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Valorization of Walleye processing by-products: Tests of enzymatic hydrolysis

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Abstract

Using walleye racks and heads, two prototypes of food grade protein powder were developed at pilot scale by Merinov. Merinov was mandated by the Great Lakes St. Lawrence Governors & Premiers (GSGP) to devise a process to valorize walleye coproduct via enzymatic hydrolysis. The resulting hydrolysate was refined in two products: a fat-rich powder made of protein extract and a low-fat powder made with purified peptide isolate. Commercially available food grade enzymes (Alcalase and Flavourzyme) were used in the hydrolysis process. Both prototypes had interesting taste, odour, colour, texture, and composition. However, yield was quite low for the peptide isolate; a large portion of the initial proteins were lost during the refining steps. We believe this is because the hydrolysis was too harsh. A harsh hydrolysis was initially chosen to attempt to completely dissolve the walleye coproducts, including their bones. Mechanical separation of the bone prior to hydrolysis followed by a milder hydrolysis might be a better strategy. Nevertheless, more than one kilogram of each powder prototype was produced. These can already be used to gauge the interest of potential clients.

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Introduction

Merinov was approached by The Great Lakes St. Lawrence Governors & Premiers (ww.GSGP.org) to develop a prototype of fish hydrolysate from walleye (*Sander vitreus*) filleting coproducts. These coproducts were sourced from the fish-processing plant in the Great Lakes area. This request was guided by prior work on the valorisation of the coproduct of lake whitefish (*Coregonus clupeaformis*). This prior work was done by Icelandic scientists and made public by the GSGP (*100% Whitefish Report*, 2023)

Objective

This project aims to maximize the value of each walleye caught by commercial fishermen in the Great Lakes region. This is done by exploring the feasibility of hydrolysing the coproducts (head and rack only) as a valorisation route. An example of hydrolysate is shown in Figure 1. The resulting hydrolysate would need to be easy to manufacture and simple to commercialize at industrial scale.

Scope

This project was limited to the processing of the largest stream of walleye filleting coproducts, the racks and the heads. Even though, at some plants, these two products may or may not be segregated, Merinov's teams consider these as a single coproduct stream. No offal, fillet skin, fillet scale, bycatch or rejected whole fish were included in this study. Two prototypes of fish hydrolysate powders were produced, along with a few liquid side streams. The method focused solely on enzymatic hydrolysis, mechanical separation, and thermal treatment.

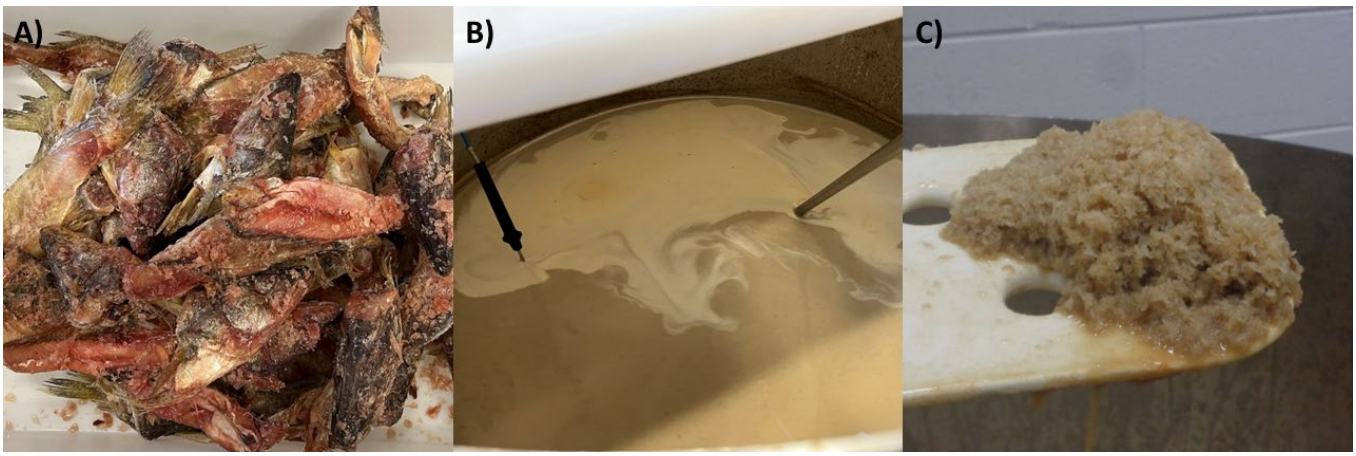

Figure 1. Summary of walleye hydrolysis used for this project: A) raw material (walleye coproduct) B) hydrolysate, C) sample of the leftover bone that resisted the enzymatic hydrolysis.

Theory

"Hydrolysate" is an umbrella term describing a liquid solution free from undissolved solids, made using originally insoluble materials. This starting material is often biomass: Meat hydrolysates, soybean hydrolysates, wood hydrolysates are common in various industries. The material can be solubilised in a few ways: chemically, by adding a base or an acid, biochemically, using various enzymes, or less commonly thermally. The chemical transformation undergone by the insoluble biomass to render it soluble is called hydrolysis and the resulting material is, therefore named hydrolysate. During hydrolysis, the molecule to be hydrolysed, often a long insoluble chain, is cut in smaller units. Water molecules are added chemically to each freshly cut ends of the chain.

Handling hydrolysate, from a food safety and equipment standpoint, is simplified because they are free from solids. There also exists a large ecosystem of suppliers for liquid handling and treatment equipment. This equipment is usually designed for the milk, beverage, brewing, and pharma industry. However, raw hydrolysate usually contains lots of water, which makes them easy to spoil and costlier to ship over long distances. To create a shelf-stable product, these hydrolysate can either be dried into a powder, salted into to create a brine, acidified by the addition of acid or alkalinised by the addition of a base (Rao et al., 2016).

During this project, our team focused on enzymatic hydrolysate, because these products generally are of higher quality and require less hazardous chemicals (bases and acids). Since walleye coproducts are mostly made of proteins, our team chose to work only with protein hydrolysing enzymes, which are called protease. In our experience, a mix of proteases is often sufficient to dissolve marine biomass almost completely, with only a few bones leftover. Table 1 briefly describes the commercial advantage and disadvantage of protein hydrolysate, according to our experience.

Table 1. Advantages and disadvantages of protein hydrolysate

Advantages	**Disadvantage**
Versatility (agri, food, pet-, feed and pharma grade)	Hard to dry
Simplicity of reaction	Needs to be concentrated
Tunable flavour	Hard to operate continuously
Low ash content	Not harsh enough to process larger bones

For protein hydrolysis, the extent of the hydrolysis is important because it dictates the flavour profile and the properties of the product. Insoluble protein can be hydrolysed to soluble protein, further hydrolysed to small peptides, or finally hydrolysed to amino acids, the building blocks of protein. The degree of hydrolysis can be controlled by the duration of the hydrolysis, and, to a lesser extent, by the size of the biomass particles, by the pH of the solution, by the vigour of the stirring as well as by the hydrolysis temperature. The type and numbers of enzymes used, and the sequence in which they are incorporated can also play a role in the degree of hydrolysis.

Fish peptides warrant a special interest because of their proven bioactivity. Fish peptides, depending on their characteristics, have health benefits in human (Ryan et al., 2011) .However, certifying that a certain peptide produced by a certain process has specific health benefits in humans is a complex endeavour.

Method

The process developed by Merinov to hydrolyse walleye coproducts was run at a pilot scale from 89 kg of raw material. However, it could be easily scalable to multiple tons per day using the same type of equipment. A simple version of the process was done, as well as a more complex version that includes 3 refining steps. Meat separation was also tested separately from the hydrolysis, to evaluate the yield of meat versus bone.

Process

The processing steps for walleye coproduct are shown in Figure 2. The walleye head and racks were processed simultaneously. First, they were cut and crushed to yield 3 mm particle of raw fish and then hydrolysed using enzymes. Two types of commercial food grade enzymes were consecutively used: Alcalase and Flavourzyme. The duration of the each hydrolysis treatment was 2 hours. Both enzymes were used at a concentration of 3.5 lb of enzyme for every 1000 lb of fish byproducts. Each enzyme has its own recommended working pH and temperature: During the first hydolysis, with Alacalase, the pH was 6.8 and the temperature was 60 C°. During the second hydrolysis, with Flavourzyme, the pH was 6.0 and the temperature was 50 C°. Before and after the two consecutive hydrolysis, the mixture was pasteurized at 85 C° for 10 min. Both pasteurizations aimed to inactivate the enzymes and microorganisms in the hydrolysate. After the hydrolysis, the undissolved bones were removed by centrifugal decantation. The raw hydrolysate, free from bone, was then split into two batches; one batch was directly spray-dried to yield a protein extract powder. The second batch was filtered to yield a partly purified, peptide isolate. This peptide isolate was also subsequently spray dried.

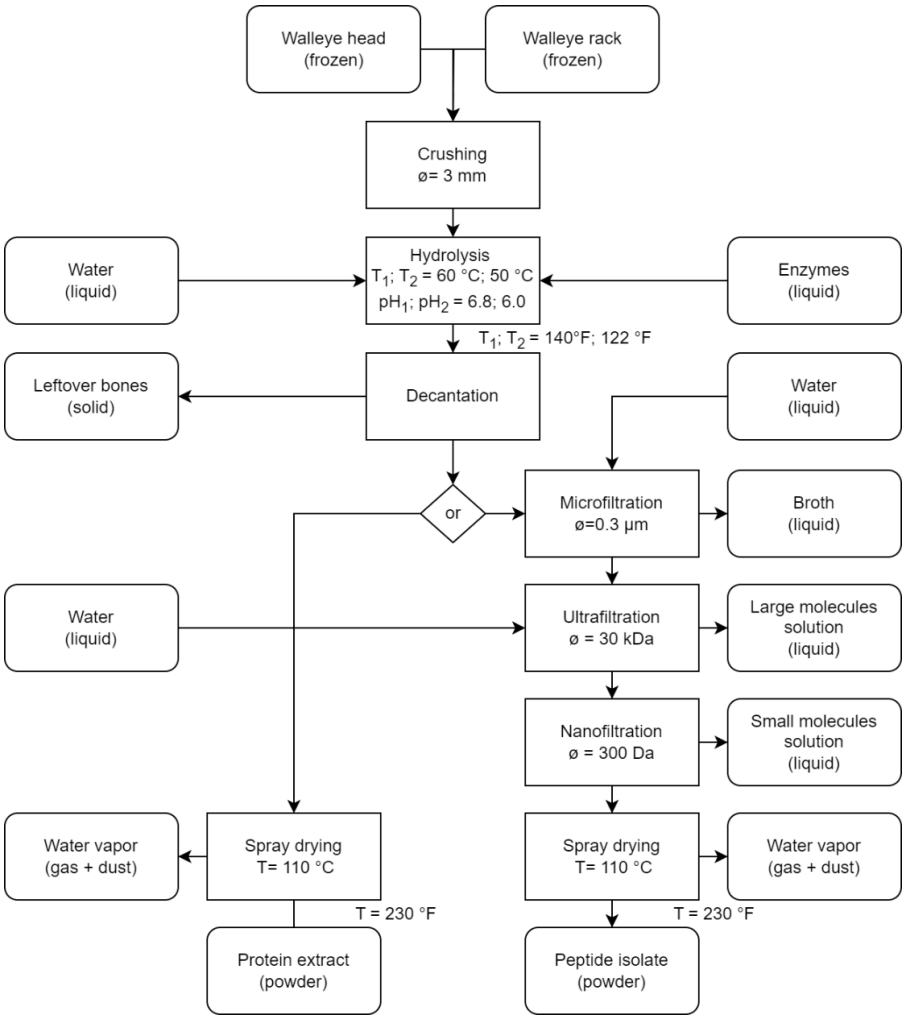

Figure 2. Process diagram of the production process of fish hydrolysate powder from raw frozen coproduct

Equipment

The equipment used for the process is shown in Figure 3. Each equipment is a scale down version of a much larger piece of equipment used in the food industry. This makes an eventual scale up and economic analysis of the process much easier. In a scaled-up processing plant, the equipment for biomass preparation (Figure 2 A, B) could be easily substituted for a variety of other equipment, if the cost of the alternative equipment is advantageous. Similarly, the centrifugal decanter (Figure 3D) could be substitute by a much cheaper vibrating sieve. Alternatives to spray drying also exist, most notably drum dryers. However, spray drying allows near-instantaneous drying. This help the product retains many of its original properties, so it is often preferred for high-value end products.

Figure 3. Process equipment used: A) Hobart chopper cutter, B) Comitrol cutter (3 mm crown) C) jacketed hydrolysis tank with stirrer, D) centrifugal decanter for solid removal, E) GEA spray dryer F) tangential filtration unit (only used for isolate production) G) Badder 601 for bone separation, equipped with 1.3 mm drum (not used in hydrolysate preparation)

Experimentation

Prior to starting the experiment, the walleye coproducts were chemically analyzed (Table 2). This analysis helps with process tuning. The walleye head and rack were not analyzed separately. Instead, it was assumed that the ratio of heads to racks in the boxes received was representative of the coproduct stream in a plant. Three samples were analyzed. The walleye hydrolysate production process outlined in Figure 2 was carried with 89 kg of walleye coproduct with the equipment shown in Figure 3. A single batch of hydrolysate was produced. It was then split into two streams. A portion of 14.44 kg (31.83 lb) of liquid hydrolysate was spray dried directly to produce 1.39 kg (3.07 lb) of protein extract. Another portion of ≈153 kg (337 lb) underwent several filtration steps to extract the peptides from the water, large protein, fat, amino acids, and minerals present in the hydrolysate. From this 153 kg of hydrolysate, about 20 kg (44 lb) of peptide solution was recovered using tangential filtration. From this portion of peptide solution, 11.24 kg (24.78 lb) were spray dried, to yield 1.50 kg (3.31 lb) of peptides isolate powder.

Table 2. Composition of the mixture of walleye head and rack

	Protein	Lipid	Carbohydrate	Mineral	Water
Sample 1	16.96%	6.90%	1.27%	6.38%	68.50%
Sample 2	17.38%	7.45%	0.62%	6.28%	68.27%
Sample 3	17.37%	6.08%	1.80%	7.02%	67.73%
Average	17.24%	6.81%	1.23%	6.56%	68.17%

Protein extract production

The biomass during each step of the protein extract preparation process is shown in Figure 4. All steps went well except for the first spray drying, in which a significant amount of powder got struck to the wall of the spray dryer. However, this does not mean this technology would not be successful to dry at larger scale and for longer operating time. The whole powder (picture F, under) was dense and a bit sticky. This is easily explained by the amount of fat in the coproducts. Nonetheless, we could readily dry it.

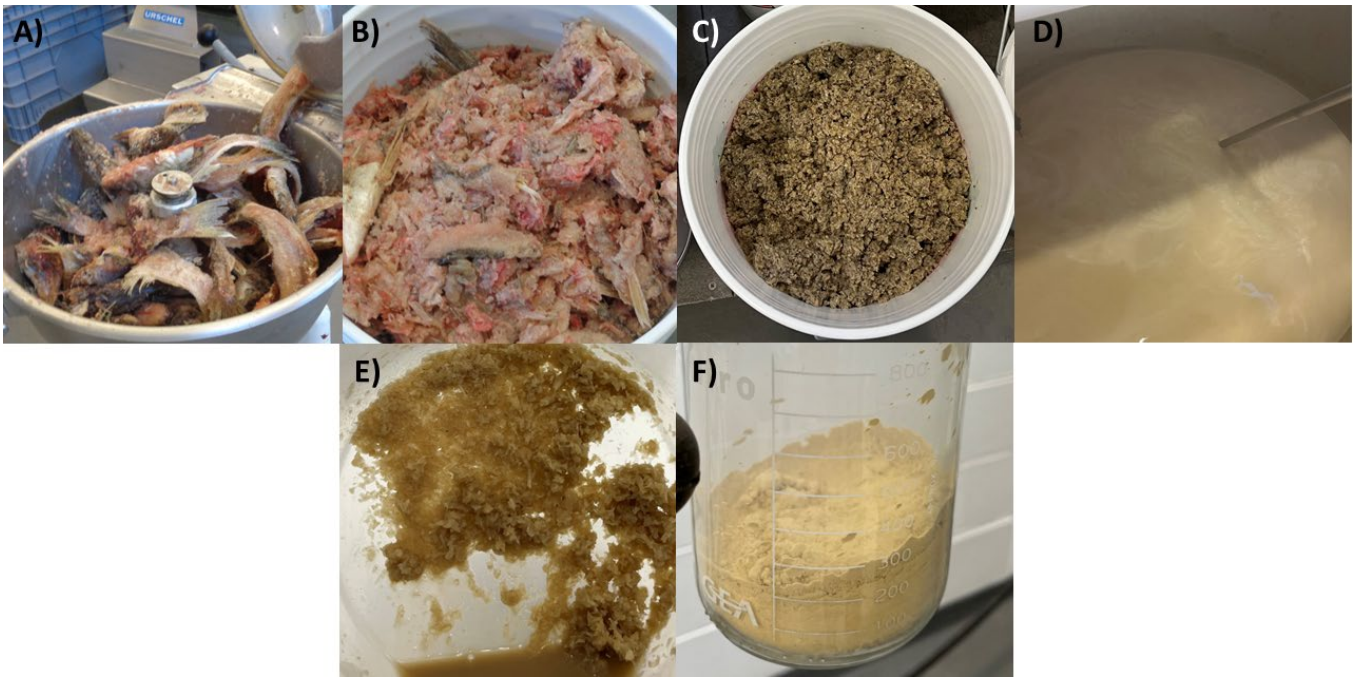

Figure 4. Different streams in the production of protein extracts: A) raw walleye coproduct (input), B) shredded coproduct, C) grinded coproduct, D) raw hydrolysate containing bones, E) leftover undissolved bones, and F) protein extract powder (prototype #1).

Peptide isolate production

The different streams produced during the production of peptide isolate are shown in Figure 5. During tangential filtration, the feed enters the system while on the permeate crosses the membrane and the retentate is retained by the membrane. The comparison between the separated streams of the hydrolysate is shown in Figure 6. At each step of the filtration, the liquid becomes clearer. The golden colour is preserved throughout the process. The discarded nanofiltration permeate (Figure 5H) is the only colourless liquid.

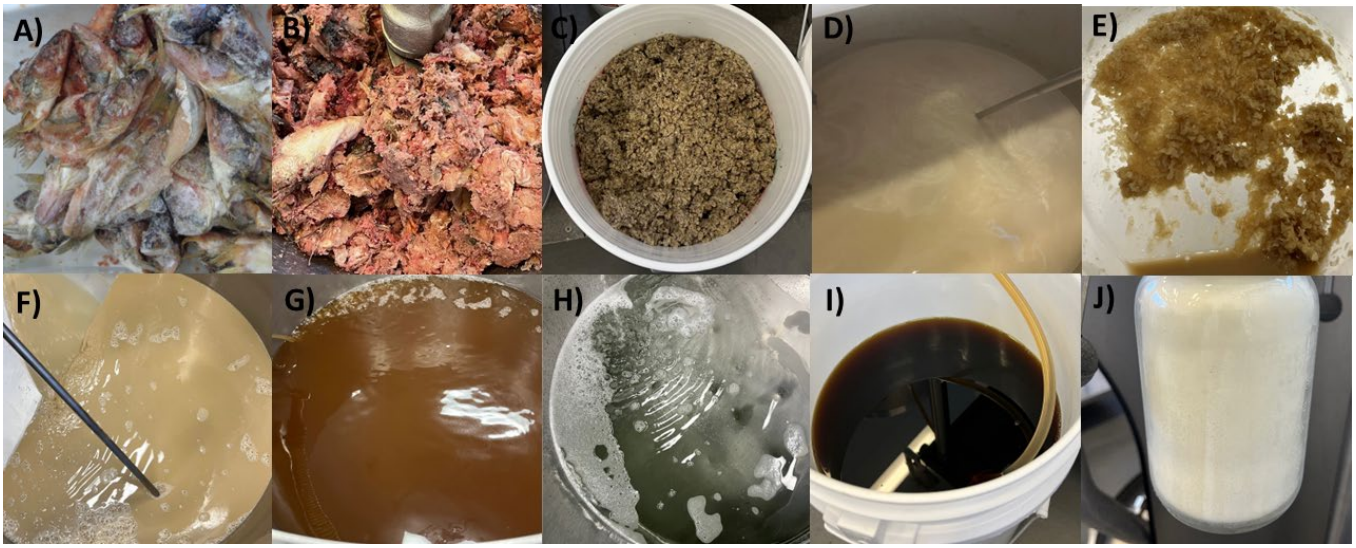

Figure 5. Different stream for the production of peptide isolate: A) raw walleye coproduct, B) shredded walleye coproduct, C) grinded coproduct, D) raw hydrolysate containing bones, E) leftover undissolved bones F) microfiltration retentate (broth) G) ultrafiltration retentate (large molecules solution), H) nanofiltration permeate (small molecules solution), I) nanofiltration retentate (peptide isolate solution), and J) peptide isolate powder (prototype #2).

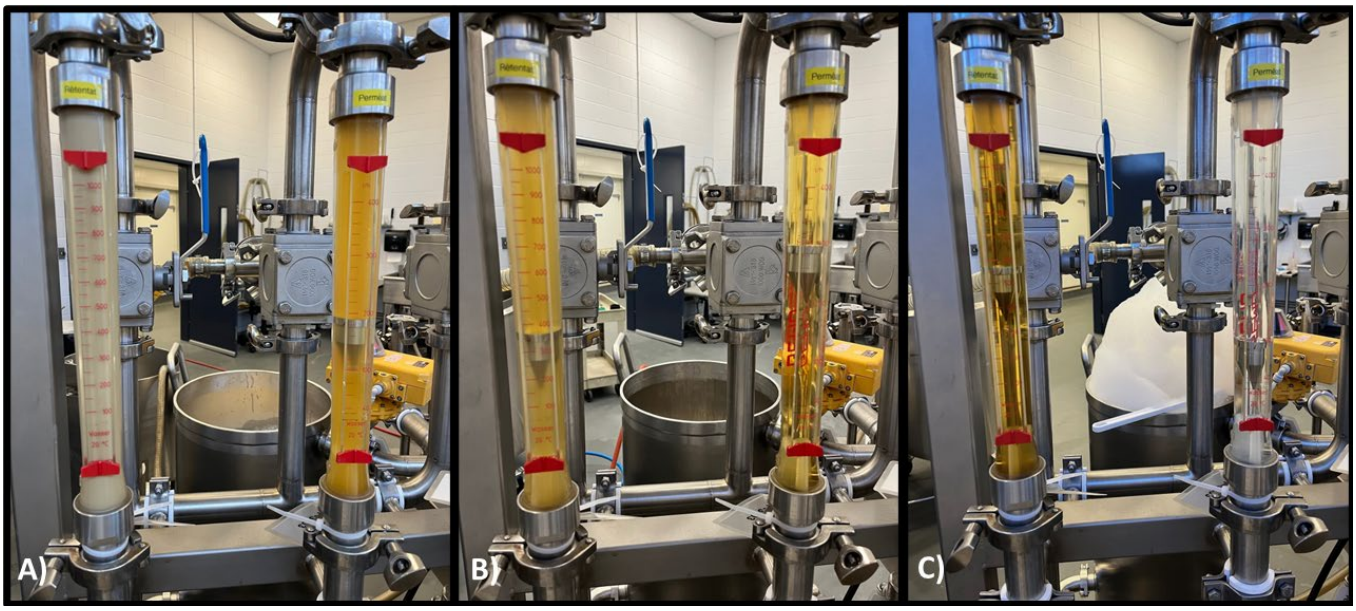

Figure 6. Retentate (left) and permeate (right) after tangential filtration. A) microfiltration (0.3 µm) to retain fat and particles, B) ultrafiltration to retain large molecules (30 kDa), C) nanofiltration to retain medium size molecules, like peptides (300 Da).

Meat and bone separation

A trial for bone separation was done to evaluate the efficacy of separating the flesh from the bone in the walleye coproduct (Figure 7). The quantitative results from this trial are presented in Table 3. A recovery of 67% boneless meat is possible. However, the mince lacked the white colour expected from walleye meat, instead it was much redder. The red coloration probably comes from the gill and brain of the fish.

Figure 7. Bone separation experiment during the first pass: A) raw material before processing, B) Baader 601 processing the raw material (1.3 mm separation drum), C) separated meat, and D) mixture of separated bone, fins, cartilages, skin, and scale.

Table 3. Yields from the bone separation of the cut walleye coproduct: after one pass and after two passes (Baader 601)

Number of passes in the machine	Mass (kg)			Cumulative yield	
	Input	Bone	Meat	Bone	Meat
1	12.45	4.40	7.60	35%	61%
2	4.40	3.35	0.78	27%	67%

Results

Two prototypes of food grade powders were produced: a protein concentrate, and a peptide isolate. Additionally, an analysis of the yield and loss incurred during the process was made, in order to understand where the process could be improved.

Prototype characteristics

The prototypes of hydrolysate powders are shown in Figure 8 and their respective composition are shown in Table 4. As expected, the isolate is almost completely made of protein, while the concentrate's composition much closely resembles that of the raw material, notwithstanding the minerals (Table 2). Both powders have satisfactory texture. However, the texture of the concentrate is much sticker than that of the isolate. This is most certainly due to the high percentage of fat and carbohydrate in the protein concentrate when compared to the isolate. The discrepancy in colour was expected when compared to the colour of the liquids (Figure 5 and Figure 6), although our team cannot ascertain what compound is chemically responsible for the colour.

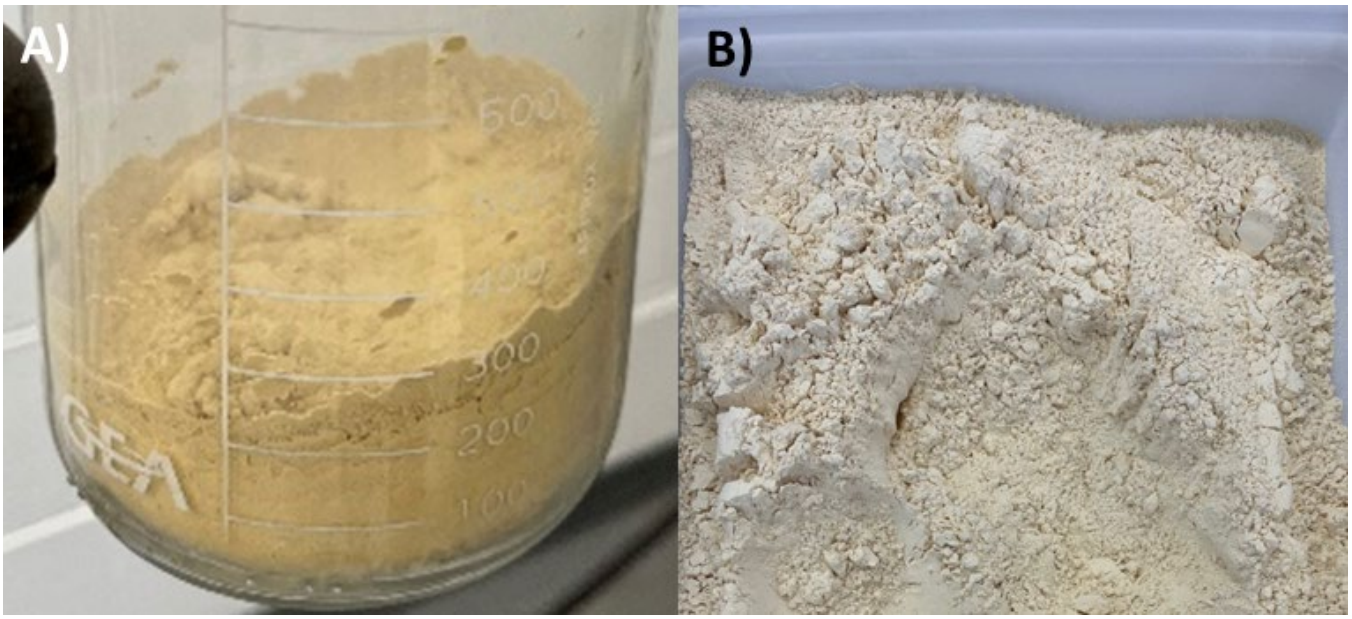

Figure 8. Prototypes of protein hydrolysate powders: a) protein extract powder b) peptide isolate powder

Table 4. Composition of hydrolysate prototypes and total mass produced.

Composition (wt/wt%)	**Protein extract**	**Peptide isolate**
Protein	62.45%	91.51%
Lipid	23.36%	0.77%
Carbohydrate	8.26%	1.13%
Mineral	3.54%	3.48%
Water	2.39%	3.11%
Total mass (g)	1.39 kg /3.07 lb	1.50 kg / 3.31 lb

Yield analysis

Table 5 compares the recovery of each component from the original biomass, using the two processes. In brief, 66% of all proteins are recovered in the extract, as opposed to only 19% in the peptide isolate. To identify where the protein is lost in the process, a mass balance was performed, using the data gathered during each experiment. Table 6 tracks the percent loss of each component during the production of the protein extract. Table 7 does the same for the peptide isolate production process. By looking at this data, it is evident that the poor protein recovery in the isolate powder hinges on the nanofiltration step, suggesting that most of the proteic molecules are lost in the nanofiltration permeate (small molecules solution).

Table 5. Yields, recovery, and conversion parameters, calculated on the basis of walleye coproduct entering the process

Yield parameter	Protein extract	Peptide isolate
Dry mass recovery	56%	11%
Protein recovery	66%	19%
Lipid recovery	62%	0%
Carbohydrate recovery	100%	3%
Mineral recovery	10%	2%
Yield of wet biomass to powder	18%	4%

Table 6. Mass losses at each step during the production of the protein extract, calculated on a process inlet basis (including enzymes)

	Loss percent	
Component	Leftover bones (decanter)	Spray dryer exhaust[1]
Dry mass	23%	24%
Protein	15%	22%
Lipid	5%	33%
Carbohydrate	24%	0%
Mineral	63%	28%

Table 7. Mass losses at each step during the production of the peptide isolate, calculated on a process inlet basis (including enzymes)

	Loss percent				
Component	Leftover bones (decanter)	Microfiltration retentate	Ultrafiltration retentate	Nanofiltration permeate[2]	Spray dryer exhaust[2]
Dry mass	23%	4%	1%	59%	3%
Protein	16%	2%	1%	58%	≈0%
Lipid	5%	13%	≈0%	81%	1%
Carbohydrate	24%	3%	≈0%	68%	1%
Mineral	63%	0%	≈0%	34%	13%

[1] These values are derived from mass balance instead of direct measurement; there might be discrepancy between the value in Table 6 and those in Table 5.

[2] These values are derived from mass balance instead of direct measurement; there might be discrepancy between the value in Table 7 and those in Table 5.

During nanofiltration, the solution is passed through a membrane with pore size of about 300 Da. Amino acids have sizes in between 89 Da and 204 Da, while almost all peptides have sizes over 300 Da (Promega, 2024; Thermofisher, 2024). Peptides should therefore be retained in the retentate of nanofiltration (Figure 6C), so only individual amino acids crossing the nanofiltration membrane could account for the observed loss of proteic material. This strongly suggests that we used too high of concentrations of one or both enzymes for hydrolysis steps and that more amino acids than peptides were produced. We had no clue about which amount to use, but tests on other biomasses with either enzyme used 0.5 to 1.5 g/kg, so with 3.5 g/kg we were probably in a upper range. The combination of the enzymes was intended to keep a pleasant, not-too-bitter organoleptic profile. However, their interaction might have been synergistic, causing an extensive hydrolysis. Hydrolysing the biomass to a lesser degree, much smaller amounts of enzymes, shall produce a hydrolysate richer in peptides and poorer in amino acids. This would increase the yield of peptides in the isolate powder, while extracting the fullest value out of the walleye coproducts and discarding the least amount of material. A milder hydrolysis would use less enzymes and/or be faster, so it would be cheaper. It might also improve the taste of the powders: The taste of the prototypes was good, as expected from the alcalase-flavourzyme process, but it was described as somewhat “fried” or “battered fish” by the panelists That could also be a consequence of over-hydrolysis. A milder hydrolysis could retain more characteristic “fishy” notes, valued by the clients.

Recommendations

Two improvements are possible to the process: exploring stabilization method other than drying (especially for the protein extract) and adding a meat separation step to enable a milder hydrolysis to be used.

Meat separation

Different tissues (bone meat, gill, brain, cartilages, skin, etc.) are hydrolysed at different speeds. To accurately control the degree of hydrolysis of the proteins, we recommend separating the harder components of the fish (scales, fins, bone and cartilage) from the softer ones. This will enable a better control over the quality of the product, namely low-fat, low-amino acid, high solubility, high peptide content, low mineral content and possibly a better taste. This can be easily done using a bone separator (Figure 7).

Interestingly, the tougher tissues of fishes are notably rich in collagen and could be used to produce collagen (Nagai & Suzuki, 2000), so as not to become a "byproduct of the byproduct". They are also rich in minerals, especially phosphates. An alternative coproduct fractionation and treatment scheme is shown in Figure 9.

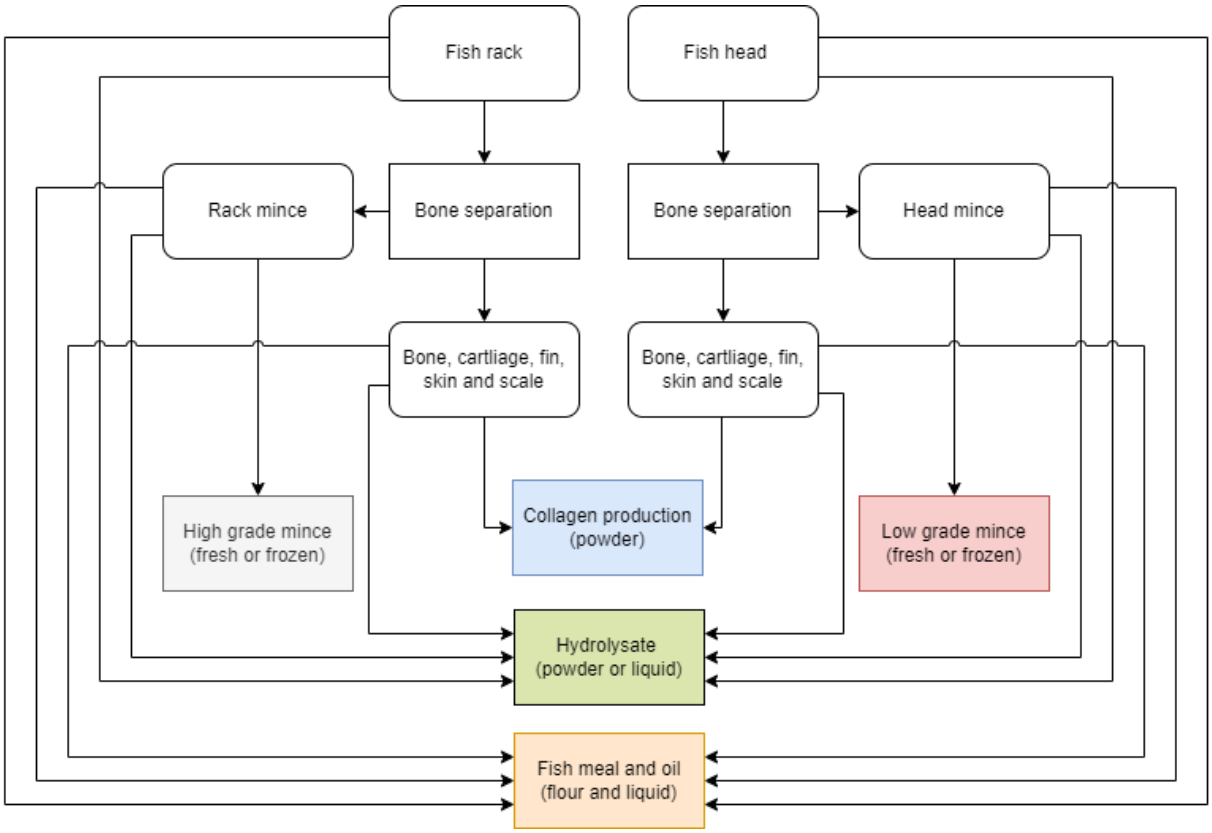

Figure 9. Walleye racks and head valorisation route

Alternative stabilisation techniques

Spray drying is a product stabilization method that creates a product of high value, with the low moisture content. However, the upfront cost of a spray dryer can quickly eat up at the profit margin, especially at smaller scale or for seasonal operations. A simpler method is salting of the product, which makes the liquid hydrolysate shelf stable. This salting process essentially creates a broth. Merinov heard of the anecdotical commercial success of this method. However, the water is still present in the product, which can increase shipping cost significantly. Similar considerations are at play for the acidification or alkalinization of the product using acid or base. These methods and other alternative drying techniques (drum dryer) warrant being explored to check their viability.

Conclusion

This project was successfully carried through a process to produce a high value fish hydrolysate from walleye coproduct was devised (Figure 2), the process was demonstrated at a pilot scale (Figure 4 and Figure 5), and two prototypes of high value hydrolysate powders were produced (Figure 8). The main conclusion of the pilot production scale is that it is possible to produce a high-quality hydrolysate using the process devised. However, a milder hydrolysis would be required to obtain a satisfactory yield for the peptide isolate. To this end, one would need to separate the meat from the bone prior to the hydrolysis or, alternatively, tolerate that a lot of bones will have to be extracted during the decantation. Overall, there is enough evidence that enzymatic hydrolysis is a potentially profitable route for the valorization of walleye coproduct. However, to evaluate quantitatively the profitability of this process, a bit more lab work needs to be done so that enough reliable data is available. The prototype powders can already be used to gauge the interest of potential clients. One must keep in mind that many of the clients for fish protein extracts and peptides are in based in Europe. Targeting shelf-stable products (salted concentrates, powders) therefore offers a better perspective of profitability. Some markets for frozen minced fish also exist overseas, carrying products containing 85% water over oceans wouldn't make a lot of sense, environmentally nor economically.

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