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RESEARCH ARTICLE

DEVELOPMENT, PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF A CUBE BROTH BASED ON "LANHOUIIN" STABILIZED USING PLANT EXTRACTS

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Abstract

The present study aims to limit the consumption of synthetic broths by developing a natural broth based on "lanhouin" flour stabilized with plant extracts for their antifungal and antioxidant activities. To do this, the essential oils of *Pimenta racemosa*, *Cymbopogon citratus* and the powder of *Curcuma longa* were used at different doses. The physico-chemical characterization of the samples of cube broths produced indicates that these latter have a water content of varied from 45.52 to 50.54% and pH varying from 6.82 and 7.58. Microbiological analyzes revealed the presence of total mesophilic flora and *Staphylococcus* spp in all samples. The Sulfito-Reducing Anaerobes (ASR) were counted in 05 samples out of 13 analyzed. The fungal flora was present in 03 samples out of 13 analyzed. On the other hand, there is a total absence of total coliforms in the different samples. It then becomes necessary to assess the acceptability of the innovative product developed with a view to its future development

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Introduction:-

Fisheries and aquaculture remain, for hundreds of millions of people around the world, activities of primary importance for food, nutrition, the acquisition of income or livelihoods (FAO, 2016; Fall et al., 2018). These activities worldwide provided 167.2 million tonnes in 2014, 93.4% of which is produced by fisheries and 73.8% by aquaculture. More than 200 million Africans regularly eat fish either fresh or most often smoked or dried (World Fish Center, 2005). In Benin, this sector plays a major role in the national economy with a contribution of 3% to GDP (Ayoubi and Failler, 2013). However, the conservation of fishery products in landing sites and sales areas, remains a major problem, due to their very perishable nature, the lack of adequate infrastructure and local climatic conditions very favorable to a degradation of the products landed in a few hours (Fall et al., 2018). The percentage of post-harvest losses and deterioration in product quality is high, with all the associated risks for consumer health (FAO, 2016). Like other countries in the West African sub-region, post-catch losses are estimated at around 20%, despite the efforts made each year, by conserving fresh fish by various traditional techniques, including fermentation to produce Lanhouin (Anihouvi et al., 2005).

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In addition, cubes are essential in most African cuisines. These culinary broths are however harmful to health. While this information is not new to some, it remains difficult for many to accept. ... The famous broths present dangers to consumers because of their high content of salt and chemicals such as monosodium glutamate, disodium inosinate and disodium guanylate ... More and more African nutritionists are trying to oppose the excessive use of these products. In Mali, in 2010, a workshop devoted to "Dangers linked to food additives" was held at the International Conference Center of Bamako. It brought together experts (veterinarians, university researchers, doctors ...), but also representatives of the Ministry of Health, health laboratories, and agencies in charge of food safety. Their conclusions are final; they reveal that the small palate seduction cubes can cause heart problems, hypo or hypertension, gastric diseases, behavioral disorders in children, swelling of the prostate, Parkinson's disease or that Alzheimer's ... They can also reduce libido, trigger bleeding and urogenital infections (Clamay, 2016).

In order to avoid the harmful consequences of cube broths on their health, more and more consumers are turning to natural products such as "lanhouin". "Lanhouin" is a natural flavor enhancer obtained by fermentation, salting and drying of the fish (Dossou-Yovo et al., 2011). It is produced on an artisanal scale by a spontaneous fermentation over which very little control can be exerted, with the obtaining of a final product often of variable quality, with risks of quality defect, in particular the formation of toxic substances such as biogenic amines including histamine (Anihouvi et al., 2006). Likewise, the use of petroleum or petrol and various insecticides against insects during production can constitute potential risks of poisoning (Anihouvi et al., 2005). It is therefore urgent to look for natural products that are slightly or not toxic in order to conserve / stabilize the "lanhouin". In this perspective, a study conducted by Anihouvi et al. (2014) allowed the improvement of the production process of "lanhouin" through, the insertion of a step of immersion of fermented salted fillets, in a solution of garlic extract and lemon juice with bacteriostatic action. However, the use of aromatic plant extracts as preservatives could constitute a credible alternative as it guarantees the preservation of consumers' health (Burt, 2004; Konfo et al., 2015). Indeed, several studies have demonstrated the antifungal and antibacterial properties of essential oils (Burt, 2004; Dègnon et al., 2013). They are known both for their antioxidant, flavoring, antimicrobial properties and their reduced toxicity compared to that of chemical preservatives (Bassolé et al., 2001). The objective of this study is to develop a technology for the production of broth (cube) from salted and dried fermented fish meal "lanhouin" stabilized and improved with essential oils and turmeric powder.

Material and Methods:-

Raw Materials:

The plant materials used in this study were *Pimenta racemosa* and *Cymbopogon citratus* essential oils and *Curcuma longa* powder. We also used cassava starch powder and samples of fish (species).

Improved food broth production manufacturing process:

Fermented, salted and dried "lanhouin" fish is obtained by spontaneous fermentation following a process including washing and or trimming, ripening, sorting, salting, fermentation and drying or draining. Washing and / or trimming consists in washing, gutting and, if necessary, scaling the fish. Depending on the case, the trimming operation can precede or succeed the maturation which consists in leaving the fish without treatment until its softening. Untrimmed fish is immersed in seawater contained in a basin (for 10 to 15 hours). The previously eviscerated fish is placed in a basin without seawater and left for the same period; the "soft fish" is obtained. The soft fish are then sorted then salted and put into fermentation. The salt is introduced into the evisceration slot under the lid, in the gills and passed over the whole body of the fish. Fermentation can last 3 to 8 days. The fish are then left to dry in the sun for 2 to 4 days. We obtain salted, fermented and dried fish "Lanhouin". Then the head of lanhouin is removed and the remaining part is ground to obtain "lanhouin" flour. To this flour, essential oil of *Pimenta racemosa* or *Cymbopogon citratus*, *Curcuma longa* powder and cassava starch powder in the appropriate proportions (Table 1) were added for each sample. The whole is then dried for 2 hours at 37 ° C.). The obtained broths were finally packed (Figure 1).

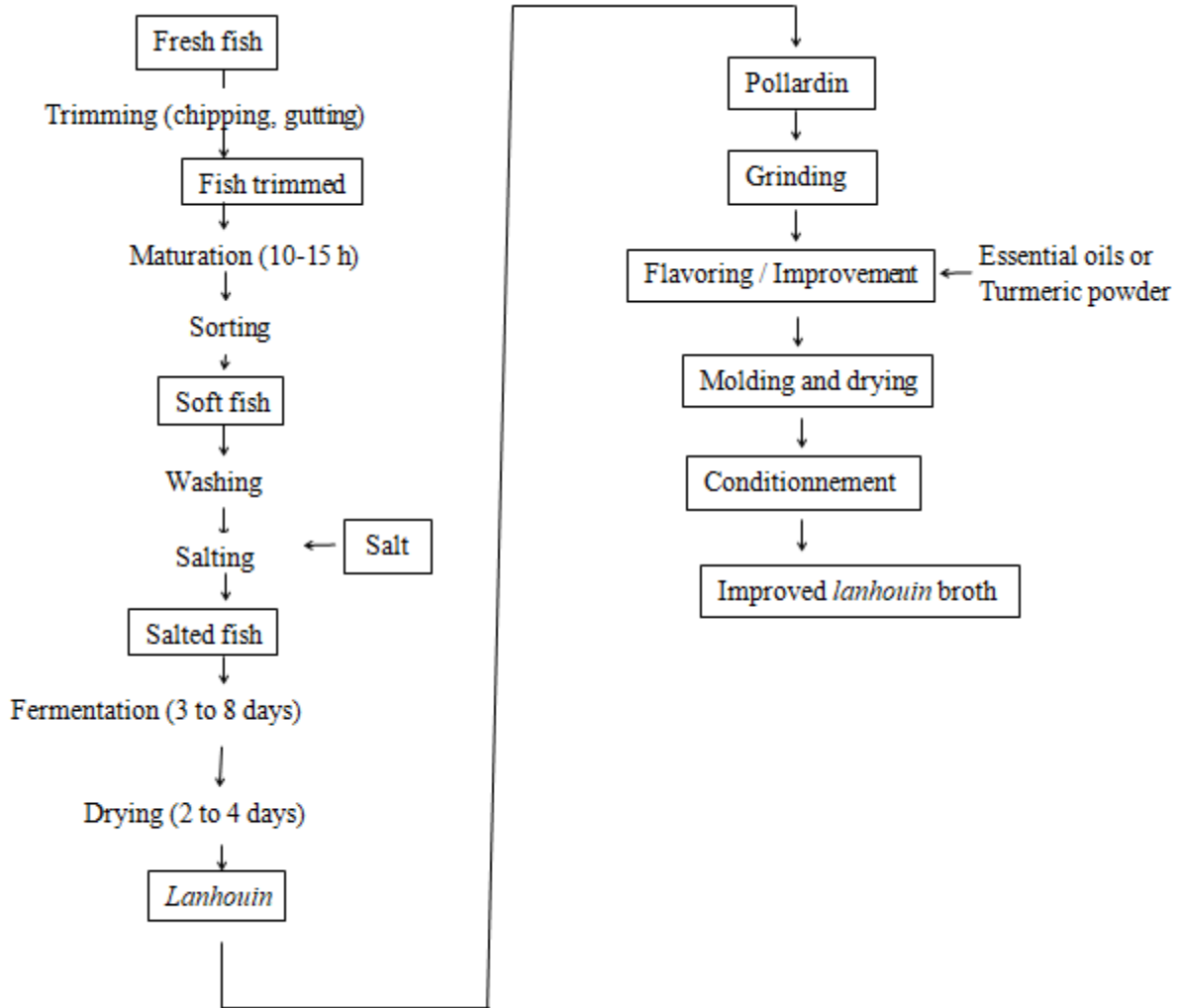


Figure1:- Manufacturing process for improved broth production based on lanhouin.

Mixing plan:

The mixing plan tested during this work is summarized in Tables 1 and 2.

Table1:- Mixing plan with *Cymbopogon citratus* essential oil.

Constituents	Propotions of Lanhouin flour(%)	Propotions of turmeric powder(%)	Propotions of Binder (%)	<i>Cymbopogon citratus</i> essential oil doses (µl/g)
Sample No.				
E ₀ : Témoïn	100	0	0	0
E _{1a}	50	10	40	1,5
E _{2a}		20	30	
E _{3a}		40	10	
E _{1b}	50	10	40	2,5
E _{2b}		20	30	
E _{3b}		40	10	

Table 2:- Mixing plan with *Pimenta racemosa* essential oil.

Constituants N° Echantillon	Propotions of « Lanhouin » flour (%)	Propotions of turmeric powder (%)	Propotions of Binder (%)	<i>Pimenta Racemosa</i> essential oil doses (µl/g)
E ₀ : Témoins	100	0	0	0
E' _{1a}	50	10	40	1,5
E' _{2a}		20	30	
E' _{3a}		40	10	
E' _{1b}	50	10	40	2,5
E' _{2b}		20	30	
E' _{3b}		40	10	

Microbiological analysis:

Samples microbiological parameters were evaluated by using standard methods. Thus, the total mesophilic aerobic flora was enumerated by inoculation on the Plate Count Agar medium (PCA) and incubation at 30 °C for 24-48 h (NF V08-051), whereas positive coagulase Staphylococci was tested on Baird Parker medium with incubation at 37°C for 24-48 (NF EN ISO 6888-1 / A1). The Anaerobic Sulpho-Reducing Bacteria were investigated on Tryptone Sulfite Neomycin (TSN) agar with incubation at 46 ° C for 20h (NF ISO 15213). The total coliforms were searched on the Violet Red Bile Lactose medium (VRBL) with incubation at 30°C for 24 h (NF V08-050), while yeasts and molds were isolated on Sabouraud medium with chloramphenicol with incubation at 25 °C for 3 to 5 days (NF V08-059).

Physicochemical analysis:

The pH was measured with a digital pH-meter (TOOGOO(R) (PH-009(I)) equipped with an electrode and a digital display screen. On a mixture obtained from 10 g of sample ground in 90 ml of distilled water. The measurements were taken by immersing the electrode of the pH meter in 10 ml of the filtrate obtained from the mixture.

Dry matter was determined according to AOAC (2008). Five (05) grams of each sample of "lanhouin" cube broth was weighed in crucibles which were then placed in an oven at 105 ° C for 48 hours. After drying, the crucibles containing the samples were cooled in a desiccator and then weighed again. The dry matter content (DMC) of each sample was obtained by the formula:

$$DMC(\%) = \frac{P1 - Po}{P} \times 100$$

- P1 (g) = weight of the sample and the crucible after passage through the oven,
- Po (g) = weight of the empty crucible,
- P (g) = weight of the sample before going to the oven;

The water content (WC) itself is determined by the formula:

$$WC (\%) = 100 - DMC (\%)$$

Statistical analysis:

Data from three independent replicate trials were subjected to statistical analysis using Microsoft Excel 2010.

Résultats:-

The pictures below show lanhouin flour and a cube broth produced

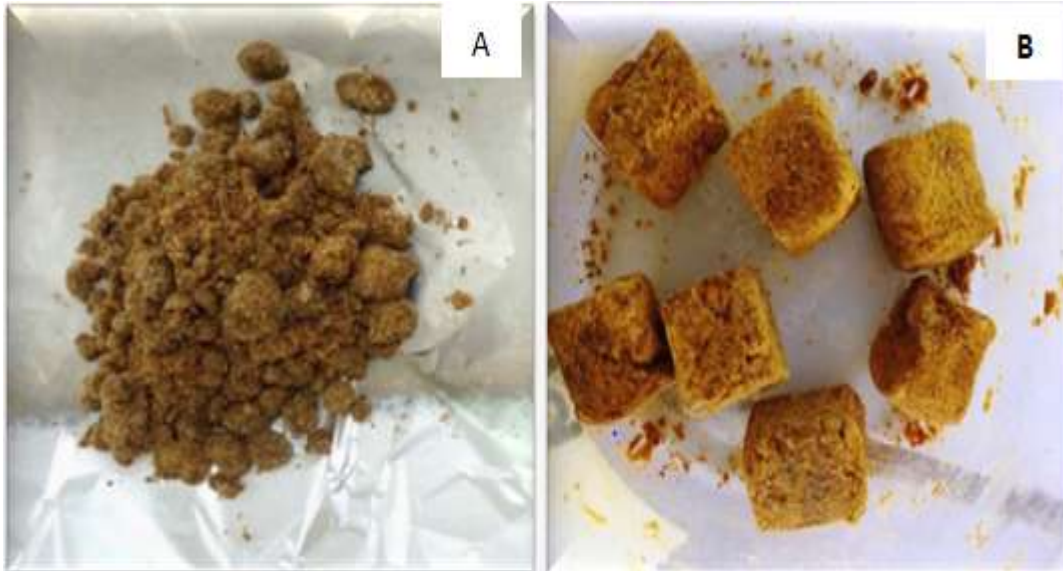


Figure 2:- Farine de lanhouin (A) et bouillon cube à base de lanhouin (B).

Physicochemical characteristics of improved food broth:

From the analysis of Figures 1 and 2, it appears that the different samples of cube broth water contents varied from 45.52 and 50.54% while the pH values obtained varied from 6.82 to 7.58.

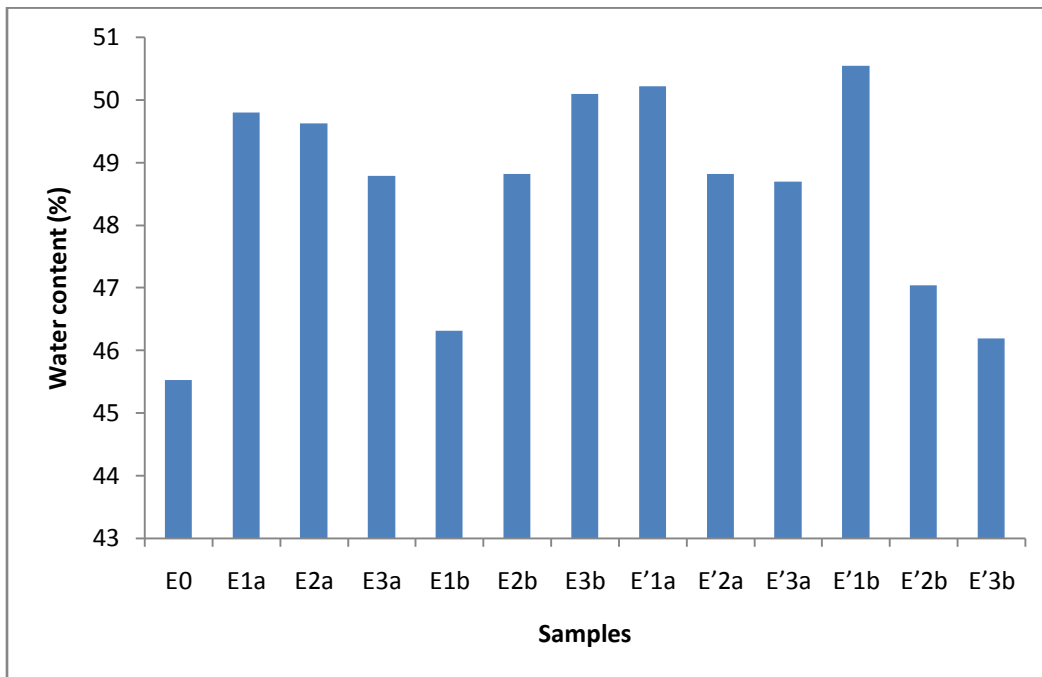


Figure 1:- Variation in water content according to the samples.

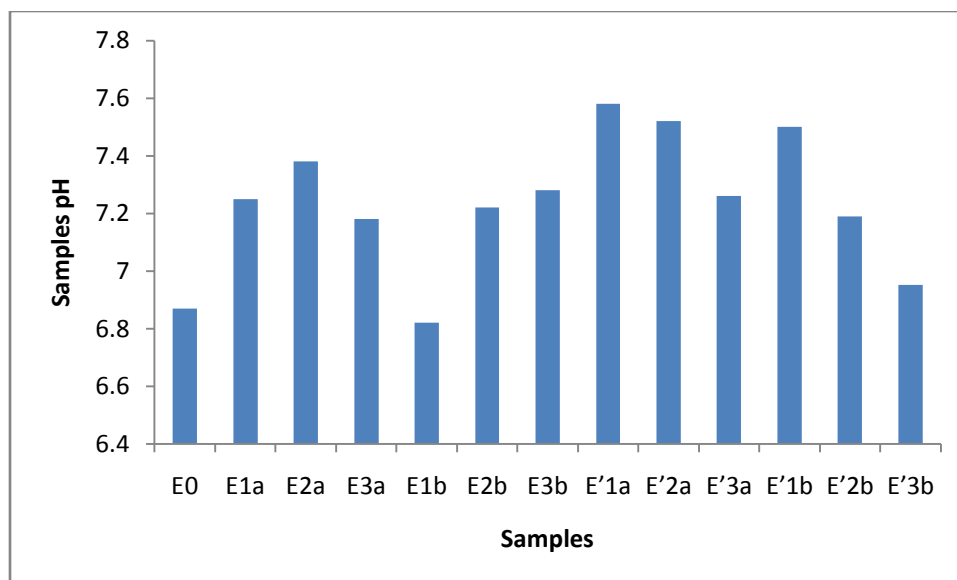


Figure 1:- Variation of pH depending on the samples.

Microbiological quality of improved food broth:

The presence of total aerobic mesophilic flora and *Staphylococcus* spp was noted in all the samples. The Sulfito-reducing anaerobes were counted in 05 samples out of 13 analyzed while fungal flora was present in 03 samples out of 13 analyzed. Total coliforms were absent in the all samples (tableau 3).

Table 3:- Microbiological characteristics (CFU / g) of the samples.

Samples	Total flora	Staphylococcus spp.	Sulphite-reducing anaerobes	Total coliforms	Yeasts and molds
E ₀	18.5x10 ³	4.5x10 ³	5.0x10 ¹	< 1	< 1
E _{1a}	>300	12.0x10 ⁴	1.5x10 ¹	< 1	< 1
E _{2a}	>300	14.8x10 ⁴	< 10	< 1	< 1
E _{3a}	>300	12.6x10 ⁴	< 10	< 1	< 1
E _{1b}	>300	14.4x10 ⁴	1.0x10 ¹	< 1	< 1
E _{2b}	>300	>300	< 10	< 1	7.0x10 ²
E _{3b}	>300	18.8x10 ⁴	< 10	< 1	< 1
E' _{1a}	>300	18.4x10 ⁴	2.0x10 ¹	< 1	1.0x10 ²
E' _{2a}	15.9x10 ⁴	5.2x10 ⁴	< 10	< 1	< 1
E' _{3a}	20.6x10 ⁴	2.0x10 ⁴	< 10	< 1	< 1
E' _{1b}	>300	>300	1.0x10 ¹	< 1	1.0x10 ³
E' _{2b}	>300	4.0x10 ⁴	< 10	< 1	< 1
E' _{3b}	>300	2.0x10 ⁴	< 10	< 1	< 1
Criteria (AFNOR)	<10 ²	<10 ⁵	<10	<10 ²	-

- : Unspecified.

Discussion:-

Lanhoun, a traditional fermented fish-based condiment is processed in the coastal areas of West African countries including Benin, Togo, Ghana, Nigeria and Côte-d'Ivoire. It is mostly used as taste enhancer and flavouring agent in many types of dishes (Anihouvi et al., 2005; Kindossi et al., 2012). The raw materials used for lanhoun production include the fish and the salt, and the fermentation is spontaneous and uncontrolled (Anihouvi et al., 2012a).

In this, this technology has been revisited and improved by the production of broths improved by the use of extracts of aromatic plants. Microbiological analyzes have shown the presence of certain germs. The frequencies of contamination observed were close to those reported by Anihouvi et al. (2005) for "Lanhoun" samples collected in the south of Benin (45.6 and 59.9%). These values were similar to those reported respectively by

Essuman (1992) on the "Momone" a fermented fish from Ghana and by Anihouvi et al. (2006) on the "lanhouin". The results indicate the presence of a total aerobic mesophilic flora in the various samples of "Lanhouin" broth. These results vary from $18.5 \cdot 10^3$ to $20.6 \cdot 10^4$ CFU/g and were higher than the threshold value recommended by the standard which is 10^2 CFU / g. Indeed, the total mesophilic aerobic flora reflects the general microbiological quality of a product. This microbial load more or less enumerated in the broth could result from the conditions of production of "lanhouin" (main component of the broth) and also from operations during the production of broths (molding of "Lanhouin" flour and drying in the open air). Indeed, according to Dossou-Yovo et al. (2011), the production of "Lanhouin" generally takes place in a relatively unclean environment. These results relating to the total aerobic mesophilic flora are lower than those obtained by Kouakou et al. (2013) which were $4.8 \pm 0.08 \cdot 10^8$ CFU / g and $6.7 \pm 0.5 \cdot 10^8$ CFU / g for fermented fish (Adjuevan) obtained using two different methods.

Concerning *Staphylococcus* spp, their presence often results from the action of factors such as wind, dust and also contamination of human origin through handling and secretions (saliva, sweat), the results obtained range from $4.5 \cdot 10^3$ to $18.8 \cdot 10^4$ CFU / g. This could always be explained by the non-compliance with hygienic rules and good manufacturing practices during the production of "lanhouin" and or manipulation during the production of broths. Thus, the presence of these germs in fermented products indicates the need to take hygienic measures capable of countering the risks linked to these types of microorganisms (Anihouvi et al., 2006; Kouakou et al., 2013). These results are all below the threshold value recommended by the standard which is 10^5 CFU / g and higher than those obtained by Adjou et al. (2017) (<10 CFU / g) on fermented fish meal improved with oil of *Pimenta racemosa*. This can be explained by the presence of plant extracts in the broths. Indeed, the antibacterial effects of *Curcuma longa* widely proven staphylococci (Teow et al., 2016; Yadav et al., 2013; Afrose et al., 2015), can also be confirmed with obtained results. Thus, with a gradual increase (10 to 40%) in the proportion of Turmeric, there is a gradual decrease in the microbial quantum ($18.4 \cdot 10^4$ to $2.0 \cdot 10^4$ and > 300 to $2.0 \cdot 10^4$ CFU / g). Indeed, at the level of the samples E'1a and E'1b, each having 10% of turmeric powder, $18.4 \cdot 10^4$ and > 300 CFU / g were observed respectively, while for the samples E'2a and E'2b, each having 20% turmeric powder, $5.2 \cdot 10^4$ and $4.0 \cdot 10^4$ CFU / g are obtained respectively. For samples E'3a and E'3b, each having 40% turmeric powder, there is again a reduction in staphylococcal germs ($2.0 \cdot 10^4$ CFU / g).

The anaerobic sulfite-reducers being ubiquitous germs, their presence in the samples would probably be related to the manipulations carried out on the ground, as well as the presence of flies on the production sites, without forgetting the recycled salt and improperly stored which is used for production. The presence of these germs could also result from the broth drying operation. These results varying from $1.0 \cdot 10^1$ CFU / g to $5.0 \cdot 10^1$ CFU / g, were higher than the threshold value recommended by the standard which is 10 CFU / g and contrary to those of Dossou-Yovo et al., (2011) who had obtained a total absence of anaerobic bacteria on improved Lanhouins obtained from two different species of fish.

The microbial quantum of yeasts and molds varies from $1 \cdot 10^2$ to $1 \cdot 10^3$ CFU / g and this on only three samples among the thirteen analyzed. In fact, out of the six samples containing *Pimenta racemosa* essential oil at different doses ($1.5 \mu\text{l} / \text{g}$ and $2.5 \mu\text{l} / \text{g}$), we count for samples E'1a and E'1b respectively of $1 \cdot 10^2$ CFU / g and $1 \cdot 10^3$ CFU / g. On the other hand, on the six samples containing the essential oil of *Cymbopogon citratus* at different doses ($1.5 \mu\text{l} / \text{g}$ and $2.5 \mu\text{l} / \text{g}$), we count for the sample E2b a value of $7 \cdot 10^2$ CFU / g. This low rate of fungal growth obtained allows us to justify the strong antifungal activity of the essential oils used and could also be justified by the antibacterial activities of *Curcuma longa*.

These results were in agreement with those of Adjou et al., 2017 (<10 CFU / g) and Dègnon et al. (2019) who respectively showed the antifungal activities of *Pimenta racemosa* oils on "Lanhouin" flours and *Cymbopogon citratus* essential oil on *Aspergillus* species isolated from fermented fish. However, the growth observed on the three samples (E2b, E'1a and E'1b) could be linked to the persistence of essential oils. This presence of fungal flora could therefore come from the production of "lanhouin" which requires drying phases which is generally done in the open air where "lanhouin" is subjected to multiple sources of contamination, in particular dust and insects which are vectors of mold spores. This mold contamination can also be the result of poor hygiene practices during production, where trimmed fish are sometimes covered with old loincloths or jute bags and left to mature. Likewise, the storage and transport of the "lanhouin" is done in baskets lined with these old loincloths or cement papers which serve to cover the "lanhouin". These results therefore show that, in the range of concentrations tested, the essential oil of *Cymbopogon citratus* is more effective on the fungal flora of cubic "lanhouin" broths, compared to the essential oil of *Pimenta racemosa*.

Conclusion:-

In order to enhance the "lanhouin", a traditional food broth from Benin and limit the consumption of industrial cube broths which are not without consequences for the health of the consumer, this work made it possible to assess the effectiveness of essential oils of *Cymbopogon citratus* and *Pimenta racemosa* as well as that of *Curcuma longa* powder as bio-preservative agents in the preservation of the quality of improved "lanhouin" product. It appears from the results obtained that the essential oil of *Cymbopogon citratus* has proven antifungal properties on the fungal flora of "lanhouin", compared to the essential oil of *Pimenta racemosa*. We can deduce that this essential oil offers new perspectives in the promotion of "lanhouin" by the use of natural plant extracts.

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