EFFECTS OF TWO DRYING METHODS ON THE COMPOSITION AND FUNCTIONAL PROPERTIES OF FISH TRIMMINGS HYDROLYTIC PRODUCTS

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ABSTRACT

Fish processing generates by-products that could be used in various fields, including food. The objective of this study is to valorize fish trimmings by enzymatic hydrolysis with pepsin and to compare the effect of oven drying and freeze drying on the composition and functional properties of the resulting products. The analyses showed that fish trimmings are good sources of protein, which are solubilized at 77.37 to 77.78% after hydrolysis with pepsin. For both types of drying, the products show functional properties including high emulsifying activities (44.18 to 48.78%) with a high level of stability (82.37% to 85.26%). Freeze-drying results in slightly higher functional properties compared to oven drying.

Keyword: *Trimmings, fish, enzymatic hydrolysis, freeze-drying, oven drying, functional properties*

1. INTRODUCTION

Fish products have an important place in the world and are sources of protein for many populations [1]. Like most food resources, fish products have uneaten parts. Different types of processing are therefore carried out in factories to prepare these products in advance. These include operations such as filleting, heading, gutting, shelling, peeling, etc. By-products are thus generated at industry level, including trimmings, which are defined as the offcuts from the various parts of the fish during filleting. These materials are considered as wastes and very few of them are recovered. However, like any other fishery by-products, they can be sources of interesting and recoverable molecules [2]. Given the problems of malnutrition combined with the rarefaction of natural resources in the world, it could be interesting to valorize these materials and use them in food. Several research projects have been carried out for this purpose, with direct use of by-products, or with preliminary extractions [3]. For environmental reasons, biological techniques are increasingly used, including enzymatic hydrolysis. Several commercial enzymes have been tested and very interesting results have been obtained, generating proteins with promising functional properties [4,5]. Several methods are used for the treatment of hydrolysis products but the most commonly used are drying. One of the most widely used and gentle methods is freeze-drying. However, this technique is more expensive and energy consuming than direct heat drying [6, 7]. Nevertheless, the characteristics of the final products may vary according to the method used.

The objective of this study is to valorize fish trimmings by enzymatic hydrolysis and to explore the differences between the nutritional and functional characteristics of the raw material and the products obtained following two different drying techniques: freeze-drying and oven drying.

2. MATERIALS AND METHODS

2.1 Biological materials

Trimmings of 3 fish species *Lutjanus* sp, *Lethrinus* sp and *Epinephelus* sp kindly provided by a processing industry based in Antananarivo, Madagascar were used. The products were collected frozen and transported in a cooler to the laboratory. They were stored at -20°C in a freezer until use.

2.2 Composition analysis

The overall composition was determined according to the methods described by AOAC [8]. Water content was determined after drying at 103°C for 24 h, crude ash content by incineration at 550°C until white or grey ash was obtained. Lipid content was determined after extraction with hexane using a Soxhlet apparatus and protein was determined according to the KJELDHAL method using the conversion factor of 6.25.

2.3 Enzymatic hydrolysis

Ground trimmings were subjected to enzymatic hydrolysis in the presence of 2% pepsin for 3h at 40°C and pH2. The pH was adjusted to 2 at the beginning of the reaction by adding 5N HCl and maintained at 2 by adding 1N HCl during the reaction. The pH was adjusted to 7 by adding 5N sodium hydroxide to stop the reaction after 3h. The preparation was cooled and centrifuged at 5000 rpm for 20 min. Two phases were recovered: the supernatant and the pellet.

2.4 Drying

The raw materials, the supernatant and pellet from enzymatic hydrolysis were dried in two different ways. The first method was oven drying at 70°C and the second consisted of freeze-drying after freezing.

2.5 Functional properties

The water absorption capacity, oil absorption capacity as well as the emulsifying property and the stability of the emulsion were determined for the different dried fractions.
The water absorption capacity was determined according to the method described by Sathivel et al. [9] as the volume of water in ml retained by g of sample after 30 min of rest alternated with stirring every 10 min, followed by centrifugation at 2560 rpm for 25 min.
For oil absorption capacity, it is the amount of soybean oil retained per g of sample after shaking a mixture of 500 mg of hydrolysate and 10 ml of oil, followed by a rest of 30 min alternated with shaking every 10 min.
The emulsifying activity was determined according to the method of Nazck [10]. The emulsion formed with 0.35g of sample, 5ml of distilled water and 5ml of oil was measured. It is expressed as the percentage of emulsion resulted over the total volume.
The determination of emulsion stability consisted of making emulsions with 250 mg of sample, 25 ml of NaCl and 25 ml of soybean oil, leaving the preparation to stand for 15 minutes and measuring the proportion of the formed emulsion [11].

2.6 Statistical analysis

Analysis were performed in triplicate. The means of the values were compared by Tukey test, using R Software.

3. RESULTS AND DISCUSSION

3.1 Enzymatic hydrolysis

After 3 h of hydrolysis with pepsin, about 13.67% of the peptide bonds were cut and a certain amount of protein passed into the soluble phase.

The yield of supernatant without enzymatic hydrolysis is significantly lower than that obtained after enzymatic hydrolysis (**Table-1**). This confirms the solubilization of the material as a result of hydrolysis. The addition of acid and base during hydrolysis may also contribute to increase the total material quantity after hydrolysis. No significant differences were found in the yields of the supernatant and pellet fractions depending on the type of drying.

Table -1: Hydrolysis fractions yields (%)

Treatment	Fraction	Oven drying at 70°C	Freeze-drying
Control	Supernatant	-	4,67
	Pellet	-	19,30
Enzymatic hydrolysis	Supernatant	26,85	26,41
	Pellet	10,89	11,54

3.2 Protein recovery

According to **Table-2**, if only 15% of proteins pass into the soluble fraction or supernatant without enzymatic hydrolysis, about 77% are found in this fraction after using pepsin, confirming their solubilization during enzymatic hydrolysis. By contrast, without enzymatic hydrolysis, 67.41% of the initial proteins were found in the pellet, whereas only 22.19% of them were found after enzymatic hydrolysis. Indeed, pepsin, which is a peptidase, cuts the peptide bonds, leading to a reduction in the proteins size and their solubilization.

Table -2: Protein recovery from hydrolysis of fish trimmings (%)

Treatment	Fraction	Oven drying at 70°C	Freeze-drying
Control	Supernatant	-	15,48
	Pellet	-	61,41
Enzymatic hydrolysis	Supernatant	77,37	77,78
	Pellet	22,20	22,19

3.3 Effect of drying technique on composition

- Moisture

The moisture content of the final products is affected by the drying method. Samples were affected differently. A higher moisture content is obtained by the raw materials freeze-drying, while the contents are lower after the supernatant and pellet freeze-drying (**Table-3**). The crystalline or amorphous structure of the material affects its behavior during freezing, whose state is very important for freeze-drying. In a more amorphous structure, more than 20% of the unfrozen water is associated with the solids and has to be removed by desorption in contrast to the frozen water which is removed by sublimation during freeze-drying [6]. This difference in consistency between the samples could be the reason for their different behavior during freeze-drying.

In all cases, moisture levels are low and allow a long shelf life of the products. The values obtained are similar to those of Abbey et al [12] on tuna co-product powders which vary from 3.5 to 8.4%.

Table -3: Moisture content in g/100g of sample

Sample	Oven drying at 70°C	Freeze-drying
Crude materials	$4,73\pm0,05^{a}$	$11,13\pm0,23^{b}$
Supernatant	$6,73\pm0,05^{a}$	$5,88\pm0,12^{b}$
Pellet	$6,57\pm0,12^{a}$	$1,87\pm0,03^{b}$

Values are presented as mean ± SD (n=3). Means followed by the same letter in the same line are not statistically significantly different from each other ($p>0.05$), by the Tukey test at 5% probability.

- Proteins

According to Table 4, the protein content of the trimmings is similar to that reported by Abbey et al. on tuna trimmings.

However, the supernatant contains a higher protein content than the initial material. This could be a dilution effect following the addition of hydrochloric acid and sodium hydroxide for enzymatic hydrolysis. Therefore, the crude ash content increases despite the protein content.

Protein contents were not affected by the type of drying (p>0.05) (**Table -4**). Similar results were obtained by Chen et al [13] on dried egg white where no difference in composition was observed between drying and freeze-drying. This was also the case for soy isolate where the protein content does not differ according to the drying technique used [14].

Table -4: Protein content in g/100g of sample on dry weight basis

Sample	Oven drying at 70°C	Freeze-drying
Crude materials	75,64±2,21[a]	73,50±0,24[a]
Supernatant	70,76±0,24[a]	69,85±0,74[a]
Pellet	49,66±0,03[a]	46,41±0,71[a]

Values are presented as mean ± SD (n=3). Means followed by the same letter in the same line are not statistically significantly different from each other (p>0.05), by the Tukey test at 5% probability

- Lipids

Lipids are affected by the drying method (p<0.05) (**Table -5**). For both pellet and raw material, freeze-dried products have more lipids than oven dried products. The ability of lipids to adhere to container surfaces could explain these differences. According to Chukwu and Shaba [15], the loss of lipids during kiln drying of catfish was attributed to the exudation of lipids with water evaporation and the long heat treatment (60-70°C for 24h).

Table -5: Lipid content in g/100g of sample on dry weight basis

Sample	Oven drying at 70°C	Freeze-drying
Crude materials	13,06±0,61[a]	16,01±0,48[b]
Supernatant	5,60±0,61[a]	4,25±0,35[b]
Pellet	20,50±1,32[a]	21,79±0,62[a]

Values are presented as mean ± SD (n=3). Means followed by the same letter in the same line are not statistically significantly different from each other (p>0.05), by the Tukey test at 5% probability

- Ash

The freeze-dried products had higher ash contents than the oven-dried products (p<0.05) (**Table -6**). Leaching during drying at 70°C could be suggested as an explanation for this phenomenon.

Table -6: Moisture content in g/100g of sample

Sample	Oven drying at 70°C	Freeze-drying
Crude materials	10,70±0,12[a]	11,41±0,29[a]
Supernatant	24,03±0,02[a]	25,74±0,45[b]
Pellet	25,75±0,08[a]	26,45±0,04[b]

Values are presented as mean ± SD (n=3). Means followed by the same letter in the same line are not statistically significantly different from each other (p>0.05), by the Tukey test at 5% probability

3.4 Effect of drying technique on functional properties

In any case, the functional properties are higher for freeze-dried products compared to oven-dried products. Proteins are primarily responsible for these functional properties. Since their contents have not been affected by the drying methods, it is the transformation in their structure during the drying operations that may their functional properties. High temperatures, for example, lead to the denaturation of proteins [17].

- Water absorption capacity

The water absorption capacity is absent for the soluble fraction. In the case of pellet, freeze-dried sample have a higher capacity than oven-dried sample (p<0.05) (**Fig-1**). The high water uptake could be attributed to the more porous structure of the freeze-dried sample [16].

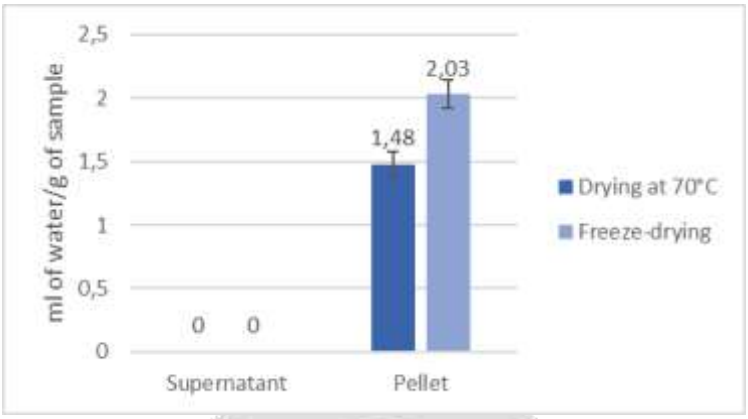

Fig -1: Water absorption capacity of hydrolysis fractions using two drying methods

- Oil absorption capacity

Both soluble and insoluble fractions show oil absorption activities. Those of the insoluble fraction are the highest. The capacities obtained with freeze-dried samples are slightly higher than those of samples dried at 70°C (**Fig-2**). The higher capacity of freeze-dried samples compared to oven drying has also been reported for white cheek shark protein hydrolysate [17], chicken skin collagen [16] and chickpea protein concentrate [18]. It is attributed to the more porous structure of freeze-dried samples which can trap oil more easily and quickly [16, 18], to the concentration of non-polar groups on the surface [17].

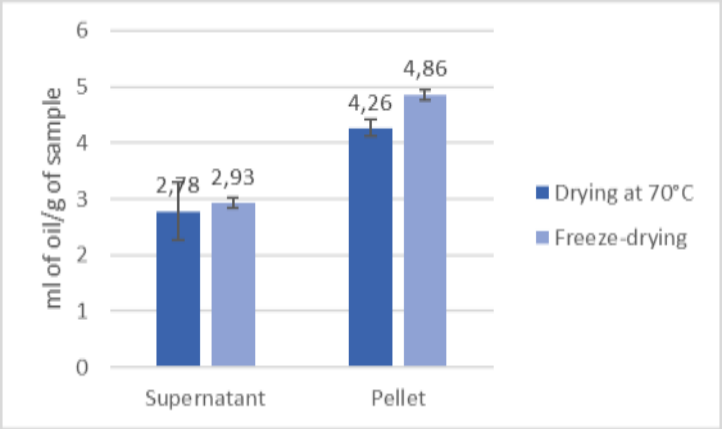

Fig -2: Oil absorption capacity of hydrolysis fractions using two drying methods

- Emulsifying capacity

In contrast to the oil absorption and water absorption activities, the supernatants show much higher emulsifying activities than the pellets (**Fig -3**). As for the other properties, compared to drying at 70°C, freeze-drying is more effective in obtaining fractions with emulsifying properties.

Linarès et al 2001 [19] reported no difference in emulsifying activity depending on the drying method, unlike Chen 2011 who found similar results to the present study. The differences are attributed to the difference in the structures of the original proteins. The hydrophilic and hydrophobic character as well as the charge of the proteins define their emulsifying capacity [13].

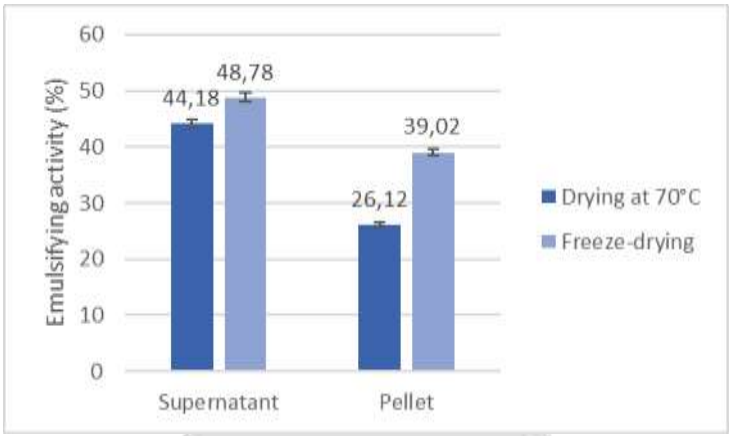

Fig -3: Emulsifying capacity of hydrolysis fractions using two drying methods

- Emulsion stability

More stable emulsions are obtained with the insoluble fractions although the soluble fractions show very interesting stabilities (**Fig-4**). If the emulsion stability obtained with sodium caseinate is 80.90%, they are 82 to 86% with the trimmings hydrolysates. These products can then be used as natural emulsifiers in the agroindustry.

Freeze-drying provides a slightly higher stability than drying at 70°C. Indeed, drying methods can affect protein denaturation, hydrophobicity, sulfhydryl groups and particle size [14] leading to differences in properties.

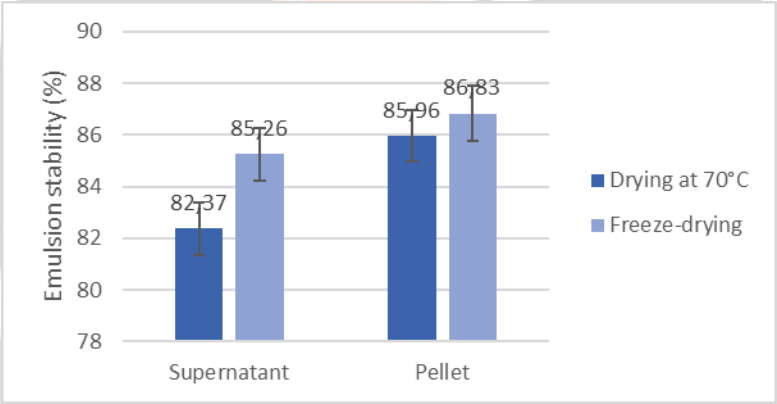

Fig -4: Emulsifying stability of hydrolysis fractions using two drying methods

4. CONCLUSIONS

Fish trimmings are important sources of protein. As expected, hydrolysis with pepsin is effective in solubilizing these native proteins. Drying at 70°C and freeze-drying provide proteins with interesting functional properties, although the freeze-dried fractions have slightly higher properties than those dried at 70°C. Further investigations on protein structure and conformational changes during drying could complement these findings. As oven drying is a less expensive method, additional research on the optimization of this technique is needed. In any case, the valorization of fish trimmings is a promising way to produce new functional ingredients for the food industry.

5. ACKNOWLEDGEMENT

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