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(RESEARCH ARTICLE)



## Salt concentration effect on yeast diversity of the Ivorian traditional fermented fish adjuévan

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### Abstract

This study aimed to investigate the yeast diversity in adjuévan fermented at the laboratory scale according to salt concentration following both traditional fermentation methods. Thus, the fish species *Galeoides decadactylus* was fermented with salt added at 10 %, 15 %, 20 %, 25 % and 30 % (w/w) for five days. Yeast identification using PCR-DDGE method revealed seven species which were *Pichia fermentans*, *Candida zeylanoides*, *Candida sp*, *Hanseniaspora osmophila*, *Kluyveromyces sp*; *Torulaspota delbrueckii* and *Kluyveromyces marxianus*. These species varied according to fermentation method used and salt concentration added with *Pichia fermentans* and *Hanseniaspora osmophila* as dominant strains. These results showed also that *Kluyveromyces marxianus* and *Torulaspota delbrueckii* were more tolerant to sodium chloride than the others. This work confirmed that yeasts were involved and participated in adjuévan production. Therefore, these yeasts should be tested for their functionality during the fermentation, and some might be useful as starter culture to produce better quality fermented fish adjuévan.

**Keywords:** Adjuévan; Fermented fish; Yeasts; PCR-DGGE; Salt

### 1. Introduction

In most tropical countries, traditional processes such as drying, salting, smoking, and fermentation are used for fresh fish preservation. Ivorian fermented fish adjuévan, like various traditional fermented fish products in the world, was produced by spontaneous fermentation and used as flavoring in foods. Adjuévan is a salted fermented fish product mostly produced according to two different traditional methods at ambient temperature (28–30 °C) by Ivorian women in the center and south of the country. In the method 1, the entire fish was allowed to deteriorate in air for 48 h, eviscerated, washed, and dry salted at around 35 % (w/w). Thereafter, the salted fish was allowed to ferment for 5 days and sun dried for 4 days at ambient temperature. For method 2, the entire fish was allowed to deteriorate in air for 24 h, gutted, filleted along both sides, washed, and dry salted at 20~35 % (w/w). Fermentation was carried out outside at room temperature, simultaneously with sun-drying on racks or nets, for at least 7 days [1]. These methods lead to visually two different end-products. Previous studies that have evaluated the microbial communities in adjuévan found the dominant species to belong to the genera *Bacillus* and *Staphylococcus* with the bacteria community varied according to the method used [2]. On the other hand, by using culture-dependent methods, Koffi-Nevry et al. [3] showed that *Lactobacillus fermentum* was the dominant lactic acid bacteria species while the report of Kouakou et al. [4-5] using PCR-DGGE showed *Lactobacillus fermentum*, *Leuconostoc lactis* subsp *lactis*, *Pediococcus pentosaceus* for method 1 and

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*Pediococcus pentosaceus*, *Lactococcus garviae*, *Streptococcus difficilis* for method 2 as the dominant lactic acid bacteria species.

Yeasts are one of the predominant groups among the microbial population throughout most of the fermentation process in several food and beverage production. Yeasts are ubiquitous microorganisms, which can grow in various environments where organic substrates are available and capable of growing and multiplying on complex substrates with high amounts of sugar, salt, low pH, low temperature and low water activity (*aw*) [6]. Previous studies that have investigated the yeast communities in adjuevan samples collected from traditional producers in the south of Côte d'Ivoire found height species belonging to several genera with variation depending to the fermentation method. These yeasts could be advantageous for adjuevan flavor like some lactic acid bacteria [7]. In fact, different yeast strains have been reported to influence the development of the flavour characteristic of dry-cured meat products, Martin et al. [8]. However, the presence of yeasts on food products may also considered as an indication of food spoilage [9]. Then yeasts may cause yeasty or bitter taste, change in colour and texture of products and may deform or blow up packaging materials and could also be an underestimated source of infections and adverse health responses [10]. Thus, yeasts found in adjuevan could be from contamination during the production by material of production, salt or others [7]. The dual role as beneficial or spoilage organisms have attracted the interests of this study to understand their significance for adjuevan product. Furthermore, the percentage of salt added during adjuevan fermentation varied according to the producer.

The purpose of this study was to investigate the yeast diversity on adjuevan produced in laboratory according to different salt concentrations following both fermentation methods.

## 2. Material and methods

### 2.1. Adjuevan production

Fresh fish (*Galeoides decadactylus*) was bought from two traditional producers of adjuevan at Vridi-port. Four samples of 4 kg of fresh fish each, was taken per traditional producer and then divided into four different sterile containers of 1 kg each. Then samples of fresh fish were taken to the laboratory for adjuevan production according to the two main traditional fermentation processing methods used by local producers as described by Kouakou et al. [1] and Montet et al. [2]. This adjuevan production was done using different concentrations of salt (w/w): 10 %, 15 %, 20 %, 25 % and 30 %. Samples were collected just after five days of fermentation for analysis. Fermentation experiments were conducted in triplicate.

### 2.2. Chemical analysis

The following physicochemical analyses were performed: pH, *Aw*, salt content and titratable acidity. Sodium content was determined by a chloride analyser (Corning Chloride analyser 926) after cold extraction in 0.3 N nitric acid [11]. Water activity was determined using a water activity meter (AQUA LAB 4TE Decagon Devices fashion). pH and titratable acidity were determined by an automatic titrimeter (TitroLine easy Schott 96) on 5 g of fermented fish dissolved in 50 ml distilled water after homogenization during 30 min, then titrated by 0.05 N NaOH.

### 2.3. Enumeration of microorganisms

For all determinations, 10 g of adjuevan sample was added aseptically to 90 ml of sterile diluent containing 0.1 % peptone (Oxoid, Basingstoke, UK) and 0.85 % NaCl (Merck, Darmstadt, Germany), with the pH adjusted to 7.2, and homogenized in a stomacher (lab blender; Seward Medical, London, UK) for 30 s at normal speed. Lactic acid bacteria (LAB) and Micrococcaceae were enumerated using Man Rogosa and Sharpe agar (MRS ; bioMerieux, Marcy l'Etoile, France) and mannitol salt agar (MSA ; Oxoid), respectively. Plates were incubated at 30 °C for 48 h. Yeast enumeration was done using malt extract agar (MEA) (Oxoid) containing 0.1 % oxytetracycline (Sigma-Aldrich Co., USA). The MEA plates were incubated at 25°C for 4 days. After the incubation, a differential count based on colony color and aspect was made.

### 2.4. Statistical analysis

Results were statistically evaluated by one-way analysis of variance (ANOVA) and Bonferroni test (Dunn) with the software Statistica, 99 Edition. Statistical differences with  $p < 0.05$  were considered significant.

## 2.5. Molecular analysis

### 2.5.1. DNA extraction from yeast

DNA extraction from yeast was done according to El Sheikha et al. [12]. About 10 g of fermented fish samples taken from skin, abdomen and head were homogenized by vortex for 30 min (Vortex Genie 2 SI-A256, USA) after addition of 16 ml of sterile peptone water (pH 7.0, Dickinson, France). Two Eppendorf tubes of 2 ml containing the homogenate were vortexed for 10 min and centrifuged at 12 000 g for 15 min, then the supernatant was discarded. The cell pellet was resuspended in 300 µl of breaking buffer [2 % Triton X-100 (Prolabo, France), 1 % SDS (sodium dodecyl sulphate; Sigma, France), 100 mM NaCl (Sigma), 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0 (Promega, France)]. A volume of 100 µl TE [10 mM Tris-HCl; 1 mM EDTA; pH 8.0 (Promega)], 100 µl lysozyme solution (25 mg/ml, Eurobio, France), and 100 µl proteinase K solution (20 mg/ml, Eurobio, France) were added and the mixture was incubated at 42 °C for 20 min. Then 50 µl of 20 % SDS were added to each tube, and then incubated at 42 °C for 10 min. 400 µl MATAB (mixed alkyltrimethyl ammonium bromide; Sigma) were added to each tube, and then incubated at 65 °C for 10 min. The lysates were then purified twice by repeated extraction with 700 µl phenol/chloroform/isoamyl alcohol (25/24/1; Carlo Erba, France), and the tubes were vortexed for 1 min and centrifuged at 12 000 × g for 15 min. The aqueous layer was transferred to an Eppendorf vial and the residual phenol was removed by extraction with 600 µl chloroform/isoamyl alcohol (24/1), and centrifuged for 15 min at 12000 × g. The aqueous phase was collected and the DNA was stabilized with 30 µl sodium acetate (3 M, pH 5), followed by precipitation by adding an equal volume of ice-cold isopropanol, and stored at -20 °C for 12 h (overnight). After centrifugation at 12000 × g for 15 min, the supernatant was eliminated, DNA pellets were washed with 500 µl 70 % ethanol, and the tubes were centrifuged at 12000 × g for 15 min. The ethanol was then discarded and the pellets were air-dried at room temperature for 3 h. Finally, the DNA was resuspended in 50 µl of ultrapure water and stored at -20 °C until analysis.

### 2.5.2. PCR-Denaturing Gradient Gel Electrophoresis (DGGE) analysis

A fragment of the D1/D2 region of the 26S rRNA gene was amplified using eukaryotic universal primers NL1GC and LS2 amplifying a 250 bp fragment [13- 12]. A 30 bp GC clamp (Sigma) was added to the forward primer. PCR amplification was performed in a final volume of 50 µl according to the protocols described by kouakou et al. [7]. Aliquots (5 µl) of PCR products were analysed first by conventional electrophoresis in 2 % w/v agarose gel with 1 × TAE buffer (40 mM Tris-HCl, pH 7.4, 20 mM sodium acetate, 1.0 mM Na-EDTA), stained with ethidium bromide (Promega) 50 µg/ml in 1× TAE and quantified by using a standard (100 bp DNA ladder; Promega). PCR products were analysed by DGGE, by using a Bio-Rad code universal mutation detection system (Bio-Rad, USA), following the procedure described by Leesing [14]. Samples containing approximately equal amounts of PCR amplicons were loaded into 8% (w/v) polyacrylamide gels in 1× TAE buffer (40 mM Tris-HCl, pH 7.4, 20 mM sodium acetate, 1.0 mM Na-EDTA). All electrophoresis experiments were performed at 60°C, using a denaturing gradient in the range of 30 to 60 % (100 % corresponded to 7 M urea and 40 % v/v formamide; Promega). The gels were electrophoresed at 20 V for 10 min and then at 80 V for 12 h. After electrophoresis, the gels were stained for 30 min with ethidium bromide and rinsed for 10 min in distilled water and then photographed on a UV transilluminator with the Gel Smart 7.3 system (Clara Vision, Les Ulis, France).

### 2.5.3. Purification and sequencing

Bands were cut from the gel and the amplicons were purified using the Wizard PCR purification kit (Preps DNA Purification System, Promega, France) followed by a new PCR but with primers without GC clamp. The PCR products were then sent at GATC Biotech (Germany) where they were submitted to a second DNA purification before sequencing. Sequence of 26S rDNA comparisons were performed using the basic local alignment search tool (BLAST) in GenBank ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)) after editing and trimming the sequences by BioEdit sequence alignment editor, version 7.0.0. The yeast isolates were identified based on a 99-100 % similarity criterion.

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## 3. Results and discussion

### 3.1. Chemical composition

Chemical characteristics of adjuvean samples were ranged in Table1. The salt content in adjuvean samples was proportional to the salt concentration used for fermentation and the method of fermentation. The salt contents of adjuvean produced with 10 %, 15 % and 20 % of salt (w/w) were between 2.89 % and 3.71 % and those obtained with 25 % and 30 % were around 6 %. Concerning the samples obtained from the second method, the salt contents observed were between 3.15 % and 4.29 % in the samples produced with the rates of 10 % and 15 % of salt (w/w) and from 5.64 to 7.36 % in the samples with 20% and 30% of salt. Titratable acidity was high in samples with 10% and 15 % at 13.81 mg/100 g and 11.17 mg/100 g, respectively for samples from method 1 and method 2. This content decreased from

25% to 10.913 mg/100 g of salt in sample of method 1 and at 09.01 mg/100 g in samples of method 2 from 20 %. The highest values of water activity (Aw) were recorded on the samples obtained from 10 % and 15 % of salt varied for 0.91 - 0.85 to decrease up to 0.76 at 30 % of salt and 0.87- 0.84 to decrease up to 0.73 at 30 % of salt respectively in samples from method 1 and method 2. Significant difference ( $p < 0.05$ ) was observed between these values. In contrast, the pH values showed a similar trend among samples, with the pH ranging from 5.54 to 6.29 (method 1) and from 6.07 to 6.62 (method 2).

**Table 1** Physicochemical compounds variation according salt percentage added in adjuvian after five days of fermentation.

Salt added (% w/w)	AW	pH	TA (mg/100g)	SC (% Nacl)
	Samples of	Method1	(n=12)	
pf (10)	0.91±0.12 <sup>a</sup>	5.54±0.04 <sup>a</sup>	13.09±0.15	2.89±0.61 <sup>a</sup>
pf (15)	0.85±1.02	5.73±0.16	13.81±0.81 <sup>a</sup>	3.20±0.44
pf (20)	0.81±0.75	6.16±0.40 <sup>b</sup>	11.23±0.57	3.71±0.19
pf (25)	0.77±0.41 <sup>b</sup>	6.42±0.22	10.91±0.71 <sup>b</sup>	5.15±0.26 <sup>b</sup>
pf (30)	0.76±1.13 <sup>b</sup>	6.29±0.06	11.52±0.24	5.94±0.47 <sup>b</sup>
	Samples of	Method2	(n=12)	
pf'' (10)	0.87±0.34 <sup>a</sup>	6.07±0.14 <sup>a</sup>	11.17±0.13 <sup>a</sup>	3.15±0.29 <sup>a</sup>
pf'' (15)	0.81±0.91	6.46±0.51 <sup>a</sup>	10.02±0.26 <sup>b</sup>	4.29±0.83
pf'' (20)	0.74±0.46 <sup>b</sup>	6.50±0.18 <sup>a</sup>	09.01±0.43 <sup>b</sup>	5.64±0.50 <sup>b</sup>
pf'' (25)	0.73±0.62 <sup>b</sup>	6.58±0.20 <sup>a</sup>	09.15±0.90	6.87±0.72 <sup>b</sup>
pf'' (30)	0.73±0.35	6.62±0.37 <sup>a</sup>	09.41±0.11	7.36±0.14 <sup>c</sup>

Means in the same row having different superscripts are significantly different ( $P < 0.05$ ), and values in the same row with same superscript are not significantly different ( $P > 0.05$ ) according each method. SC: Salt content, TA: Titratable acidity; Aw: water activity; pf: adjuvian from method1; pf'': adjuvian from method2

### 3.2. Microbiological content of adjuvian

The microbiological status of samples is summarized in Table 2. Variation in the load of microorganisms with predominance of micrococci was observed on all bacteria regardless of the fermentation method and the percentage of salt added. Strong growth of micrococci was observed from the beginning (first day) to the end (five day) of fermentation in samples from the both methods regardless the percentage of salt added.

**Table 2** Mean load of the bacterial and yeast communities determined in the adjuvian according to the two methods of fermentation.

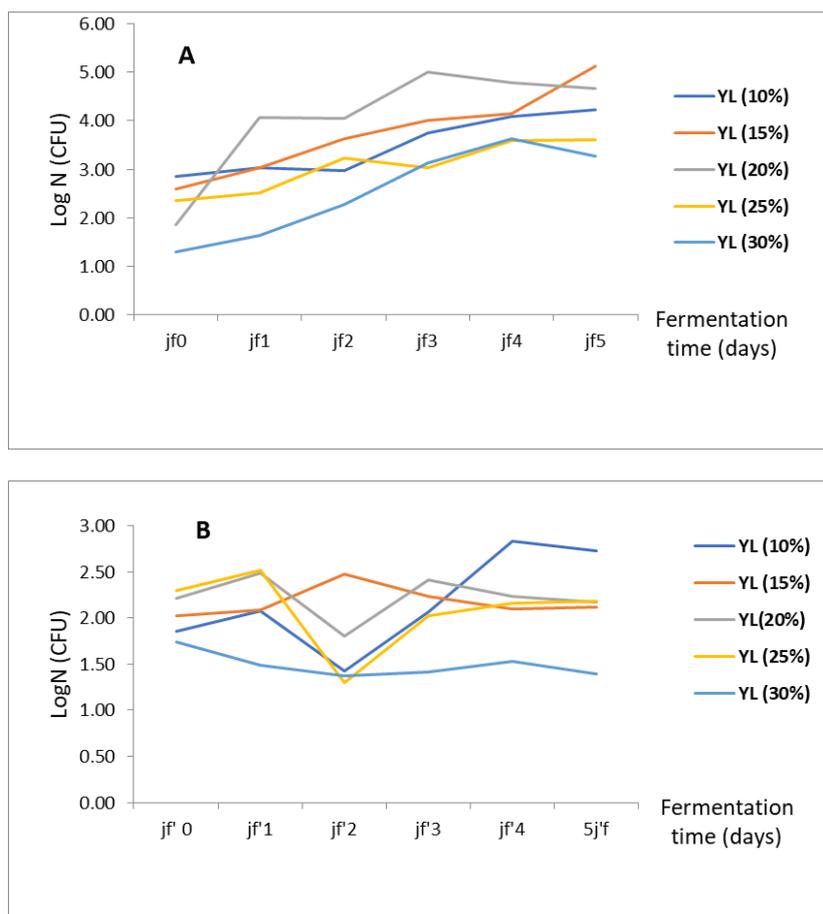
Salt added (% w/w)	LAB	yeasts	Micrococci
	Samples of	Method 1	(n =12)
pf (10)	3.91±0.02 10 <sup>5a</sup>	3.02±0.12 10 <sup>1b</sup>	6.02±0.03 10 <sup>4b</sup>
pf (15)	5.44 ±0.11 10 <sup>5a</sup>	5.12±0.22 10 <sup>2a</sup>	4.28±0.01 10 <sup>6</sup>
pf (20)	7.41±0.31 10 <sup>4</sup>	3.6 ±0.04 10 <sup>1</sup>	7.61±0.02 10 <sup>6a</sup>
pf (25)	6.13 ±0.01 10 <sup>4</sup>	4.67±0.02 10 <sup>1</sup>	3.51 ±0.06 10 <sup>5</sup>
pf (30)	5.14±0.23 10 <sup>3b</sup>	4.08 ±0.05 10 <sup>2</sup>	5.09±0.15 10 <sup>5</sup>
	Samples of	Method 2	(n =12)
pf'' (10)	5.35 ±0.04 10 <sup>4a</sup>	2.52±0.05 10 <sup>2</sup>	4.58±0.33 10 <sup>6a</sup>
pf'' (15)	3.95±0.01 10 <sup>2b</sup>	4.71±1.03 10 <sup>1</sup>	5.62±0.0110 <sup>5</sup>
pf'' (20)	6.98 ±0.24 10 <sup>2</sup>	3.57±0.04 10 <sup>2a</sup>	6.93±0.03 10 <sup>5</sup>
pf'' (25)	5.93±0.30 10 <sup>3</sup>	4.64 ±0.13 10 <sup>1</sup>	4.46±0.20 10 <sup>6</sup>
pf'' (30)	4.91±0.21 10 <sup>2</sup>	2.43±0.07 10 <sup>1b</sup>	2.89±1.01 10 <sup>4b</sup>

Means in the same row having different superscripts are significantly different ( $P < 0.05$ ), and values in the same row with same superscript are not significantly different ( $P > 0.05$ ) according each method.

pf : adjuvian from method1. ; pf' : adjuvian from method2 ; LAB : lactic acid bacteria

The lactic acid bacteria (LAB) load increased at 15 % of salt from  $5.44 \pm 0.11 \cdot 10^5 \log$  (CFU / g) to decrease to  $5.14 \cdot 10^3 \log$  (CFU / g) at 30 % of salt and  $5.35 \pm 0.04 \cdot 10^4 \log$  (CFU / g) in sample with 10 % of salt to decrease to  $4.91 \pm 0.21 \cdot 10^2 \log$  (CFU / g) at 30 % of salt, respectively in samples of method 1 and method 2. The loads of lactic acid bacteria decreased after 15 % of salt and 10 % of salt respectively in the samples of method 1 and method 2. The highest values of Lactic acid bacteria (LAB) loads were recorded on the samples from method 1 obtained with 10% and 15 % of salt. However, yeasts loads were very little with the higher of  $5.12 \pm 0.22 \cdot 10^2 \log$  (CFU / g) in samples with 10 % of salt and  $3.57 \pm 0.04 \cdot 10^2 \log$  (CFU / g) 15 % of salt, respectively following method 1 and method 2.

Population growth dynamics of the yeast during the fermentations were monitored from first days to the five days (Fig. 1A; 1B). Results indicated that, according the method 1, generally, a lag phase was observed until to three days or four days according to samples with 10 % to 20 % of salt. But concerning samples of the second method, yeast loads decreased at the second day of fermentation then increased with an exponential growth in sample fermented with 10 % of salt (w/w). The variation in yeast populations during fermentation was more influenced by the methods used than by the salt concentrations added.

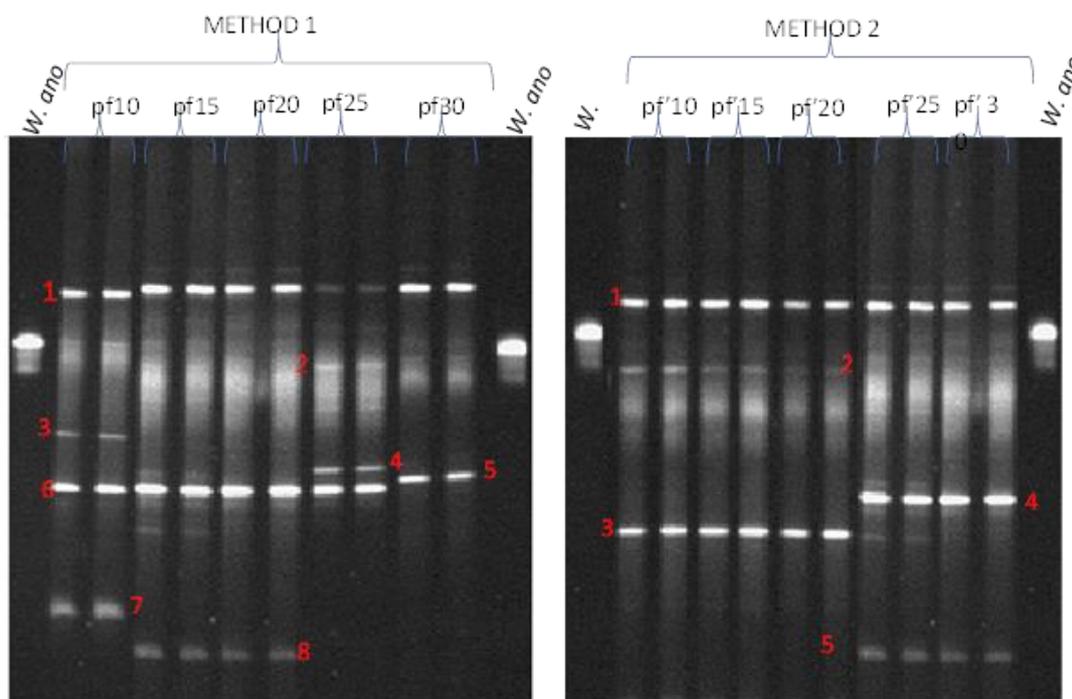


**Figure 1** Yeasts evolution during adjuvan fermentation according salt concentration added

A=Yeasts growth during adjuvan fermentation for method 1; B=Yeasts growth during adjuvan fermentation for method; YL: yeast load; %: salt concentration added for fermentation; jf: fermentation following method1; jf': fermentation day following method2

#### 4. DGGE profiles of the yeast community

The PCR-DGGE profiles of the fermented fish adjuvan revealed the presence of 5 to 8 DNA bands from yeasts (Fig. 2) following method used and percentage of salt added for the fermentation. So, 8 bands were present in the samples of method 1 and 5 bands in the samples of method 2. In sample from method 1, on the bases of these yeast profiles, three sample groups could be distinguished: (i) samples produced with 10 % and 25 % salt which gave two more different bands; (ii) those with 15 % and 20 % salt gave the same profile with one more band and (iii) samples of 30 % of salt with only 2 bands.



**Figure 2** DGGE profiles of the yeasts community from DNA directly extracted from *adjuvant* produced with two traditional processing methods at laboratory scale.

Bands indicated by numbers were excised and, after re-amplification, subjected to sequencing. Yeasts diversity were: For Method: 1- *Pichia fermentans*; 2. *Candida zeylanoides*; 3. *Candida sp*; 4. *Kluyveromyces marxianus*; 5. *Kluyveromyces sp*; 6. *Hanseniaspora osmophila*  
For Method2 : 1. *Pichia fermentans* ; 2. *Candida zeylanoides* ; 3. *Hanseniaspora osmophila* ; 4. *Torulaspora delbrueckii*.

However, DGGE profile of the samples of method 2 showed five bands in general which could be grouped in two groups according to salt concentration. The first group composed of samples of 10 %, 15 % and 20 % salt showed the same profile of three bands while the second group, those of 25 % and 30 % of salt, showed identical profile of three bands. These yeasts bands on the DGGE profile were sequenced and the species were identified (Table 3). According to samples from method 1, six closely species were found: *Pichia fermentans*, *Candida zeylanoides*, *Candida sp*, *Hanseniaspora osmophila*, *Kluyveromyces sp* and *Kluyveromyces marxianus*. On the bases of these yeast species profiles, four samples groups following salt concentration could be distinguished : (i) samples of 10 % of salt showed three species which were *Pichia fermentans*, *Hanseniaspora osmophila* and *Candida sp* ; (ii) samples of 15 % and 20 % of salt with showed only two species: *Pichia fermentans* and *Hanseniaspora osmophila* ; (iii) samples of 25 % of salt with three species : *Pichia fermentans*, *Hanseniaspora osmophila* and *Kluyveromyces marxianus* ; (iiii) samples of 30 % of salt with three species : *Pichia fermentans* and *Kluyveromyces sp*. Concerning samples of method 2, four species were identified: *Pichia fermentans*, *Candida zeylanoides*, *Hanseniaspora osmophila* and *Torulaspora delbrueckii*. Only two sample groups following salt concentration, on the bases of these yeast species profiles, could be distinguished: (i) samples of 10 %, 15 % and 20 % of salt showed three species named *Pichia fermentans*, *Candida zeylanoides* and *Hanseniaspora osmophila* and (ii) samples of 25 % and 30 % of salt with *Pichia fermentans* and *Torulaspora delbrueckii*. The most frequently identified species from adjuvant samples of the both methods used, without salt added were *Pichia fermentans* and *Hanseniaspora osmophila*. An exception to these grouping, among species, difference was made between the both methods again. Then two genera such as *Candida sp* and *Kluyveromyces sp* were observed only in samples for method 1 contrary to method 2 which was characterized by the presence of one specie (*Torulaspora delbrueckii*) seen exceptionally in samples of 25 % and 30 % of salt.

**Table 3** Identity of bands obtained from the denaturing gradient gel electrophoresis analysis of adjuvane yeasts communities.

Closest relative	Identity %	Source	Band number	
			Method 1	Method2
<i>Pichia fermentans</i>	100	EU019222	1	1
<i>Candida zeylanoides</i>	98	AF374610	2	2
<i>Torulaspora delbrueckii</i>	99	AB436465	X	4
<i>Candida sp</i>	97	EU651854	3	X
<i>Hanseniaspora osmophila</i>	100	HQ149312	6	3
<i>Kluyveromyces marxianus</i>	99	DQ139802	4	X
<i>Kluyveromyces sp</i>	98	EU019220	5	X

X: not detected

## 5. Discussion

Chemical compounds varied following salt concentration added during fermentation. The pH and salt content were low with highest water activity and titratable acidity in all samples of 10 % and 15 % salt. The salt concentration increase induces the increase of pH around 6 but a decrease of water activity especially in sample from method 2. This is favored by the large contact surface between the salt and the flesh of the fish by the fact that the fish were open. Our results are in agreement with those of Barat *et al.* [15] who showed that the larger the surface of contact with salt, the greater the penetration with a significant effect on the driving forces in the system (activity of the water, osmotic pressure and salt gradient).

More attention was given to understand the dynamic of fish salting [16]. These authors showed the relationships between salt diffusivity and fish fillet compound under various conditions. NaCl values measured in this study varied following salt concentration and fermentation method used which is in accordance with those reported by Anihouvi *et al.* [17]. Regarding the analysis of microbial flora, this study showed in general a dominance of micrococci, high presence of lactic acid bacteria and especially lowest count of yeasts in fermented fish samples. LAB counts were ranged between  $10^3$ - $10^5$  Log UFC/g according method 1 and  $10^2$ - $10^4$  Log UFC/g for method 2. The dominance of the micrococci in the present study may indicate that the fermentation was proteolytic and lipolytic. Such results have also been reported by Achinewhu *et al.* [18] and Anihouvi *et al.* [19] in the case of fermented sardines from Nigeria and lanhouin from Benin. In contrast of LAB, yeast enumeration showed a low load in samples generally around  $10^1$ - $10^2$  Log UFC/g regardless the method used. This result has been reported in momoni [20] and the plaa-som [21]. In this study, these lowest loads of yeasts varied exclusively according to the fermentation method used. This result can also be explained by the fact that yeasts are capable of growing and multiplying on complex substrates with high amounts of sugar, salt, low pH, low temperature and low water activity [6].

Identification of yeasts revealed seven (7) species as *Pichia fermentans*, *Candida zeylanoides*, *Candida sp*, *Hanseniaspora osmophila*, *Kluyveromyces sp*, *Kluyveromyces marxianus* and *Torulaspora delbrueckii*. The yeast diversity found using both traditional fermentation methods in adjuvane was similar. Therefore, the use of both approaches is considered more comprehensive for a full description of the yeast populations in these products. These yeasts were present on adjuvane produced at the laboratory scale as well as those produced by traditional producers. Kouakou *et al.* [7] have reported, having identified the species such as *Pichia fermentans*, *Hanseniaspora osmophila* and *Kluyveromyces marxianus* on the adjuvane collected with traditional producers in the south of Côte d'Ivoire. It showed that these species could come from the marine origin of the fresh fish but not from contamination linked to fermentation materials used or the environment as reported by Sanni *et al.* [20] and Kouakou *et al.* [7]. There could therefore contribute positively to fermentation of the adjuvane such as the development of the characteristic flavour. *Pichia fermentans* and *Hanseniaspora osmophila* were found dominant strains in all samples regardless salt added, then the same yeast species have been found important in other adjuvane samples from traditional producers by Kouakou *et al.* [7]; which suggests a distinct contribution of the species to the fermentation of adjuvane. These yeasts growth may play either a beneficial role, to generate arrays of volatile and non-volatile metabolites [22], so positive contribution for the development of sensory qualities of adjuvane. Their metabolites can affect the chemosensory properties of the adjuvane products, important to consumers. Yeasts on the surface of adjuvane could also be used as probiotics and serve as bio-

control agents against pathogenic microorganisms like in the case of dry-cured meat products and cheeses according to Alvarez-Martin et al. [23]; Jacques and Casaregola [9]; Asefa et al. [24]. *Candida zeylanoides* was found in adjuevan from the both methods. This specie has been found dominate from the Italian and spanish dry-cured meat products during fermentation [25-10]. However, Nielsen et al. [26] and Asefa et al. [24] have taxed this specie to be an emerging pathogen. So, it could have a pathogenic potential in adjuevan, dangerous for consumers. Moreover, *Torulaspora delbrueckii* which had never be described previously in adjuevan and *Kluyveromyces marxianus* have been found only in adjuevan produced at 25-30% salt with method 2 and method 1, respectively. These yeast species frequently associated with meat products spoilage [27] could participate to adjuevan spoilage. The selection in this study can be linked not only to the origin of the fresh fish but these species could be more resistant to the high salt content, influenced to the fermentation method condition.

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## 6. Conclusion

This study is the first to describe yeasts associated with adjuevan according to salt concentration added for fermentation by employing both traditional methods. Seven species were identified, varied following fermentation method used and salt concentration added with *Pichia fermentans* and *Hanseniaspora osmophila* as dominants species. So, the yeast diversity found here is similar to as those identified in adjuevan samples collected with traditional producers. The results also showed that *Kluyveromyces marxianus* and *Torulaspora delbrueckii* were more tolerant to salt than the others. This work confirmed that yeasts were involved in adjuevan production co-existing with LAB. These yeasts should be tested for their functionality during the fermentation, and some might be useful as starter culture to produce better quality fermented fish adjuevan.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interests regarding the publication of this paper.

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