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100% fish in the Great Lakes region

Cisco (*Coregonus Artedi*) full utilization

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Skýrsla Matís 23-24

July 2024

ISSN 1670-7192

DOI nr. 10.5281/zenodo.13454076



Report Summary

Icelandic Food and Biotech R&D

ISSN 1670-7192

<i>Title</i>	100% fish in the Great Lakes region Cisco (<i>Coregonus Artedi</i>) full utilization		
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<i>Report no.</i>	23-24	<i>Date</i>	July 2024
<i>Project no.</i>	62740		
<i>Funding:</i>	Great Lakes St. Lawrence Governors & Premiers (GSGP)		
<i>Summary in English:</i>	<p>This report presents a comprehensive biotechnical analysis of fillets and byproducts (heads, bones/frames, and skin) from Cisco (<i>Coregonus Artedi</i>) harvested in the Great Lakes region of the United States. Conducted by Matís for the Conference of Great Lakes St. Lawrence Governors & Premiers (GSGP), the study aims to identify higher-value alternative uses for Cisco byproducts.</p> <p>Key findings include:</p> <ul style="list-style-type: none"> • Whole Body Mass Balance and Proximate Composition: Detailed analysis of fillets, heads, bones, and skin. • Amino Acid and Hydroxyproline Content: Evaluation of amino acid content in heads and hydroxyproline content in skins and scales, with scales showing notably high hydroxyproline at 2.45 g/100g protein. <p>Utilization Alternatives</p> <ol style="list-style-type: none"> 1. Protein Isolate and Fish Oil from Heads: The heads showed significant amino acid content, making them suitable for protein isolate production and fish oil extraction. 2. Gelatine Production from Skins and Scales: High hydroxyproline levels indicate potential for high-quality gelatine production. 3. Protein Hydrolysates from Viscera and Roes: Though promising, these transformations require substantial investment and adequate raw material supply. <p>Conclusion and Recommendations</p> <p>This initial identification of utilization alternatives highlights the potential for value-added products from Cisco byproducts. Further analysis and feasibility studies are recommended to optimize and implement these alternatives.</p>		
<i>English keywords:</i>	Lake Cisco, full utilization, heads, bones, skin, scales, FPH, FPI, gelatine		

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Introduction

Cisco also named as Lake Herring (*Coregonus Artedi*) is an endemic specie found in the lakes of Canada and the United States. They are mainly found in the Great Lakes region in the Lake Superior waters of Ontario, Canada; Minnesota, Wisconsin and Michigan, United States. The samples received at Matis were wild caught in the Minnesota waters of Lake Superior. It is the most widespread of the cisco-type fishes in North America (U.S. Fish & Wildlife service, n.d.). Cisco has an adult length between 25 and 45cm and has a distinctive silvery color with pink and purple iridescence. They have a white belly.

Cisco have been extensively analyzed by the Monterey Bay Aquarium's Seafood Watch Program which ranks Lake Superior Cisco as a "best choice" for consumers. Resource managers have extensive biological and spawning stock biomass data for Lake Superior Cisco and anticipate a huge influx of adult biomass to the population in two years when the 2022 banner year-class becomes mature.

To maximize the value of this fish, it is of interest to use it fully. Nowadays, the fish are mainly used for their fillets and roe as caviar. The use of their skins and scales as well as the heads and frames for further added value would be of interest. The aim of this report is to evaluate the proximate composition from the side streams from Cisco from Lake Superior and evaluate the potential further utilization.

Material and Methods

Proximate composition and chemical analysis of the samples

10 *Coregonus Artedi* fish from Lake Superior were shipped frozen to Iceland. Prior to mass balance and separation of the pieces of interest, the fish were defrosted overnight at 4°C on a tray cover with a plastic sheet to prevent drying.

The mass balance of the whole fish and the heads, skins, frame, fillets, roe (if present) and viscera was done on 6 fishes. The whole-body weight was taken on the 10 fishes. For the fillets, skins, bones, scales and heads, the proximate composition (water (ISO 6496-1999), protein (ISO 5983-2 (2005)), ash (ISO 5984 (2022)) and fat (Soxhlet method AOCS Ba 3-38 (2017))) was measured. Analysis of amino acid composition (method EU 152/2009 (F), ISO 13903:2005, AMSUR, IC-UV for the amino acid composition and method EU 152/2009 (F), ISO 13903:2005, IC-UV for cysteine and methionine) on the heads and hydroxyproline content (SO 13903:2005, IC-UV) on the skins and scales.

For economic reasons, a pool of the 6 fish fillets and 6 fish skins was created and measured once for proximate composition. For the frames, two pools of 3 fish frames were made and analyzed. For the heads, 3 pools of 2 fish heads were made and analyzed.

Gelatine extraction from skins, scales and frames

Gelatine from skins

The gelatine (water soluble collagen) was extracted according to the method of Phanturat et al. (Phanturat et al., 2010) with slight modifications. The skins were cut in 3 squared cm and then rinsed for 5 min in cold water to remove the impurities (left scales, pieces of muscles...). They were then transferred into a 0.1M NaOH solution (ratio 1:10) for 3 times 30 min at room temperature. This step removed the non-collagenous proteins. After that, they were washed under cold water to have a pH close to 7. They then went into a 0.05M acetic acid solution (ratio 1:10) for 2 times 45 min to remove the rest of the impurities and after that for 3h to allow the swelling of the protein matrix. They were then rinsed again with cold water to bring the pH back to close to 7. The gelatine was extracted in

water at 45°C for 14h at 100rpm. The solution was then filtered through 50µm cheese cloth and then freeze dried.

Gelatine from scales

Fish scales were washed in 10% NaCl solution for 24h with a ratio scales: solution 1:10 to remove the non-collagenous proteins. They were then rinsed with water until the pH became neutral. A demineralization step was then conducted by stirring the scales for 90min in a 0.4mol/L HCl solution (ratio 1:15). The scales were then rinsed again to bring back the pH to around 7. The scales were soaked back in water with a ratio of 1:3. The gelatine was extracted by heating the solution at 80°C for 14h. The solution was then filtered through a 50µm cheese cloth and then freeze dried.

Gelatine from frames

The frames were soaked in 1M HCl solution with a ratio of 1:5 for 36h (frames were soft after – meaning that most of the minerals were gone, only leaving ossein). The demineralized frames were rinsed with water until the pH went over 5. After that the gelatine was extracted with hot water, with a ratio frames: water at 1.3. It was extracted for 24h at 80°C. The solution was then filtered through a 50µm cheese cloth and then freeze dried.

Hydrolysates of viscera and roes

Viscera and roes were tested to evaluate if they could work to produce protein hydrolysates. Two enzymes were tested: Neutrase and Flavourzyme. Neutrase is a high-quality broad-spectrum endo-protease which provides a mild hydrolysis. Flavourzyme is a high-quality blend of endo and exo-peptidase, which provides an extensive hydrolysis. The samples were mixed with a ratio of 1:2 of water for the roes (high water percentage) and 1:3 for the viscera (lower water content). Around 1 part of enzyme for 50 parts of protein was used as both neutrase and flavourzyme are working for the conditions 50°C, pH between 6 and 8. All the samples were prepared with those conditions, heated for 2 hours. Then, the enzymes were stopped by heating the solutions at 90°C for 20 minutes and put on ice to stop the reaction afterwards. The samples were filtrated through the 50µm cheese cloth, and the liquid part was then weighed and freeze dried.

pH-shift of heads and roes: recovery of oil, protein isolate and rest raw materials

The heads and roes were weighed and mixed with cold water with a ratio 1:3 and homogenized at 15000rpm for 90s with the IKA Ultra-Turax T25. Once the samples were homogenized, the pH of the solution was adjusted to pH 11 by slowly adding 2M NaOH. If the solution became too thick, water was added. The solution was then centrifugated for 20 minutes at 5000rpm. 3 layers were formed: the top layer which was mainly fat, the middle layer with water and soluble proteins and the bottom layer with all the insoluble proteins and the rest pieces. The three layers were separated. To the middle layer was added 3M HCl solution, until the pH reached 5.3. The protein isolate was drained through the 50µm cheese cloth and was then freeze dried.

The top layer was weighed for an estimation of the fat content recoverable. The bottom layer could then be submitted to the gelatine extraction process mentioned earlier.

Methods to analyze the different products

Color analysis

The color of the samples was determined with a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) using the CIE Lab system. The instrument recorded the L*-value, indicating lightness on the scale from black to white, 0 to 100 respectively, the a*-value, ranging from (+) red to (-) green, and the b*-value, ranging from (+) yellow to (-) blue. The Whiteness and Yellowness of the samples could also be calculated with the following formulas:

$$\text{Whiteness (Judd)} = 100 - ((100 - L)^2 + (a)^2 + (b)^2)^{0.5}$$

$$\text{Yellowness (Francis and Clydesdale)} = \frac{142.86 * \text{abs}(b)}{\text{abs}(L)}$$

Bloom strength of gelatine

The bloom strength was measured according to the standard method of gelatine manufacturers of the Europe association (gelatine Manufacturers of Europe, 2020). This method shows the response of how gelatine could be used in the industry depending on its gelling properties.

Results and discussion

The analysis of whole-body weight of the samples received at Matis showed that the Cisco – Lake Herring from Lake Superior had an average whole-body weight of 656.5g ± 193.8g. The smallest fish received weighed 388g while the biggest weighed 1070g. Some of the fish (4 out of 6) had roes in them, representing a significant part of their weight. The percentage of each part was different depending on the size of the fish, the fillet representing a lower percentage of the whole body mass for the smaller fish and a more important one for the bigger size fish.

Mass balance

For Cisco, fillets represent the majority of the weight (Figure 1). For the average weight mentioned earlier, fillets would weigh 199±78g. The second largest part would be the head with an average weight of 86.5±32.1g. The third biggest would be the roe (when present) with an average weight of 84.2±69.7g (quantities of roe varied a lot between fishes). After the frames represented on average 54.8±16.6g, the viscera with a weight of 43.0±18.7g. The two lowest were the skin with an average of 30.2±9.2g and the scales with an average weight of 17.5±8.5g. Despite their lower weight, those two groups are the ones with the highest potential added value. The rest is a mix of blood, parts of muscles and skins from the fins, tail and some ice left on the fish.

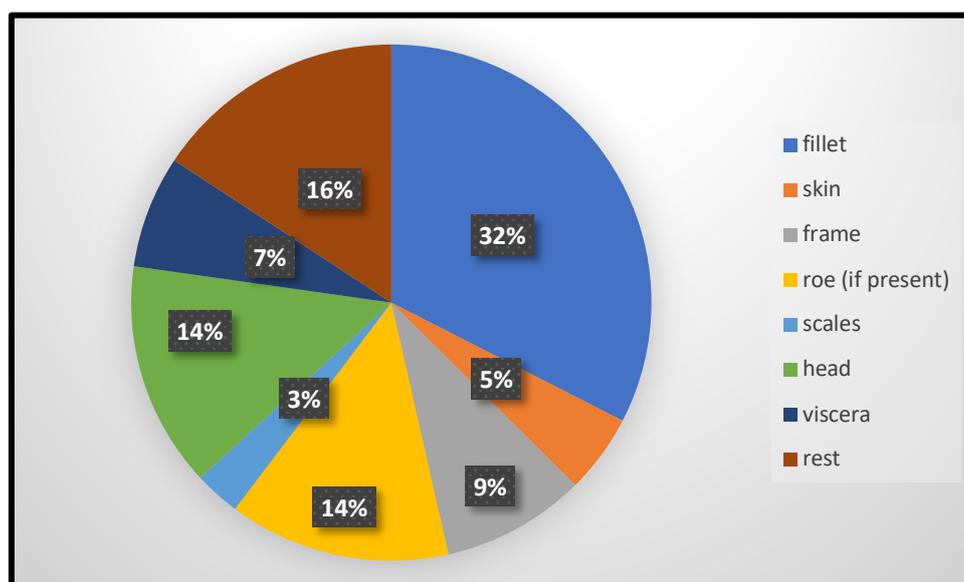


Figure 1: Mass balance of the different parts of the fish

Proximate composition and chemical composition of the fillets and side streams

Cisco fillets

The fillets had on average a water content of 74.5%, a protein content of 20.6%, a fat content of 3.4% and an ash content of 1.3%. Cisco could therefore be considered as a lean fish (under 5% of fat in its fillet).

Cisco head

The Cisco heads were analyzed both for the proximate composition and their amino acid content. The heads were chosen for the amino acid content as they are an average representation of the whole body. The water content of the heads was $69.5\pm 1.2\%$, the protein content was $17.2\pm 0.7\%$, the fat content was on average 6.9%, and the ash content was $7.2\pm 0.2\%$. Compared to Herring (*Clupea harengus*), Cisco heads were slightly lower in water and fat content but slightly higher in protein and ash. Those changes could be linked to the feeding pattern that would be different in a lake versus at sea (where more biodiversity could be found).

The amino acid composition of the heads from Cisco were studied due to their importance for human nutrition to synthesize proteins. As some amino acids (Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine) are only provided through food intake, it was of interest to see if those would be present in high quantity in those fish and therefore specifically of interest for human health. Those values will be used for comparison with the data from (Mohanty et al., 2014) to evaluate the levels of the individuals amino acids.

The amino acids have been classified in different categories for their importance in human nutrition. They were traditionally classified as essential amino acids (EAA), conditionally essential (CEAA), and non-essential (NEAA). A fourth category has been developed to classify amino acids which have a role and impact in key metabolic pathways, therefore improving health, development and other essential functions in the body--they are named functional amino acid (FAA).

In the Cisco samples, some amino acids were considered high compared to other species (Mohanty et al., 2014). In Table 1, the main amino acid (the ones detected over or around 1 g/100g protein) are detailed.

Table 1: Amino acids in medium/high quantity in the Heads of Cisco from the Great Lakes

Amino acid	Quantity (in g/100g protein)	Levels analysis	Classification
Alanine	1.19	low	NEAA
Aspartic acid	1.48	low	NEAA
Glutamic acid	1.91	Medium	EAA
Glycine	1.75	Medium	EAA
Leucine	1.06	Medium-high	EAA
Lysine	1.22	Low-Medium	EAA
Proline	0.93	High	EAA

Proline is one of the amino acids that on a per-gram basis for human nutrition has the highest requirement as it is used as a key regulator in multiple biochemical and physiological processes of the cells like signaling molecule and superoxide anion participating in redox reactions. It also has a role in cell differentiation and serves as a major amino acid in the synthesis of polyamines (regulator of DNA and protein synthesis) of the small intestine and placenta (Wu et al., 2011). In Cisco, it is higher than in most fresh water fish species from Mohanty et al. article.

Leucine is the only amino acid which can stimulate muscle protein synthesis and plays a therapeutic role in stress conditions such as burn or trauma. It had also been highlighted that it could be useful to help reduce obesity and achieve some weight loss (Layman, 2003). Levels of Leucine in Cisco are considered good compared to some other fish species such as *Sardinella longiceps* (0.6 ± 0.1) but low compared to others like *Rastrelliger kanagurta* (10.3 ± 0.4).

Glutamic acid has an important role in metabolism due to its role in transamination reactions and its necessary presence in the synthesis of key molecules such as glutathione. Compared to most fish, the glutamic acid content of Cisco was medium, except in *Catla catla* where it reached 13.8 ± 3.5 (Mohanty et al., 2014).

Overall, in Cisco 5 essential or conditionally essential amino acids have been found in medium to higher levels than in most fresh and seawater fish which is making it a good candidate for fish protein hydrolysate.

Cisco frames

The frame (and other small bones) of the Cisco were analyzed for proximate composition. In the frames the spinal cord was still inside with the bone marrow, therefore, it was not only bones analyzed, but more the skeleton system.

The water content of the bones was $49.3\pm 1.3\%$, the protein content was $21.2\pm 0.7\%$, and the ash content was $17.8\pm 2.8\%$. As a deduction, the fat content in the bones should be around 12%.

As the bones seem to be high in fat, when the extraction of gelatine from them is done, it would be necessary to add an extra step for defatting to ensure a not too high fat content in the final product.

Cisco Skin

The skin measurements were realized on the skin with the scales removed. The water content was 60.9% and the protein content was 26.1%. The 14% left could be both fat and a bit of ash.

The hydroxyproline content in the skin is measured to know how much collagen/gelatine is present in it and could therefore be extracted. In most of the fish skins, the quantity of hydroxyproline detected is around 1% (Skierka & Sadowska, 2007) and in Cisco those levels were confirmed with hydroxyproline content being $0.72\pm 0.14\%$. As 13 to 14% of the fish skin collagen is made of hydroxyproline, we could expect an extraction yield of 10 to 13% of the whole skin.

Cisco scales

The water content of the scales was 40.7% and the protein content was 43.4%. The rest (15.9% would be mainly ash and potentially a bit of fat). The hydroxyproline content of the scales was $2.45\pm 0.49\%$ which is really high compared to most fish species (for example in Tilapia where the hydroxyproline content was only 0.71% - according to Zhang et al., 2019).

This high level of hydroxyproline in scales suggests that they would be an interesting material to extract significant amount of gelatine from.

Gelatine made from the skins, bones and scales

Gelatine from the skins

The gelatine (water soluble collagen) extracted from the skins gave promising results for further development. The yield (ratio of freeze-dried product, from initial wet weight of the skins) was at 9.8%. The dry yield was 25.1%. The color of the freeze-dried product was white with an average L-value of 84.2 ± 5.3 , an a-value of -0.5 ± 0.2 and a b-value of 6.7 ± 1.5 . The whiteness (Judd) was 82.87 and

the Yellowness was 11.29. As shown in Figure 2, the overall appearance of the gelatine was white. It was soft and the smell was not strong.



Figure 2: Freeze-dried gelatine made from Cisco skins

The bloom strength of the gelatine at 4°C was 91.8g which is lower than commercial gelatine (200g). This means that this gelatine could only be used for softer texture material and aimed at being stored at cold temperatures (under 15°C).

Gelatine from the frames

The gelatine extracted from the frames needed a lot of improvement compared to the one done with the skins. As mentioned, due to the high fat content in the frame and that no step was added to remove that fat, the fat content was really high on the gelatine (visible with its yellow color and a strong fatty smell). Due to a less quality extraction, the yield (wet) was only 4.9% and dry yield was only 9.6%. The color of the freeze-dried product was yellow with an average L-value of 77.56 ± 4.9 , an a-value of -0.8 ± 0.3 and a b-value of 13.2 ± 1.7 . The whiteness (Judd) was 73.97 and the Yellowness was 24.26. This gelatine definitely needs some improvement due to the high fat content as seen Figure 3.



Figure 3: Freeze-dried gelatine made from Cisco frames

A defatting step must be added to ensure a better quality. Both the low yield and low quality indicate that the frames might not be the best candidates for gelatine extraction and might be better suited for dual protein - mineral powder source. Therefore, this gelatine was not tested for bloom strength.

Gelatine from the scales

The gelatine made from the scales gave promising results. The wet yield of scales gelatine was 15.5 %; when the dry yield was measured, it was 26.2. It is consistent with the high hydroxyproline content found in the scales, showing a high yield of extraction. The color of the freeze-dried product was white with an L-value of 82.9 ± 7.5 , an a-value of -0.3 ± 0.2 and a b-value of 2.0 ± 0.8 . The Whiteness (Judd) was 82.75, which was comparable to the gelatine from the skins. On the other hand, the Yellowness was 3.40, which is the lowest from the 3 gelatines extracted, corroborating the idea that the scales with their low fat content would produce a good gelatine quality. As shown in Figure 4, the gelatine extracted was white and the texture of the dry gelatine was less soft than the one from the skins, indicating that probably some minerals got extracted as well in there.

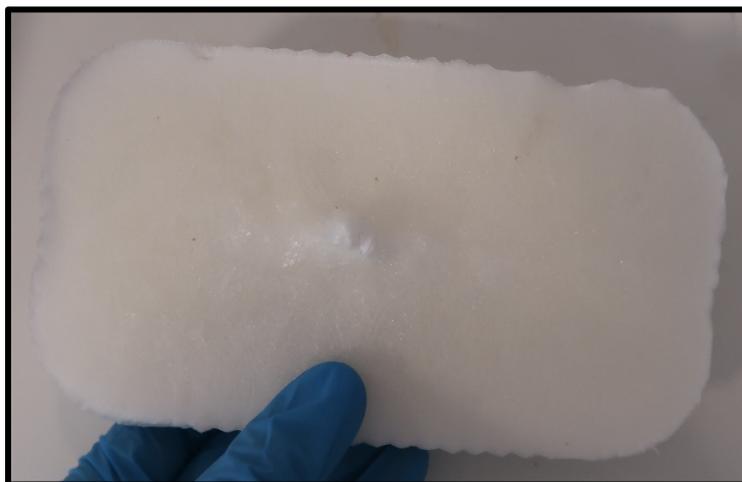


Figure 4: Freeze-dried gelatine made from Cisco scales

The bloom strength of the gelatine at 4°C was 37.3g. This low bloom strength could be due to two main reasons: either the extraction method needs to be changed to increase the bloom strength (early process making), or the process needs to be more refined to ensure a better final quality of the product.

Protein hydrolysates from viscera and roes

Both the viscera and the roe when extracted for protein hydrolysates were expected to get higher yields when flavourzyme was used compared to neutrase, as flavourzyme is a harsher enzyme (Table 2).

Table 2: Yield obtained from the hydrolysis of viscera and roes

Sample used	Enzyme used	Yield obtained (in % of dry final results : wet initial sample)
Roe	Neutrase	12.7 %
Roe	Flavourzyme	22.7%
Viscera	Neutrase	23.4%
Viscera	Flavourzyme	26.9%

The yields are always higher for Flavourzyme as expected, but the difference is most noticeable for the roes where Flavourzyme was clearly better than Neutrase, as the yield was close to twice more.

Flavourzyme also produced more white hydrolysates than neutrase with a more visible difference for the roe protein hydrolysates. As seen in Figure 5, it was visible from the extraction process that roe hydrolysates had a lighter color than viscera ones and it was confirmed once the samples were freeze-dried.



Figure 5: Protein hydrolysates from Cisco roe and viscera

Regarding the sensory properties, the roe protein hydrolysates made with neutrase had a softer smell than the protein hydrolysate made with neutrase. For the viscera, where the color was darker than in the roes, both the ones extracted with flavourzyme and neutrase had a pronounced smell (chemical, acid characteristic – not fishy).

From this test, it seems that roes could be used as protein hydrolysates when treated with Flavourzyme and adding extra steps to allow a whiter and odorless product. It would also be necessary to get the amino acid profile and the composition of the hydrolysate to understand more deeply how they could be valorized.

pH-shift process on heads and roes

From both the heads and roes during the pH shift process, three layers were formed. On the top one, the oil layer, the yield recovered showed that the process worked well to recover the oil. However, extra steps would be needed to end fully the separation between the oil and the protein isolate layer as, as seen in Figure 6. For both samples, the top-fatty- layer was not clear – especially for the head.

The yield obtained was 15.9% for the head for the fat extraction (which is a mix of fat and protein isolate – so probably half of the weight or less would be pure fat) and it was 8.1% for roes (layer seemed more homogeneous – probably around 80% of it was fat).

Once those extra steps to separate the fat properly would have been made, those two rest raw material parts could be good candidates for valorization.

For the protein isolate, the isolates recovered were of a really small size, with a high-water content in them. It was therefore decided to dry them to get an approximate yield for the isolates. The wet yield (in percentage of dry isolate to the wet initial sample) was 16.9 for the roe and 20.80 for the proteins. As the heads were measured with 17% protein, the extra 3% recovery could be linked to some fat (contained within the bones and not separated from the isolate during the process due to a too low spinning) and water that was left in the drying process (freeze dry samples can contain under 5% of water).

The rest pieces were not processed further into gelatine as the gelatine extraction from the frames showed the high fat content it had and the extra steps that would be needed to get a good quality of gelatine extracted. Once an improved process would be found for how to get a good valorization pattern for the bones, then this third layer from the heads could be valorized as well. For the roes, the last layer left was similar to the one found with the hydrolysates process.

Overall, the pH shift process would be good to use on the heads to get as much added value from it as possible, as it is one of the biggest side streams from the processing.

Possible utilization of rest raw materials

Numerous possible utilizations of rest raw materials exist -from lower value utilization as fish oil and meal to higher end products as additives in food and drinks to even higher-end value valorization in health products.

Fish meal and fish oil

Fish meal and fish oil are produced from the unused part of fish and/or from fish that are unfit from human consumption. In recent years, due to a large increase of aquaculture facilities in China, the demand for both fish meal and oil has increased. On the other hand, the production of fish meal and fish oil was similar or even lower than the previous years due to bad weather and the temporary closure of the fishery in Peru, which led to an increase of the price of those products on the market. On top of that, the increase of price of alternative proteins like soya due to the war in Ukraine, has led to an even tenser market and higher prices (FAO, 2022). In 2022, the market for fish meal reached 1800 USD/ton (increased by 200 USD/ton since 2021). The latest information gave fish meal prices at 1600USD/ton in April 2023. The market for fish oil reached 3000 USD/ton in 2022 (increased by 700



Figure 6: Fat form the pH shift process of heads and roe

USD/ton since 2021) as shown in Figure 7 and continued to rise. In September 2023, food grade oil was sold for 3700USD/ton.

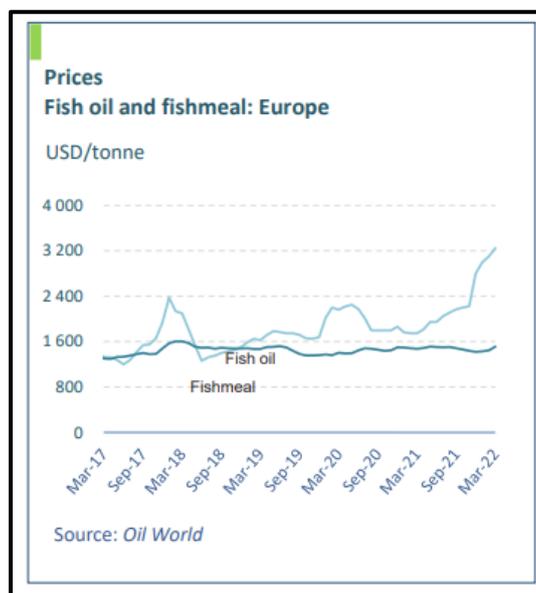


Figure 7: Price evolution (in USD/ton) of fish meal and fish oil from 2017 until 2022 in Europe

It is usually accepted that when full fish or mixed rest raw materials (RRM) are used to produce fish meal and oil, from 100kg of material, 21kg of fish meal and 3 to 6 kg of fish oil can be produced (European Commission. Directorate General for Maritime Affairs and Fisheries. & EUMOFA., 2021). As the amount of Cisco fished per year in Lake Superior is 1108.3 tons/year (2021 data), the revenue of transformation of Cisco rest raw material (heads and frames) into meal and oil would be 152,910 USD per year as shown in Table 3.

Table 3: Expected revenue from fish meal and fish oil production from Lake Whitefish rest raw material per year

	Rest raw material (heads and frames)
Cisco (ton/year)	1108.3
Heads and frames RRM (ton/year)	254.9
Fish meal (ton/year)	53.5
Fish oil (ton/year)	15.3
Revenue (USD/year)	152 910

Fish protein hydrolysate (FPH)

FPH are of interest thanks to their antioxidant properties as well as interesting sensory properties. They can also improve water holding capacity and texture of the food products they are used in. Nowadays, fish protein hydrolysate prices vary depending on the quality (amino acid composition). Price per ton could range from 1,500 USD to 10,000 USD. The recovery of raw material after transformation is known to be between 60 and 70% of the initial proteins (Ghaly et al., 2013). Other articles have mentioned a maximum recovery yield from fish heads through hydrolyzation of 50% of the raw material (wet yield)(Nurdiani et al., 2022).

As a dry product, the yield of fish protein hydrolysate is around 10% (Thankamma et al., 1979). But from the first test conducted with flavourzyme, the FPH obtained was around 22%. From the total

amount of viscera and roe available per year in the Great Lakes region, a total of between 76,800 USD and 512,000 USD can be produced as shown in Table 4, depending on the quality of the FPH produced.

Table 4: Expected revenue from fish protein hydrolysate from Great lakes Cisco in a year

	<i>Rest raw material (viscera and roe)</i>
<i>Cisco (ton/year)</i>	1108.3
<i>Viscera and roe (ton/year)</i>	232.7
<i>Fish Protein Hydrolysate (Dry ton/year)</i>	51.2
<i>Revenue (USD/year)</i>	256 000 (average)

Fish Protein Isolate (FPI)

Fish protein isolate is a purified fish protein (at least 90% of the dry material is protein). These protein isolates are usually used as food complements thanks to their properties (good gelation properties and some foaming-emulsion properties). They can also be used for surimi production. The advantage of this process is a high recovery of protein for a relatively low cost (Kristinsson & Liang, 2006). The market for fish protein isolate is similar to the one of fish protein hydrolysate.

Mineral bone powder

As bones are known to be rich in minerals and collagen, they are of interest for numerous products. Creation of mineral rich (calcium mainly) powder to use as supplement for humans or to include in food as an additive to create superfood like sausages (Hemung et al., 2018) or biscuits (Ananda & Anggraeni, 2021) is becoming more common as the world is seeking healthier products. To create the bone powder, the bones are usually cleaned with mild chemical and/or heat treatment, then dried and mixed in a fine powder as described in (Yin et al., 2016). As a feed ingredient (which has a low market price, around 1 USD/kg), the fish bone powder is interesting to be sold if it is low quality and a lot has to be used. As a food additive and supplement, as it is food grade, the price can range between 50 to 100 USD per kg (when put in capsules as from traditional food). From 100kg of wet bones, 50% is water, therefore around 40% of the initial weight could be recovered (during the mild chemical and heat treatments some proteins could be lost), making approximately 40kg of powder. Therefore, considering the frames from the Great Lakes region, a total of around 3,000,000 USD per year could be made as shown in Table 5.

Table 5: Expected revenue from fish bone powder from Great lakes Cisco in a year

	<i>Rest raw material (frames)</i>
<i>Cisco (ton/year)</i>	1108.3
<i>Frames RRM (ton/year)</i>	99.7
<i>Fish Bones powder (Dry ton/year)</i>	39.9
<i>Revenue (USD/year)</i>	2 992 410

Gelatine from skins and scales

Gelatine (partially hydrolyzed collagen) was extracted. Collagen is one of the structural proteins present in different parts of the body, mainly in the skin, scales and bones of fish. Collagen and gelatine from fish have gained interest in the last years for both social (following the bovine spongiform encephalopathy disease that touched cows in the 90s, people tend to be more cautious)

and religious reasons (pork and beef collagen are only acceptable if prepared in a specific way in Islamic culture). Collagen and gelatine have numerous applications from the food processing industry to health industry. In the food industry, it is mainly used to improve rheological properties of food products like sausage. It can also act as a good emulsifier in acidic products or as a meat replacer in some formulations. It could also be added in drinks to work as healthy drinks to give active people better recovery (Hashim et al., 2014). In health products, it is often added in cream or in pills/tablets to improve textural properties or as a complement to help regenerate damaged bone for example (Jafari et al., 2020). Gelatine is the water-soluble part of the collagen, and it is usually extracted from cold water fish. It has extremely good emulsifying and film forming properties (Jónsson & Viðarsson, 2016).

When gelatine is produced from Cisco skin, a yield of around 10% is recovered. When produced from scales, a yield of 15.5% is recovered. The market price of fish gelatine is between 20 and 60 USD/kg. From the skins and scales of the Cisco produced per year, 10.7 tons of gelatine could be produced for around 500,000 USD/year as shown in Table 6.

Table 6: Expected revenue from fish skin collagen from Great lakes Cisco in a year

	<i>Rest raw material (skin and scales) per Year</i>
<i>Cisco (ton/year)</i>	1108.3
<i>Expected skin (ton/year)</i>	55.4
<i>Skin gelatine (Dry ton/year)</i>	5.5
<i>Expected scales (ton/year)</i>	33.3
<i>Scales gelatine (ton/year)</i>	5.2
<i>Revenue (from skin and scales) (USD/year)</i>	428 000

Conclusion

This report provides chemical analysis of both fillets and rest raw materials (heads, skin, scales and bones) from Cisco from Lake Superior.

The proximate composition of the rest raw materials showed good opportunities of valorization into gelatine (skin and scales), fish protein isolates and oil for the heads, a combined mineral-protein powder from the frames, and fish protein hydrolysates from the roe and viscera. However, those transformations include high investment costs, which would be of interest only if enough raw materials were provided.

This report is to be considered as an initial identification of utilization alternatives. Further analysis is needed to determine the applicability of the alternatives identified.

References

- Ananda, S., & Anggraeni, A. A. (2021). Substitution of fishbone powder in the development of choco chips cookies. *IOP Conference Series: Earth and Environmental Science*, 672(1), 012062. <https://doi.org/10.1088/1755-1315/672/1/012062>
- European Commission. Directorate General for Maritime Affairs and Fisheries. & EUMOFA. (2021). *Fishmeal and fish oil: Production and trade flows in the EU*. Publications Office. <https://data.europa.eu/doi/10.2771/062233>
- FAO. (2022). *GLOBEFISH highlights—International markets for fisheries and aquaculture products*. FAO. <https://doi.org/10.4060/cc1350en>
- gelatine Manufacturers of Europe. (2020). *STANDARDISED METHODS FOR THE TESTING OF EDIBLE GELATINE*. https://www.gelatine.org/fileadmin/user_upload/gme_content/GME_Statements/GME_gelatine_Monograph-version_15_-_October_2020_-_short_version.pdf
- Ghaly, A., Ramakrishnan, V., Brooks, M., Budge, S., & Dave, D. (2013). Fish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review. *Journal of Microbial & Biochemical Technology*, 05(04). <https://doi.org/10.4172/1948-5948.1000110>
- Hashim, P., Mohd Ridzwan, M., Bakar, J., & Mat Hashim, D. (2014). Collagen in food and beverage industries. *International Food Research Journal*, 22(1), 8.
- Hemung, B.-O., Yongsawatdigul, J., Chin, K. B., Liphirat, W., & Siritapetawee, J. (2018). Silver Carp Bone Powder as Natural Calcium for Fish Sausage. *Journal of Aquatic Food Product Technology*, 27(3), 305–315. <https://doi.org/10.1080/10498850.2018.1432733>
- Jafari, H., Lista, A., Siekapen, M. M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H., & Shavandi, A. (2020). Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering. *Polymers*, 12(10), 2230. <https://doi.org/10.3390/polym12102230>
- Jónsson, Á., & Viðarsson, J. R. (2016). *By-products from whitefish processing* (08–16). Matis. <https://www.matis.is/media/matis/utgafa/08-16-By-products-from-whitefish.pdf>
- Kristinsson, H. G., & Liang, Y. (2006). Effect of pH-Shift Processing and Surimi Processing on Atlantic Croaker (*Micropogonias undulates*) Muscle Proteins. *Journal of Food Science*, 71(5), C304–C312. <https://doi.org/10.1111/j.1750-3841.2006.00046.x>
- Layman, D. K. (2003). The Role of Leucine in Weight Loss Diets and Glucose Homeostasis. *The Journal of Nutrition*, 133(1), 261S–267S. <https://doi.org/10.1093/jn/133.1.261S>
- Mohanty, B., Mahanty, A., Ganguly, S., Sankar, T. V., Chakraborty, K., Rangasamy, A., Paul, B., Sarma, D., Mathew, S., Asha, K. K., Behera, B., Aftabuddin, Md., Debnath, D., Vijayagopal, P., Sridhar, N., Akhtar, M. S., Sahi, N., Mitra, T., Banerjee, S., ... Sharma, A. P. (2014). Amino Acid Compositions of 27 Food Fishes and Their Importance in Clinical Nutrition. *Journal of Amino Acids*, 2014, 1–7. <https://doi.org/10.1155/2014/269797>
- Nurdiani, R., Ramadhan, M., Prihanto, A. A., & Firdaus, M. (2022). Characteristics of Fish Protein Hydrolysate from Mackerel (*Scomber Japonicus*) By-Products. *Journal of Hunan University Natural Sciences*, 49(1), 75–83. <https://doi.org/10.55463/issn.1674-2974.49.1.10>
- Phanturat, P., Benjakul, S., Visessanguan, W., & Roytrakul, S. (2010). Use of pyloric caeca extract from bigeye snapper (*Priacanthus macracanthus*) for the production of gelatin hydrolysate with antioxidative activity. *LWT - Food Science and Technology*, 43(1), 86–97. <https://doi.org/10.1016/j.lwt.2009.06.010>

- Skierka, E., & Sadowska, M. (2007). The influence of different acids and pepsin on the extractability of collagen from the skin of Baltic cod (*Gadus morhua*). *Food Chemistry*, *105*(3), 1302–1306. <https://doi.org/10.1016/j.foodchem.2007.04.030>
- Thankamma, R., Gopakumar, K., Nair, A. L., Shenoy, A. V., & James, M. A. (1979). Protein hydrolysate from miscellaneous fish. *Fishery Technology*, *16*, 71–75.
- Traditional food. (n.d.). *Fish bones powder capsules*. <https://www.traditionalfoods.org/fish-bone-calcium-mchc-powder.html>
- U.S. Fish & Wildlife service. (n.d.). *Cisco*. <https://www.fws.gov/species/cisco-coregonus-artedi>
- Wu, G., Bazer, F. W., Burghardt, R. C., Johnson, G. A., Kim, S. W., Knabe, D. A., Li, P., Li, X., McKnight, J. R., Satterfield, M. C., & Spencer, T. E. (2011). Proline and hydroxyproline metabolism: Implications for animal and human nutrition. *Amino Acids*, *40*(4), 1053–1063. <https://doi.org/10.1007/s00726-010-0715-z>
- Yin, T., Du, H., Zhang, J., & Xiong, S. (2016). Preparation and Characterization of Ultrafine Fish Bone Powder. *Journal of Aquatic Food Product Technology*, *25*(7), 1045–1055. <https://doi.org/10.1080/10498850.2015.1010128>
- Zhang, Y., Tu, D., Shen, Q., & Dai, Z. (2019). Fish Scale Valorization by Hydrothermal Pretreatment Followed by Enzymatic Hydrolysis for Gelatin Hydrolysate Production. *Molecules*, *24*(16), 2998. <https://doi.org/10.3390/molecules24162998>