



Impact of Storage Conditions on the Stability of the Physicochemical and Microbiological Characteristics of Animal Feed Made from Fish Waste

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ABSTRACT

Background: This study was conducted to evaluate the effect of storage time and temperature on the stability of the physicochemical and microbiological characteristics of animal meal derived from fish waste collected in the northern central region of Algeria (fisheries of Algiers and Boumerdes).

Methods : The obtained meal sample was stored in the refrigerator, at room temperature and in an oven at 55°C for 7, 15 and 21 days. The analyses were performed at the Laboratoire National de Contrôle et Analyse des Produits de la Pêche et d'Aquaculture et de la Salubrité des Milieux (L.N.C.A.P.P.A.S.M) in Algiers.

Result: The results on the fresh meal reveal a satisfactory physicochemical composition, with a pH close to neutrality and low humidity, but high levels of ash and fat. After the storage period, it was observed that keeping the flour at low temperatures helped preserve its quality better. However, refrigeration allowed for the stabilization of humidity and lipids, while limiting microbial proliferation. Conversely, high temperatures (55°C) promoted the degradation of nutrients, especially in the case of prolonged storage, thus increasing the risks of lipid oxidation and protein degradation. The microbiological results indicate significant contamination from the outset, particularly from the total aerobic mesophilic flora as well as yeasts and molds. These results underscore the importance of strict control of technological processes to ensure the nutritional and hygienic properties of flour made from fish waste.

Key words: Animal flour, Fish waste, Microbiological quality, Physicochemical quality, Storage, Temperatures.

INTRODUCTION

Fishmeal is a highly nutritious food product, widely used in animal feed formulation, particularly for fish (Nguyen and Tran, 2025), as well as for poultry and pigs. It is also being explored for its pharmaceutical and industrial potential due to its by-products, collagen and gelatin (Kim and Mendis, 2006). The parts of fish not consumed by humans (heads, entrails, skin, scales, bones, etc.) contain potentially exploitable molecules, such as proteins, lipid fractions, especially according to Foroutani *et al.* (2020), in polyunsaturated fatty acids, vitamins, minerals, as well as bioactive compounds.

Algeria has strong aquaculture potential due to its natural resources. According to the Centre National de Recherche et de Développement de la Pêche et de l'Aquaculture (C.N.R.D.P.A, 2024), aquaculture production increased from 351 tons in 2000 to 5436 tons in 2020, with an estimated annual growth rate of 14.68%, surpassing regional and global averages.

Marine perciforms (*Percoidea*) dominate this production, accounting for over 60% of the total. Tilapia, catfish, mussels, freshwater perch, river eels and carp are the most raised and studied species in Algeria, according to the same report. However, yields have remained insufficient to meet the demand for animal protein (Boudjellal and Laskar, 2023) due to various technological, ecological and/or economic constraints. It is in this context that this study on the valuation of fish waste into animal

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meal was conducted to analyze the factors affecting the stability of its nutritional and hygienic qualities under different storage conditions based on the (temperature/ storage duration) pair.

MATERIALS AND METHODS

The present study was conducted from November 2023 to June 2024. The sampling was carried out on a total live weight of 2.2 kg of waste mixture from different species of fish (merlon, cuttlefish, sardine, dogfish, swordfish, squid, shrimp and red tilapia), collected from fishmongers and

restaurants in the region of Zemmouri (Boumerdes province) and from the Algiers central fishery.

All physicochemical and microbiological analyses, as well as stability tests on our flour sample, were conducted at the Laboratoire National de Contrôle et Analyse des Produits de la Pêche et d'Aquaculture et de la Salubrité des Milieux (L.N.C.A.P.P.A.S.M). The preparation steps before laboratory analyses are as follows :

- Weighing the fresh state of the different parts of the fish in our sample.
- Grinding with the grinder of the parts (C: skin, D: heads, E: intestines, F: remnants and G: branches) and with the mortar of the parts (A: fins, B: swim bladder) to reduce their sizes and facilitate drying.
- Weighing after drying in a ventilated oven at a temperature of 50°C for 48 hours, then at 65°C for the same period.
- Final grinding of the mixture to obtain fish flour with a dry weight of approximately 900 g.

Physicochemical analyses

Determination of pH

The pH is measured using a calibrated pH meter. 10 g of fishmeal sample is added to 100 ml of distilled water and stirred for 10 minutes in a shaker. After resting for 30 minutes, the solution is filtered and its pH is read. (ISO 2917: 1999).

Moisture content determination (%)

The dry matter content is estimated by drying 5 g of the sample in an oven at 103±2°C for 6 hours. Cool and weigh (AOAC, 2000), No : 934.01.

Ash content determination (%)

The ash content was determined according to the AOAC method (2000), No : 942.05. Ashes are obtained by incineration. Place 5 g of the sample in a muffle furnace, calcine for 4 hours at 550°C. After cooling, weigh the remaining ashes.

Fat content determination (%)

The fat content is obtained by extraction using a Soxhlet according to the AOAC method (2000), No : 920.39 using, hexane as the solvent.

Total nitrogenous matter determination (%)

The protein content is determined by the Kjeldahl method (AOAC, 2000), No : 984.13. It is based on the principle of mineralizing the sample in sulfuric acid. Thus, nitrogen is converted into ammonium sulfate. The content of ammonium sulfate is measured by adding an excess of soda to the mineralized substance. The ammonia released is distilled and titrated. The percentage of protein is obtained by multiplying the nitrogen content by 6.25.

Microbiological analyses

Five microbial groups are searched for and enumerated. They are studied using classical culture methods for counting and isolation on specific or enrichment culture media.

- The total mesophilic aerobic flora (TMAF) is counted on PC agar (Plate Count Agar) by deep inoculation of 1ml of the dilutions and incubation at 30°C for 72 hours (ISO 4833-1 : 2013).
- Coliforms are sought on lactose and sodium citrate agar (DLC) at 1%, incubated for 24 hours at 37°C for total coliforms and at 44°C for fecal coliforms, according to protocols (ISO 4832: 2006).
- Yeasts and molds are counted on 4% glucose Sabouraud medium and incubated for 5 to 7 days at a room temperature of 25 to 27°C (ISO 21527-2 : 2008).

Evaluation of the stability of the physico-chemical and microbiological properties of fishmeal during storage

The physico-chemical parameters (humidity, fat content, protein content) as well as microbiological characteristics were tested for stability at different storage times (7, 15 and 21 days) and at distinct temperatures : 4°C, room temperature and 55°C. Regarding the protein content, the analysis was only repeated after 3 weeks of storage at room temperature and 55°C, due to the complexity of the analytical method and the limited availability of chemical reagents. All measurements were performed in triplicate to calculate reliable averages.

Statistical analyses

A descriptive statistical analysis was performed for the evaluation of means. The values of the microbiological variables were transformed to Log 10 in order to conduct parametric statistical tests.

RESULTS AND DISCUSSION

The results of the weighings carried out on our sample of flour made from fish waste reveal a significant reduction in weight between the fresh state and the dry state, particularly for the intestines (-70.93%), with a minimum weight loss noted for the swim bladders (-26.64%) (Table 1).

As for the fresh-weight fish sample, it experienced a decrease of more than half of its weight (-58.75%). This indicates a significant loss of water due to evaporation.

Evaluation of the physicochemical and microbiological quality of the fishmeal

The results obtained reveal an overall significant physicochemical composition (Table 2), characterized by low moisture (4.07%) and a pH close to neutrality (6.65). However, the product showed high ash (29.46%) and fat (40.33%) levels, along with low protein compared to FAO (2018).

Furthermore, the FAO (2018), recommends maintaining fishmeal moisture below 10% to limit microbial development, prevent oxidation and ensure proper preservation. The ash content in the fish waste meal analyzed in this study is relatively high, reaching 29.46% (Table 2). This reflects a significant concentration of minerals, mainly due to the presence of bone fragments, particularly the spines, contributing to the enrichment of the product in calcium and phosphorus.

Rahim *et al.* (2017), report a fishmeal ash content varying between 12.32 and 18.32%. These results also remain lower than ours. Regarding fat content, our sample stored at room temperature showed a high value of 40.33%. This value can be beneficial by constituting a source of energy for the animal, but these lipids can be subject to oxidation, thus affecting the nutritional quality of the meal in the long term, following the appearance of unpleasant flavors during storage. Finally, the protein content of our sample is 19.32%, it is considered significantly lower than the standard recommended by the FAO (2023), which fluctuates between 68 and 70% for fishmeal intended for animal feed. As reported by Tiwari *et al.* (2023), the prepared fish powder from *Labeo bata*, a minor carp species from Assam, recorded moisture, protein, fat and ash contents of 4.62, 63.87, 13.04 and 2.96 g/100 g respectively. This product also demonstrated good shelf life, as assessed through changes in moisture, free fatty acids, total plate count and pH during 90 days of storage.

The total aerobic mesophilic flora was found to be too numerous to count (Table 3), suggesting a high level of microbial contamination in the fishmeal. Similar findings have been reported in previous studies, where high microbial loads were associated with poor handling and storage conditions of fish (Hatha *et al.*, 2013).

In contrast, coliforms were not detected, indicating the absence of fecal contamination and pathogenic bacteria of intestinal origin in the marine environment. However, the high counts of yeasts and molds indicate favorable environmental conditions for fungal growth and proliferation, consistent with earlier observations by Vieira *et al.* (2020).

Results of physicochemical and microbiological quality stability tests during storage

Data analysis (Table 4), indicates that samples exposed to room temperature and the incubator (55°C) have a high dry matter content of approximately 95%, a level close to that obtained initially. This content is followed by a significant

Table 1: Results of the different stages of weighing fishmeal.

Parts of fish	Fresh weight (g)	Weight after cutting and grinding (g)	Weight after drying (g)	Difference (fresh weight / after drying) (%)
Fins (A)	380.62	380.62	180.94	52.46
swim bladder (B)	168.00	165.65	123.24	26.64
Skin (C)	250.98	227.78	148.13	40.97
Heads (D)	348.56	332.24	123.2	64.65
Intestines (E)	324.70	223.39	94.36	70.93
Remaining fish (F)	181.69	162.49	58.84	67.61
Branches (G)	545.38	509.74	178.63	67.24
Total	2199.93	1991.91	907.34	58.75

Table 2: Results of the analyses of the physicochemical quality.

Parameters	pH	Humidity	Ash content	Fat content	Protein content
Results (%)	6.65	4.07	29.46	40.33	19.32
FAO (2018)	/	<10	14-16	8-11	68-70

Table 3: Results of microbiological analyses.

Germs	Total aerobic mesophilic flora	Total coliforms	Fecal coliforms	Yeasts and molds
Colonies/g	Uncountable	Absence	Absence	900

Table 4 : Results of the stability tests performed on the physicochemical parameters

Shelf life (days) / Storage temperature (°C)	0	7	15	21	Parameter analyzed (%)
4	4.07	6.50	6.58	6.25	Moisture
Ambient		4.11	5.37	4.22	
55		4.01	4.11	4.49	
4	40.33	13.33	13.66	16.33	Fat
Ambient		13.66	10.66	18.66	
55		41	13.00	7.66	
Ambient	19.32	/	/	13.67	Protein
55		/	/	4.10	

decrease in the average moisture content of our flour sample, thus limiting alterations in the product's nutritional quality and promoting its better preservation.

According to Biswas (2024), the fish preservation in a portable solar cooler is practical and economical for up to 7 days, showing similar results to household refrigeration at controlled temperature.

It should be noted that at a temperature of 55°C, increased evaporation of moisture from fishmeal may occur. However, under these conditions, the meal may reach a new hygroscopic equilibrium, different from that initially achieved during storage at room temperature. However, prolonged storage at 55°C is generally not recommended due to the increased risk of deterioration of the chemical, physical and organoleptic qualities of fishmeal, which is mainly manifested by altered odor and taste, often perceived as indicators of poor storage. Conversely, at 4°C, the fishmeal produced experienced a gradual increase in moisture content, estimated at approximately 2% compared to its initial content.

This increase is likely due to moisture absorption from the surrounding air, inadequate packaging conditions, or the intrinsic hygroscopic properties of this meal. However, slight moisture retention under control may be relevant for specific applications. For comparison, Ponce and Gernat (2002) reported a value of 5.4% for the moisture content of Tilapia flour, which is close to our estimated results after 2 weeks of storage at room temperature. The results of the work of Hariyono *et al.* (2021) indicate that if the moisture content of the fillet is too high, it becomes vulnerable to deterioration during storage. This instability is caused by the water vapor produced during the metabolic activity of the fungi and the longer the fermentation time, the higher this moisture content, leading to a degradation of the product quality.

After 7 and 15 days of storage, the fishmeal exposed to different temperatures showed changes in fat stability, as shown in Table 4.

At low temperatures (4°C and room temperature), the average fat content remained relatively stable at around

13%. Over the weeks (1st, 2nd and 3rd), with increasing storage temperatures, the fat content of the fishmeal gradually decreased due to oxidation and hydrolysis phenomena. This was observed during the high temperature (55°C) where this content significantly decreased ($p \leq 0.05$) from an initial 41% to 7.66% after the 3rd week of storage. It should be noted that heat accelerates the action of lipolytic enzymes, particularly phospholipase, according to Kaneniwa *et al.* (2000), even when they are present in small amounts. Lipids can then undergo hydrolysis, leading to the breakdown of triglycerides into free fatty acids and glycerol, thus compromising product stability.

This is in contrast to low-temperature storage conditions, where the fat present in fishmeal is less likely to oxidize rapidly. According to Ashton (2002), lipolysis is a common post-mortem characteristic in fish muscle, resulting from the enzymatic hydrolysis of esterified lipids. However, these changes generally remain limited if fishmeal is stored properly at 4°C under airtight, dry conditions, thus preventing enzymatic activity and oxidation.

In the study conducted by Hariyono *et al.* (2021), it was found that the lowest lipid content was obtained during the 50 hours fermentation process, dropping from 19.38% to approximately 0.9%. This result suggests that fermentation treatment results in a significant reduction in the fat fraction. Furthermore, seasonal variations in the chemical composition of capelin flour were observed.

Bragadóttir *et al.* (2004) report that the lowest lipid content was recorded in spring (8.4%), but it reached between 10.9 and 11.9% during other seasons ($p < 0.05$), with an inverse relationship to moisture content. However, the lipid content of cappel flour did not vary from one season to the next in another study by Bragadóttir *et al.* (2002), unlike that of cappelin, which decreased from approximately 14% in autumn to nearly 3% in spring. Regarding the effects of different heat treatments, Hilmarsson *et al.* (2020) studied the impact of cooking on lipid composition during the production of pelagic fishmeal,

Table 5: Results of stability tests of microbiological parameters carried out.

Shelf life (days) / Storage temperature (°C)	0	7	15	21	Parameter analyzed
4 Ambient 55			uncountable		Total aerobic mesophilic flora
4 Ambient 55	Absence	Absence	Absence	Absence	Total coliforms
4 Ambient 55	Absence	35 colonies/g Absence	Absence	Absence	Fecal coliforms
4 Ambient 55	Absence 9 colonies/g Absence	4 colonies/g 11 colonies/g uncountable	Uncountable	Uncountable	Yeasts and molds

demonstrating that cooking temperatures of 85°C reduce the final lipid content while preserving the quality of the remaining lipids.

Furthermore, research conducted by Ruyter *et al.* (2003) on lipid metabolism in Atlantic salmon showed that temperatures of 5°C combined with the presence of linoleic acid promote the synthesis of certain essential fatty acids in hepatocytes. Furthermore, Sissener *et al.* (2017) highlighted the effect of temperature on lipid accumulation in Atlantic salmon liver. Low temperatures (6°C) lead to a greater accumulation of reserve lipids compared to 12°C.

Pourashouri *et al.* (2013), when evaluating the quality loss in ready-to-eat fish-based foods during refrigerated storage, highlight a significant development of lipid oxidation despite low microbial growth, hence the sensitivity of lipids even at low temperatures.

A decrease in protein content after 3 weeks of storage at different temperatures was observed compared to the initial value. The drop exceeded 5% at room temperature and more than 15% at 55°C. However, it should be noted that storage at room temperature allows for the preservation of a relatively large amount of protein (13.67%), while high temperatures (55°C) lead to a marked degradation of proteins (4.1%) in the fish waste meal analyzed in this work.

This protein alteration could be linked to chemical reactions such as oxidation or to enzymatic activity favored by storage conditions (temperature and duration) (Poojary and Lund, 2022). In addition, Maillard reactions occur between amino acids and reducing sugars, thus causing a loss of essential amino acids and a loss of the nutritional value of the meal (Xiang *et al.*, 2021). Furthermore, proteins subjected to high temperatures tend to denature or coagulate, making the drying process, when poorly controlled, responsible for the deterioration of their nutritional and functional properties (swelling capacity, water retention) (Poojary and Lund, 2022).

Analysis of weekly measurements of microorganisms present in fishmeal stored under different temperature and duration conditions highlights various aspects related to its microbiological quality. Table 5 reveals significant contamination, particularly by total aerobic mesophilic flora, regardless of the experimental storage conditions.

The same is true for yeasts and molds, whose contamination only progressed from the first week of storage, with a notable intensification at 55°C. This rapid fungal growth results from inadequate storage conditions or insufficient initial quality of the resulting flour.

Indeed, yeasts and molds in "piracuí" fishmeal were only observed after 15 days of storage, with a rate of 3×10^2 CFU/g in the study conducted by Lourenço *et al.* (2011). As for coliforms, their total absence was noted under the various storage conditions tested. A marginal appearance of total coliforms was observed after one week of storage. However, the absence of fecal coliforms is a good indicator of the sanitary quality of our fishmeal sample, indicating its effective exposure to heat treatments, thus eliminating all

environmental bacteria and ensuring good microbiological quality of the fishmeal (FAO, 2019).

Asif *et al.* (2019) highlighted the presence of various pathogenic bacteria in samples of raw, cooked and frozen fish, with the presence of fecal coliforms in raw fish, highlighting the health risk caused by poor hygiene.

Juliana *et al.* (2021) study of dry salted shrimp marketed in Brazil showed that yeasts and molds, including *Aspergillus*, *Penicillium* and *Cladosporium*, reached significant levels, requiring increased hygiene measures and sanitary controls. Nguyen *et al.* (2018) made the same observation on the microbial risks associated with refrigerated catfish fillets. They observed a significant growth ($p < 0.05$) in aerobic bacterial populations (7.446 log CFU/g) after only 4 days of storage. Yeasts and molds also proliferated, reaching 2.97 log CFU/g after 3 days of refrigerated storage, while pathogenic bacteria such as *Listeria monocytogenes* and *Clostridium sporogenes* were not detected until the 6th day of refrigerated storage at 5 to 7°C.

Furthermore, Albaris *et al.* (2022), in their research on microorganisms responsible for the deterioration of aquatic products, highlighted that yeasts of the genera *Candida* and *Rhodotorula* are frequently encountered spoilage agents, particularly at low temperatures. A fungal load assessment conducted by Ibrahim *et al.* (2015) on 100 samples of imported frozen fish revealed the predominance of molds of the genera *Aspergillus spp* and *Penicillium spp*. In addition to the presence of other mold and yeast isolates, according to Malimon *et al.* (2018), high levels of mesophilic microorganisms were present in 25% of the samples analyzed. Furthermore, psychrotrophic organisms were detected in 60 to 70% of samples with a load exceeding 50000 CFU/g, often exceeding the accepted microbiological standards for fresh fish.

CONCLUSION

The results of the study on the stability of the physicochemical and microbiological parameters of fishmeal obtained from fish waste under different storage conditions highlights the importance of refrigerated conditions in a controlled atmosphere to ensure optimal preservation of the product. Storage at 4°C stabilizes moisture, lipids and proteins, while limiting microbial proliferation, which helps preserve the nutritional and hygienic qualities of the meal ; While storing at high temperature (55°C) promote increased water evaporation and accelerate the process of nutrient degradation through lipid oxidation and protein denaturation. Knowing that fishmeal is a perishable product, its storage at room temperature makes bacterial growth faster than under refrigerated conditions, which could reduce its stability and shelf life.

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Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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