Bighead Carp: Effect of Drying Methods, Protein Hydrolysis Using Enzymes and Technical Methods and Study Fraction of Protein Peptides

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Abstract In last few years, there has been an increased effort to develop some advanced technologies of protein hydrolysis and fractionation in protein aquatic products. Using different protein enzymes with different extraction methods can lead to improve the yield of protein hydrolysis. In this review we studied the effect of different drying methods on chemical composition of bighead carp. Ficin enzyme was studied as proteolytic enzyme of fig latex. Whereas the properties of papain and bromelin have been quite thoroughly studied, relatively little is known about the properties of ficin. Also used different methods of extraction to show the effect of extraction on protein hydrolysis, this study highlights many of protein and peptides fractionation, such as electrophoresis and high-performance liquid chromatography (HPLC), which have experienced significant development over the past few years.

Keywords: drying, bighead carp, enzyme proteolytic, hydrolysis, extraction, fractionation

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1. Introduction

Fish is one of rich protein food which provides very nutritional meals in our daily life and many species can live in fresh water especially in Asia and North America. River fish species have certain requirements [1], many of which are required by all animals, but others are specific to life in flowing water. Physiological [2], behavioral and morphological characteristics give an indication of the needs of a fish with respect to its habitat [3].

Fish are cold-blooded animals, meaning their bodies are heated by their surrounding environment rather than being heated internally as humans and many other animals are. Being cold-blooded is generally an advantage living in water because it uses less energy and the daily and even seasonal temperature changes in water are much smaller compared to the changes in air temperature land animals must endure [4,5]. This means that fish can become well adapted to living at a relatively constant temperature. If water temperature within a stream changes too much, too quickly, it can end up killing fish as their bodily functions may not be able to withstand the drastic changes in their metabolism as a result of the changes in temperature.

2. Requirements for Life in a Stream

Living in water, obviously river fish need a certain depth and volume of water and the water must also be of a certain quality. As water levels rise and fall, fish must move to suitable habitats where water levels are safe for them to survive. In some cases, for example when the water level decreases after a flood event, fish can get caught in spots that are disconnected from the main stream or river and, unless there is another flood soon, they face an unpleasant end as the water dries up around them [6,7].

Nutrient fluctuations are another concern for many fish populations. Increases in farming and many other practices have led to an increase in nutrients finding their way into streams and rivers. If nutrient concentrations become too high in a stream it can lead to the water becoming depleted of oxygen due to algal blooms which consequently ends in the death of the fish within the stream.

As with any animal in nature, freshwater fish need protection from predators. This could come in the form of structures in the water such as logs, boulders or undercut banks or it may be an issue of timing. For example, certain fish species have developed behavioral patterns that help
them avoid predators by only coming out to forage for food at night [8]. Many fish have a cryptic camouflage to help them blend into their environment and reduce the chances of being spotted by a predator.

3. River Fish Species

Species of river fish differ significantly from marine fish. One big difference between freshwater and marine fish communities is the prevalence of eels in freshwater environments. Eels are found in the ocean but they are far more common in most of the world’s rivers and streams and are all believed to have originated from the tropics around south-east Asia. All freshwater eels belong to a single genus called Anguilla [9].

Common fishing species are trout and salmon which are also found through most parts of the globe, either naturally or due to being introduced by humans. Catfish are another common group of fish found in many parts of the world. Some species of catfish can grow into giant freshwater monsters and reach a length of 2.5 m (8 ft.) long and weigh more than 100 kg (220 lbs.) [10].

Carp are a family of freshwater fish that have become a massive problem in many parts of the world after being introduced and then completely taking over many rivers and streams through extremely prolific reproduction. Once carp have established themselves in an environment they often then go and completely alter the quality of the water through their eating methods and can entirely change the ecosystem. Despite intensive efforts to control carp populations in a number of streams and rivers, attempts more often than not appear to be unsuccessful as carp numbers continue to increase.

4. Fresh Water Fish

Fresh water fishes those that spend some or all of their lives in fresh water, such as rivers and lakes, with a salinity of less than 1.05%. These environments differ from marine conditions in many ways, the most obvious being the difference in levels of salinity. To survive fresh water, the fish need a range of physiological adaptations [11].

41.24% of all known species of fish are found in fresh water. This is primarily due to the rapid speciation that the scattered habitats make possible. When dealing with ponds and lakes, one might use the same basic models of speciation as when studying island.

Freshwater fish differ physiologically from salt water fish in several respects. Their gills must be able to diffuse dissolved gasses while keeping the salts in the body fluids inside. Their scales reduce water diffusion through the skin: freshwater fish that have lost too many scales will die. They also have well developed kidneys to reclaim salts from body fluids before excretion [12].

Many species of fish do reproduce in freshwater, but spend most of their adult lives in the sea. These are known as anadromous fish, and include, for instance, salmon, trout, sea lamprey and three-spined stickleback. Some other kinds of fish are, on the contrary, born in salt water, but live most of or parts of their adult lives in fresh water; for instance the eels [13]. These are known as catadromous fish, Species migrating between marine and fresh waters need adaptations for both environments; when in salt water they need to keep the bodily salt concentration on a level lower than the surroundings, and vice versa. Many species solve this problem by associating different habitats with different stages of life. Eels, anadromous salmoniform fish and the sea lamprey have different tolerances in salinity in different stages of their lives [14].

5. Classification of Freshwater Fish in US

Among fishers in the United States, freshwater fish species are usually classified by the water temperature in which they survive. The water temperature affects the amount of oxygen available as cold water contains more oxygen than warm water [15].

| Classification | Description |
|----------------|-------------|
| Coldwater      | Coolwater fish species survive in the coldest temperatures, preferring a water temperature of 50 to 60 °F (10-16°C). |
| Coolwater      | Coolwater fish species prefer water temperature between the coldwater and warmwater species, around 60 to 80 °F (16-27°C). |
| Warmwater      | Warmwater fish species can survive in a wide range of conditions, preferring a water temperature around 80 °F (27°C). Warmwater fish can survive cold winter temperatures in northern climates. |

6. Exotic Species

The introduction of exotic fish species into ecosystems is a threat to many endemic populations. The native species struggle to survive alongside exotic species which decimate prey populations or out compete indigenous fishes. High densities of exotic fish are negatively correlated with native species richness. Because the exotic species was suddenly thrown into a community instead of evolving alongside the other organisms, it doesn't have established predators, prey, parasites, etc. which other species do, and the exotic species thus has a fitness advantage over endemic organisms [16].

One such example is the destruction of the endemic cichlid population in Lake Victoria via the introduction of the predatory Nile perch (Lates niloticus). Although the exact time is unknown, in the 1950s the Ugandan Game and Fisheries Department covertly introduced the Nile perch into Lake Victoria, possibly to improve sport fishing and boost the fishery. In the 1980s, the Nile perch population saw a large increase which coincided with a great increase in the value of the fishery [17]. This surge in Nile perch numbers restructured the lake's ecology. The endemic cichlid population, known to have around 500 species, was cut almost in half. By the 1990s, only three species of sport fish were left to support the once multispecies fishery, two of which were invasive. More recent research has suggested that remaining cichlids are recovering due to the recent surge in Nile perch commercial fishing, and the cichlids that are left have the greatest phenotypic plasticity and are able to react to environmental changes quickly [18].
7. Freshwater fish of China

China has a wide range of marine and inland fisheries which support a similarly wide variety of marine and freshwater fish that are native to the country. There are more than 700 freshwater species and thousands of marine species in China. China is the world’s leading exporter of fish. Extensive fishing has resulted in a decline of most of these species in their native habitats leaving some at a risk of extinction. Others are exploited in commercial aquariums and ponds [19].

8. Native Fish Species of China

8.1. Balkhash Perch (Perca Schrenk)

The Balkhash is a freshwater fish native to China and Kazakhstan. The perch has an elongated body with medium-sized scales. The fish is mainly found in the waters of two lakes: Lake Balkhash and Alakol. The fish is an introduced species to some countries such as Uzbekistan [20]. The populations of the fish have been on a persistent decline making the species rare in its habitat mainly due to the introduction of predatory species such as the Zander.

8.2. The White Cloud Mountain Minnow (Tanichthys Albonubes)

The fish has been listed as an endangered species by the Chinese government due to its reduced population, pollution, and restricted range. The freshwater fish has silver-green color and red marks on the fins [21]. The species in their natural habitat are thought to be restricted to the Pearl River and Hainan Island. The white cloud minnow is an important species in the aquarium trade.

8.3. Silver Carp (Hypophthalmichthys Molitrix)

The silver carp has a laterally compressed body with a silvery color that changes to greenish on the top during adulthood. The scales of the fish are small with unusually placed eyes. The mouth of the fish faces upwards while its eyes face downwards and are placed in the middle of the body [22]. The fish is distributed in parts of the Pacific drainage in China, Pearl River, Amur River and some parts of Vietnam. Silver carp feeds on phytoplankton and zooplankton and can live up to 20 years [23].

8.4. Japanese Seerfish (Scomberomorus Niphonius)

The fish is native to the north-western Pacific coasts of China, Korea, Japan, and Russia in the subtropical and temperate waters. Due to heavy fishing in their native ranges, the populations of the fish have declined rapidly. In China, the species is listed as data deficient due to lack of information about the population of the species in China’s fisheries [24]. The fish migrates in the spring during the spawning period as well as in September to November during the feeding season. The species has an estimated lifespan of six years [25].

9. Native Fish Species of China

| Species                          | Scientific Name               |
|---------------------------------|-------------------------------|
| White Cloud Mountain Minnow     | Tanichthys albonubes          |
| Silver Carp                     | Hypophthalmichthys molitrix   |
| Japanese Seerfish               | Scomberomorus niphonius       |
| White Amur Bream                | Parabramis pekinensis         |
| Chinese Rice Fish               | Oryzias sinensis              |
| Kanning Nase                    | Xenocypris yunnanensis        |
| Eastern Viviparous Blenny       | Zoarces elongatus             |
| Amur Goby                       | Rhinogobius similis           |

10. 4 Species of Asian Carp

There are 4 species of Asian carp that threaten the Great Lakes. Bighead and Silver are considered by many to be the most dangerous threat to the ecosystem [26].

Bighead carp - black carp - silver carp - grass carp.

11. Description of Chinese Carp

| Carp name                  | Origin                  | Size                           | Life       | Key Issues                                                                 |
|----------------------------|-------------------------|-------------------------------|------------|---------------------------------------------------------------------------|
| Bighead Carp               | Eastern China and far East Russia, large rivers and lakes.       | Mature in 2-3 years, weight 18 kg, max 40 kg (rare), 1.5 m | 16+ years  | Ability to cross breed with Silver Carp.                                   |
| Black Carp                 | East Asia, South Russia, South China, Vietnam.                    | Mature at 6-11 years, Maximum 35 kg, 1.8 m | 15 years   | Reduces food, shelter and spawning areas for native fish                  |
| Silver Carp                | East Asia, South China, N. Korea, Southern China                 | Mature in 2 to 4 years, weight 9 kg, Max 40 kg, 1.2 m | 15 to 20 years | Ability to cross breed with Bighead Carp.                                  |
| Grass Carp                 | East Asia, South Russia, N. Vietnam                             | Average is 2 to 14 kg, up50kg, 1.5 m, mature in 1- to 10 years | 5 to 11 years | Reduces food, shelter and spawning areas for native fish                  |

12. Description of Bighead Carp

12.1. Common Name

Bighead Carp, This fish may also be referred to as noble fish, speckled amur, or lake fish. SCIENTIFIC NAME: Hypophthalmichthys nobilis

The bighead carp was formerly known as Aristichthys nobilis but that is no longer its accepted scientific name. It belongs to the Cyprinidae family, which is the carp and minnow family.
Asian bighead carp

In worldwide aquaculture, bighead carp (Hypophthalmichthys nobilis) ranks fourth in production (2.8 billion pounds in 1995). Bighead was introduced into the southern United States from China (the largest producer) in the early 1970s [27]. Because the word “carp” has a negative connotation to some consumers, alternative names proposed for bighead include “noble fish,” “speckled amur” and “lake fish.” In the United States, bighead is polycultured with channel catfish in approximately 5,100 acres of earthen ponds, including an estimated 4,000 acres in Arkansas, 500 in Mississippi, and 600 in Alabama [28]. Bigheads are an important source of additional income that, at times of low catfish prices, has kept some fish farmers in business. The primary market is to live-haulers who sell to specialty markets in large cities. The current U.S. market is limited and easily saturated. Wide fluctuations in price and unique marketing requirements for bighead have prompted farmers to seek alternative markets. Bighead has a pleasant, mild tasting flesh, but is too bony for most U.S. consumers [29].

12.2. Biology

The bighead is aptly named, as this fish has a large head with a protruding lower jaw and eyes that “look down. They are deep-bodied fish with tiny scales and gray to black blotches on the body, which gives them a speckled appearance. While bighead can reach 60 pounds or more and 4-year-old fish may weigh 20 to 25 pounds, the primary market is for 6- to 12-pound fish [30]. Bighead can survive in a wide range of temperatures, but in one study their preferred temperature was 78°F and the thermal death point were 100 to 102°F. The natural food of the bighead is zooplankton, along with larger phytoplankton. Bighead is filter feeders and uses their fine, comblike gill rakers to strain tiny animals and large algae from the water. If zooplankton is scarce, bighead may feed on detritus (organic matter and associated bacteria that accumulate on the pond bottom) [31]. Pond bottom organisms are not a normal food item; in one study, bighead were not found to have a significant impact on the benthic (pond bottom) community. Under favorable conditions, bighead grows rapidly. After reaching 1 to 2 pounds they can gain 1 pound or more per month. Growth is largely dependent on the fertility of the water and the stocking density. Market size fish (6 to 12 pounds) are usually 2 to 3 years old [31].

12.3. Harvesting and Marketing

The preferred market size for bighead is 6 to 12 pounds. Larger or smaller fish bring much lower prices. Bigheads are gentle and relatively easy to capture with a seine. They must be separated from catfish by hand at harvest, unless a very large mesh net is used. Where pond seining is done by crews from a catfish processor, stocking of bighead is discouraged because the sorting process slows the catfish harvest [32]. Some producers have learned the art of “swimming off” bighead. This involves using an aerator to create a current through the net containing the bighead and catfish. A 10- to 15-foot section of the float line of the live car (sock) is then submerged slightly (6 to 8 inches) so that the bighead will swim against the current and out of the live car, thus separating themselves from the catfish [33]. New advances in grading technology designed to select only market size fish from multiple-batch catfish ponds may speed sorting of bighead as well.

13. Specific Description of Bighead Carp

The body of a bighead carp is laterally compressed with the top being a dark gray color which grades down to off white on its belly. It has many dark blotches on its sides. Its head is comparatively large with no scales and a large terminal mouth. The bighead has no teeth and its lower jaw protrudes out farther than its upper jaw. The eyes are situated low on its head and are positioned downward. The scales of a bighead carp are small and resemble the scales of a trout [34]. This species is very similar to another exotic Asian carp found in the United States, the silver carp. The bighead carp has a keeled belly from approximately its pelvic fins to the anal fin, whereas the silver carp has a sharp keel from the anal fin to the throat [35]. They can also be distinguished by the fact that the bighead carp has many dark blotches on its sides and the silver carp does not. Indiana’s record bighead carp weighed 53 lbs and was caught in 2000.
14. Impacts of Bighead Carp

The main fear that biologists have in regards to the bighead carp is that it consumes the exact food that our native filter feeders eat, as well as what most juvenile fish eat. Because the bighead carp can reach such a large size they put extreme pressure on zooplankton populations. This loss of food for our native species could result in their population declines. Bighead carp especially affect paddlefish, bigmouth buffalo, gizzard shad and native mussels [36]. Also, declines in the zooplankton population can result in dense planktonic algae blooms. These fish pose an economic threat. In areas where bighead carp are so numerous, they are fouling the nets of commercial fisherman to the point where they can no longer lift their nets and are forced to abandon those fishing spots. Bighead carp is a preferred food for the Asian community [37]. Worldwide bighead carp ranks fourth in total production. With the Asian American population on the rise there is more and more pressure for Asian markets in the U.S. to supply bighead carp.

In March 2011 the U.S. Fish and Wildlife Service included bighead carp on the list of injurious fish species. This law prohibits importation into the United States and interstate transport of the fish. Indiana prohibits the importation, possession, or release of bighead carp into public or private waters. If a bighead carp is caught in Indiana, it must be killed immediately and not returned to the water alive. An aquaculture permit may be provided for medical, educational or scientific research purposes [10].

15. Morphological Compare between Asian Carp

Fish drying: an excellent method for food preservation

Drying or dehydration is, by definition, the heat and mass transfer process for removal of water by application of heat, from a solid or liquid food, with the purpose of obtaining a solid product sufficiently low in water content. Where removal of water takes place by virtue of a difference in osmotic pressure and not by evaporation. The main objectives of food dehydration are: Preservation as a result of lowering of water activity; low transport and storage cost as a reduction in weight and volume; Transformation of a food to a form more convenient to store, package, transport and use, e.g. transformation of liquids such as milk or coffee extract, to a dry powder that can be reconstituted to the original form by addition of water (instant products) [38].

16. Methods of Drying

Mainly, there are two types of drying which are given below
1) Natural drying
2) Mechanical drying

1. Natural Drying

Natural drying is the method of drying, in which we are using the natural source (viz. Sun) for drying of food products. It is also known as sun/solar drying. It has been used to dry fish, meat, cloth, grains and has proved to generate food stuffs of high quality and low spoilage though solar drying is cheap easy and popular method, its application is restricted by the long drying time and need for favorable weather. A research in (1994) showed that 6 - 9 weeks were required to dry grapes to a water content of 25-30 % and further steps were required to dry them completely [39]. Sun drying is cheaper method due to natural source of drying. While it is slow process, very prone to contaminants as well as weather dependent. That is why it is not most common in commercial scale [40].

1.1- Sun Drying:

The high sugar and acid content of fruits make them safe to dry in the sun. Vegetables and meats are not recommended for sun drying. Vegetables are low in sugar and acid. This increases the risks for food spoilage [41]. Meats are high in protein making them ideal for microbial growth when heat and humidity cannot be controlled. To dry in the sun, hot, dry, breezy days are best. A minimum temperature of 86°F is needed with higher temperatures being better. It takes several days to dry foods out-of-doors. Because the weather is uncontrollable, sun drying can be risky. Also, the high humidity in the South is a problem. Humidity below 60 percent is best for sun drying. Often these ideal conditions are not available when fruit ripens. Fruits dried in the sun are placed on trays made of screen or wooden dowels. Screens need to be safe for contact with food [42]. Outdoor drying rack most woods are fine for making trays. However, do not use green wood, pine, cedar, oak or redwood. These woods warp, stain the food or cause off-flavors in the food. Place trays on blocks to allow for better air movement around the food. Because the ground may be moist, it is best to place the racks or screens on a concrete driveway or if possible over a sheet of aluminum or tin [43].

1.2- solar drying:

Recent efforts to improve on sun drying have led to solar drying. Solar drying also uses the sun as the heat source. A foil surface inside the dehydrator helps to increase the temperature. Ventilation speeds up the drying time. Shorter drying times reduce the risks of food spoilage or mold growth.
2) Mechanical drying
  2.1- Oven Drying:
  Everyone who has an oven has a dehydrator. By combining the factors of heat, low humidity and air flow, an oven can be used as a dehydrator. An oven is ideal for occasional drying of fruit leathers, banana chips or for preserving excess produce like celery or mushrooms. Because the oven is needed for every day cooking, it may not be satisfactory for preserving abundant garden produce. Oven drying is slower than dehydrators because it does not have a built-in fan for the air movement. (However, some convection ovens do have a fan). It takes about two times longer to dry food in an oven than it does in a dehydrator. Thus, the oven is not as efficient as a dehydrator and uses more energy [44].

2.2- microwave drying:
Sun-drying of fish is a traditional practice. However, sun-drying often has various disadvantages (e.g long time to dry under non-sterile conditions often couple with poor handling during processing, resulting in a poor quality and unattractive finished product). Other drying techniques are being developed to improve the process such as solar drying, convective drying, vacuum drying, freeze drying, infrared and microwave drying. Although widely used, hot air drying has many disadvantages such as lower energy efficiency and long drying times [45].

To minimize these disadvantages, several drying methods have been studied in the literature. Microwave drying has been studied during the last two decades [46,47]. For industrial applications a frequency of 2450 MHz is widely applied for heating and drying. Microwaves work because of the quick absorption of energy by the water molecules, which causes rapid evaporation of the water and results in high drying rates for foods using less energy and giving a better quality of dried food [48]. According to [49], microwave drying is cost-efficient, controlled, rapid and safe when used properly.

The microwave drying of grass carp [50], tilapia fish fillets [49], are some examples. Infrared drying is also viewed as an alternative drying method for foods. When infrared radiation is used to warm up or dry moist materials, it penetrates the material where the energy of radiation is converted into heat through vibration of water molecules [51]. It also can reduce the drying time, lower energy costs, maintain a uniform temperature in the product and provide a better quality-finished product [52]. This drying method is particularly suitable for thin layers of material with large surface areas exposed to the radiation. Infrared drying of mullet fish [53] have been studied. There are several factors influencing the quality parameters of dried product. Some chemical and biochemical reactions such as browning reactions and lipid oxidation may alter the final color. Temperature, power level and drying time are the main factors affecting the color changes during drying [54].

However, the infrared drying of rainbow trout has not been studied. The main objectives of this study were to investigate the effect of microwave and infrared drying methods on the drying rate, time and color, to fit the experimental data to seven different mathematical models, and to compute effective diffusivity and activation energy of rainbow trout [55].

17. Fish Protein Hydrolysis
Most of the initial work on fish protein hydrolysis was performed in the 1960s. Emphasis was on fish protein concentrates an inexpensive nutritious protein source for developing countries [56]. Enzymatic hydrolysis of fish protein has been employed as an alternative approach for converting underutilized fish biomass into edible protein products, instead of animal feed or fertilizer [57]. Fish meat (a horse mackerel) was liquefied with hydrolysis in a reactor under subcritical (10-3kg fish meat, 3.36 × 10-<6cm3Milli-Q water) and supercritical (10-3kg fish meat, 3.06 × 10-<6(280°C, 30MPa) or 1.76 × 10-6 (400°C, 30MPa) cm3Milli-Q water) conditions at 200-400°C (Yoshida et al.,1999, 2000). At 200°C, 3.35 MPa and 5 min reaction time, lactic acid (0.03 kg/kg of dry meat), phosphoric acid (0.12 kg/kg of dry meat) and histamine (0.01 kg/kg of dry meat) were formed. At 280°C, 6.42 MPa and 30 min reaction time, pyroglutamic acid (0.095 kg/kg of dry meat) was produced. On the other hand, amino acids (cystine, alanine, glycine and leucine) were obtained at 270°C and 5.51 MPa. Oil was extracted from a water-insoluble phase with hexane and was rich in fatty acids, such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) at 200-400°C and 5 min reaction time, oil, amino acids (cystine, alanine and glycine) and organic acids (pyroglutamic acid) were formed under subcritical conditions [58].

Homogenized minced Atlantic salmon (Salmosalar) muscle was treated with one of four alkaline proteases (Alcalase 2.4L, Flavourzyme 1000L, Corolase PN-L and Corolase 7089) or endogenous digestive proteases were added in order to obtain 5, 10 and 15% degrees of hydrolysis (DH). The enzymatic hydrolysis was conducted at pH 7.5, 40°C and 7.5% protein content. Lipid content of fish protein hydrolysates (FPH) was low, while protein and nitrogen recovery was 71.7-88.4% and 40.6-79.9%, respectively. The highest protein and nitrogen recovery were reported when the sample was treated with Alcalase and Corolase 7089 proteases. Furthermore, proteases with high nitrogen recovery were produced at 15% DH, whereas the protein content of the hydrolysates did not differentiate with regard to DH. On the other hand, hydrolysates solubility increased at high pH (pH 7) and varied between 92.4 and 99.7%. Fish protein emulsification capacity and stability were higher at 5% DH and with Corolase PN-L and Corolase 7089 treatment.

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Fish protein emulsification capacity was 75-299 ml of oil emulsified per 200 mg of protein and fish protein emulsification stability varied from 50 to 70%. Finally, fat absorption was higher at 5 and 10% DH and ranged from 2.86 to 5.98 ml of oil/g of protein [56].

Northern shrimp (Pandalus borealis) waste (heads and scales) was mixed with water (1:1) and 5% (v, w) protease (Alcalase) was added to the mixture. The sample was hydrolyzed at 40°C for 2 h in an open stirred reactor, whereas solid tissues and crude hydrolysates were separated with pressing. The obtained press cake was processed to chitosan by demineralization, deproteinization, deacetylation and drying. Suspended solids from the crude hydrolysate were centrifuged and protein hydrolysates and astaxanthin were recovered. According to the conventional method, 12.8% Kjeldahl nitrogen and 8.8% total dry matter were recovered, whereas following this method 68.5% nitrogen and 33.2% total dry matter were retrieved. The obtained chitosan from this process had higher viscosity and similar dry matter ash and Kjeldahl nitrogen content to the one obtained from the conventional process. The protein hydrolysate was rich in essential amino acids, thus indicating potential use as a feed in salmonid fish or microorganisms [59].

18. Nutritional Value of Seafood Proteins in Human Health

The nutritional value of seafood muscle proteins is relatively high because of their favorable EAA content [60]. Although marine fish tend to have slightly higher protein content, there are no significant differences in the AA composition of freshwater and marine fish. However, certain marine fish such as mackerel and tuna may be exceptionally rich in the EAA, histidine. As compared with red meat, fish proteins are considered nutritionally equivalent or slightly superior. Fish proteins are easily broken down in human gastrointestinal tract by proteolytic (ie, protein digesting) enzymes, resulting in their digestibility exceeding 90%. The in vivo digestibility of proteins from raw fish and shell fish meat is in the range of 90-98% and 85%, respectively. The enhanced digestibility of seafood as compared to dietary protein derived from terrestrial animals is largely due to the absence of strong collagenous fibers and tendons as well as lower content of stroma proteins (mainly connective tissue) in seafood, which are common in terrestrial animals [61]. Since the human body utilizes protein from different sources with different efficiency in terms of incorporating the protein into the body, the PER makes it easy to compare the quality of protein from different dietary sources. The PER of fish muscle protein is slightly above that of casein, the major milk protein. Casein is commonly used in human nutrition for comparison purposes. The net protein utilization (NPV) reflects the ratio of AAs used to synthesize protein in the human body to AAs consumed [61]. Egg protein is considered a standard for protein nutritional quality and it is often used for comparison purposes. Friedman (1996) provided a comprehensive comparison of various indices of nutritional quality for dietary proteins from different sources, including seafood [62].

Ficin Enzyme

The name “ficin” was coined by Robbins in 1930 to apply to a protein powder he prepared from the latices of fig trees of the genus Ficus. Yet, by historical usage, one must consider ficin to include all preparations ranging from the crude latex to a single crystalline enzyme isolated from the complex of as many as ten proteinases found in the various latices. This, of course, complicates any definitive consideration of ficin. The literature on ficin rivals that of papain, extending to more than three hundred publications. It antedates the literature on papain by more than a millenium. In the Iliad (ca. 850 B.C.) Homer refers to the wild fig-tree sap which curdles milk, a property of the latex frequently mentioned in subsequent literature for the preparation of cheese. This is accomplished by stirring warm milk with a split fig branch.

Leonard da Vinci is reported to have fixed “the temper for the color (of paints) with the yolk of an egg and the milky ‘sap of the young branches of fig trees, mixed with water and wine.’” [63]. In Gabon, West Africa, the latex of Ficus dusenii Warb has been used as birdlime for catching or snaring birds. In Malaya the wax of the latex is employed in batik dying and for making candles. In Venezuela the natives use the latex as a glue or varnish [64].

A large part of the literature relates to the use of the latex, both fluid and dried, as a vermifuge. The use of “fig milk” for this purpose was first recorded by de Prifontaine in 1762 and was expounded upon by Bajon, the Surgeon General of French Guiana. Bajon (1770) conducted in vitro experiments on living worms and demonstrated the safety of the latex by rubbing it into his skin, tasting it, giving it to puppies and applying it to ulcers - all with little or no deleterious effect.

19. Properties of Commercial Ficin

Proteolytic enzymes of plant origin have been used quite extensively in industry during recent years. Papain, the latex of the green fruit of Carica papaya, and bromelin, the papainase of pineapple juice, has been used to clarify beverages and to tenderize meats. Most recently proteolytic enzymes derived from other sources such as the latex of the fig tree, bacteria, and fungi have been used to broaden the range of industrial usage. Rhozyme P-11 (Rohm and Haas Co.) is a potent proteolytic enzyme of fungal origin [65]. Whereas the properties of papain and bromelin have been quite thoroughly studied, relatively little is known about the properties of ficin. It is usually stated that papain and ficin are similar in properties. The known properties of ficin are summarized by Greenberg and Winnick [66].

20. Gelatin Hydrolysis Using Proteolytic Enzymes (Papain, Bromelin and Ficin)

As shown by following Figure the data on the hydrolysis of gelatin by papain and bromelin can be reduced to a linear form in the same manner as for ficin.
Papain and bromelin were found to have the same proteolytic activity and to be only about one-half as active as the same concentration of ficin in decreasing the viscosity of gelatin. The specific reaction rate constants at 33.9°C for papain and bromelin were found to be 2.50 and for ficin 5.10 mg.2 per min. The viscosity reduction method thus offers a convenient method for measuring the proteolytic activities of papain and bromelin as well as ficin.

Sonication is usually recommended for pretreatment of solid environmental samples for the extraction of nonvolatile and semi volatile organic compounds from solid, such as soils, sludges and wastes. When comparing the different methods available for analyte extraction from solid samples, sonication is considered as an effective method since unsophisticated instrumentation is required and solid-liquid separations can usually be performed in a short time using diluted reagents and low temperatures. To date, most of applications of ultrasonic extraction have been carried out for organic compounds, but the usefulness of ultrasound for element extraction is still to be explored. Some examples of solid-liquid extraction of some elements with the use of ultrasound are shown in coming table.

21. Extraction Methods

1. Ultrasound-Assisted Extraction:
Extraction techniques are widely accepted as a prerequisite for analytical determination of both organic and inorganic analytes in a large variety of samples. As a part of an analytical process, sample preparation is considered to be an essential step so that the entire process can be simplified. In this case, the ability of many analytical systems to handle liquid samples has brought about the development of separation methods which fulfill a main objective, i.e. to obtain quantitative analyte leaching from the solid matrix using a suitable solvent, with little or no matrix release, so that matrix effects can be kept to a minimum during the measurement steps. For speciation applications, a last condition of a solid-liquid extraction method must be the maintenance of the species integrity during treatment.

Comparison of the rates of hydrolysis of 200 mg. of gelatin by 16.0 pg. of ficin, papain and bromelin at pH 7.50 and 33.9°C C

For best results with this method as an assay method the following conditions should be observed. The gelatin solution (2.24%) buffered at pH 7.50 by 0.22 M phosphate buffer should age for at least one hour at 40° C. in a water bath maintained at rt0.1°. Determination of the activity should be carried out at 40.0°C with a ficin solution containing the activity equivalent to 4-10 pg. of the commercial ficin used in this work. The results would be even more significant if they were expressed in terms of the activity of crystalline ficin [67].

Solid-liquid extraction of some elements with the use of ultrasound

| Sample         | Element & percentage of extraction | Sonication system |
|----------------|-----------------------------------|-------------------|
| Bovine liver   | Cd (111%)                         | Bath              |
| Bovine liver   | Mn (100%), Fe (72%)               | Probe             |
| Cabbage leave  | Cd (89%), Pb (1%)                 | Probe             |
| Carbon         | Cr (14%)                          | Probe             |
| Lemon leaves   | Cd (67%), Cu (88%)                | Bath              |
| Rice flour     | Cd (100%)                         | Bath              |
| Sediment       | Cu (60%), Cr (10%)                | Probe             |
| Spinach        | Cu (98%), Cr (74%)                | Probe             |
| Tomato leaves  | Mn (70%), Fe (70%)                | Probe             |
| Wheat flour    | Mn (97%), Fe (88%)                | Probe             |

The extraction efficiency obtained with ultrasound could be increased by addition of glass beads which promote particle disruption by focusing the energy released by cavitation, and by physical crushing. Particle disruption could also be enhanced by increasing hydrostatic pressure and viscosity. The use of a bubbling gas during sonication gives rise to an enhanced formation of H2O2 and hydroxylradicals (OH-) thus aiding analyte extraction from oxidizable materials. In general, the use of probe-type sonicators at the appropriate vibrational amplitude and sonication time is required so that extraction efficiency can be improved for strongly-bound elements.

2. Microwave assisted extraction:
Microwave-assisted extraction technology is now becoming an emerging technology to obtain useful compounds from plant biomass [68]. Microwaves directly generate heat by initiating molecular motions of water and electrolytes in plant biomass; therefore, the irradiated materials are quickly heated from within. In addition, microwaves enhance the diffusion of the target compounds by directly heating biomass from within and facilitating non equilibrium mass transfer from inside of the plants to the extracting solvent [68]. The figure below shows the recent trends in the numbers of published papers regarding “microwave-assisted extraction” and “microwave water extraction.” The number of papers regarding microwave-assisted extraction has tripled within a decade reflecting the increasing attention to this process. Microwave-assisted extraction has been widely applied for extraction of polysaccharides, phenolic compounds, and for extraction of polysaccharides, phenolic compounds,
oils, and proteins from terrestrial plants, algae (seaweeds and microalgae), agricultural and food wastes, and lignocellulosic biomass.

The table below shows Microwave-Assisted Water Extraction of Polysaccharides, Phenolic Compounds, Oils and Others

| Feedstock       | Product          | Equipment                | Reference |
|-----------------|------------------|--------------------------|-----------|
| Mushroom        | -Glucan          | Microwave extra          | [69]      |
| Jujube          | Polysaccharide   | MLS Ethos 1600 (Milestone)| [70]      |
| Cordyceps sinensis | Polysaccharides | Anton microwave equipment | [71]      |
| Flammulina velutipes | Polysaccharides | (SINEO Microwave Chemistry & Technology) | [72] |
| Fucus vesiculosus  | Fucoidan        | MDS-2000 (CEM)           | [73]      |
| Rice bran       | Rice bran protein | LG MC8088HRC (LG Electronics) | [74] |

**Microwave assisted extraction (MAE):** MAE utilizes microwave energy to facilitate partition of analytes from the sample matrix into the solvent [75]. Microwave radiation interacts with dipoles of polar and polarizable materials (e.g. solvents and sample) causes heating near the surface of the materials and heat is transferred by conduction. Dipole rotation of the molecules induced by microwave electromagnetic disrupts hydrogen bonding; enhancing the migration of dissolved ions and promotes solvent penetration into the matrix [76]. In non-polar solvents, poor heating occurs as the energy is transferred by dielectric absorption only [77]. MAE can be considered as selective methods that favour polar molecules and solvents with high dielectric constant.

1. Maceration, infusion, percolation and decoction:
Maceration is a technique use in wine making and has been adopted and widely used in medicinal plants research. Maceration involved soaking plant materials (coarse or powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation [77]. The processed intended to soften and break the plant’s cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or strained by filtration. In this conventional method, heat is transferred through convection and conduction and the choice of solvents will determine the type of compound extracted from the samples. Infusion and decoction uses the same principle as maceration; both are soaked in cold or boiled water. However, the maceration period for infusion is shorter and the sample is boiled in specified volume of water (eg. 1:4 or 1:16) for a defined time for decoction [77].

**22. Protein Fraction**

Protein fractionation utilizes the varied properties of proteins to separate a complex biological sample into more basic, enriched and concentrated samples. There are numerous properties of protein that can be utilized to fractionate proteins, including size, shape, sedimentation velocity, ability to bind to various ionic groups, affinity for substrates or pseudo-substrates, solubility, stability, and many more. Basically fractionation of a protein of interest can use any protein property that differs from unwanted proteins. For the fractionation of a protein of interest from a complex biological sample, numerous fractionation properties are routinely used.

**23. Fractionation Procedures**

Proteins can be fractionated into different groups having similar physical and chemical properties by a variety of different analytical methods, as shown in Table 1. Fractionation of proteins from a cell lysate by chromatography or electrophoresis is achieved by manipulations of either (i) the mobile-phase properties such as slope (time) of the gradient, organic modifier concentration, buffer pH, salt concentration, and/or ampholyte pH range; or (ii) type of column, such as reversed-phase (e.g., C-18), ion exchange, size exclusion, normal phase (e.g., silica), or affinity (e.g., antibody, lectin, aptamer, metal, DNA, etc.), and (iii) gel properties [78].

| Fractionation method       | Physical/chemical property |
|----------------------------|----------------------------|
| Ultracentrifugation        | Density                    |
| Isoelectric focusing       | Isoelectric point          |
| Hydrophobic chromatography | Hydrophobicity             |
| Affinity chromatography    | Specific interaction       |
| Gel electrophoresis        | Stoke’s radius             |

List of various fractionation methods used to separate proteins based on a particular physical or chemical property.
24. Affinity Chromatography

Selective separation of a specific protein or group of proteins can be achieved using affinity HPLC or affinity CE. The principle of affinity is based on the ability of a biologically active molecule to bind specifically and reversibly to complimentary molecule, often bound to a solid support. These ligand molecules may include antibodies, metals, lectins, biotin, aptamers, etc. In addition, affinity methods have been developed to select peptides containing specific types of residues such as cysteine, tryptophan or methionine [79,80].

25. Chromatographic Methods

The principle of chromatographic fractionation is based on the interaction of the proteins or peptides with the solid support (stationary phase) and the mobile-phase. The interaction may be adsorption on silica surfaces, partitioning on reversed-phase materials or ion exchange based on effective charge of the proteins and peptides. Moore and Lee [81] described a procedure for the fractionating of water-soluble proteins from liver into 15 fractions by chromatography on diethylaminoethylcellulose by elution with a parabolic gradient to 1 M sodium chloride. A defect of the parabolic gradient, according to Moore and McGregor [82] is that much of the proteins appeared as a large broad peak and a number of enzyme activities overlapped in the region of 0.1M chloride during the elution. Moore and McGregor used a complex nine-chambered gradient device to improve their gradient development.

26. Multidimensional Separation Approaches

As mentioned, no single chromatographic or electrophoretic procedure is likely to resolve all the proteins in a cell or tissue. A multidimensional (i.e., 2-D or 3-D) method that employs orthogonal separation techniques, slab-gel electrophoresis (SGE), HPLC or CE, or separation methods with different separation mechanisms, ion exchange, partition, adsorption, affinity, size exclusion, etc., will significantly improve the chances of resolving a complex mixture of proteins into its individual constituents. An added advantage is that an extensive fractionation lowers the dynamic range requirements on the instrumental technology. According to Giddings [83], the peak capacity of a multidimensional separation is the product of the peak capacities of its component 1-Dmethod. While increasing the number of separation dimensions can continually increase the peak capacity, it is always important to remember that the overall separation technology must strive to meet and integrate with the demands of proteomic analysis.

27. Conclusion

Bighead carp has been studied for several years, in this review we tried to study the effect of ficin enzyme on protein hydrolysis process extracted from bighead carp. Ficin enzyme used long time before but few researches study effect of ficin enzyme on protein hydrolysis. Different extraction methods such as ultrasonic and conventional extraction method have been studied to show the effect of these methods on protein hydrolysis.

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