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Chemical Composition and Lactic Microflora of Adjuevan, A Traditional Ivorian Fermented Fish Condiment

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Abstract: Adjuevan, a traditional Ivorian naturally fermented fish prepared from the Atlantic bumper Chloroscombrus chrysurus, was assessed for its proximate and lactic acid microflora compositions in order to establish its nutritive and technological usefulness. Significant differences were observed between the fresh and the fermented fish for all the chemical substance analyzed except for the lipid content. Adjuevan contained between 69.65 and 71.25% moisture, 12.16 and 12.36% lipid, 21.21 and 26.81% protein. The mean value for the ash, Ca, P, Na and K content of the adjuevan were 10.5%, 4.16 mg/g, 3.82 mg/g, 0.72 mg/g and 0.67 mg/g respectively. There were no significant differences between samples from the different processors. The genera and species of lactic acid bacteria isolated and identified from the fresh fish and the adjeuvan samples were Leuconostoc lactis, Lactobacillus fermentum, Pediococcus sp., Streptococcus sp. The highest load was obtained with Lactobacillus fermentum, 9.4x10³ to 3.7x10⁴ cfu/g. The fermentation does not adversely affect the composition of Chloroscombrus chrysurus. Lactic acid bacteria found could participate in the technological processing and contribute to keeping the quality of the adjuevan.

Key words: Adjuevan, fermentation, fish, composition, lactic bacteria

INTRODUCTION

Fish is a rich source of easily digestible protein that also provides polyunsaturated fatty acids, vitamins and minerals for human nutrition. Although some species of fish are used industrially for fish meal manufacture, a need for their preservation and utilization for human consumption has been recognized in order to prevent post-harvest fishery losses (Venugopal and Shahidi, 1995). In many African countries, animal protein consumption patterns depend on ecological factors such as animal husbandry practices and traditional beliefs as well as urbanization, income levels and the type of animal protein available in a community (Gomna and Rana, 2007; Essuman, 1992). Meat and fish form an integral part of the diet of Ivorian people and are considered to be essential protein foods, also serving as a focal point for the family meal. The relative contribution of each of these protein sources, however, may vary depending on the livelihood activities of the family, their income and fish availability.

In Cote d'Ivoire, fish is known to play a significant role in the diet. In many developing countries, the dependency on fish remains high as substitutes in the form of other animal foods are inaccessible to the poor. At the household level, the consumption patterns may depend on the availability of hard currency, the primary activity of the household and the social structures and customs. Essuman (1992) reported that fish consumption is

relatively higher in the coastal countries of West Africa, especially in Cote d'Ivoire where fish constitutes the main source of animal protein. Fresh fish is a highly perishable product due to its biological composition. Thus, if the fish is not immediately utilized or preserved after harvest, it spoils. While in developed countries the practice of cold storage limits the problem posed by the extreme perishability of fish, in tropical regions, particularly in West Africa, traditional processes such as drying, salting, smoking, fermentation and combinations of these treatments are used for fresh fish preservation (Essuman, 1992; Oulai et al., 2007).

Fermented fishery products from Africa usually remain whole and firm after processing and the fermentation period varies from 2 to 6 days (Anihouvi et al., 2007; Sanni et al., 2002; Anihouvi et al., 2006) unlike South Asian types of fermented fish obtained after 3 to 9 months fermentation until the fish flesh becomes liquefied or turned into a paste (Kopermsub and Yunchalard, 2010; Paludan-Muler et al., 2002). Fermented fish products are generally good sources of nutriments (Cho et al., 2004; Asiedu and Sanni, 2002) and are specific to each country and their chemical and microbiological compositions are quite known. That is the case of patis (Phillipines), garum (Europe), momone (Ghana), lanhouin (Benin) and adjuevan (Cote d'Ivoire). According to Essuman (1992), fermented fish consumed on a large scale as food fish in the diet contributes substantially to protein intake instead of its use in small quantities as condiments that renders its contribution less important. Adjuevan, a fermented fish product made in Cote d'Ivoire, is processed through a spontaneous and uncontrolled fermentation. Adjuevan, very appreciated by the population of Cote d'Ivoire, is used as a condiment but not eaten as food fish because of the strong smell. However, the uncontrolled fermentation process of during adjeuvan production could lead sometimes to a product with variable qualities with occasional public health hazards as indicated by Anihouvi et al. (2006) from their work on lanhouin, a fermented fish produced in Benin. So far, nutritional and microbiological quality defects are often associated to fermented fish and linked to process technology (Sanni et al., 2002).

To our knowledge, no nutritional study has been done on fermented fish in Cote d'Ivoire. It seems important to investigate the potential nutritional value of *adjuevan* where no published work has been done. The aim of the present study was therefore to assess the nutritional characteristics and the fermentative microflora by examining a proximate composition of *adjuevan* and lactic acid bacteria involved in its processing.

MATERIALS AND METHODS

Chloroscombrus chrysurus, a small size fish from the Carangidae family was used for its high usage in fermented fish production in Cote d'Ivoire. This Atlantic bumper is an ecologically important species in the Gulf south to Cote d'Ivoire. It occurs along the West African coast. It's available on all the markets all year long. This fish has a lateral flat elongated body (around 15 cm in length and 8 cm for the height). Fresh fish were obtained from Abobodoume market, one of the marketplaces for fish commerce in Abidjan. A part of these fresh fish were transported to the major site of fermented fish processing in Abidjan, Vridi Zimbabwue, in the commune of Port-Bouet, a southern township of Abidjan to be fermented. Therefore some of the same fresh fish were used for the adjuevan processing.

Sampling procedure: Seventy two samples of fresh Chloroscombrus chrysurus and seventy two of adjuevan obtained from fermented Chloroscombrus chrysurus were collected. A total of 144 samples of fresh fish were collected from which, 72 were analyzed fresh and the other 72 were fermented into adjuevan. Adjeuvan samples were taken from three processors (Processors A, B and C) twice a week over a period of 6 months. Samples were taken per processor at the end of the fermentation process, from a basket containing the fermented fish, one day of a week and the same day of production. A sample consisted of 3 fresh fish or 3 fermented fish (200-300 g) from the same basket. The samples were packed in sterile plastic bags and stored

in icebox filled with ice and transported to the laboratory for immediate use.

Chemical analysis: All the samples (fresh and fermented fish) were analyzed fort their chemical characteristics. The moisture content was obtained by the difference between the fresh and the dry weight of the samples, dried at 105±1°C until constant weight (AOAC, 1990). The proteins were determined through Kjeldahl method and the fat by Soxhlet method (Unid Tecator, System HT2 1045, Sweden) (BIPEA, 1976). The ash fraction was obtained by the incineration of the organic matter at 550°C (AOAC, 1990), minerals by atomic absorption (Pelkin Elmer (PE 3110, Norwalk USA) but phosphorous according to the method describe by Taussky and Shorre (1953). The pH of samples was measured with a pH-meter (Hanna Instrument HI 9318) on a mixture of 20 g of blended fish meat and 80 ml of distilled water. The energetic density was calculated with the specific coefficients of Atwater and Rosa (1899). NaCl content was determined according to AOAC (1990). All the determinations were made in triplicate, the results were expressed on dry weight basis and mean values were given.

Microbiological analysis: Minced adjuevan samples were ground in a stomacher into a homogeneous mixture. To analyze the samples of adjuevan, the methods stated in Compendium of Methods for the Examination of Foods (Vanderzant and Splittstoesser, 1992) and Food and Drug Administration (FDA) (Anonymous, 1998) were used. Ten (10) grams of each mixture were suspended in 90 mL of sterile peptone buffer and homogenized again for 30s at normal speed. 1 ml of the homogenate was serially diluted in aseptic conditions and used for the enumeration of microorganisms. Streptococcus was determined on Slanetz and Bartley agar and TTC (Triphenyl Tetrazolium Chlorure), plates incubated at 37°C for 48 h. We investigated lactic acid bacteria on Man Rogosa and Sharpe Agar (MRS) (Biomérieux, France) with the pH adjusted to 5.5 and the plates were incubated anaerobically at 30°C for 48 h. Pure cultures of the isolates were Gram-stained, while the cell morphology was examined through phase contrast microscopy. Duplicate agar plates of between 30 and 300 colonies were counted and mean counts calculated. The mean number of colonies counted for all count types was expressed as Colonies Forming Units (CFU) per gram. All the tests were carried out in duplicate.

Statistical analyses: The statistical package for Social Science (SPSS version 10) was used. Data analysis involved one-way Analysis of Variance (ANOVA). The mean differences were determined using Duncan's Multiple Range Test. A significant difference was established.

Table 1: Chemical composition of the fresh and the fermented fish Chloroscombrus chrysurus according to processors

	Processor A		Processor B		Processor C	
	Fresh fish	Adjuevan	Fresh fish	Adjuevan	Fresh fish	Adjuevan
Protein (g/100 g)	26.58±0.26	25.96±0.45	27.48±1.49	26.81±0.1	26.83±0.48	24.21±0.55
Lipid (g/100 g)	12.64±0.05	12.26±0.13	12.63±0.13	12.36±0.25	12.41±0.02	12.16±0.3
pH	6.50±0.1	6.10±0.1	6.50±0.5	5.20±0.5	6.60±0.2	5.90±0.1
Moisture (%)	75.11±0.9	70.99±0.5	73.65±2.1	71.25±0.3	73.23±1.2	69.65±2.4
Salt (NaCl) (%)	0.52±0.1	2.00±0.3	0.70±0.1	1.51±0.2	0.59±0.1	1.55±0.1
Ash (%)	12.15±0.5	10.02±0.1	11.05±0.15	10.12±0.1	11.45±0.4	10.01±0.2
Energy (Kcal)	326.93±7.92	317.61±12.14	329.14±8.07	318.42±11.09	327.78±9.03	317.91±11.36
Ca (mg/g)	5.50±1.3	4.32±0.02	5.22±0.11	4.05±0.15	5.03±0.39	4.10±0.01
P (mg/g)	4.30±0.3	3.83±0.03	3.95±0.4	3.84±0.11	5.03±0.39	4.10±0.01
Na (mg/g)	0.77±0.02	0.47±0.04	0.80±0.3	0.70±0.02	0.78±0.03	0.72±0.02
K (mg/g)	0.94±0.1	0.68±0.02	0.90±0.03	0.69±0.01	1.12±0.2	0.65±0.05

Table 2: Chemical composition of the fresh and fermented fish Chloroscombrus chrysurus

Parameters	Freshfish	Adjuevan	
Protein (g/100 g)	53.93±1.77ª	25.66±02.41b	
Lipid (g/100 g)	12.47±0.14°	12.26±00.21°	
Energy (Kcal)	327.95±8.34°	317.98±11.53b	
pH	6.60±0.30°	5.73±00.50b	
Moisture (%)	73.99±0.98°	70.63±00.85 ^b	
Salt (NaCl) (%)	0.60±0.10°	1.68±00.27 ^b	
Ash (%)	11.55±0.35°	10.05±00.10 ^b	
Ca (mg/g)	5.25±0.36°	4.16±00.06 ^b	
P (mg/g)	4.10±0.10°	3.82±00.03b	
Na (mg/g)	0.78±0.01°	0.72±00.02b	
K (mg/g)	0.98±0.12°	0.67±00.02 ^b	

Means with the same superscript letter in the same line are not significantly different (p>0.05)

RESULTS AND DISCUSSION

Chemical characteristics of the samples: The chemical composition of fresh fish and adjuevan samples collected is showed in Table 1. A decrease was observed in the pH value, energy, protein, lipid, moisture, ash and mineral contents of all the fermented fish samples analyzed. However, the lipid content was similar in the fresh and fermented fish samples. The moisture contents of the samples varied between 69.65 and 71.25% for adjuevan and between 45.3 and 61.6% for fresh fish. A significant difference (p<0.05) was observed within and between fresh and fermented fish. The highest moisture contents were recorded on adjuevan samples from processor B. The pH values of all the samples were below 7. It was 6.5 to 6.6 for fresh fish and varied between 5.2 and 6.1 for the fermented fish with a mean value of 5.73 (Table 2). There was a statistically significant difference (p<0.05) between pH values of fresh and fermented fish. The lipid contents of adjuevan samples varied between 12.16% and 12.36%, whereas that obtained from fresh fish varied between 12.41% and 12.64%. The protein contents in fresh Chloroscombrus chrysurus samples varied between 26 and 26.5% while that of adjuevan ranged from 24.21-26.81% with a mean value of 25.66% (Tables 1 and 2). Significant difference (p<0.05) was noted between fresh and fermented fish (Table 2). The salt (sodium chloride) concentrations determined in the samples ranged between 0.52 and 0.7% and 1.51 and 2% for fresh fish

and fermented fish respectively. The differences between these values were statistically significant (p<0.05). There were significant differences (p<0.05) in the ash and mineral content recorded (calcium, phosphor, sodium and potassium) for fresh and fermented fish (Table 2). The mineral content decreases during the fermented fish processing.

Microbiological analysis: The load in lactic acid bacteria of the samples is summarized in Table 3. The lactic acid bacteria load was 1.2 x 10⁵ cfu/g in adjuevan samples. The lactic acid bacteria isolated of these samples were Leuconostoc lactis. Lactobacillus fermentum. Pediococcus sp., Streptococcus sp. and other lactic acid bacteria. Their numbers ranged between 2.4x103-1.4x 10^4 , $9.4x10^3-3.7x10^4$, $5.2x10^3-6.5x10^3$, $1.6x10^2-3.7x10^2$, 4.1x10⁴-6.9x10⁴ CFU/g respectively. These same general of lactic bacteria were also isolated from the fresh fish used for adjuevan processing. Lactic acid bacteria load increases during adjuevan processing in all the samples examined. No significant differences (p>0.05) were observed for Leuconostoc lactis between the types of fish analyzed.

A decrease in chemical composition of adjuevan compared to fresh Chloroscombrus chrysurus was observed. Gradual decreases in moisture, fat and protein contents were also reported by various workers who studied momone, a Ghanaian fermented fish product (Yankah, 1988; Abbey et al., 1994). The moisture value of fermented fish (adjuevan) in the present work (69.65-71.25%) was closed to value reported by Asiedu and Sanni (2002) who obtained 77.8% for naturally fermented Enam Ne-Setaakye, a west African fermented fish, but disagreed with the 50-56% moisture reported by Sanni et al. (2002) on momone and Anihouvi et al. (2006) on Lanhouin (a fermented fish from Benin). The variation in the moisture contents of the samples could be the result of variable drying methods, time and amount of salt used for the curing. The pH values for all the adjuevan samples examined were below 7. Similar values of pH were reported on momone (6.5) (Sanni et al., 2002) and Pedah siam a fermented fish processed in Thailand (FAO, 1971). However, the pH values obtained in this study disagreed with those reported for

Table 3: Mean load of lactic acid bacteria determined in the fresh and fermented chloroscombrus chrysurus according to processors

(CFU/g)	Fresh fish	Adjuevan	Fresh fish	Adjuevan	Fresh fish	Adjuevan
Lactic acid bacteria	4.0x10⁴±2.6x10⁴	1.2x10°±8.6x10°	6.3x10 ⁴ ±2.0x10 ⁴	1.2x10°±1.3x10°	6.2x10⁴±2.7x10⁴	1.3x10°±0.0x10°
Leuconostoc lactis	2.1x103±2.4x102	3.1x10°±5.9x10°	1.9x10°±6.5x10°	2.4x10°±1.2x10°	6.0x10°±1.1x10°	1.4x10°±2.2x10°
Lactobacillus fermentum	5.7x10°±2.4x10°	9.5x10°±5.9x10°	7.2x10°±2.1x10°	9.4x10°±8.4x10°	8.0x10°±0.0x10°	3.7x10°±1.8x10°
Pediococcus sp.	6.3x10 ² ±1.3x10 ²	5.5x10°±2.5x10°	1.5x10°±1.4x10°	6.5x10°±1.9x10°	2.2x10 ² ±2.1x10 ²	5.2x10°±2.1x10°
Streptococcus sp.	1.2x10 ² ±1.1x10 ¹	3.7x10 ² ±1.1x10 ²	1.1x10 ² ±2.0x10 ¹	1.6x10 ² ±0.0x10 ²	8.0x101±0.0x101	2.6x10 ² ±0.0x10 ²
Other lactic acid bacteria	1.2x10°±2.0x10°	6.9x10°±2.0x10°	7.6x10°±1.7x10°	4.1x10°±2.3x10°	1.2x10°±1.1x10°	4.3x10 ⁴ ±2.1x10 ²

lanhouin, where the pH values were above 7. A gradual decrease of pH values of fish or other food during Fermentation is well documented (Coulin et al., 2006; Paludan-Muler et al., 2002). No literature on the recommended pH range of adjuevan is available as also indicated by Anihouvi et al. (2006) for lanhouin.

Considering fish as a major source of protein, a general observation of Table 2 and 3 shows that the fermentation does not adversely affect the crude protein content of fishery products such adjuevan. This is in accordance with the results reported by other authors (Sanni et al., 2002; Anihouvi et al., 2006). The results indicated that there was a weak proteolytic activity during the adjuevan processing. This result is due to the fact that Adjuevan is obtained after only 3 days of fermentation and the texture of Adjuevan was not significantly affected by the fermentation compared to fermented fish such as Norvegian rakefisks, Suedish surchominas and Vietnamese fermented (Essuman, 1992; Nwabueze and Nwabueze, 2010). The fermentation may contribute positively to the flavour development of the product. The degradation process was initiated by proteolytic bacteria such as Pseudomonas, Micrococcus and Bacillus. These micro organisms produce amines, ammoniac, organic acids, methylmercaptan, responsible of the characteristic odour of fermented fish products (Anihouvi et al., 2006; Mensah, 1997).

The level of lipids remained basically stable during the seven-days period of the fish processing. This was expected, since the enzymatic curing process acts mainly on proteins rather than on lipids (Chang et al., 1992). The lipid content (12%) was below the one found by Hjamarsson et al. (2007) in Mallotus villosus, a fermented fish sauce with 16.3%. Usydus et al. (2009) stated that, the fundamental nutritive benefit of processed fish lies in its highly beneficial fatty acid composition, which is what imparts them healthy nutritive qualities. The high content of Long-Chain Polyunsaturated Fatty Acids (LC-PUFAs), which is not noted in other food products, is particularly important. Adjuevan has a low energy value. That suggests that it can't meet the daily energy requirement which is 3000 kcal for an adult man and 2200 kcal for an active woman (Anonymous, 1972). Therefore adjuevan is not an energetic food product and should be consumed as food fish in the diet, instead of using it in small quantities as condiments that renders its contribution less important.

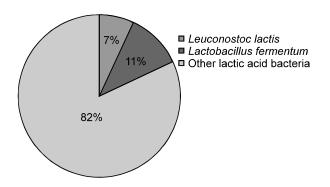


Fig. 1: Distribution of Leuconostoc lactis and Lactobacillus fermentum in the lactic flora of fresh Chloroscombrus chrysurus

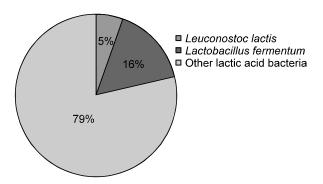


Fig. 2: Distribution of Leuconostoc lactis and Lactobacillus fermentum in the lactic flora of adjuevan

The salt contents of all adjuevan samples appeared lower when compared to the values of 10 and 15% found in momone (Nerquaye-Tetteh et al., 1978). However, salt concentrations of 4-6% were also reported in momone. The salt concentrations of 1.68% recorded on adjuevan samples are too low to inhibit the microbial histidine decarboxylase activity since histamine production by bacteria may occur at 8% salt and up to 12% (Ababouch, 1990). The salt acts on the muscles, viscera, microorganisms and enzymes, developing microorganisms which produce lactic acid, lowering the pH and making the product resistant to the development of putrefying bacteria (Oetterer and Pescado, 2003). The variations observed in the mineral content could probably be due to some micro organisms capable of using them during their metabolism such as nitrogen

Table 4: Mean load of lactic acid bacteria determined in the fresh and fermented Chloroscombrus chrysurus

Lactic bacteria (UFC/g)	Fresh fish	Fermented fish (<i>adjuevan</i>)	
Flore lactique	6.2 x 10⁴±2.2 x 10⁴a	1.2 x 10⁵±9.4 x 10³b	
Leuconostoc lactis	4.3 x 10 ³ ±2.4 x 10 ² *	5.7 x 10 ³ ±1.2 x 10 ^{2a}	
Lactobacillus fermentum	6.9 x 10 ³ ±1.7 x 10 ²	1.9 x 10 ⁴ ±1.4 x 10 ^{3b}	
Pediococcus sp.	7.8 x 10 ² ±1.6 x 10 ²	6.5 x 10 ³ ±2.2 x 10 ^{3b}	
Streptococcus sp.	1.0 x 10 ² ±1.4 x 10 ¹ a	2.6 x 10 ² ±0.0 x 10 ^{2b}	
Other lactic acid bacteria 5.1 x 10 ⁴ ±2.1 x 10 ⁴ a		9.4 x 10 ⁴ ±7.9 x 10 ^{3b}	

Means with the same superscript letter on the same line are not significantly different (p>0.05)

and phosphorous cycles. In the fermented fish, Ca (6.76±0.06%) and P (3.82±0.3%) contents were in accordance with the Ca and P values reported by Petenuci *et al.* (2008) in tilapia (*Oreochromis niloticus*) which are 2.7% et 1.1% respectively.

The counts of lactic acid bacteria on adjuevan samples showed a high lactic bacteria population (1.2 10⁵ ufc/g) similar to the values reported by Sanni et al. (2002) on momone. The genera and species of lactic acid bacteria isolated and identified from the adjeuvan samples were lactis. Lactobacillus Leuconostoc fermentum. Pediococcus sp. and Streptococcus sp. Their mean numbers were 5.7×10^3 , 1.9×10^4 , 6.5×10^3 , 2.6×10^2 cfu/g. The highest load was obtained with Lactobacillus fermentum, 9.4 10³ to 3.7 10⁴ cfu/g. The predominant lactic acid bacteria were Lactobacillus followed by Pediococcus. These genera of lactic bacteria were also isolated on the fresh fish used for adjuevan processing. The lactic acid bacteria load was higher in the adjuevan samples examined. No significant differences (p>0.05) were observed for Leuconostoc lactis between the type of fish analyzed. The implication of these bacteria in the production of fermented food products has been reported in previous work (Paludan-Muler et al., 2002; Kopermsub and Yunchalard, 2010). The isolation of these bacteria agreed with the findings of Sanni et al. (2002), who reported the isolation of Lactobacillus, Pediococcus among others from momoni, a Ghanaian fermented fish condiment. Kopermsub and Yunchalard (2010) reported in their study that plaa-som, a traditional fermented fish product from Thailand harboured similar organisms as found in this study. These authors concluded that a mixture of these LAB species could be considered as species for development of a starter culture for plaa-som fermentation. This finding is in accordance with the results found in this study, except Lactococcus which was not found. Surano and Hosono (1994) reported a presence of Lactobacillus. Pediococcus, etc. during the fermentation of terasi, a traditional Indonesian fermented fish and/or shrimp paste.

Conclusion: Adjuevan is a traditional fermented fish known, consumed and appreciated in Cote d'Ivoire as a flavour exhauster. The fermentation does not adversely affect the chemical composition of *Chloroscombrus chrysurus*. Therefore, *adjuevan* processed from

Chloroscombrus chrysurus species kept most of the nutritional content of the fresh fish. Lactic acid bacteria generally considered as a normal microflora of such products were present in all the samples and participate in the technological processing. These bacteria could contribute to keeping the quality of such products; inhibit some pathogens and participate to the fermented fish maturation. Fermented fish remains a good source of animal proteins.

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