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Influence of Salt on the Biochemical Characteristics of Fermented, Salty and Dried Catfish (*Clarias gariepinus*) in Benin

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Abstrat: The aim of the current study is to assess the influence of salt on the chemical composition of fermented, salty and dry catfish (*lanhouin*) in Benin. Different flours of fish were produced and their chemical composition was evaluated based on conventional methods of analysis. From the results obtained, the Catfish is rich in dry matter (76.640 ± 0.162 to 88.050 ± 0.085%), total ash (3.660 ± 0.064 to 23.000 ± 0.020%), total protein (5.425 ± 0.194 to 64.405 ± 1.785%) and total lipids (11.495 ± 0.009 to 1 7.875 ± 0.357%). Essential amino acids are in abundance in the fermented catfish. From the quantitative point of view, it was noticed that at high concentration (salting to more than 20%) salt is the chemical composition of the catfish. This resulted in the decrease of makers in lipids, protein and amino acids on the one hand, and on the other hand, by the high concentration of markers in dry matter and total ash. Statistical analyses showed a significant difference with of protein, ash, dry matter and amino acids; where as lipids there is no significant difference at 5%. Qualitatively, the catfish contains all nutrients (proteins, amino acids, lipids, etc.).

Keywords: Chemical Composition, Fermented Catfish, Proteins, Amino Acids, Salt

1. Introduction

The global fisheries and aquaculture production will reach 191 million tonnes in 2024, with the prevalence of freshwater fish. The majority of fishing and of the world aquaculture production will be provided by aquaculture [1]. Thanks to fast the development of aquaculture in freshwater in sub-Saharan Africa, the global contribution of Africa to the aquaculture production increased from 1.2 percent to 2.2 percent over the last decade [2].

Several research studies have shown the importance of fish in food and the beneficial effects of eating the fish on health. Despite the main role of the fish in the diet, Benin has a fish deficit. To address this deficit, the Beninese State has made the aquaculture a priority. Thus, aquaculture is in its infancy and is carried out on two species of fish: the African catfish (*Clarias gariepinus*) and the Nile tilapia (*Oreochromis niloticus*) with a production of 667.07 tonnes in 2013. In Benin, the fresh clarias of aquaculture is exported in its living form in cans of water to Nigeria; but also the smoked clarias is exported. In addition, the volumes of Clarias sold in Nigeria are estimated at 284 tons in fresh form and 710 tons in smoke form [3].

Various techniques have been adopted for the conservation of the fish mainly: fermentation, salting, drying, etc. Fermentation of the fishery products is a very old practice. It is customary to think that fermented fish foods belong to the cultural areas of the Southeast Asia, whose nuoc-man of the Viet Nam, the most known [4], or the of Thailand [5]. Yet, several types of food made from fermented fish exist in Africa and particularly in the countries of West Africa, such as *momoni* in Ghana, *lanhouin* in Benin, *Guedj* in Senegal and *adjuevan* in Ivory Cost [6, 7].

Clarias conservation technics depend on the culinary habits of each country. For example in Asian countries (Viet Nam, Thailand, Indonesia, Philippine, Malaysia, etc.) the clarias undergoes longer fermentation [8-14]. However in Nigeria it is often smoked or dried [15-19].

If several works of research, in Benin, have dealt with farming clarias [20-22] however no literature deals with its fermentation.

The aim of this work is to study the impact of the salting on the biochemical parameters of fermented catfish fishmeal, salted and dried in Benin.

2. Material and Methods

2.1. Material

The catfish, *Clarias gariepinus*, bought at the Center of Research Incubation and aquaculture of Benin (CRIAB) at Ouedo in the commune of Abomey-Calavi was transported in a cooler at the Research Laboratory in Fishery Products treatment and conservation, Faculty of Science and Technology, University of Abomey-Calavi. The fish were gutted and subjected to four processes of transformation and conservation based on the improved *lanhouin* production method of Dossou-Yovo in 2002 (view figure 1) [6].

2.2. Chemical Analysis

From the transformation of fish meal have been analyzed in the industrial organic chemistry unit of the Faculty of Agricultural Sciences of Gembloux. The biochemical parameters identified are: dry matter by method NREL [23], the total ash by method NREL [24], crude protein (Kjeldahl method), lipids [25], the fatty acids profile, and amino acids (European directive no. 98/64/CE, HPLC [26].

2.3. Statistical Analysis

Data were analyzed in the SAS software version 9.1.2 using the General Linear Model (GLM) procedure. Analysis of variance by the Newman Keuls (SNK) and method was carried out and the averages were presented with the standard errors (ES) and probabilities (P) from the comparison of averages. Significant differences have been mentioned on the threshold of 5% ($P < 0.05$).

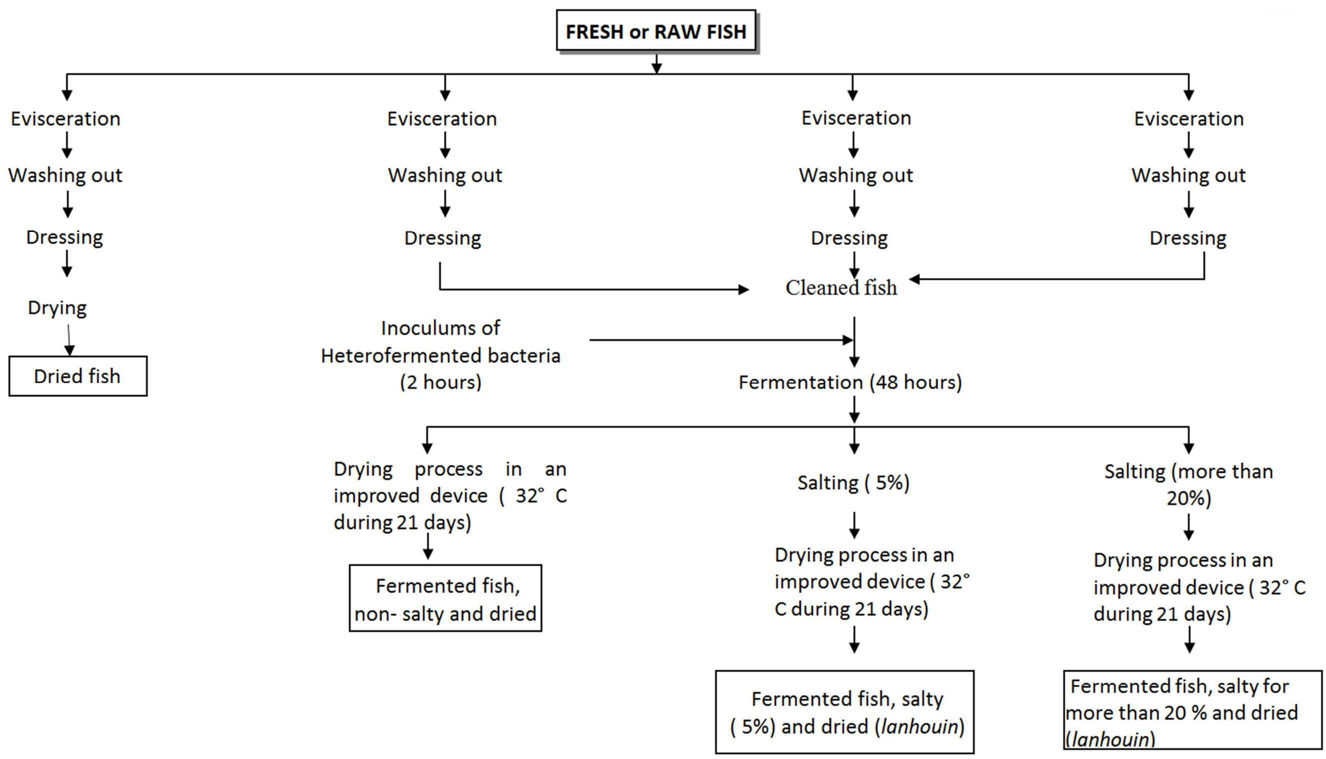

Figure 1. *Different technological diagrams of non and fermented fish transformation.*

3. Results of the Analysis

Figure 2 shows the physico-chemical composition of fishmeal.

The results obtained show a significant proportion of dry matter in fermented, salted to > 20% and dried fish meal (88.05% ± 0.085) compared to the other flours. It is followed respectively by fermented, salted to 5% and dried fish meal (82.755% ± 0.017), and the one of fermented fish, not salted and dried (78.430% ± 0.517) and finally not fermented, not salty and dry fishmeal (76.640% ± 0.162) (figure 2). It was also found that the contribution of salt has increased the concentration of dry matter, so there is an increase in organic matter. The statistical results show that there is a highly significant difference ($p = 0.0001 < 0.05$) at the level of the analyzed samples at the threshold of 5%.

As for the total ash, the same trend has been observed; fish flour fermented, salty more than 20% and dried (23.000% ± 0.020) seems to be richer in total ash in comparison with the proportions of the other flours (flour of fish not fermented, not salty and dried fish flour fermented, salty to 5% and dried and fish flour fermented, not salty and dried at concentrations of 10.895% ± 0.152, 4.405% ± 0.122 and 3.660% ± 0.064 respectively) (see figure 2). The observation of the results shows that fermented, salty fish meal to more than 20% contains more than total ash than the other 3 meals.

The values obtained in ashes for fermented, non- salty and dried fish meal and flour of fermented fish, salty to 5% are lower than the one of witness flour (non- fermented fish meal, non- salty and dried) so there is loss of minerals. But with 5% salt added to the fermented fish, it was noticed a slight increase in ash compared to the one of fermented, not salty and dried fish meal. The addition of salt to more than 20% to the fermented fish led to a noticeable increase the total ash content. Statistical analysis revealed a highly significant difference (p = < 0.0001 < 0.05) on the threshold of 5% from the results obtained.

A lot of lipid proportion was recorded at the level of fermented, salted to 5% and dried fishmeal (17.875% ± 0.357) compared to those of the other three flours whose proportions are respectively 14.860% ± 0.184 for the fermented fish meal, salty more than 20% and dried, 14.215% ± 0.155 for the fermented fish meal, non- salty and dried and, from 11.495% ± 0.009 for the fish meal non- fermented, non- salty and dried (figure 2).

Fish flour fermented, salted to 5% and dried seems to be oilier than others; then comes the fermented fish flour, salty to more than 20%, then the one of fermented, non- salty and dried fish and finally, flour of fish non- fermented, non- salty and dried (flour witness). Indeed, it was also found that more the salt is in high concentration (salting more than 20%), the less concentration of lipids. The statistical analysis reflect that there is a highly significant difference (p = 0.0006 < 0.005) between the fat matter obtained at 5% threshold.

The assessment of the amount of total protein in fishmeal show that fish fermented, not salty and dry flour contains more protein (64.405% ± 1.785) compared to the levels obtained in descending order of the other samples of fish meal (see figure 2). In fact, fermented, salted to 5% and dried fishmeal shows less total protein compared to fermented, non- salty and dried fish (61.7 percent compared with 64.4%). Then, we find that as soon as there is a lot of salt composition in proteins is reduced. Statistically point of view, there is a significant difference between protein levels (P = 0.0256) to 5% threshold.

Table 1. *Amino acids of different flours of catfish (grams of amino acids per 100 g of dry matter).*

Parameters	**Non- fermented fish meal, non salty and dried**	**Fermented fish meal, non- salty et dried**	**Fermented fish meal, salty at 5% and dried**	**fermented fish meal, salty more than 20% and dried**	**Probability**
Amino acids					
Non- essentials					
Aspartic acid	5.028^{b} ±0.137	5.520^{b} ± 0.113	6.180^{a} ± 0.092	5.133^{b} ±0.058	0.0004
Serine	2.025^{b} ± 0.060	2.230^{b} ± 0.35	2.573^{a} ± 0.017	2.20^{b} ±0.068	0.0024
Glutamic acid	10.350^{c}±0.202	11.003^{b}±0.116	11.775^{a} ±0.103	8.650^{d} ±0.125	<0.0001
Proline	2.265^{b} ± 0.138	2.027^{b} ±0.139	3.075^{a} ± 0.090	1.580^{b} ±0.042	0.0102
Glycine	2.810^{a} ± 0.070	2.903^{a} ±0.125	3.055^{a} ± 0.059	2.685^{a} ±0.019	0.2545
Alanine	4.243^{b} ± 0.086	5.063^{a} ± 0.091	4.305^{b} ± 0.035	3.577^{c} ±0.050	<0.0001
Tyrosine	1.845^{b} ± 0.153	2.425^{a} ±0.095	2.323^{a} ± 0.102	1.763^{b} ±0.065	0.0163
Essentials					
Valine	3.305^{a} ± 0.136	3.713^{a} ±0.054	3.580^{a} ± 0.084	2.860^{b} ±0.040	0.0015
Leucine	4.828^{b} ± 0.033	5.208^{a} ± 0.066	5.085^{a} ± 0.022	4.657^{b} ±0.055	0.0002
Isoleucine	2.988^{ab}± 0.099	3.388^{a} ± 0.065	3.203^{ab} ± 0.098	2.843^{b} ±0.121	0.0318
Phenylalanine	2.247^{b} ± 0.121	2.838^{a} ± 0.12	3.003^{a} ± 0.048	2.345^{b} ±0.108	0.0096
Methionine	2.140^{a} ± 0.143	1.767^{a} ± 0.037	2.025^{a} ± 0.117	1.798^{a} ±0.111	0.2476
Threonine	2.418^{bc}± 0.126	2.757^{ab}±0.026	2.927^{a} ± 0.059	2.307^{c} ±0.044	0.0112
Histidine	1.210^{a} ± 0.060	1.310^{a} ± 0.091	1.425^{a} ± 0.044	1.307^{a} ±0.064	0.4035
Lysine	4.110^{c} ± 0.050	4.478^{b} ± 0.088	5.223^{a} ± 0.103	4.933^{a} ±0.035	0.0002
Arginine	2.593^{b} ± 0.150	2.710^{b} ± 0.102	3.323^{a} ± 0.105	2.610^{b} ±0.063	0.0124

Average values with the same letter on the same line are not significantly different at the 5% threshold.

Table 1 it is shown in the composition in amino acids of fishmeal. The results obtained from this table show an acceptable amino acid profile. Sixteen (16) amino acids were detected and quantified. The protein profile includes seven essential amino acids (lysine, valine, leucine, isoleucine, threonine, phenylalanine, methionine) whose average proportions vary between a minimum of 1.767 ± 0.037 g for methionine and a maximum of 5.223 ± 0.103 g for lysine. We also have the so-called semi-critical amino acids group which is composed of histidine and arginine with medium-sized proportions of the order of 1.210 ± 0.060 g and 3.323 ± 0.105 g respectively. Values of the seven acids amino nonessentials (aspartic acid, serine, glutamic acid, proline, glycine, alanine, and tyrosine,) quantifiable fluctuate between 1.580 ± 0.042 g for proline and 11.775 ± 0.103 g for glutamic acid. Glutamic acid shows the largest value. When you look at serine, aspartic acid, glutamic acid, proline, glycine, threonine, phenylalanine, arginine, lysine and histidine, we find fermented fish flour, salty 5% has a high content acids amino. It is followed by the flour fermented, non-salty and dry fish and flour of fish non-fermented, non- salty and dried (flour witness) and finally fermented fish flour, salty to more than 20%.

It was found that the more there is salt, the amino acid content decreases. Statistical analyses show a highly significant differencefor acid glutamic and alanine (p = < 0, 0001) while there is a significant difference to amino acids such as arginine, isoleucine, leucine, lysine, aspartic acid, phenylalanine, threonine, valine, tyrosine, arginine, and proline. However the histidine, glycine, and methionine present no significant difference to the 5% threshold.

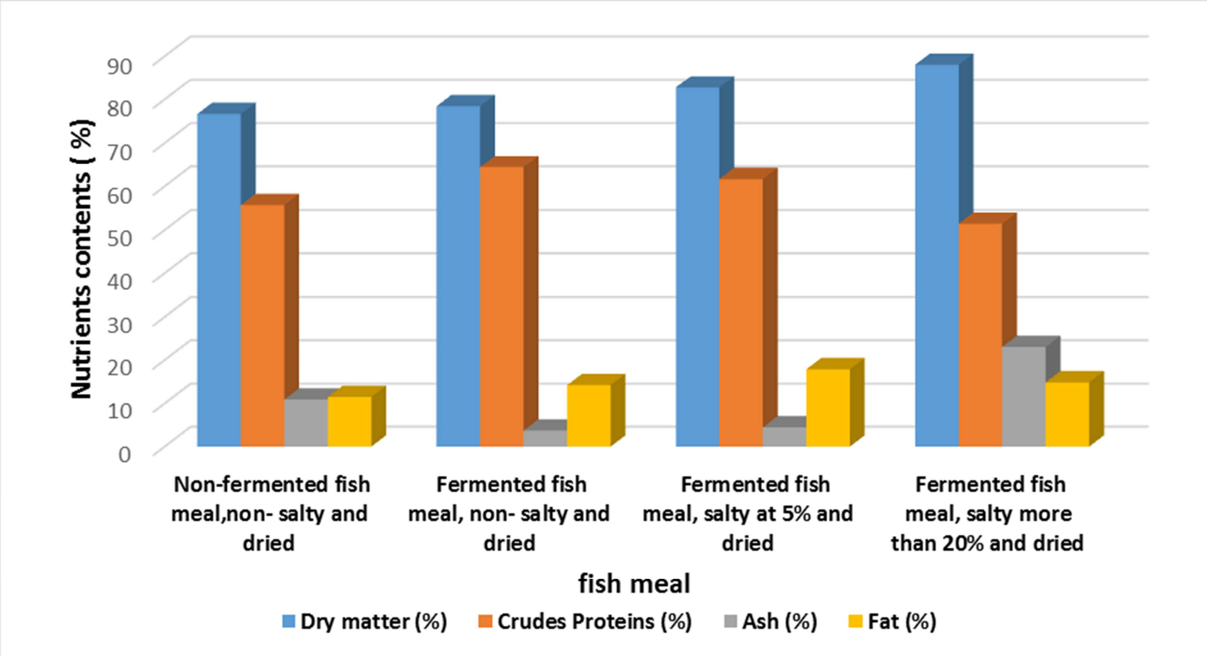

Figure 2. *Chemical composition of fish flour.*

4. Discussion

The increase in dry matter in fish meal fermented compared with the non-fermented flour of fish, non-salty and dried (flour witness) is probably due to the fermentation which has an action on the composition in dry matter (Figure 2). This increase can also be explained by the concentration of organic elements. When there is a lot of salt in the fish fermented the dry matter get consentrated. The set of the three technics (fermentation, salting and drying) conservation of fish in the presence of a high concentration of salt has led to a significant increase in dry matter. This would mean that the contribution of salt lowering the activity of water is explained an apparent increase in the mass of the product. The Observed values with the dry matter of our fish meal are superior to those obtained by Dossu-Yovo *et al.* [27] respectively for the fish fermented, salty and dried traditional (artisanal *lanhouin*) and fermented, salty and dried fish improved (improved *lanhouin*). This superiority of results is explained by changes in the chemical composition of fish species considering the production area, age, food, sex, etc. Which confirms that the methods of conservation of fish would have a positive impact on the nutritional composition of the fish.

The ashes are the minerals in the sample. Loss of total ash recorded for samples of fermented fish meal, non-salty and dried and fermented fish flour, salted to 5% would be due to a decrease in minerals during the fermentation which is explained by drainage of minerals of fish that will find themselves in the autolysat from the fermentation. In General, it has been noticed that the more there is salt, the more there is concentration of minerals. This concentration of mineral salts could be explained by a contribution of the minerals specific salt such as iodine and fluorine.

So the salt has a positive action on the total ash composition. These results are the same with those of Dossou-Yovo *et al.* [28] which confirmed that the increase in the rate of total ash in salted fish net is justified by the addition of salt and its penetration in the flesh. The penetration of the salt into the flesh of the fish by the phenomenon of osmosis lowers the water content and increases the total ash content [29].

Whole fish with bones used as raw material first in the fermentation process as well as minerals of salt may increase the ash content of the final products [30]. These obtained results are above those recorded by studies on the fresh catfish, the smoked and / or dried (*Clarias gariepinus*) in Nigeria [15, 16, 31-33]. This means that patterns of conservation in particular the association of fermentation and salting have a positive influence on the chemical composition of the fish. However, total ash content of the fermented catfish, non-salty and dried, is lower to the result mentioned by Oluwaniyi *et al*. [34]. This contradiction would be due to the loss of nutrients during the fermentation.

During the fermentation, fermented fish meal have a high fat content (figure 2). This could be explained by a probable hydrolysis of lipids during the fermentation. Fish lipids are mechanistic through various present lipases in the flesh [35]. This occurs in various degrees during salting and fermenting fish. Our samples of fermented fish were salted. Following a contribution of high rate of salt (salting above 20%) to the fermented fish, a decrease in the concentration of lipids have been noticed. We can deduce that salting to high salt content creates a decrease of the composition in the fermented fish lipids. This decline in the rate of lipids after the salting with rate high would be probably due to their drainage by the phenomenon of osmosis in the autolysat from the fermentation. This reduction in lipid content is due to the correlation with the addition of salt and this same salt penetration in fish muscle [36, 37].

The Concentrations of lipid in our samples of fermented fish salty and/ or non- salty (figure 2) are high compared to the reported concentrations by Sangjindavong *et al.* [38] on the Nham Pla, fermented and salted catfish sauce. In addition, these results are always superior to the studies of Dossou-Yovo *et al.* [27, 39, 40], Fall *et al.* [41] and Kouakou *et al.* [7] respectively for fermented sea fish (bass and mackerel) as *lanhouin* in Benin, the *Guedj* in Senegal and the *Adjuevan* in Ivory Cost. This difference in lipid content would be explained by changes in the lipid composition of fish species which is a function of the production area, of age, food, sex.

Moreover, we find that fish fermented, non- salty and dry flour has a protein concentration higher than for the other flours (figure 2). This increase in protein results in degradation of the protein-like molecules (amino acids) by the body during the fermentation of fish. The increase in the rate of protein is explained in the dissociation of protein with other membrane constituents links, and also, in the release of non protein nitrogen. In addition, a high salt rate concentration high after fermentation, leads to a decrease in the protein content.

The same observation was made by Amano [42], who mentioned that the high concentrations of salt slow enzymatic activity which slows the fermentation and therefore induces a low degradation of the proteins so the decrease of protein recorded after salting. During the fermentation, we are witnessing a proteolytic activity of the proteins in the tissues of the fish. Moreover, it has been showed that the high salt content causes the disintegrate of protein materials in amino acids free and volatile nitrogenous bases such as: ammonia, trimethylamine, urea and creatine [43].

However, our study shows that fermented fish salty or non-salty have a high protein content. These values remain higher compared to the work of Sangjindavong *et al*. [38] on the composition in protein of Nam pla, catfish fermented and salted sauce. In addition, Thailand, Thanonkaew *et al*. [9] reported a low concentration of protein from catfish fermented, salty and dried compared to our values. These results are higher than those of Dossou-Yovo *et al*. [27, 39, 40], Fall *et al.* [41] and Kouakou *et al.*[7] respectively for fermented and salty sea fish (bass and mackerel) respectively as *lanhouin* in Benin, the *Guedj* in Senegal and the *Adjuevan* in Ivory Cost. On the one hand, we could deduce that the conservation methods used affect the composition of the catfish. Indeed, they enrich the product fermented in nutrient (protein). Positive action noticed with the proteins can be explained by dissociation and the release of organic elements as amino acids. And on the other hand, this difference in protein concentration would be due to the change in the protein composition of fish species which is a function of the production area, of age, food, sex.

Table 1 shows the profile of amino acids of fishmeal. The presence of essential and non-essential amino acids leads us to say that fermentation has no much influence on the amino acid profile. Indeed, studies showed that the protein hydrolysates from fish contain all the amino acids essential and non-essential [44-46]. Our samples contain more essential amino acids than non-essential amino acids. This is accordance with previous studies on the sauces of fermented fish [13, 47].

However, in our study we noticed the absence of tryptophan, which is an essential amino acid. This lack of tryptophan in fishmeal could be explained on the one hand, by a probable loss of this amino acid during the fermentation, which would be drained in the autolysate oozing fermented fish and on the other by his likely absence in fresh catfish transformed.

However, the work of Oluwaniyi *et a*l. [34] also mentioned the absence of tryptophan in the fresh catfish. These remarks show that fresh catfish contains no tryptophan before their transformation. This means that the composition in amino acids of the fish depends on the origin of fish and the technique of fermentation or transformation. Our study revealed that glutamic acid is in high proportion among the identified amino acids. This result is the same with the results obtained by Seniman *et al.* [48] which showed that the enzymatic hydrolysate of protein from catfish contains a significant amount of glutamic acid. The same remark was made for fish sauces [4, 11] and the protein hydrolyzate of fish [44, 45]. Furthermore, it was noticed that a high rate of salt has an impact on the amino acids content. This would mean that salt has an impact on the content of amino acids of fishmeal in high concentration. In actually, amino acids are the result of the enzymatic transformation of proteins. However, we noticed the decrease in protein and by corollary of amino acids content.

In general, salt has an influence on the biochemical composition of the fish. This influence can be positive (ex: concentration of some elements such as the dry matter or ash) or negative (dwindling of lipids or proteins) to the fresh of the fish. It increases or decreases the amount in nutrients. Thus, salt is an essential raw material essential for the production of fish sauce because it contributes to the prevention of the deterioration of fish [49]. Indeed, a high concentration in salt (20% to 30%) slows down bacterial proliferation and thus the alteration of fish. However, too high concentrations in salt may be inhibitory to the enzyme activity in general and particularly proteasic [50]. On the other hand, a reduction of the salt content accelerates proteolysis [50-52]. But, too low concentrations can be harmful because bacterial development is no more limited, notably resulting in the appearance of unwanted odors.

If despite the influence of salt on the biochemical composition of fermented fish, we find protein, dry matter, total ash, lipids and in particular essential amino acids, we can say that the salt does not have much impact on the nutritional quality of the fermented fish. So conservation modes do not affect the chemical composition of the fish.

5. Conclusion

It is carried out from our study that the catfish fermented, salty and dried in Benin is full of nutrients such as proteins, lipids, and amino acids, ash (minerals). The application of high rate of salt, after fermentation can lead to an increase of concentration in chemical elements (total ash or dry matter) or a reduction of the chemical composition (lipids, proteins or amino acids). It would be better to do a moderate salting (10-15% of salt) for the benefits of fish consumption. Quantitatively point of view, the chemical composition of fish is influenced by salting. The presence or the determination of the chemical parameters in particular essential amino acids shows that salt and fish conservation techniques have not effect on the composition of the fish.

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