodents, fish, birds and other animals account for a further 200 million pets.

Increasing attention is being paid to meet the nutritive needs of pets, which have different nutritional requirements. The pet food industries consider these requirements when formulating feeds, which are made by mixing various raw materials. Annual sales in the United States have risen to $53 billion (Kvamme 2006) and the pet food industry has become a specialized sector.

There are many studies on pet nutrition. For example, some breeds of dogs may require dietary taurine (Fascetti *et al*. 2003). Supplementing cat and dog diets with β-carotene and lutein will enhance their immune system response (Chew and Park 2004). Consumable forms of omega-3 fatty acids are important in dogs and cats diets (Aldrich n.d). Both cats and dogs require metabolic glucose and obtain it by converting dietary proteins to metabolic glucose in the gluconeogenic pathway. Fish oils can be added to the surface of the extruded and dried pet food that are extruded and dried and dogs find diets that include meals, hydrolysates and some components of salmon very palatable (Folador *et al*. 2006).

Fish are excellent sources of protein and can be used to prepare aquafeed (Dabrowski and Kozak 1979). Koi carp has potential as a cheap source of protein and dietary omega-3 fatty acids for the pet food industries. To obtain a stable, convenient product for formulated feed, the fresh fish must be preserved. One process is rendering, which produces oil and fish meal. The fish oil requires very little or no preservative against oxidation (Aldrich n.d). The high bone content of Koi carp make it a good source of calcium for animals such as pigs (Malde *et al.* 2010).

2.3.2 Processed Non-Food Applications

- ***Fish Oil***

Dietary fish oil from oily fish contains high amounts of omega 3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These accumulate in fish because they consume microalgae that produce these fatty acids. The high EPA and DHA levels in fish oil account for its popularity and health benefits, and is widely used as a dietary supplement and medicine.

Dietary imbalance is considered to be the major cause of many diseases that affect humans. Improper balance of ratio of dietary omega 6 to that of omega 3 is common in western. The ratio of omega 6 to omega 3 should be 3:1. Studies show that omega 3 fatty acids plays an important role in protection against cardiovascular diseases. However it is not clear if it is achieved by modifying the risk factors of cardiovascular diseases through mechanisms not directly related to lipids or by the mechanisms directly related to anti-atherogenic functions of these omega 3 fatty acids (Nestel 2000).

The benefits of fish oil consumption include reducing post-prandial lipemia, reducing concentration of triacylglycerol-rich lipoprotein and remanant proteins, and increasing concentrations of HDL_2- cholesterol. Fish oil also enhances endothelial functions, improves arterial compliance, reduces risk of some cancers, and reduces risk of Alzheimer disease and many other mental illnesses (Phillipson *et al*. 1985; Nestel 2000; Kimura 2002). Including fish oil in diets of animals farmed for human consumption could be beneficial. For example, feeding algae or fish extracts increased DHA level in chicken muscle seven fold, in eggs three to six fold, and in fish twenty fold. However, DHA levels in beef were less than doubled (Bourre 2005).

Carp is a good source of the omega 3 fatty acids EPA and DHA and have less cholesterol than salmon and tuna. The EPA and DHA are balanced (Yang 1994; Crexi 2010). The amount of omega 3 fatty acids in two different carp is given in Table 2.1.

Table 2.1: Omega 3 fatty acid content in different carp

Type of carp	EPA (g/100 g fresh fish)	DHA (g/100 g fresh fish)
Silver carp	0.187	0.246
Big head carp	0.229	0.325

There were no significant differences in the quality of refined carp oil obtained from fish meal processing and from ensilage (Crexi 2010). Fatty acids such as oleic, palmitic, palmitoleic, linoleic and linolenic acids made up approximately 70% of the total fatty acids in the both the crude and refined oils. The refined oil had n-3/n-6 ratio of 1.05 (Crexi 2010).

There is increasing international demand for fish oil. The product does not have the disadvantage of having a muddy taste because this is removed during processing. Fish oil obtained from koi carp could be a possible source of nutrients in various animal feeds and is therefore a product with high market viability and demand.

- ***Bioplastics***

Plastics are non-biodegradable polymers of high molecular mass made from petroleum-based chemicals. Plastics are convenient and durable and hence millions of tonnes of materials made from plastic are manufactured on a daily basis throughout the world. Plastics have a deleterious effect on the environment because they are made from petrochemical, which are non-biodegradable. Disposing of such plastics has become a major pollution issue. Research efforts to develop biodegradable polymers have resulted in the development of bioplastics (Swift 1992), derived from renewable sources of biomass. Bio-composites are being developed from biofibres with renewable resource-based biopolymers such as poly actides, starch plastics, cellulosic plastics, bacterial poly esters and soy-based plastics (Mohanty *et al*. 2002). Many vegetable proteins such as wheat gluten, corn zein, soy protein and animal proteins such as keratin, gelatin, collagen and myofibrillar proteins can be used to manufacture bioplastics (Pommet *et al.* 2003).

Carp can be a source of keratin as both epithelial and mesenchymally derived tissues from carp expresses keratins. Carp is also a source of collagen, which can be obtained from fish skin using an acetic acid extraction method. This collagen can be used to manufacture biofilms (Sullivan *et al.* 2006). Carp have flexible cycloid scales that are thin, large, round or oval in shape (DPI 2005). Protein in scales from carp could be used to manufacture bio-plastics.

However, there is no published information on using scales from koi carp to manufacture bioplastics. As lot of research and development needs to be done, this option was not considered as viable.

- ***Fish Collagen***

Collagen is the most abundant fibrous protein in mammals and makes up the major part of tendons, ligaments, organic matrix in bone and dentin. It is also present in skin, arteries and cartilage. Collagen type I is the most abundant of all the collagen types and confers mechanical stability, strength and toughness to a range of tissues (Fratzl 2008). Collagen is most commonly extracted from bovine or porcine and used in functional foods, cosmetics and pharmaceuticals. The crisis caused by bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) reduced use of collagen and collagen-based products derived from animal skin and led to a shift to fish collagen (Venugopal 2008). Fish muscle and skin collagen have a significantly higher content of essential amino acids but lower hydroxyproline content (Alasalvar and Taylor 2002).

Fish processing wastes such as filleting frames, scales and skin are a good source of collagen. Type I collagen makes up 90-95% of extracellular matrix, along with other non-collagenous proteins (Venugopal 2008). Collagen could be extracted from fish scales by decalcification and disaggregation followed by pepsin digestion. These collagens are heterotrimers with a denaturation temperature of about 45°C, which is much lower than land-animal collagen. Collagen could be isolated from fish bones and used in cosmetic materials in which the process involves acetone treatment, followed by decalcification and protease treatment, and finally filtered, lyophilised and collagen peptides separated via ultra-filtration (Venugopal 2008).

Carp is a rich source of collagen. Carp (*Cyprinus carpio*) skin can yield 41.3% of acid-soluble collagen but scale and bone only have a 1.35% and 1.06% (dry weight basis) acid-soluble collagen respectively (Duan *et al*. 2009). The acid-soluble collagen from carp skin, scale and bones are all type I collagen, with a denaturation temperature of around 28°C (Duan *et al*. 2009). Type I collagen from the carp scales and bone (skull) consist of two different molecular forms. Most is (alpha 1)2 alpha 2, with alpha 1 alpha 2 alpha 3 as a minor constituent (Kimura *et al*. 1991). An immunogenic study of carp collagen indicates it contains at least two different antigenic determinants resembling calf collagen (Wolff *et al*. 1970).

Even though carp is a good source for collagen extraction, the extraction is laborious, expensive and requires technically skilled labour. Collagen extraction only uses only specific parts of the fish, which does not suit the aim of finding an application that uses the whole fish in an economic way.

- ***Fish Gelatin***

Collagen is present in animal skin, bone and connective tissue. The biopolymer gelatin is produced by partial hydrolysis of fibrous protein collagen. Gelatin is used widely in food, cosmetic, pharmaceutical industries and also in photographic applications (Sadler and Horsey 1987; Johnston-Banks 1990; Pollack 1990; Rao 1995; Schrieber and Gareis 2007). Most of the gelatin is obtained from cows and pigs although some religions do not allow consumption of these animals. Bovine spongiform encephalopathy crisis has been attributed to feeding ruminant animal-based products to ruminants and hence helping the transfer of the disease-causing agent prion. This crisis, along with the religious concerns and the increasing demand for gelatin has resulted in an intensive research to find new sources for gelatin or alternative products.

Fish gelatin is considered a possible alternative for bovine gelatin (Rustad 2003; Kim and Mendis 2006). Intensive research has been done to use collagenous fish waste to produce gelatine. This also reduces the pollution caused by fish waste (Gilsenan and Ross Murphy 1999; Holzer 1996; Nagai and Suzuki 2000; Badii and Howell 2006; Wasswa *et al.* 2007).

Collagen is converted into gelatin by heating it in alkali or acid. The main stages are pre-treating the raw material, extracting the gelatin, and then purification and drying. Depending upon the pre-treatment method, two different types of gelatin can be produced from fish. Type A gelatine, produced from acid-treated collagen, has isoelectric point of pH 6 to 9; type B gelatine, produced from alkali-treated collagen, and has isoelectric point of approximately pH 5 (Stainsby 1987).

For food applications; viscosity, gel strength, gelling and melting points are the most important properties of gelatin. Several factors such as gelatin solution concentration, gel maturation temperature and time, salt content and pH affect these properties (Karim and Bhat 2009). In the last decade, extensive research has been done on the physicochemical and functional properties of fish gelatin (Choi and Regenstein 2000; Dickinson and Lopez 2001; Gudmundsson 2002; Jamilah and Harvinder 2002; Muyonga *et al.* 2004; Badii and Howell 2006; Surh *et al.* 2006; Zhang *et al.* 2007; Zhou and Regenstein 2007).

Collagen from fish skin has a wider range of amino acid composition than mammalian collagens (Karim and Bhat 2009). It has lower hydroxyproline and proline and higher serine and threonine levels (Balian and Bowes 1977). Fish collagen denatures at a lower temperature than mammalian collagens due to its lower imino acid content (Grossman and Bergman 1992). Fish gelatins have impressive film forming properties (Avena-Bustillos *et al.* 2006). Water vapour permeability of cold water fish gelatin films is significantly lower than films from warm water fish gelatins (Avena-Bustillos *et al.* 2006). Choi and Regenstein (2000) claim that fish gelatin releases better aroma and hence can provide new opportunities to the product developers. Fish gelatin has a low gelling temperature than mammalian gelatin (Norland 1990) and can be used for processes such as microencapsulation of pharmaceutical additives and vitamins (Karim and Bhat 2009) and in frozen products that are readily consumed after being removed from the refrigerator.

Even though fish gelatin is a useful product, it makes up only 1% of the annual world gelatin production (Arnesen and Gildberg 2006). The main factor contributing to the low annual production of fish gelatin is the inferior rheological properties of the gel produced compared to gelatin from land mammals (Norland 1990; Leuenberger 1991). The high production cost, low yield and variable quality of fish gelatin makes it a less preferable product for manufacturers in current situation. Therefore, producing fish gelatin from koi carp in New Zealand probably will not be economically viable.

- ***Acetylcholinesterase (AChE)***

There are high amounts of the enzyme AChE in carp muscles and brain (Golombieski *et al.* 2008). This enzyme hydrolyses the neurotransmitter acetylcholine at cholinergic synapses and neuromuscular junctions (Bigbee *et al.* 1999). The AChE, present at the myoneural junction is the true ChE, and hydrolyses acetylcholine to re-establish the junction and prepare it for additional signals (Whittaker 1983). Myoneural AChE is present in red blood cells (Soetan *et al.* 2010). Patients suffering from Alzheimer's disease (AD) show reduced AChE activity in the hippocampus and cortex (Fishman *et al.* 1986; Hammond and Brimijoin 1988). The decreased AChE activity in AD patients, with concurrent loss of cognitive function demonstrates the role of AChE (Hammond and Brimijoin 1988). Aluminium toxicity, through long-term post natal exposure to aluminium, decreases AChE activity (Ravi *et al*. 2000). AChE is also highly correlated with neurite outgrowth, cell survival and growth (Appleyard 1992). Several studies have shown that chronic stress leads to depression, anxiety and learning and memory impairment. Restraint stress is concurrent with decreased AChE activity (Sunanda *et al*. 2000). Park *et al*. (2004) propose that AChE also plays a major role in apoptosome formation (protein structures formed during apoptosis). AChE reactivators show favourable effects in experimental tetanus and also clinically in human beings (Aleksevich 1977).

The highest AChE activity in carp (*Cyprinus carpio* L.) is found in serum (900 U/L). Brain (1110 U/L), heart (90 U/L), and trunk muscle (35 U/L) have lower AChE levels (Szabo *et al*. 1992). Korami and Eric (1998) showed that 85% of AChE extracted from common carp brain was in soluble form and 15% was membrane-bound. The presence of AChE in fish has been used for bio monitoring and as a diagnostic tool for various insecticides (Dembélé *et al*. 2000), pesticides (Nemcsok *et al*. 1984; Szabo *et al*. 1992) and heavy metal pollution.

It would be beneficial if this useful enzyme could be extracted from carp brain and muscles, purified and used for any medical purposes. AChE can be extracted using isotonic sucrose solutions (Sung and Ruff 1982), but yields are low. For commercial purposes, methods to extract large amounts of the enzyme need be developed. Researches on compatibility of carp AChE in the human body also need to be done before it can be used as a pharmaceutical product. This will involve a lot of funding and also will not use whole carp, and thus do not help utilise the fish that are caught by any eradication programme.

- ***Fish Peptides***

Peptides are short polymers of alpha amino acids. The link connecting one amino acid to the next is called a peptide bond. Peptides are being widely used in medical diagnostics and as therapeutic agents. Antimicrobial peptides act as the first line of mucosal host defence by exerting a broad spectrum of microbicidal activity against foreign pathogenic microbes (Cole *et al*. 2000). A new antimicrobial peptide called Pleurocidin isolated from skin secretions of winter flounder (fish) showed broad microbicidal activity against bacterial and fungal clinical isolates and has potential as a beneficial therapeutic agent (Cole *et al.* 2000). Fish peptides can also lower the blood pressure. An enzyme called ACE (angiotensin converting enzyme) converts angiotensin I to angiotensin II, a compound that increases both fluid volume and degree of blood vessel constriction. Fish peptides inhibit ACE, thus reducing fluid volume and relaxing arterial walls, and in turn lowering blood pressure levels.

The different carp peptides, their specific uses and compatibility in human body have not been studied in detail. Therefore, much more research is needed before carp peptides can be used in medical applications. Also, the infrastructural requirements and skill labour will be high. Our aim is to produce a viable product that can either act as a substitute for other products or to produce products that can be removed from the market once the raw material source is used up. Fish peptide is not suitable to be produced from koi carp in New Zealand.

2.3.3. Miscellaneous Uses

- ***Biomarkers***

A biomarker is any biological compound that has a biological response to a chemical or chemicals and thus measures attributes such as time of exposure, current state and toxicity. There is a growing interest in using of biomarkers for environment assessment and biomarkers have a wide range of uses including pollution indicators (Peakall 2004), for assessing the health and environmental impacts of chemicals (Travis 1993) and even for detecting land mines.

Aquatic system plays a very important role in the life of humans, plants and animals. To resolve the problems of pollution, it is necessary to determine the extent of pollution affecting each aquatic body. Fish can be used as a biomarker to analyse the level of pollution in an aquatic system. Concentration of chemicals, metals and insecticides can be determined by this process. For example, the concentration of various metals in the Nasser Lake was determined using *Tilapia Nilotica* as a biomarker. The maximum concentration of more than half the metals studied were in the fish scales (Rashed n.d). The principle of the whole process was to measure and analyse the excess amount of metals in the fish tissues. Common carp is considered and widely used as an excellent biomarker to study the environmental quality. The environmental quality of two sites was determined using common carp as a biomarker. The results indicated that MT, AChE and TBARS in the liver were the most sensitive biomarkers to the pollution (Falfushynska and Stolyar 2009). Carp exposed to diafuran (insecticide) become lethargic or immobile because AChE activity in the brain and muscle is inhibited (Golombieski 2008). This characteristic feature, along with other behavioural changes, allows carp to be used as an early biomarker of insecticides such as diafuran. Caged juvenile carp were used to study bioaccumulation of micropollutants in the aquatic ecosystems in Belgium. Assessing pollutant accumulation in the fish tissues, condition factor, weight change, hepatosomatic index, Ache activity and blood biochemical parameters were related to the metal load in the ecosystem (van der Oost 1998; Bervoets *et al.* 2009).

Using carp as live biomarkers pose a threat of accidental introduction to waterways that have not previously been invaded by carp. Furthermore, the legal designation of carp in New Zealand demands the fish is killed as soon as it is caught. Some native fish species may be useful biomarkers. Therefore, using carp as a biomarker in New Zealand is not a viable option.

- ***Biogas***

Wastes from food industry are a major environmental contamination source. Various studies have investigated ways to utilize these waste materials and to turn those into useful products (Guerard *et al.* 2001). Most parts of fish are normally wasted and add to environment pollution (Kristinsson and Rasco 2000). These parts can be the raw material in biogas plant. Biogas is a mixture of carbon dioxide and methane produced by anaerobic digestion of organic matter. Biogas is an alternative fuel source and is widely accepted as being a comparatively clean fuel. Biogas plants help control pathogenic diseases, which are mainly caused from decayed organic matter.

Biogas can be obtained from cattle manure, by-products of aquaculture, piggery waste waters, agro-industries and urban wastes (Lo *et al*. 1986; Lo and Liao 1986; Ng and Chin 1987). In East African regions, biogas from fish wastes is an approach for controlling pollution and for obtaining energy. The biogas can either be burnt directly or used to produce electricity. Research by Mshandete *et al.* (2004) showed that co-digestion of fish wastes and sisal pulp increased biogas yield and digestibility of the materials. Digestion of sisal pulp produced 0.32 m^3 CH_4/kg volatile solids at total solids loading 5% and digestion of fish produced 0.39 m^3 CH_4/kg at a total solids loading of 5%. However, co-digesting two parts sisal pulp with one part fish waste increased biomass yield to 0.62 m^3 CH_4/kg volatile solids at a total solids loading of 16.6%, increasing methane yield by 60% to 95% (Mshandete *et al.* 2004).

Koi carp captured from the aquatic systems of New Zealand could be used to produce biogas, either as whole fish or fish parts. The fish material can also be co-digested with other organic wastes.

However, studies by Heubeck (2008) indicate that biogas is not a viable option in New Zealand due to the technology barrier and economic barriers. The cost of logistics (planning, procurement, transportation, supply and maintenance) for biogas is high (Heubeck 2008).

- ***Biodiesel***

There is an increasing interest in alternatives to petrochemicals for energy. One among the alternative is biodiesel. Biodiesel is animal fat or vegetable oil based diesel fuel that consists of long chain alkyl esters (Mittelbach 1996; Fukuda *et al.* 2001). They are non toxic and biodegradable. The main advantage of this fuel is that the raw materials used for its production are natural and renewable (Marchetti *et al.* 2007).

Kato *et al.* (2004) evaluated the possibility of using ozone-treated fish waste oil as diesel. Characteristics such as density, pour point, heating value, flash point, distillation test and sulphur content were measured for direct use of the oil in diesel engines. Absence of soot and sulphur oxides, polyaromatic and carbon dioxide emissions indicated that the oil had beneficial properties compared to methyl-esterified vegetable oil waste. The oil was suitable for diesel engines, especially at low-temperatures. There are reports on the production of biodiesel from waste salmon oil and also from vegetable oils like soybean oil, sunflower oil (Antolin *et al.* 2002; Cao *et al.* 2005). However, a preliminary economic analysis by Cao *et al.* (2005) showed that the cost of biodiesel production from salmon oil was double to that produced from soybean oil.

Koi carp may be a potential raw material for the biodiesel production. However, lack of any research on using koi carp as a starting material for biodiesel is an eminent disadvantage.

- ***Fish Hydrolysate and Silage***

Fish hydrolysate (sometimes called fish protein hydrolysate or FPH) is produced by enzymatic or acid breakdown of the fish proteins into smaller peptides and amino acids (Wright 2004). It has been used as organic fertilizer, as animal and fish feed, and in the medical sector. Large yellow croaker fed diets supplemented with 10 or 15% FPH significantly grew faster and had higher immunity (measured as lysozyme activity, complement C4, IgM and complement C3 levels) than croaker fed unsupplemented diets (Tang *et al*. 2008). Picot *et al*. (2005) reported that various fish protein hydrolysates were significant growth inhibitors on two human breast cancer cell lines. FPH may act as an intestinal health food. Adding 1 mg/ml FPH made from pacific whiting fish increased cell proliferation and cell migration by three-fold (Fitzgerald *et al.* 2005). Adding 25/ml reduced gastric injury by almost two-thirds, indicating FPH is an inexpensive way for preventing the injurious effect of certain drugs and treating ulcerated bowel (Fitzgerald *et al*. 2005).

There are reports on enzymic hydrolysis of carp. Optimum conditions for using the commercial proteinase alcalase to produce FPH hydrolysate from grass carp skin were 84min at 60°C and pH 8.25, using a enzyme/substrate ratio (as % v/w of minced skin) of 1.70% (Wasswa *et al*. 2008). The freeze dried hydrolysate (FDH) contained 90% protein, was very highly water soluble, and had good water holding, oil binding and emulsifying properties (Wasswa *et al*. 2008).

Fish silage production is similar to fish hydrolysate and involves liquefying the whole fish or parts of fish using indigenous enzymes, sometimes with added acid. The enzymes hydrolyse the proteins while the acid increases their activity and prevents bacterial spoilage (Tatterson and Windsor 2006).Fish silage is used as an organic fertilizer and animal feed. Fish oil can be extracted from the silage. Adding 10% silage (g silage per kg dry matter) increased the growth rate of pigs (Green *et al*. 1988).

The fish silage process is a simple process and can use whole fish or fish wastes. The acid added for preservation is the main cost. More research is needed to determine which acid or acid combination is the best for effective ensiling.

- ***Biofertilizer***

Using fish fertilizers dates back to ancient Egypt, where farmers used fish to increase soil fertility. The early indigenous people of North America used to put whole fish in the soil and plant their crops over the top. The idea of fish fertilizer was lost with the introduction of chemical fertilizers to provide the nitrogen, phosphorous and potassium. Recently studies have focused on the benefits organic fertilizers on soil and plant health, increasing the awareness and use of materials such as fish fertilizers.

The major drawback of synthetic fertilizers is that they provide too much nitrogen to the plant, which can make the plant be more vulnerable to weather fluctuations, insects and diseases (GPB 2010). Another disadvantage is that the nutrients are in salt form; once dissolved in water, these salts can evaporate, gasify or leach out via runoff. Nitrogen runoff into water systems causes eutrophication. Fish fertilizers supply a controlled level of nitrogen beneficial for producing chlorophyll and maintaining plant health. Another major advantage of using fish fertilizers is that they stimulate the commonly found soil microorganisms such as nitrifying bacteria. The increase in microbial number produces more available organic nitrogen for root uptake. Having a vital soil ecosystem can improve the vitality of the crop and increase six-fold (Great Pacific BioProducts 2010).

Synnes and Opstad (1995) compared the efficiency of chemical fertilizers and salmon fish silage fertilizer in Western Norway. The silage, which had 25.3% dry matter, was found to be an effective fertilizer for Italian ryegrass.

Using carp as a commercial organic fertilizer in the form of hydrolysate or silage needs further research on factors such as the unpleasant odour, spreading of pathogenic organisms, and effect of crop yields. Biofertilizer from koi carp is a potential marketable product. However there is a need of intensive research on its effects and this might be a time consuming procedure.

- ***Fish Bait***

Baits are used to attract animals or birds, in large-scale for fishing and for trapping of pests. Baits are mainly made from various foods or food combinations.

Fishmeal wastes were used as a bait to attract economically important flies of agricultural crops, such as moringa fruit fly (*Gitona* sp.), sorghum shoot fly (*Atherigona soccam* Rond.), and the Indian uzifly (*Exorista bombycis*) attacking mulberry silkworm (Mohan *et al*. 1993). The fishmeal was dried, powdered, moistened with a piece of cotton dipped in an insecticide and placed in fields and the observations indicated that the female flies were attracted and nearly half of them were with eggs. Fish baits are useful in luring ants into traps (Perfecto and Vandermeer 1996). The bait shape and size could affect capture rate of fish; industrially-prepared pellets seemed to have benefits over baits in block form (Kutka *et al*.1992).

Market viability for large scale utilization of koi carp needs to be investigated. There are better options available for possible use.

2.4. Rendering

Only about one-third of a slaughtered animal or fish is edible. Skins and pelts can be used for leather but stable products need to be made from the remaining 40 – 50% to ensure viability and reduce pollution. Until late nineteenth century, most of this material was wasted (Meeker and Hamilton n.d; Swan 2000). Meat industries then realized the possibilities of manufacturing by-products (or co-products) such as meals and fats (Meeker and Hamilton n.d; Swan 2000). The process used to manufacture by-products such as meals and fats is called rendering (Aldrich n.d; Meeker and Hamilton n.d; Swan 2000).

Recently, the use of food wastes as animal feed is an option of increasing interest, as it provides environmental and public benefit along with reducing the cost of animal production (Samuels *et al*. 1991; Westendorf 2000).

Offal and wastes from the fish industries could be used as a feed ingredient in the same way as the wastes from animal processing. It represents a beneficial source of high-quality protein, nutrients and energy (New 1996; Gabrielsen and Austreng 1998).

Raw material for rending usually has up 50-60% water, 10-20% protein, 10-20% fat and some minerals. The rendering process can produce a wide range of products such as meals for animal feeds (poultry, ruminants, fish and pets) and fertilizers and fats that can be used in human and animal feeds or as a feedstock for further processing (Fig. 2.4). It is estimated that 25% of the total US production of rendered materials is utilized in the pet food industries (Aldrich n.d; Swisher 2005). This estimate clearly indicates the mutual reliance of rendering and pet food industries.

The rendering process

This process involves separating water, fat and protein from the raw material and producing stable products. To ensure product safety, the industry has developed best practice procedures involving HACCP (Hazard analysis and critical control point). In the United States, these are also overseen by the FDA (Food and Drug Administration) (Meeker and Hamilton n.d).

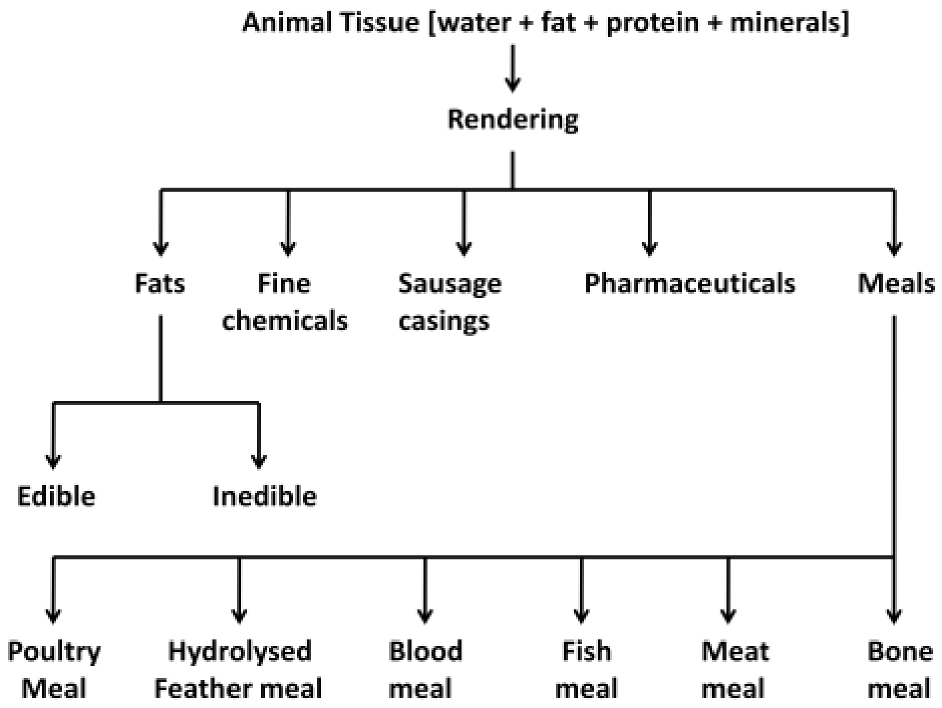

Figure 2.4: Rendering By-products

Food industry wastes are a massive environmental contamination source. Research has been carried out to develop techniques to convert these wastes into useful products (Perea *et al*. 1993; Kristinsson and Rasco 2000; Guerard *et al*. 2001). The meat industry produces enormous amounts of waste. For example, 24,500 to 27,220 tonnes of raw material are generated in the United States annually (Meeker and Hamilton n.d). Modern products like table-ready meat products and pre-packed products have increased amount of raw 'waste' material available. Similarly more than 50% of the remaining material from the total fish capture is not used as food. This involves about 32 million tonnes of fish waste (Kristinsson and Rasco 2000). These organic materials contain many microorganisms and various contaminants. Hence they need to be processed as soon as possible. Otherwise, treatment costs escalate and there is increased possibility of developing unpleasant odours, spreading pathogenic diseases and contaminating water supplies.

Rendering offers a safe system of handling these raw materials, with the possibility of producing economical products whilst maintaining the fundamental requirement of environmental protection and disease control (Meeker and Hamilton n.d; Swan 2000; Kinley *et al*. 2010). Rendered products are considered as safe, as the process eliminates microorganisms and pathogens (Meeker and Hamilton n.d). A study across two years (Trout *et al.* 2001) showed that the heat used effectively removed all pathogenic bacteria (Table 2.2).

A major setback to using the rendered products in animal feeds was the outbreak of bovine spongiform encephalopathy (BSE or mad cow disease) in the 1980s. This is caused by prions; an infectious agent composed primarily of protein, and induces a mis-folded protein state. This syndrome is related to Creutzfeldt Jakob disease (CJD) in humans (Meeker and Hamilton n.d), a fatal syndrome.

Prions are not destroyed by current rendering processes, although their infectivity is reduced (Taylor *et al*. 1995). Even the solvent extraction process used by renderers in Britain cannot effectively destroy prions (Taylor *et al*. 1998). Due to these factors, rendered products made from ruminant by-products are not used to make ruminant feed. However, the meals can be used to make fish feeds.

Table 2.2: Efficacy of rendering for reducing pathogenic bacteria (adapted from Trout *et al.* 2001, in Meeker and Hamilton n.d).

	% positive samples	
Pathogen	Raw material	Post processing
C. jejuni	20.0	0
Campylobacter species	29.8	0
Clostridium perfringens	71.4	0
L. monocytogenes	8.3	0
Listeria species	76.2	0
Salmonella species	84.5	0

Principle of rendering

The aim of the rendering is to produce stable products of commercial value free of disease-bearing organisms, from raw materials (Fig. 2.5) usually considered unsuitable or unfit for human consumption (Meeker and Hamilton n.d; Swan 2000).

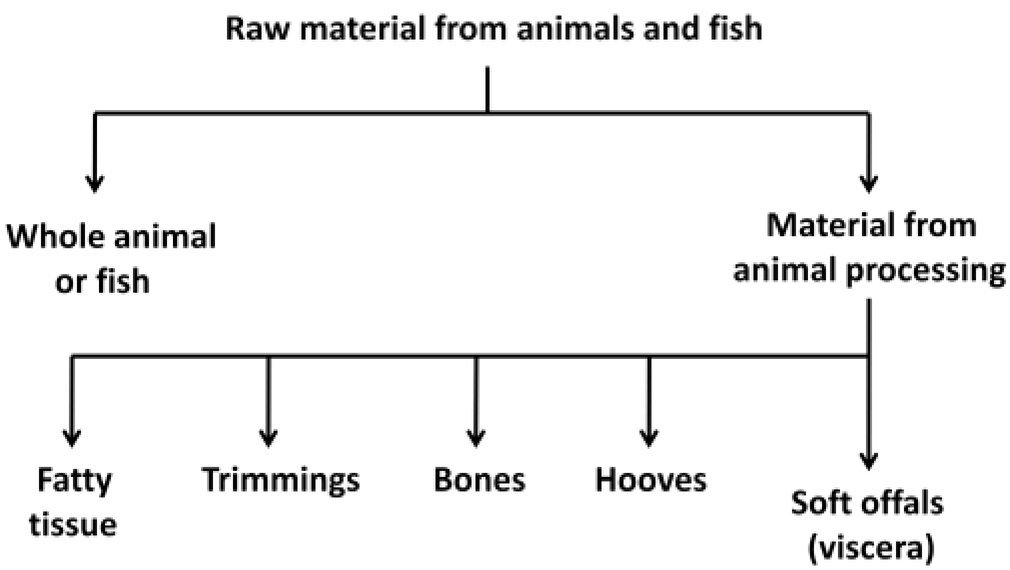

Figure 2.5: Raw materials used in the rendering process

Animals store surplus dietary energy as fat in the fat cells. This energy reserve can be used when needed. Reticular fibres around the fat cells also act as a support structure for the fat cells. To release the fat, these structures need to be broken. Edible raw materials also can be used to produce edible fats and proteins for human consumption (Meeker and Hamilton n.d; Swan 2000; Pearl 2005).

The basic process in rendering involves applying heat, separating the various phases and then extracting moisture. Heat is the main method used to rupture the fat cells although enzymatic and solvent extraction can also be used (Swan 2000).

Rendering processes can be classified as dry or wet. In a dry rendering process, the fat is removed after the removal of moisture. In a wet rendering process, fat is removed before the meal is dried. The rendering process can involve high heat but some processes, called as low temperature rendering process, use comparatively less heat.

The preliminary steps are same for all rendering processes and involve thorough washing to remove contaminants that may affect product quality, followed by size reduction to help mass and heat transfer. The rendering process can operate in a batch, continuous or semi-continuous mode. Each method has its own advantage and disadvantage. The most appropriate method will always depend upon the application and production requirements. The temperature used and the time material is exposed to high temperatures are the two primary factors affecting product quality (Swan 2000).

Dry Rendering

In dry rendering (Fig. 2.6), pre-ground raw material is heated in a steam jacketed vessel until all water has been evaporated This is energy intensive, so most dry rendering plants recover heat energy by condensing the evaporated water. An agitator in the vessel ensures uniform mixing and facilities heat distribution.

Dry rendering processes can operate in batch and continuous mode. In batch systems, the material can be sterilized and any wool, hair or bones hydrolyzed by applying 200 to 500 kPa for a specified time (Meeker and Hamilton n.d; Swan 2000). The time required for the complete process depends on factors such as quantity and type of material (Swan 2000). Under normal circumstances it takes up to three hours to produce dry material and the end point temperature is often

$120^oC$to 140^oC. Most of the water is removed at around 100°C and then the temperature rises.

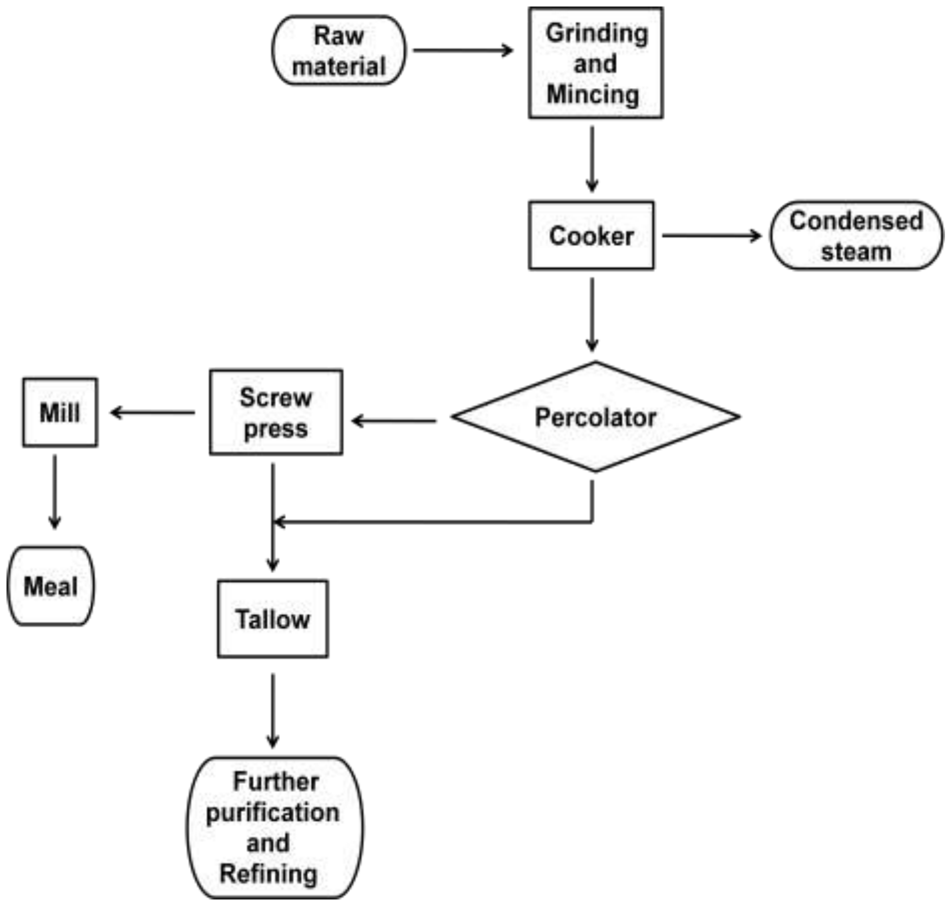

Figure 2.6: Dry rendering process

Skurray and Cumming (1974) report that availability of essential amino acids and nutritive value of the product decreases during this stage. There is also a simultaneous decrease in total reducing sugars and glucose.

Batch and continuous dry rendering systems have advantages and disadvantages (Table 2.3).

Cooking time in continuous systems depends on factors such as characteristics and feed rate of the raw material, cooker volume and heat transfer capability of the vessel (Meeker and Hamilton n.d; Swan 2000). Free draining fat is removed by discharging the contents of the cooking vessel into a percolator. Depending upon the system used; additional fat is then removed by either centrifuging the solid material (batch system) or pressing (continuous system). The meat meal produced is then ground. Any fines and moisture in the fats can be removed in a disc centrifuge.

Table 2.3: Advantages and disadvantages of dry rendering process

Advantages	Disadvantages
Batch Dry Rendering: • The same vessel can be used for cooking, pressurizing and sterilizing • Less material lost • Heat can be recovered from the vent stream • Different cookers can be assigned for processing different raw material, and hence for producing different grades of fat	• The fat is usually of poorer quality than from wet or low-temperature rendering • High temperatures produce fines, reducing fat quality and product yields • Meal has higher fat than from wet and LTR systems • High protein, low fat material is difficult to process • Energy usage is high, especially if heat is not recovered. • Cooking end-point is difficult to control. • The process is labour intensive.
Continuous Dry Rendering: • Uses less labour. • Requires less space. • Posses most of the advantages of batch dry rendering.	• System cannot be pressurized, so cannot sterilize and hydrolyse. • Fat quality tends to be lower than batch operations as the end point temperatures are often higher.

Wet Rendering

In the original wet rendering process, raw material is cooked in a closed vertical tank under 380 kPa to 500 kPa pressure using direct steam injection (Fig. 2.7) for three to six hours. At the end point, based on previous practical experience, the pressure is released slowly and the phases are allowed to settle before draining off the fat and water. The solids are pressed or centrifuged and dried [Meeker and Hamilton n.d; Swan 2000].

Wet rendering can be operated in batch, semi continuous and continuous low-temperature modes. The semi-continuous wet rendering process varies slightly from the wet rendering process.

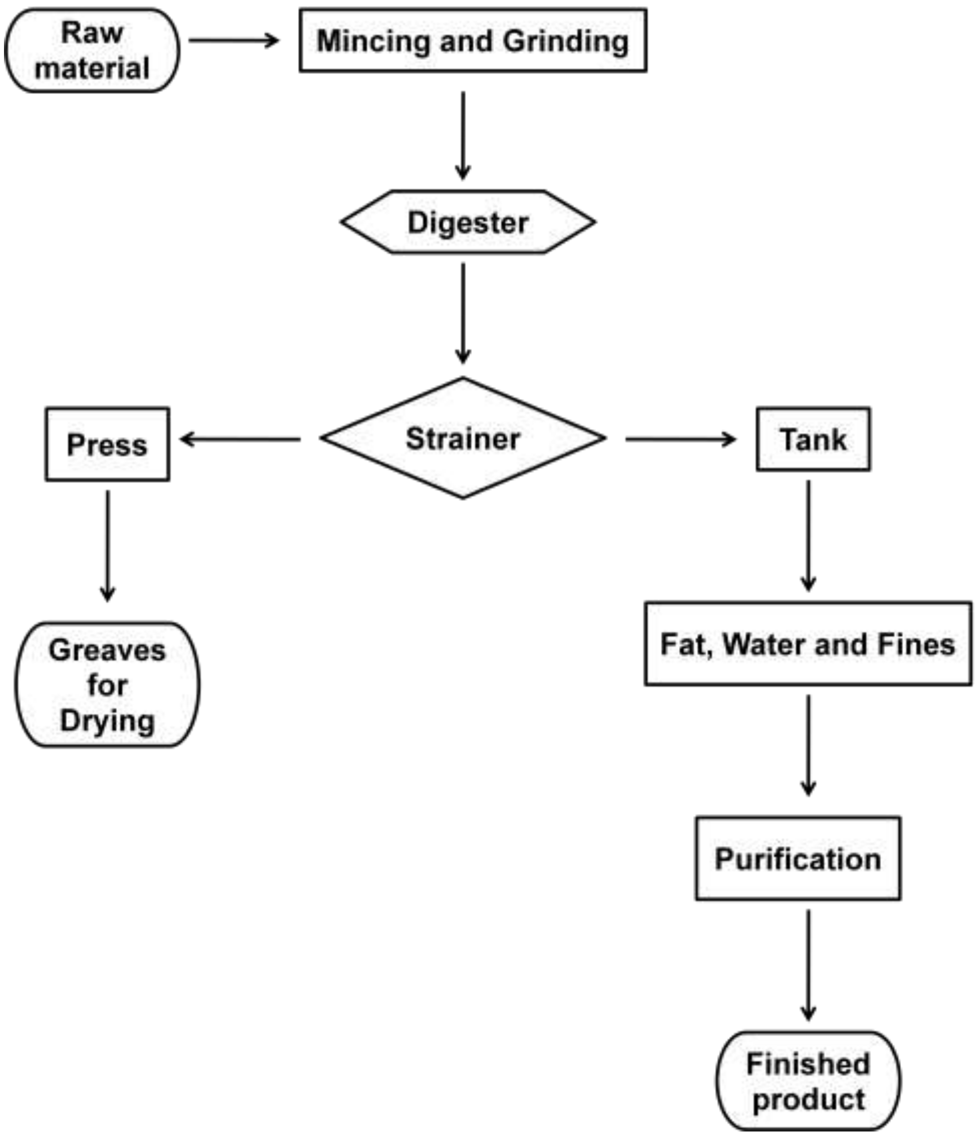

Figure 2.7: Wet rendering process

In semi-continuous systems (Fig 2.8), raw material is cooked under pressure for a short time and then the phases are separated. Fat is separated using disc centrifuges but it may be difficult to get a precise separation, so three or four layers may form - clear fat, an emulsified layer, occasionally a small water phase, and a heavy protein tissue phase (Meeker and Hamilton n.d). The meal can be dried using continuous dryers. The advantages and disadvantages of wet rendering systems are given in the Table 2.4.

Table 2.4: Advantages and disadvantages of wet rendering system

Advantages	Disadvantages
• Produces high quality fat and low fat meal • Uses less energy than conventional wet- or dry- rendering systems	• High capital costs, repair costs and maintenance costs

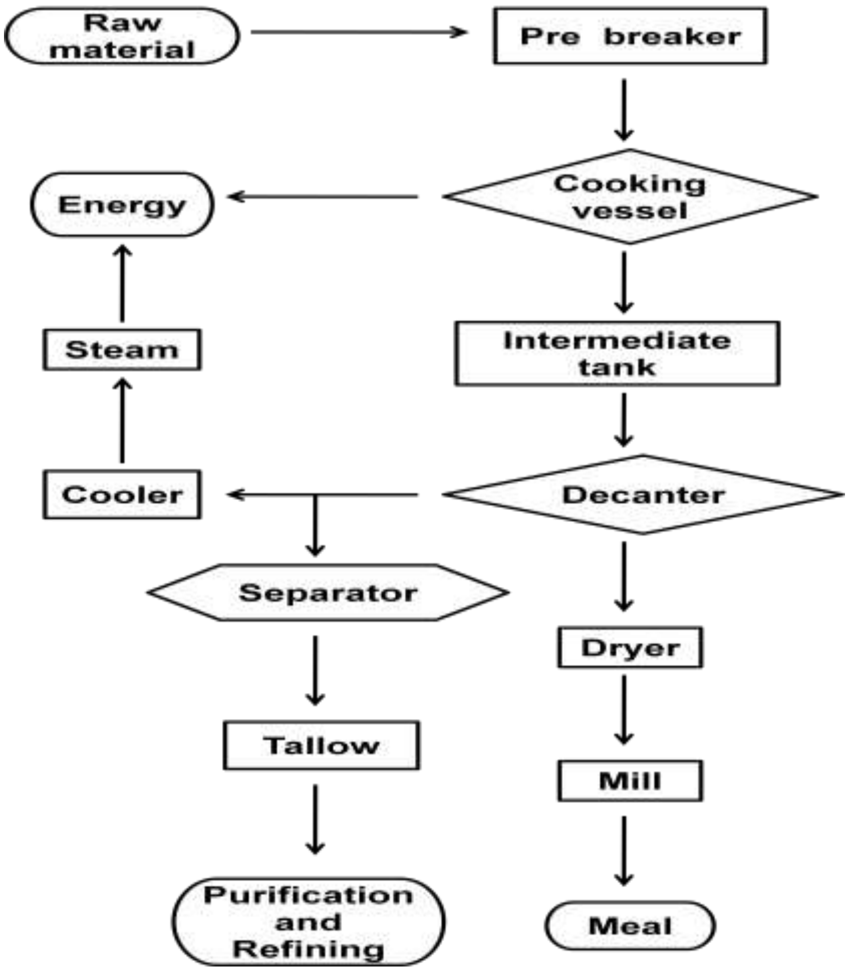

Figure 2.8: Semi continuous wet rendering process

Low temperature rendering system (LTR):

Low Temperature Rendering system is a comparatively modern system that subjects the raw material to less heat than either the wet or dry rendering processes. Phase separation is carried out between 70^{o}C and 100 ^{o}C. Low temperature rendering systems, which include mincers, heating units, phase separating equipments, evaporators, dryers and surge bins, are mostly continuous

(Swan 2000). The major advantages and disadvantages of low temperature rendering systems are summarized in Table 2.5.

The various factors that affect the colour of fats include source of raw material and presence of contaminants. Various parameters are used to indicate fat quality such as free fatty acid (FFA), FAC colour (Standard set by Fat Analysis Committee of American Oil Chemists Society), bleach colour, moisture, peroxide value, iodine value, saponification number, insoluble impurities, unsaponifiable matter (MIU) and smoke point(Swan 2000). Commercially-important fat includes tallow (fat from sheep and beef), lard (fat from pigs) and oil (from fish) (Meeker and Hamilton n.d; Aldrich n.d; Swan 2000). Fish oil is gaining in importance because tallow and lard are implicated in coronary diseases. However, fish oil is known and widely consumed for its health benefits (Wang et al 2006).

Table 2.5: Advantages and disadvantages of LTR system

LOW TEMPERATURE RENDERING	
Advantages	**Disadvantages**
• Energy requirements about half those of dry-rendering systems. • Uses low heat treatment, so high quality fat produced. • Raw material does not need be washed. • Meal has low fat content and high nutritional value because heat treatment is minimal. • Low labour requirements. • System easily automated.	• High capital costs. • High repair and maintenance costs • Require highly trained technical operators

Meal composition from rendering processes depends on the source of raw material and the process method. The names of the meals indicate the material used to produce them such as fish meal, feather meal, poultry meal, meat meal, blood meal, bone meal, etc. These main uses of these meals are to provide cheap protein, fat and minerals in formulated animals and fish feeds (Aldrich n.d; Meeker and Hamilton n.d; Swan 2000; Pearl 2005).

Waste material from fish processing can be rendered. The resultant meal tends to have higher protein content than meals made from animal by-products. It finds immediate demand in formulated pet foods and for production of fish feed. The main disadvantages are that there can be substantial compositional differences in the fatty acid profile, stability, and ash levels between fish species (Palstinen *et al.* 1985; Pike and Miller 2000). There are very little available data in the literature about the nutrient utilization of the fish meal by canines and felines (Aldrich n.d).

The fish oil could be used for human and animal consumption (Aldrich n.d; Meeker and Hamilton n.d; Swan 2000; Pearl 2005; Wang *et al*. 2006). These oils are derived mainly from pelagic fish like anchovy, menhaden, mackerel and herring. They have a strong oily taste and aroma, which is not appreciated by most people. However, this does not appear to be a considerable problem for dogs, but some cats seem to prefer one fish oil over another (Aldrich n.d). Fish oils are generally added to the surface of the pet food, post-extrusion and drying. Adding fish oil to meet the required omega-3 fatty acid level usually requires less than one to two percent of the formula and hence can be challenging to accurately measure without properly designed equipment. Palatability concern is a major disadvantage for surface application of fish oil (Aldrich n.d).

The fatty acid profile of the different fish oils can vary substantially. Most of the fish oil utilized in the pet food industry is cold pressed and refined. Greater processing increases cost. However, the trade-off is improved handling, better shelf life and animal acceptability. Very little to no preservative is needed to stabilize bulk fish oil against oxidation and oil in canned pet foods. Once consumed, the way the body uses oil is similar to that of other fat sources. The omega-3 fatty acids circulate within hours of consumption and pass their benefits along for weeks.

2.5. Aims of the research

The three most common and effective methods for using aquatic waste (from wild stock or aquaculture) are manufacturing fishmeal/oil, organic fertilizer or silage (Arvanitoyannis and Kassaveti 2008). This matches the preliminary requirement of my research as these processes seems to have the potential to reduce the costs of capturing koi carp from the environment. They could also generate additional revenue.

However, there is little available information on rendering koi carp and the quality of the products obtained. The motive of this research is to investigate whether koi carp can be rendered and to compare the yields using bench-scale, simulated dry and wet rendering processes, autoclave method and solvent extraction. Koi carp (whole fish) will be weighed, minced, froze and then rendered. The results will be used to ascertain whether this is a workable method of processing koi carp caught in the eradication programme.

3: MATERIALS AND METHODS

A series of trials were done to investigate the yield of oil and meal under different rendering processes and also to analyse the ash and moisture content in the koi carp. Preliminary trials were done to identify the end point of various rendering processes. Further trials were done to analyse suitable methods for separating the oil from the dried meal.

3.1 Materials

3.1.1 Fish

The koi carp (average weight 1.3 kg and length 30 cm) used in the study came from Lake Waikare during the summer. Lake Waikare is a shallow lake (1.5-1.8 m deep) in the lower Waikato catchment system. This aquatic system has no large submerged aquatic plants and has poor water quality (EW 1999-2010). Since 1993, there have been significant increases in the level of total nitrogen, total phosphorus and chlorophyll a. The levels of suspended sediment are high and water quality is highly variable.

The fish were selectively caught and killed with the help of staff from the Department of Biological Sciences at Waikato University using an electrofishing unit. The electrofishing unit emits electric pulses at a frequency of 60 pulses per second with 2-4 amp root mean square. A fishing field 4 metre wide is created using two adjustable 1-m long anodes. The electric pulses stun all the fish in the fishing field, making them to rise to the surface. Koi carp were then selectively caught using nets with long handles and killed (Hicks *et al*. 2005). These fish were then stored in boxes with ice, transported to the Waikato University and later weighed and stored in a freezer at -21^{o}C.

The fish was prepared for the trials by chopping the whole frozen fish into pieces (7.5 cm x 5 cm) with knives and an axe. The fish were chopped and minced individually through a 5-mm hole plate in a commercial mincer and packed individually into resealable plastic bags (27cm x 18cm). The packed fish were then weighed and stored in the freezer at -21^{o}C. When required, the minced fish was taken from the freezer, thawed and used for the trials (Figure 3.1).

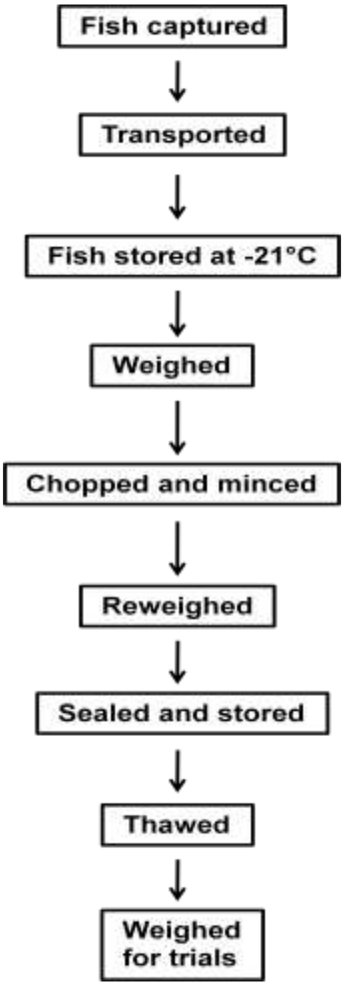

Figure 3.1: Initial preparations

3.1.2 Chemicals and Additives

- Sulphuric acid: 98%, 18.4 M, 36.8N (supplied by Ajax Finechem Pty Ltd) was used to adjust pH to 4.5 during some of the trials.
- n-hexane (supplied by Ajax Finechem Pty Ltd) was used for solvent extraction of oil from dried meal.

3.1.3 Other Materials and Equipment

Knives and an axe were used for chopping frozen fish. Fish were stored in re-sealable plastic bags. The heat source was 1.5 KW laboratory hot plates. The minced fish was heated in a saucepan and stirred using wooden spoons. Filter paper (No 1 grade Whatman filter paper), cheese cloth and garlic press were used to separate oil from the meal. A laboratory centrifuge was used to separate oil from the meal. Trials above 100^{o}C were done in a pressurized laboratory autoclave. Pipettes, eye droppers, measuring cylinders, centrifuge tubes, desiccator and oven were used in the analytical tests.

3.1.4 Analytical Methods

Ash: Triplicate 3-g samples of dried meal were transferred to weighed porcelain dishes and weighed accurately. The samples were then carbonized over a flame before being placed in a muffle furnace at 525^{o}C for 72 hours to produce a white ash. After being cooled in a desiccator at room temperature, the dishes were reweighed. The process was repeated until a constant weight was obtained. Ash was expressed as percentage of whole fish

Temperature: Thermocouple probes with digital display were used to measure temperature.

pH: Glass electrodes were used to measure pH

3.2. Trials

3.2.1. Preliminary experiment

To investigate conditions for simulated dry rendering, 1 kg minced koi carp was weighed and transferred to a weighed saucepan. The pH was measured using a glass electrode before heating the minced fish using a hot plate. The material was stirred continuously with a wooden spoon and the temperature was monitored throughout the process. The minced material was heated and stirred until a dried meal was obtained. The endpoint temperature and the total processing time were recorded. This trial was repeated to ensure the endpoint temperature.

Various trials were done to separate the oil from dried meal including: squeezing by hands using cheese cloth, filter paper and a garlic press. Separating oil by keeping the dried meal placed in filter paper in funnel in an 80°C oven for 24 hours was also investigated.

In a further trial, 100 g of dried meal was mixed with 20 ml of hot water and boiled for 5 min. This mixture was then centrifuged for 10 min at 4000 rpm to separate the oil. The separated oil was measured and weighed. The solid phase was dried, weighed and stored.

To simulate the conditions for wet rendering, 1 kg minced koi carp was weighed and transferred to a saucepan along with an equal weight of water. The pH of the mixture was recorded. The mixture was heated on a hot plate and stirred continuously with a wooden spoon. Temperature was monitored throughout. Heating continued until oil drops were visible. The endpoint temperature and processing time were recorded. The mixture was centrifuged at 4000 rpm for 10 min. The supernatant obtained was re-centrifuged at 4000 rpm for a further 10 min. The oil was then separated, measured and weighed. The solid phase was weighed, heated and dried thoroughly

Trials were repeated for different heating times (initial heating was to facilitate oil separation) and different centrifuging rpm and times to identify optimum conditions to separate oil from the dried meal.

3.2.2. Dry Rendering

Minced koi carp was weighed and then heated on a hot plate. The minced fish was continuously stirred using a wooden spoon (about 3 hours) until a completely dry material was obtained (Figure 3.2). The end point of the process was determined using experience from the preliminary trials.

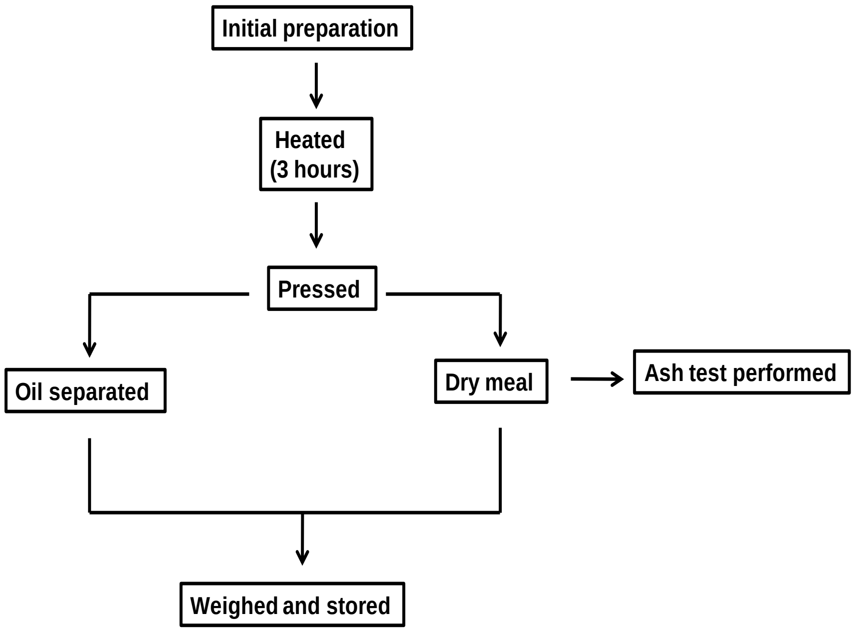

Figure 3.2: Dry rendering process

Once the meal appeared dry, it was weighed. The oil was separated from dried meal using a garlic press. A layer of filter paper was placed inside the garlic press to stop dried meal contaminating the oil separation. The oil was measured and weighed. As only small samples could be processed in the garlic press, mild heat was constantly supplied to meal in the saucepan to prevent it cooling. The meal obtained after the oil had been pressed out was weighed and the ash determined on a subsample. The dry rendering trial was repeated five times.

In a slight variation of the dry rendering process, the meal (with oil) that had been dried was not pressed but mixed with 10% boiling water, heated for two minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant was re-centrifuged and the oil was separated and weighed. All other phases were heated again in the sauce pan to obtain a dry meal. The meal was weighed and the ash determined on a small subsample.

3.2.3 Wet Rendering

Samples of thawed minced koi carp were weighed and transferred to a saucepan and an equal weight of water was added. The mixture was heated on a hot plate (Fig. 3.3).

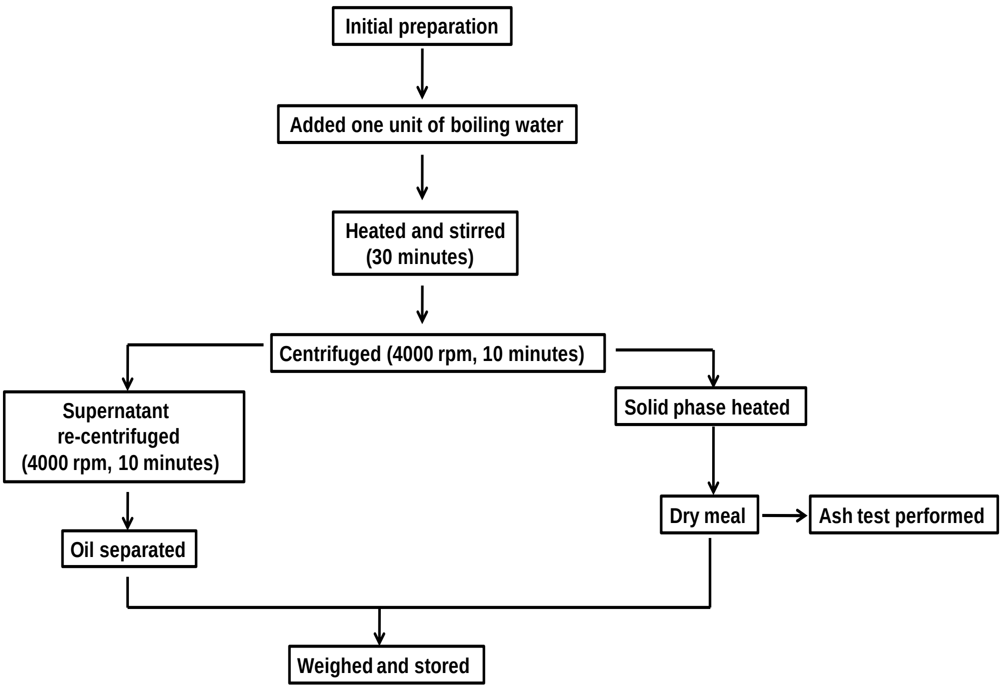

Figure 3.3: Wet rendering process

The mixture was continuously stirred using a wooden spoon and the temperature was constantly monitored. After 30 min, the mixture was weighed and centrifuged at 4000 rpm for 10 min. The supernatant was separated and re-centrifuged at 4000 rpm for 10 min to separate the oil. The oil was measured.

The solid phase from centrifugation was weighed and then heated and stirred continuously to remove moisture and obtain the dry meal. The end point temperature was noted. The meal was weighed and the ash of sub-samples determined.

The wet rendering trials were repeated several times. Some were done with minced fish that had been adjusted to pH 4.5 using concentrated sulphuric acid, or a combination of adjusted pH (4.5) and an equal volume of boiling water.

3.2.4 Pressure Processing Method

Samples of thawed minced koi carp was weighed, transferred to a glass beaker, covered with aluminium foil and autoclaved at 1 psi (120^{o}C) for 20 min. After the heat treatment, the sample was centrifuged at 4000 rpm for 10 min. The supernatant separated and re-centrifuged to obtain the oil, which was separated and weighed (Fig. 3.4).

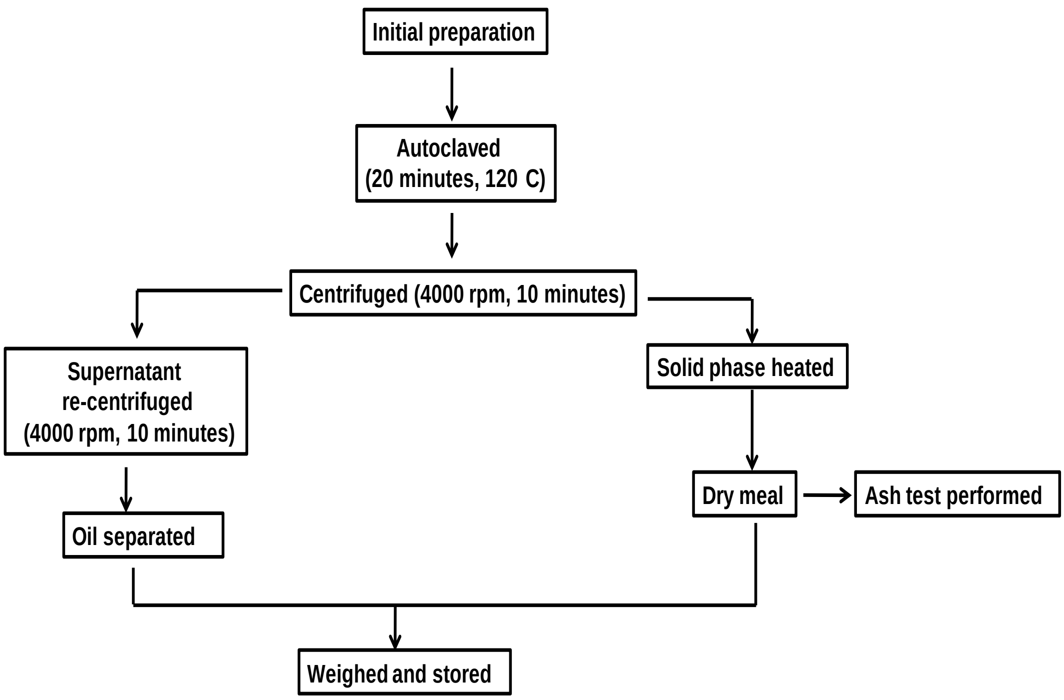

Figure 3.4: Pressure processing method

The solid phase from the first centrifugation was then weighed and transferred to a saucepan, then heated and stirred continuously until the meal was dried. The dried meal was weighed and the ash of a sub-sample determined. The method was repeated 5 times.

Some trials were done in which the pH of the minced fish had been adjusted to 4.5, and with minced fish adjusted to pH (4.5) with an equal weight of boiling water.

3.2.5 Solvent Extraction

Minced koi carp was weighed, transferred to a saucepan and heated on the hot plate. The minced fish was continuously stirred and the temperature monitored until completely dried (endpoint temperature 130-135^{o}C). Five 10-g samples of dried meal was mixed with 10 ml of n-hexane and vigorously stirred. The oil separated from the meal and then the n-hexane was evaporated. The oil was measured.

3.3 Calculations

The amount of moisture in the fish was calculated from repeated trials by measuring the initial weight (minced fish) and the final weight (dried meal with oil).

$$\% \text{ Moisture} = \frac{\text{final weight after drying, g} * 100}{\text{initial sample weight, g}}$$

The following was used to calculate ash content

$$\% \text{ Ash} = \frac{\text{weight of ash , g} * 100}{\text{weight of sample for moisture, g}}$$

The following was used to calculate the mass balance.

$$\text{Input} = \text{Output} + \text{Loss}$$

The mass balance was done on total weight and also on components in the process. The equation was also used to calculate the theoretical yield.

4: RESULTS AND DISCUSSION

From the literature review on possible uses for koi carp, using whole fish to produce meal and lipid was selected as being the most suitable. Fish contains moisture, protein, lipid and ash and can be rendered to obtain lipid and meal from minced koi carp. Two different rendering processes were simulated to estimate the meal and lipid yields that could be obtained from minced whole koi carp.

Preliminary trials were done to understand the conditions and processing time. Other preliminary trials were used to identify suitable methods for separating oil from the meal. Conditions for the main trials were based on results obtained from the preliminary trials.

4.1. Preliminary Trials

The aim of the first dry rendering trial was to identify the temperature of the minced koi carp at different stages during the heating process. This would help in determining the end point of the process and prevent charring the product.

Initially the temperature rise was slow and uniform (Table 4.1). After 30 min, the temperature remained at 102°C as the water evaporated from the minced fish. The maximum rate of weight loss occurred during this constant temperature period. It took some time to evaporate the water as a large proportion (70%) of the fish is water. After the bulk of the water has evaporated, the temperature rises further and the end point of the dry rendering process was noted as 133°C (Table 4.1).

This end point was confirmed by heating the dried meal for a further 30 min but there was minimal weight loss during this period. This extended heating charred the meal. Replicates of this trial corroborated that the end temperature for dry rendering was between 130°C to 133°C. The total time for the heating, evaporation and further heating was 180 to 210 min. It was important to stir the minced fish to distribute the heat uniformly and prevent material charring at the bottom of the saucepan. The average yield (n=3) of meal from drying rending 1-kg batches of minced koi carp was approximately 30% (Table 4.1). This material would contain protein, minerals, lipid and any residual moisture.

Table 4.1: Determining endpoint temperature for dry rendering minced koi carp

Time (min)	Weight (g)	Temperature (°C)
0	1000	23
30	891	102
60	570	102
90	480	108
120	348	117
150	303	122
180	2940	133

The next stage in dry rendering is to separate oil from the meal so an approximate estimate of oil yields can be made. It was also necessary to understand the best method for this process. Visual observations during heating indicate that oil drops were present. Visual examinations showed the dried meal had an oily texture. However, the first attempt to separate oil using manual filtration with filter paper did not work. The filter paper absorbed the oil and applying manual pressure meal tore the filter paper.

In the next trial, the meal was wrapped in cheese cloth and squeeze to extrude the oil. However, the oil absorbed onto the cheese cloth. Also, the mesh in the cheese cloth was comparatively large (even though it had been folded to give four layers) and pieces of meal came through so clarified oil could not be obtained. The next trial involved placing dried meal in a funnel lined with filter paper and keeping it in an 80°C oven for 24 hour. The method was also unsuccessful got separating oil from the meal.

Using a domestic garlic press resulted in separating oil from the meal. However, small particles of dried meal escaped through the pores in the garlic press and contaminated the oil so a layer of filter paper was placed inside the press to prevent the contamination. The first 70-g batch of dried meal produced 10.4 g oil but only 1.4 g was obtained from a second 70-g batch. Attempts to separate oil from the rest of dried meal failed.

Repeated trials showed that oil can only be separated if the meal is prevented from cooling. Cooling increases viscosity of the oil, making separation difficult. Further trials were done while maintaining the dried meal temperature between 80°C and 90°C. This allowed oil to be separated using the garlic press so this method was used in the main trials.

Adding water to the warm meal will decrease viscosity of the mix and may help oil recovery. However, any added water increases processing cost so trials were done to identify the minimum amount of water that needed to be added but which helped oil recovery. The oil was recovered by centrifuging the meal/water mix at different speeds.

Adding 20% water to the dried meal, mixing it for 5 min at 80^{o}C, followed by centrifuging at 4000 rpm for 10 min was found to be optimum. Centrifugation had to be done in two stages to produce an oil phase. In the first centrifugation step, three phases were formed. The bottom phase was made up of protein, bones and scales; the middle phase was a water/oil emulsion; and the top phase contained water and oil. The top two layers were removed and re-centrifuged to further separate an oil phase.

Wet rendering was simulated by adding an equal volume of water to the minced fish and then heating it quickly to 100^{o}C. A small oil layer appeared at the top of the heated material after 30 min to 45 min. The phases were allowed to settle for approximately 10 min. The liquid layer was removed and centrifuged. Only traces of oil were obtained so the hot mixture was centrifuged and a better oil yield was obtained.

Different centrifugation speeds and times were tried to determine the best possible combination. Unlike centrifuging only the liquid phase produced from heating the fish, only two phases were obtained after centrifugation the cooked fish. The bottom phase was made of protein, bones, scales and water and top phase was an oil layer. The same centrifugation speed and time used when separating oil from dry rendered meal was used (4000 rpm and 10 min). The yield of oil from wet rendered meal was 1.5% of original minced fish (wet weight basis).

Several trials were done to determine the best heating times and heating for 30 min to 45 min was found to be optimum. Investigations on the amount of water to add indicated that adding and equal weight of boiling water gave slightly better oil yields than heating with cold water.

The pH of minced fish was 6.6. Adjusting the pH to 4.0 or 4.5 before it was heated increased the oil yield. The oil yield from mince adjusted to pH 5 was similar to that of mince that had not been acidified. A pH of 4.5 was selected for the main trials.

A further preliminary trial simulated wet rendering under pressure. Batches of minced fish and an equal volume of water were cooked under pressure (100°C and 130°C) for various times in a laboratory autoclave. The minced fish was acidified to pH 4.5 before being cooked. The autoclaved sample was mixed and centrifuged. The maximum oil yield of 1.8% of wet fish weight was obtained from fish heated for 20 min at 120°C and the optimum centrifugation speed and time for oil separation was 4000 rpm and 10 min.

Two layers were produced when the sample was centrifuged. The bottom layer was dark and made of protein, scales, bones and water. There was a small layer of oil at the top. The optimum conditions were then applied in the main trials. The solids produced by autoclaving were very fine compared with the meal that had been heated only in the saucepan, which would make it difficult to separate efficiently without major losses in a commercial process.

Trials on conditions for ashing dried defatted meal showed that a constant weight was obtained by heating the 3-g samples at 525°C for 72 h (Table 4.2).

Table 4.2: Preliminary trial for calculating optimum time for ashing dried defatted meal at 525°C

Time (h)	0	24	48	72
Material (% original sample)	100	10.8	9.5	8.8

4.2. Main Trials

The optimum processing conditions determined in the preliminary trials were applied on a laboratory scale to simulate the various rendering processes. All results are the mean value of five replicates and ash data are the mean value of three replicates.

4.2.1. Dry Rendering

The temperature of minced koi carp rapidly rose from room temperature (24°C) to about 100°C (Table 4.3). After remaining at this temperature for 30 min while the large amount of moisture was evaporated from the tissue, the temperature then rose to an endpoint of 132°C after 180 min total heating. The yield of dried meal represented about 30% of the initial material.

Table 4.3: Changes in weight when dry rendering minced koi carp

Time (min)	Weight (g)	Temperature (°C)
0	1325	24
30	1231	102
60	1080	102
90	880	106
120	521	116
150	432	125
180	402	132

Heating the minced fish for 3 h completely ruptured the cells and evaporated the moisture, thus releasing the oil (Section 2.4). There was a strong fishy smell during the heating process, which may be not acceptable to some people. The aroma of the end product was much more pleasant.

Pressing dried meal in the garlic press provided sufficient mechanical force to separate the oil. The oil yield was 17% of the dried meal, which represents 5.2% (w/w) of the original minced koi carp. The pressed meal was a light brown powder with some tiny bone pieces. It appeared free of oil. The separated oil was brown and translucent (Figure 4.1).

The visual observations indicate that it had a poorer quality than oil obtained from wet rendering (Section 4.2.3), probably due to presence of fines produced at the high process temperatures used (endpoint of 132°C). The process is labour intensive because it required continuous stirring. The pressing also was labour intensive.

Figure 4.1: Oil obtained from koi carp

Using the reported data on the chemical composition of whole fish herring (Section 2.3.1), which is regarded as an oily fish, the maximum yields obtainable by dry rendering method can be calculated (Table 4.4). These calculations are done by assuming the composition of whole fish is 71% moisture, 18% protein, 8% lipid and 3% ash (Section 2.3.1). The dried meal, which has a residual moisture content of 5% w/w, is 30.5% of the original minced fish. After being pressed, the resultant meal contains 15% lipid (Table 4.4) and rest is separated. It is assumed that 5% of product (based on dry matter) is lost during the process via spillages. The remaining dry matter ends up in the dried solids.

Therefore, dry rendering 1 tonne of minced koi carp will produce 305 kg of dried material with a moisture content of 5%, a protein content of 59% .a lipid content of 26.2% and an ash content of 9.8% (Table 4.4). After the lipid has been separated and assuming 5% product loss, 251 kg of dried defatted meal is produced. Information on the composition of dry rendered meal (Swan, pers. comm.) indicates this product is likely to have a lipid content of 15% with the remainder being water, protein and ash.

The lipid separated after pressing represents 4% of the original weight of fish. The amount of moisture, which is removed as steam, represents 70% of the original weight of fish. These calculations on the theoretical yields by rendering fish indicate that oil yields are likely to be low and that a lot of energy will be needed to evaporate the moisture. Fat yields from rendering meat wastes are often up to 20% of the original material (Swan 2000).

Table 4.4: Mass balance on unit operations for dry rendering 100 kg fish

	Raw fish by component (%w/w)	**Drying Operation**		**Pressing operation**		**Meal**	
		Dried meal (kg)	**Water evaporated**	**Pressed meal (kg)**	**Free lipid (kg)**	**kg**	**% w/w**
Water	71	1.5	69.5	1.5		1.4	5.7
Protein	18	18		18		17.1	68.0
Lipid	8	8		4.0	4.0	3.8	15.0
Ash	3	3		3		2.9	11.3
Total	100	30.5		26.5		25.1	100

Data from measuring and analysing the phases produced in the various trials indicate a composition of 69.6% moisture, 23% protein, 5.2% lipid and 2.2% ash and is similar to the value for the composition of herring (Section 2.3.1).

4.2.2. Dry rendering with centrifugation

In a further dry rendering trial, a similar temperature profile was observed during the process (Table 4.5). The endpoint temperature was 134°C and the dried meal yield was 29%. After heating this dried meal with 20% w/w water at 80°C for 5 min, it was centrifuged to give a 20% yield of lipid (percentage of dried material). Some of the added water added evaporated during the heating process.

Calculations showed that the composition of the minced koi carp was 70.8% moisture 21% protein, 6% lipid and 2.2% ash, which was similar to the previous dry rendering trial. The oil yield obtained by centrifuging dried meal after it had been mixed with hot water was higher than obtained by pressing dried meal but it had a similar appearance and colour.

Table 4.5: Changes in weight when dry rendering minced koi carp followed by separating lipid by centrifugation'

Time (min)	Weight (g)	Temperature (°C)
0	1280	26
30	1185	104
60	1017	105
90	830	112
120	534	116
150	403	128
180	376	134

The comparatively higher oil yield observed during this process may be due to the centrifugal force used on the sample, which was completely dry after being heated for 3 hours. This process should have ruptured the cells, making separating the lipid easier. The meal obtained seemed free of lipid and light brown.

4.2.3. Wet Rendering

In the wet rendering process, the raw material is heated to rupture the cells and then the solid and liquid phases are separated, usually by centrifugation (although gravity settling can be used). When the minced koi carp with an equal volume of hot water was heated, the temperature increased from room temperature to 100°C in 20 min (Table 4.6). About 30% of the moisture evaporated after the mixture had been heated for 45 min. Centrifuging the mixture produced a thin layer of oil, which represented 1.4% of the original minced koi carp.

Temperature of the defatted mixture rapidly rose from 28°C to about 100°C in 30 min and then rose to an end point temperature of 132°C after 180 min. The yield of defatted dried meal represented 27% of the initial material (minced koi carp).

The oil yield from wet rendering was less than dry rendering minced fish. However, oil quality seemed higher than obtained by dry rendering because the oil was a lighter colour and free from the fines that can be produced at high process temperatures (end point 132°C). The reason for the better quality is probably because it had been separated after only 45 min of heating and had not been exposed to high temperatures for extended times.

Table 4.6: Changes in weight when wet rendering minced koi carp mixed with an equal weight of cold water

Weight represents 1430 g minced koi carp with an equal weight of hot water		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	2861	24
20	2623	100
45	2021	100
Centrifuged at 4000 rpm for 10 min to separate liquid phase		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	2000	28
30	1680	102
60	1203	103
90	980	106
120	603	109
150	407	125
180	388	132

The solids phase obtained during centrifugation contained a mixture of protein, bones, scales, water and oil. Therefore, the dried meal would contain oil. The fishy smell during the heating process after the separation of oil was acceptable and the aroma of the end product was also pleasant.

The meal obtained was slightly darker than obtained from dry rendering and was powdery in nature and contained small white pieces of bones (Figure 4.2).

Figure 4.2: Defatted dried meal

Using data on the chemical composition of fish (Section 2.3.1), the theoretical yield obtainable from wet rendering can be calculated. After heating and centrifuging, approximately 44 kg of solid phase is obtained from 100 kg minced fish. It contains 21.9 kg water, 17.1 kg protein, 2 kg oil and 2.85 kg ash from the original fish. It is assumed that about 5% of the original protein and ash will be solubilised during the heating process and transferred to the liquid phase. The liquid phase, with soluble material, contains is 49.1 kg water, 0.9 kg protein, 6 kg oil (85% on wet basis) and 0.15 kg ash from the original fish (Table 4.7). The yield of meal, produced by drying the solids, contains 1.9 kg water, 17.1 kg protein, 2% oil and 2.85 kg ash from the original minced fish. The dried meal has 8% residual moisture, 71.7% protein, 8.4 % lipid, and 11.9% ash. About 1% of lipid in the liquid phase is lost and the rest is recovered as free oil.

Therefore, wet rendering if 1 tonne of minced koi carp will theoretically produce 439 kg wet solids with 50% of dry moisture and 561 kg liquid phase. The liquid phase contains any protein and ash that has been solubilised during the heating process. Centrifuging the liquid phase produces 50 kg of lipid. After the solids have been dried, 239 kg of meal is produced.

Table 4.7: Mass balance in unit operations for wet rendering 100 kg fish

	Raw herring by component (%)	Fish	Heating and centrifuging (kg)		Meal drying (kg)		Meal composition (%)	Liquid phase separation (kg)	
		kg	Solids phase	Liquid phase	Dried meal	Steam		Oil	Water phase
Water	71	71	21.9	49.1	1.9	20.0	8.0		49.1
Protein	18	18	17.1	0.9	17.1		71.7		0.9
Lipid	8	8	2.0	6.0	2.0		8.4	5.0	1.0
Ash	3	3	2.85	0.15	2.9		11.9		0.15
Total	100		43.9	56.1	23.9	20.0	100	5.0	51.1

Data from measuring and analysing the phases obtained in the trials indicate that the composition of the minced koi carp was 71.5% moisture, 24.9% protein, 1.4%

lipid and 2.2% ash. This does not compare well with the calculated compositions from drying rendering minced koi carp (Section 4.2.2), which had a higher lipid content and a lower protein content. The differences are probably due to the higher product loss via the liquid phase produced in the wet rendering process as well as losses when material was being transferred between different process steps. The oil yield obtained by laboratory wet rendering trials was only 1.5% of the original fish, which is much lower than the theoretical yield of 5%. The protein content of the meal is much higher than the theoretical 17.1%. The moisture and ash content also varied slightly from the theoretical values.

4.2.4. Wet Rendering with boiling water

The high losses may have been due to the relatively long time it took to heat the sample from room temperature to 100^{o}C, which would allow material to be solubilised and therefore lost via the aqueous phase. In the next wet rendering trial, the temperature of the minced koi carp was raised quickly to 62°C by adding an equal volume of boiling water. The temperature then quickly rose to about 100°C (Table 4.8). After centrifuging, an oil yield of 1.7% was obtained, which was slightly higher than when an equal volume of cold water had been added. However, it was still much lower than obtained in the dry rendering trials.

The temperature profile during the wet rendering process (Table 4.8) was similar to the previous wet rendering trial, with an endpoint temperature of 132°C

The dried meal yield was 27% of the initial minced koi carp. The defatted meal and oil obtained from this trial had a similar appearance and aroma to that obtained when cold water had been used.

Data from measuring and analysing the phases obtained in this trial indicate that the composition of the minced koi carp used in the trial was 71.2% moisture, 24.9% protein, 1.7% lipid and 2.2% ash. The results obtained by using hot water were similar to that from using cold water. In comparison with the previous trial (using cold water) an increase of 0.3% of lipid yield was obtained. However it is a minimal increase.

Table 4.8: Changes in weight when wet rendering minced koi carp with an equal weight of hot water

Weight represents 1210 g minced koi carp with an equal weight of hot water		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	2421	62
20	2102	100
40	1632	100
Centrifuged at 4000 rpm for 10 min to separate liquid phase		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	1612	28
30	1160	104
60	830	109
90	505	109
120	364	115
150	328	132

4.2.5. Wet rendering with boiling water and acid

To see if product losses could be reduced and to reduce formation of emulsions (which made it difficult to recover the oil), the minced fish was acidified to pH 4.5 with sulphuric acid before being wet rendered. The temperature of minced koi carp rose from 60°C to about 100°C in 20 min (Table 4.9). Centrifuging the mixture produced a thin layer of oil but the oil yield obtained was only 1.9% of the initial minced koi carp. The temperature of the defatted mixture rapidly rose from 26°C to 100° in 30 min and then rose to an end point of 133°C after 180 min of heating. The yield of defatted dried meal represented 26.20% of the initial material (minced koi carp).

The 1.9% oil yield obtained after centrifugation the acidified fish was higher than from unacidified fish. Lowering of the pH helped to coagulate the tissue proteins and free the oil so it was more easily separated. However, the dried meal is believed to have some oil content as it had a similar appearance to that obtained in the previous wet rendering trials. The aroma during the process and of the end product was also similar to the previous wet rendering processes.

Table 4.9: Changes in weight when wet rendering minced koi carp, acidified to pH 4.5, with an equal weight of hot water

Weight represents 1356 g minced koi carp with an equal weight of hot water		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	2713	60
20	2420	100
40	1830	100
Centrifuged at 4000 rpm for 10 min to separate oil		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	1804	26
30	1500	100
60	1115	102
90	871	104
120	588	105
150	390	115
180	358	133

4.2.6. Pressure processing

Material that is heated under pressure will reach a higher temperature, which will help break down the tissue. It was thought that this may increase the oil yield. Pressure treatment will also kill any micro organisms present.

After being heat treated under pressure, the meal looked very cooked. There was no loss of product during autoclaving because the vessel had been covered with aluminium foil. The oil yield after the cooked material was centrifuged represented 1.5% of the original fish weight, which was similar to that obtained when wet rendering process at atmospheric pressure. The reason for oil yields being lower than from dry rendering, even though the minced material from the same batch of fish had been used, is not known. Further work should be done to investigate whether the time the fish is subjected to the high pressure (and therefore high temperature) affects oil recovery,

The endpoint temperature when drying the dewatered solids to meal was 132°C (Table 4.10) and the yield of meal represented 26.3% of original fish weight.

The process was not as labour intensive as the other processes investigated as it did not involved stirring in the initial stage (autoclaving). However, this lack of stirring may have contributed to the low oil yield. The aroma during the process was milder than the other processes (and especially the dry rendering process) investigated as the initial stage was done in an enclosed vessel (the autoclave). Also, before the dewatered material was dried in the saucepan, the oil phase had been extracted. The meal obtained contained some lipid and was dark brown.

Table 4.10: Changes in weight and temperature when minced koi carp is heated under pressure and the dewatered solids are then dried at atmospheric pressure

Time (min)	Weight (g)	Temperature (°C)
0	1361	23
20	1361	120
40	1361	120
Centrifuged at 4000 rpm for 10 min to separate oil		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	1340	28
30	1109	100
60	980	100
90	621	100
120	540	100
150	381	120
180	357	133

Data from measuring and analysing the phases indicate that the minced koi carp contained 72.3% moisture, 24% protein, 1.5% lipid and 2.2% ash. As the overall procedure used is very similar to wet rendering (but under pressure), the theoretical yield from wet rendering (Section 4.2.3) can be used for comparison process.

4.2.7. Pressure processing with boiling water

When the minced fish with added water was pressure treated, the temperature carp rose to 120°C (Table 4.11). The oil yield from centrifuging the cooked material represented 1.5% of the original fish. The endpoint temperature of the meal after the dewatered material had been dried was 132°C and the meal yield represented 27.9% of the original fish. The dried meal has high protein content, along with ash and some oil (Table 4.14).

Table 4.11: Changes in weight and temperature when minced koi carp with an equal weight of hot water is heated under pressure and the dewatered solids are then dried at atmospheric pressure

Weight represents 1180 g minced koi carp with an equal weight of hot water		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	2360	65
20	2360	120
40	2360	120
Centrifuged at 4000 rpm for 10 min to is separate the oil		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	2341	29
30	2008	100
60	1483	100
90	1014	102
120	760	102
150	431	115
180	370	125
210	329	133

The aroma during the process and of the end product was similar to the product obtained when minced fish had been pressure treated with no added water. However centrifugation was labour intensive as there was a greater volume of liquor layer (due to the added water). The liquor had a thin layer of oil. The fish seemed properly cooked. The meal and the lipid obtained had a similar in texture and colour to the products obtained by pressure treatment without added water.

Data from the product weights, along with analysis of the phases indicate that the minced koi carp contained 70.6% moisture, 25.7% protein, 1.5% lipid and 2.2% ash.

4.2.8. Pressure processing acidified fish

In a further trial of pressure rendering, the pH was adjusted to 4.5 with sulphuric acid. The temperature of the material increased to 120°C during pressure treatment (Table 4.12) and the yield of oil after centrifuging the liquid phase produced an oil yield of1.8% of original fish weight. The dried meal represented 27.1% of the original weight of fish and contained has protein, ash and some lipid. The endpoint temperature was 132°C (Table 4.12).

Table 4.12: Changes in weight and temperature when acidified minced koi carp is heated under pressure and the dewatered solids are then dried at atmospheric pressure

Time (min)	Weight (g)	Temperature (°C)
0	1306	24
20	1306	120
40	1306	120
Centrifuged at 4000 rpm for 10 min to separate oil		
Time (min)	**Weight (g)**	**Temperature (°C)**
0	1283	27
30	1013	100
60	801	100
90	549	105
120	436	108
150	380	125
180	354	132

The colour and texture of the meal was similar to that of meal from other variations of the pressure treatment and the colour of the oil was similar to oil from other pressure treatments. The pressure cooked flesh seemed well cooked and thin layer of liquor with oil and water had separated from the flesh. This liquor layer was darker than that obtained in the previous pressure treatments (i.e., with and without added water). The meal still contained some oil. This process was not labour intensive and the aroma during processing was acceptable. The oil yield was higher (1.8% of original fish weight) than from the other pressure treatments investigated, which may have been due to acidifying the fish before processing it.

Data from measuring and analysing the phases indicate that the minced koi carp contained 71.1% moisture, 24.9% protein, 1.8% lipid and 2.2% ash. The lipid% and ash% obtained is less than the theoretical yield and the percentage of moisture, protein and ash are higher than the theoretical yield.

4.2.9. Solvent extraction

The low oil yields indicated that much of the oil had been retained in the dried meal. Some commercial rendering processes use solvents (Section 2.4), especially if a low fat meal is required. Solvent extraction helps estimate the maximum amount of lipid that could be obtained from dried meal. However this process is not industrially viable process because solvents are expensive and there are high risks associated with using flammable solvents.

Dried meal for solvent extraction trials was obtained by heating minced koi carp to complete dryness for 180 min and an endpoint temperature of 133^{o}C. Sub-samples were then mixed with an equal volume of n-hexane for 120 min. A mass balance on the products and their composition indicated that the original fish contained 70.6% moisture, 6.7% lipid, 20.5% protein and 2.2% ash. The results from this trial indicated that the lipid content of the koi carp is low, even though they are often classified as oily fish (Section 1.3). It is possible that the variety caught in Lake Waikare is different from the type referred to in the literature. This also means that rendering this fish is likely to produce only a meal only and that any commercial operation can be set up without the need to include the oil separation phase (whether a press or a centrifuge). Both of these pieces of equipment are expensive.

4.2.10 Ash samples from the main trials

The ash content of triplicates 3-g defatted samples from each process (dry rendering, wet rendering, high pressure and solvent extraction) were determined. All meal samples had an ash content of 2.1-2.2% (wet basis). The ash obtained after the test was white and powdered (Figure 4.3).

Figure 4.3: Ash obtained after ash test

The low ash percentage is advantageous as the protein source in pet food industries require low level of ash.

4.3. Summary

Results from all the processes investigated are summarised in the Table 4.13. The mean value was calculated to show that the koi carp used for the trials had an average composition of 71% moisture, 23.8% protein, 3.2% lipid and 2.2% ash. The protein content compares well with published values (Section 2.1). However, the lipid and ash contents are lower and the moisture content is higher than reported. Variations may be due to factors such as seasonal variations in feed availability, feed composition, the migratory cycles of the fish, and sexual maturation. Wild koi carp were used in this study so these factors cannot be controlled.

The yields obtained from all the process investigated are summarised in the Table 4.13

The mean value from the different processes indicates that 3.2 kg free oil and 26.0 kg of dried meal can be produced from 100 kg of minced koi carp. These low oil yields indicate that it is probably not commerically feasible to extract oil from koi carp. Instead, the fish should be rendered to a dried meal with a fat content of approximately 9%, which is lower than many commercial meals. Eliminating the press or centrifuge will reduce capital cost, processing cost and processing time.

Table 4.13: Summary of calculations to estimate koi carp composition by mass balance of data from the different rendering processes

	Dry rendering		Wet rendering			Pressure treatment			Solvent
	Oil separated by pressing	Oil separated by centrifuging	No additives	Added boiling water	Added boiling water and acid	No additives	Added boiling water	Added boiling water and acid	
Moisture%	69.6	70.8	71.5	71.2	71.7	72.3	70.6	71.1	70.6
Protein%	23.0	21.0	24.9	24.9	24.2	24.0	25.7	24.9	20.5
Lipid%	5.2	6.0	1.4	1.7	1.9	1.5	1.5	1.8	6.7
Ash%	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2

Table 4.14: Summary of products (kg) from using different processes to render 100 kg of fresh koi carp

	Dry rendering		Wet rendering			Pressure treatment			Solvent
	Oil separated by pressing	**Oil separated by centrifuging**	**No additives**	**Added boiling water**	**Added boiling water and acid**	**No additives**	**Added boiling water**	**Added boiling water and acid**	
Free oil (kg)	5.2	6.0	1.4	1.7	1.9	1.5	1.5	1.8	6.7
Dried meal (kg)	25.2	23.2	27.1	27.1	26.4	26.2	27.9	27.1	22.7
Total product (kg)	30.4	29.2	28.5	28.8	28.3	27.7	29.4	28.9	29.4

5: CONCLUSIONS AND RECOMMENDATIONS

Koi carp are a pest fish that destroy the natural habitat in New Zealand waterways. Finding a use for the captured fish would help offset eradication costs. A literature search helped identify possible options. Important criteria for selecting an appropriate method included factors such as having a developed market, using a relatively cheap and/or simple process, and not developing a product that required a long-term supply of fish. Rendering the koi carp into oil and a fish meal that could be used in formulated animal or fish feed was identified as a most feasible option.

Koi carp is made of protein, fat, ash and moisture. Laboratory trials were carried out to simulate both dry rendering and wet rendering processes to separate these components. A high pressure process was also used. To determine the maximum oil yield dried fish was extracted with solvent.

The literature reported that wet rendering systems produce high quality fat and a low-fat meal. Dry rendering has minimal material loss but the oil has lower quality.

Data from the trials indicated that oil yields from koi carp were low. The maximum oil yield of 6.7% was obtained by solvent extraction. However solvent extraction is not an industrially viable process. The oil yield from wet and dry rendering processes was 1.4% and 5.2% respectively.

The yields of defatted meal from the wet and dry rendering processes were 27% and 25% respectively and the ash content was 2.2%. The pressure processing yielded 1.5% of oil and 24% of defatted meal. Koi carp appeared to be a good source of protein and may be suitable as an ingredient in formulated animal and fish feed.

Koi carp could be processed in established rendering facilities. A satisfactory meal could be produced using either of the main commercial rendering processes.

It is recommended that further work be done on scaling the rendering process to pilot scale before going to commercial production.

More research is required on the quality of the meal and oil produced. This would include determining if there are any heavy metals or toxic components in the meal. Other factors include the effect of season on meal and oil quality, composition and yields. The omega fatty acid content of the resultant meal should also be determined. The eradiation process is carried out periodically. Therefore, it is recommended that negotiations with commercial rendering plants are initiated to discuss factors such as frequency of supply, quantities that could be caught and processed, and now to maintain the quality of the fish (which have to be killed immediately they are caught) in the period before they are rendered. Further trials should also be done on incorporating the resultant meal into formulated animal feeds, which could then be used in acceptability trials with species such as poultry, pets and in fish farming. Lastly the processing costs and economic viability of a rendering industry based on processing the captured koi carp needs to be done.

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