

Microbiological Composition, Processing and Consumer's Characteristics of Adjuevan, a Traditional Ivorian Fermented Fish

Rose Koffi-Nevry¹ & Marina Koussémon^{1*}

Keywords: Adjuevan- Fermented fish- Microbiological composition- Processing- Ivory Coast

Summary

Adjuevan is a traditional Ivorian fermented fish product. This study which lasted 6 months was carried out on the unique collective processing site of adjuevan production in Abidjan, to determine the microbiological composition and the processors and processing characteristics of this product. Only non-native women (100%) between 30 and 60 years old (93.33%) processed adjuevan. Ninety eight percent of housework people know adjuevan and 96% consumed the fermented fish, but only 8% know the processing techniques, and 22.66% the processing site in the communities surveyed. Adjuevan is used for its flavour (73.33%). The mean pH value, the moisture and protein contents of adjuevan were 5.31%, 68.91% and 49.27% respectively. The total aerobic count, Enterobacteriaceae, coliforms, yeast-mould, and lactic acid bacteria were counted at the average of 8.0×10^5 , 5.27×10^2 , 10^2 , 34 and 10^4 cfu/g, respectively. A total of 38 isolates of Gram negative bacteria belonging to 7 genera were obtained from the adjuevan samples analyzed. *Enterobacter* sp. (9.72%) was the most predominant Enterobacteria. *Pseudomonas* sp. as found in 8.33% of the analyzed samples and *Klebsiella* sp. in 5.55%, *Pseudomonas fluorescens* and *E. coli* (4.16%), *Salmonella* sp. and *Serratia marcescens* (2.77%). *Salmonella arizonae* and *Proteus* sp. were less represented (1.38%). The unhygienic handling of the fish can affect the ultimate quality of the fermented fish. Consuming such contaminated products can represent a public health problem.

Résumé

Composition microbiologique, caractéristiques de la fabrication et de la consommation de l'adjuevan, un poisson fermenté traditionnel ivoirien

L'adjuevan est un poisson fermenté traditionnel de Côte d'Ivoire. Cette étude réalisée pendant 6 mois a porté sur l'unique site de production de l'adjuevan à Abidjan, sur la détermination de la flore bactérienne de ce produit ainsi que sur les producteurs et le processus de fabrication de l'adjuevan. L'étude a indiqué que 100% des producteurs d'adjuevan sont des femmes d'origine étrangère dont 93,33% sont âgées de 30 à 60 ans. Au total 98% des ménages enquêtés connaissent l'adjuevan, 96% consomment cette denrée mais seulement 8% des ménages connaissent le processus de fabrication de l'adjuevan et 22,66% connaissent le site de production de cette denrée. L'adjuevan est apprécié par 73,33% des consommateurs pour sa saveur. Les valeurs moyennes du pH, de l'humidité et des protéines de l'adjuevan sont respectivement de 5,31%; 68,91% et 49,27%. Les teneurs en flore totale aérobie, entérobactéries, coliformes, levures-moisissures et bactéries lactiques de l'adjuevan sont respectivement de $8,0 \times 10^5$; $5,27 \times 10^2$; 10^2 ; 34 et 10^4 ufc/g. Au total, 38 souches de bactéries Gram négatif appartenant à 7 genres ont été isolées des échantillons d'adjuevan analysés. Parmi les Entérobactéries, le genre *Enterobacter* (9,72%) est prédominant. *Pseudomonas* sp. est retrouvé dans 8,33% des échantillons analysés et *Klebsiella* sp. dans 5,55% de ces échantillons. Les espèces *Pseudomonas fluorescens* et *E. coli* (4,16%), *Salmonella* sp., *Serratia marcescens* (2,77%), *Salmonella arizonae* et *Proteus* sp. (1,38%) sont les moins représentées. Les manipulations non hygiéniques du poisson au cours de sa transformation en adjuevan peuvent affecter la qualité du produit final. La consommation d'un tel produit pourrait ainsi représenter un problème de santé publique.

Introduction

Animal proteins such as meat, meat products, fish and fishery products are generally regarded as high risk commodities with respect to pathogen contents,

natural toxins and other possible contaminants and adulterants (25). Fresh fish is a highly perishable product due to its biological composition. Thus, if this food is

¹University of Abobo-Adjame, Department of Food Science and Technology (UFR-STa), Laboratory of Biotechnology and Food Microbiology, 02 BP 801, Abidjan 02, Ivory Coast.

*Corresponding author: Marina Koussémon, Department of Food Science and Technology (UFR-STa), Laboratory of Biotechnology and Food Microbiology, 02 BP 801, Abidjan 02, Ivory Coast. E-mail: marinakoussemon@yahoo.fr

Received on 13.07.11 and accepted for publication on 24.11.11.

not immediately utilized or preserved after harvest, it spoils. The preservation of fish is usually accomplished through a combination of many preservation methods which greatly lengthen the storage conditions of this product. The principal methods are smoking, sun-drying, salting, fermentation, grilling and frying (10, 20).

Fermentation develops a distinctive flavour in the final product (3). In Africa, the popularity of fermented fish products has been influenced by the fish consumption pattern, which has been reported to be relatively higher in the coastal countries such as Ivory Coast, due to the proximity to the source of fish than in the interland (12). Traditional fermented fish are called *lanhouin* in Benin, *momoni* in Ghana and *adjuevan* in Ivory Coast. *Adjuevan* is a fermented fish condiment produced by using a high amount of salt leading to a final product highly concentrated in salt. This product is widely used and appreciated as a condiment in many types of flavourings and cuisines to season sauces for the consumption of yam, plantain, *attieke*, etc. (15, 16).

Improper handling, unhygienic conditions during the processing and the processing procedures can cause contamination of fermented fish. Among the microorganisms responsible for these contaminations are Gram negative Enterobacteria, some of which can represent a risk for the consumers and also a public health problem. It has been reported that Gram negative bacteria account for approximately 69% of the cases of bacterial food borne diseases (9). Members of the Gram negative bacteria e.g. *E. coli* and *Salmonella* are widely distributed in the environment and contaminated food and water are the major sources by which the bacteria are spread (9).

Despite the fact that *adjeuvan* is appreciated in Ivory Coast (17), there is no report in the literature on *adjeuvan* regarding the consumption patterns, the personal and environmental hygiene of the processing sites. Then, it became necessary to give useful information about the *adjeuvan* processing environment in Abidjan and the bacterial load of this product. This study therefore focuses on assessing the bacteriological level, isolating and identifying Gram negative Enterobacteria in *adjeuvan*, processor characteristics and the hygienic conditions during the process in order to highlight the public health risk and provide useful information where necessary to the general public and create awareness among the consumers.

Material and methods

1. Material

The study was carried out on the fermented fish called *adjeuvan* in Ivory Coast, obtained by vomer

(*Chloroscombrus chrysurus*) and collected from the unique collective fish fermentation processing site, Vridi Zimbabwue (Abidjan, Ivory Coast).

2. Methods

2.1. Survey

The survey population comprised mainly householders and *adjeuvan* processors. The questionnaire was divided into two sections. The first section solicited information on age, sex, level of education, country of origin and sanitary practices/hygiene of *adjeuvan* processors and the types of fish used. The second section obtained information on the knowledge and the consumption of *adjeuvan*, etc. The study which lasted 8 weeks was carried out in 4 randomly selected communes in the district of Abidjan (Cocody, Koumassi, Abobo and Yopougon). A total of 300 householders in Abidjan and 30 *adjeuvan* processors were interviewed.

2.2. Sampling procedures

The samples were obtained from 3 processors randomly selected from the processing site. Samples were taken at the end of the fermentation process which lasted 7 days, one day of a week but the same day of production from a basket containing the fermented fish. A sample consisted of 3 to 5 fermented fish (200 to 300 g) from the same batch. A total of 72 samples of *adjeuvan* were randomly and aseptically collected at a week interval and transported to the laboratory for analysis.

2.3. Chemical analysis

The following composition characteristics were determined from the samples taken from each processor. The moisture content was obtained through the difference between the fresh and the dry weight of each sample, dried at 105 ± 1 °C until constant weight (1). The proteins were determined through Kjeldahl method, and the fat by Soxhlet method (Unid Tecator, System HT2 1045, Sweden) (2). The pH of the 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.

2.4. Microbiological analysis

2.4.1. Culturing, enumeration, and isolation

To analyze the samples of *adjeuvan*, the methods stated in Compendium of Methods for the Examination of Foods (24) and Food and Drug Administration (FDA) (5) were used. The count performed were total aerobic mesophilic, coliforms, Enterobacteriaceae, lactic acid bacteria, Yeasts and mould, *Pseudomonas*.

2.4.2. Identification of Gram negative bacteria

Representative colonies of the Gram negative bacteria (coliforms and Enterobacteriaceae) were

picked up randomly, purified by repeated streaking on appropriate agar medium, and identified using the classical tests and by comparing their characteristics with those of known taxa, as described by Bergey's Manual for Determinative Bacteriology (8).

2.5. Statistical analysis of the data

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
Characteristics of *adjuevan* processors, type of fish and usage pattern of *adjuevan*

<i>Adjuevan</i> processors	Characteristics of respondents	Percentage
	Age (years)	
<30		06.66
30 – 60		93.33
> 60		00
Sex		
Male		00
Female		100
Level of educational		
No formal education		86.66
Primary school		13.33
Secondary school		0
Country of the processors		
Native		00
Non-native		100
Awareness of hygienic conditions		
none		90
poor		10
Hygienic practices		
none		100
Poor		00
Processors hygiene		
Poor		100
Types of fish used for <i>adjuevan</i> production	Ceinture, sardine, tuna, vomer, pelamida, flat sardine	
Consumers	Usage pattern	Percentage
<i>Adjuevan</i> knowledge		98
<i>Adjuevan</i> consumption pattern		96
Usage per week		
<2 days		14.33
2-3 days		59
3-5 days		26.66
Every day		0
Reason for <i>adjuevan</i> usage		
Flavour		73.33
Odour		26.33
Processing site knowledge		22.66
Process of production knowledge		8

Results

1. Survey

The results of the survey, presented in table 1, were divided into two sections; one reflecting the educational characteristics and types of fish used, and the other the pattern of *adjuevan* consumption. The survey revealed that the respondents were all women (100%) between the ages of 30 and 60 (93.33%). Only 6.66% were below the age of 30 and none of them was over 60 years old. About their education status, 68.66% of them attested not to have gotten any formal education, 13.33% said they attended primary school and none of them attended secondary education. All the women involved (100%) in the *adjuevan* processing are non-native of Ivory Coast. As far as hygiene is concerned, 90% of the respondents attested not to have gotten any formal education on hygiene. Since they were not familiar with hygiene, none of them observed hygienic practices during the *adjuevan* processing and handling. Therefore they could not observe personal hygiene as it could be seen on their clothing, their dirty hands and the surrounding environment. The types of fish most used for *adjuevan* production are: "ceinture", "flat sardine", "tuna", "vomer", "sardine" and "pelamida".

The household usage patterns indicated that 98% of the householders interviewed know *adjuevan*, 96% use or consume *adjuevan* but only 8% know the process of fabrication and 22.66% the processing site. Fifty nine percent of the householders said they use *adjuevan* in hot cooking with a frequency of 2 to 3 times a week, and 26.66% 3 to 5 times a week. Only 14.33% of them use the fermented fish less than twice a week and none of them use *adjuevan* everyday. It comes out of this survey that the majority (73.33%) of the householders selected for this study consumed *adjuevan* for its flavour and only 26.33 admitted using it for its odour. The householders surveyed also indicated that between the types of fish used for *adjuevan* production, tuna, vomer and sardine give *adjuevan* of best exhaustive quality.

2. Chemical analysis

The mean pH, the protein and moisture contents during the *adjuevan* processing are presented in table 2. The pH values of all the samples examined were below 7 with a mean value of 5.31. The moisture content was relatively high between 67.35 and 70.11% and the protein between 48.11 and 50.24%.

Table 2
Chemical characteristics of *adjuevan*

	Processor A	Processor B	Processor C	Mean value
pH	6.03 ± 0.4 ^a	5.11 ± 0.44 ^b	4.8 ± 0.41 ^b	5.31 ± 0.4 ^c
Protein %	50.24 ± 0.12 ^a	48.11 ± 0.88 ^b	49.48 ± 0.88 ^b	49.27 ± 0.55 ^d
Moisture %	70.11 ^a	69.27 ^a	67.35 ^b	68.91 ^b

In a line, values with the same letter are not significantly different.

3. Microbiological analysis

The load of microorganisms found in *adjuevan* and their frequency of isolation are summarized in table 3. The total aerobic count, Enterobacteriaceae, coliforms, Yeast-Mould, and lactic acid bacteria were counted at the average of 8.0×10^5 , 5.3×10^2 , 1×10^2 , 34 and 1×10^4 cfu/g, respectively.

Table 3
Bacterial load and frequency of occurrence of microorganisms isolated from *adjuevan*

Bacteria	Bacterial load (cfu/g)	Frequency (%)
Total aerobic count	$8.0 \times 10^5 \pm 7.5 \times 10^9$	100
Enterobacteria	$5.3 \times 10^2 \pm 58$	100
Coliforms	$1 \times 10^2 \pm 32$	16.66
Faecal coliforms	<15	1.66
Yeast and mould	34 ± 8	7.14
Lactic acid bacteria	$1 \times 10^4 \pm 2 \times 10^3$	100

Enterobacteria and lactic acid bacteria were found in all the samples analysed (100%). Coliform bacteria were isolated with a frequency of 16.66%, followed by yeast and mould, 7.14%. Table 4 shows the distribution pattern and the frequency and percentage incidence of Gram negative bacterial isolated from *adjuevan* produced in Abidjan. A total of 38 isolates of Gram negative bacteria belonging to 7 genera were obtained from the natural fermentation of *adjuevan*. Those isolates were identified as *Escherichia coli*, *Enterobacter* sp., *Proteus* sp., *Klebsiella* sp., *Serratia marcescens*, *Salmonella arizonae*, *Salmonella* sp., *Pseudomonas fluorescens*, *Pseudomonas* sp. by comparing their morphological and biochemical characteristics with the standard reference of the organisms. *Enterobacter* sp. 7 (9.72%) was the most predominant enterobacteria isolated. This was followed by *Pseudomonas* sp. 6 (8.33%) *Klebsiella* sp. 4 (5.55%), *Pseudomonas fluorescens* and *E. coli* 3 (4.16%), *Salmonella* sp. and *Serratia marcescens* 2 (2.77%). *Salmonella arizonae* and *Proteus* sp. (1.38%) were less represented.

Table 4
Frequency and Incidence of Gram Negative bacterial in *adjuevan*

Gram negative bacteria	Frequency	Number of isolates	Percentage
<i>Escherichia coli</i>	3/72	5	4.16
<i>Enterobacter</i> sp.	7/72	10	9.72
<i>Klebsiella</i> sp.	4/72	5	5.55
<i>Proteus</i> sp.	1/72	1	1.38
<i>S. marcescens</i>	2/72	2	2.77
<i>Pseudomonas fluorescens</i>	3/72	4	4.16
<i>Pseudomonas</i> sp.	6/72	7	8.33
<i>Salmonella arizonae</i>	1/72	1	1.38
<i>Salmonella</i> sp.	2/72	3	2.77
Total		38	

Discussion

The results obtained show that the fermented fish is known and accepted by the people of Ivory Coast. *Adjuevan*, a fermented fish produced in Ivory Coast for preservation and as food exhauster appears as a traditional product processed from large stocks of fresh fish. This justifies the location of the processing site along the coastal waters of the Gulf of Ivory Coast such as Abidjan. In Ivory Coast, men do not partake to the processing of fish fermentation because it is regarded as housework for women. The study shows that it is only the women who produce and commercialize *adjuevan*. This activity constitutes the main source of income for most of them. These women are between 30-60 years old and all of them are Ghanaan (100%). Similar results were obtained by Oulaï *et al.* (20) who reported the age of the smoked fish processors to be between 21 and 60 and between 21 and 40 by Baryeh *et al.* (7). However, our results disagree with those of these last authors who reported that 88, 8% of people working in the fish preservation domain are men against only 11.2% women. Eyo *et al.* (11) also stated that fish preservation is dominated by men in northern Nigeria. In Ivory Coast, smoking, sun-drying, salting, fermentation are mainly exercised by women. Men do not partake to those activities. They preserve the fish by freezing them. In Burundi, Sudan and Uganda, fish processing is performed by men (10).

Most of the fermented fish processors have no formal education so that they are not familiar with hygienic practices. This finding agrees with the work of Essuman (10) and Oulaï *et al.* (20). The lack of hygiene, probably related to their level of educational generally low, can explain their ignorance of the impact of poor sanitary conditions on the quality of their products (13). The Processors, the equipment, the water, the fish and the ingredients are of low hygienic conditions at the artisanal level of fish handling and processing. This may be attributed to a lack of education, facilities, standards, and economic support from the quality products' market. Salihu *et al.* (21) stated that the hygienic conditions are poor when foods are produced in non-industrial establishments. According to Anihouvi *et al.* (4), the surrounding environment in which fermented fish are processed in Africa is generally unhygienic, paving the way to a possible microbial contamination and evolution of food toxicants.

Salted water or fresh water fish can be used for *adjuevan* production depending on the season. According to the processors, the species of fish most used are "ceinture", "flat sardine", "tuna", "vomer", "sardine" and "pelamida" but tuna, vomer and sardine give *adjuevan* of best exhaustive quality according to customers. This argument confirms the one from Oetterer *et al.* (19) who reported that the fish used for fermentation are usually small, with a low commercial

value and seasonally abundant. Post-harvest handling, processing and marketing of fish are divided along the gender lines but the roles vary from one region to another. In the coastal countries of West Africa, women dominate the processing and marketing of fishery products. In Ivory Coast salting, fermentation, sun-drying and smoking are women's occupations. Fish processors and traders are not organized into formal associations or cooperatives. They operate individually or as family units and occasionally hire labourers to assist them in the operations.

The high moisture content of fermented fish found in this study agrees with the work of Asiedu and Sanni (6) who reported a value of 77.8% on naturally fermented Enam Ne-Setaakye, a west African fermented fish-carbohydrate product, but disagrees with the 50 to 56% moisture reported by Sanni *et al.* (22) on Monomi and Anihouvi *et al.* (4) on Lanhouin. The discord between these values could be due to the variable drying times, the amount and the types of salt used for the curing. The pH values for all the *adjuevan* samples analysed were below 7. Similar values of pH were reported on *monomi* (6.5), a fermented fish from Ghana (22) and *Pedah siam* a fermented fish processed in Thailand (12). However, the pH values obtained in this study disagree with those reported for *lanhouin*, a fermented fish from Benin where the pH values were above 7. The variations of the different fermented fish pH values could be explained by the duration of the fermentation process which determines the degree of lactic acid production. Protein values obtained for different samples agree with those reported by other authors (4, 22). The variations observed in protein levels could probably be due to the different species used, the amount of salt used and the duration of fermentation, which determine the degree of proteolytic activity during the processing.

This study reveals that *adjuevan* is contaminated with bacteria. One should pay attention to the presence of some of them (e.g. *Salmonella*, *E. coli*) because that indicates a public health hazard and sends a warning signal for the possible occurrence of a food borne intoxication as also indicated by Sanni *et al.* (22). A total of 38 isolates comprising 7 different genera of Gram negative bacteria were isolated in this study with an incidence rate below 50%. The study shows that all the processors contributed equally to the microbial diversity reported in this study. Microorganisms isolated from *adjuevan* samples in this study have

been earlier found in fermented fish (22), foods, environment and other places, and their pattern is similar to previous reports (9, 18, 22)

The findings of this study agree with the reports from Asiedu and Sanni (6) where they isolated almost similar organisms from a fermented fish named *Enam ne-Setaakye*. In our study, *Salmonella arizonae*, a serovar of *Salmonella enterica*, has been identified. *Salmonella arizonae* is often found in the intestine of cold blooded animals like the lizard, the gecko and the frog (14). Its presence in the fermented fish could be linked to the large number of these animals on the processing site due to the high level of insalubrity since the fermented fish is dried on the soil where these animals can crawl over the fish. During the drying process, the fermented fish are also exposed to insect infestation and microorganisms contamination. The presence of these Enterobacteria is related to a post contamination. Species of *Pseudomonas* are also isolated. This genus is often found in foods and is known to be a strong agent of food spoilage (20). The results of this microbiological analysis could be explained by the poor sanitary conditions during the process and at the selling places. The processors can contaminate the fermented fish from the mouth, the nose or the skin by talking, coughing or sneezing over the fish, or handling it with dirty hands or skin. This unhygienic handling of the fish can affect the ultimate quality of freshly fermented products. Our results are similar to previous reports (23) indicating that the lack of hygiene is the major source of food contamination.

Conclusion

It comes out of this survey that the majority of the householders selected for this study consume *adjuevan* for its flavour. The *adjuevan* processors do not have any formal education on hygiene and they do not apply hygienic practices to the *adjuevan* processing and handling. Such conditions can contribute to the high level of contamination of the fermented fish. The samples were contaminated by Gram negative bacteria. The presence of some of them such as *Salmonella* spp., *E. coli*, *Klebsiella*, and *Pseudomonas* represents a health risk associated to the consumption of contaminated fermented fish. The unhygienic handling of the fish can affect the ultimate quality of the fermented fish. To reduce the health risk associated to the consumption of contaminated fermented fish, safer processing and hygienic practices are required.

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Rose Koffi-Nevry, Ivorian, Scientist Professor of Microbiology (Maître de Conférences), University of Abobo-Adjame, Department of Food Science and Technology (UFR-STA), Laboratory of Biotechnology and Food Microbiology, 02 BP 801, Abidjan 02, Ivory Coast.
E-mail: rosenevry2002@yahoo.fr

Marina Koussémon, Ivorian, Scientist Professor of Microbiology (Maître de Conférences), University of Abobo-Adjame, Department of Food Science and Technology (UFR-STA), Laboratory of Biotechnology and Food Microbiology, 02 BP 801, Abidjan 02, Ivory Coast.
E-mail: marinakoussemon@yahoo.fr