



**Full Length Article**

# Effect of Controlled Lactic Acid Bacteria Fermentation on the Microbiological and Chemical Quality of Moroccan Sardines (*Sardina pilchardus*)

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## ABSTRACT

Lactic acid bacteria strains (LAB) were assayed for the conservation of fresh sardine "*Sardina pilchardus*". *Lactobacillus delbrueckii subsp. delbrueckii* was used of sardine fillets at NaCl (5% w/w) and glucose (4% w/w) concentration. Microbiological counts including standard plate count (SPC), lactic acid bacteria (LAB), yeasts, coliforms, *Salmonella*, staphylococci and *Clostridium* were followed during two weeks of storage at 30°C. Determination of chemical parameters including pH, dry matter, fat matter, ash, total nitrogen (NT), total volatile basic nitrogen (TVB-N) and trimethylamine (TMA-N) was carried out under the same conditions. Chemical determinations showed a net pH decrease from an initial value of 6.08 in raw sardine fillets to 4.3 after 25 days of fermentation. Microbiological control showed that LAB counts increased during fermentation process. After two weeks, fermented fish was free of coliforms. No growth of *Staphylococcus aureus*, *Salmonella spp.* and sulfite-reducing *Clostridia*. The inhibition of spoilage microflora including standard plate count and yeasts was also observed. The results indicated that controlled LAB fermentation could be used as a successful process for biopreservation of sardines produced in huge quantities.

**Key Words:** Fermentation; Fish; Lactic acid bacteria; *Sardina pilchardus*; Quality control

## INTRODUCTION

Fresh fish is an extremely perishable food compared to other food commodities. The hygienic quality of fish and fishery products decline rapidly due to microbial cross contaminations from various sources leading ultimately to spoilage (Gram & Huss, 1996). Microbiological, biochemical and sensory methods have been used to assess freshness and fish quality during handling and storage, with the main attributes of freshness being aroma, taste, texture and appearance response (Koutsoumanis *et al.*, 2002). Biochemical methods based on nucleotide metabolism, production of trimethylamine (TMA), hypoxanthine (Hx), total volatile basic nitrogen (TVB-N) and biogenic amine, have also been commonly used to assess the fish quality (Hasegawa, 1987). The development of new fish products, which are stable during storage, free of the undesirable odour and taste and retaining all the nutritional advantages of fish, would expand the range of applications of health-giving, fish-based foods as fish sauce and silage fermentation. A promising approach to the creation of such fish products seems to be through the use of lactic acid

bacteria (LAB). These bacteria have long been used for changing the aromatic and textural properties of food and for extending the shelf-life of various products such as milk, meat, poultry, fruits, vegetables and cereals (De Roissart & Luquet, 1994). However, LAB have not been used to any great extent in fish products (Jeppesen & Huss, 1993), with the exception of fish sauces in South East Asia (Paludan-Muller *et al.*, 1999) and silage fermentation (Van Wyk & Heydenrych, 1985). Antimicrobial activity of LAB against pathogens and spoilage bacteria has been reviewed by Gambas (1989) and Lindgren and Dobrogosz (1990). Metabolic by-products of LAB have been shown to increase product shelf-life (Abee *et al.*, 1995).

The production of lactic and acetic acids and resulting pH decrease are considered responsible for inhibition of pathogens. Lactic acid bacteria have been found to be the dominant microorganisms in many fermented fish products (Ostergaard *et al.*, 1998). The primary role of LAB is to ferment available carbohydrates and causing a decrease in pH. The combination of low pH and organic acids (mainly lactic acid) is the main preservation factor in fermented fish products. In addition, salt and spices (such as garlic, pepper

or ginger) may be added to improve the organoleptic characteristics and to ensure products safety. Also, in some products garlic may serve as a carbohydrate source for the fermentation (Paludan-Muller *et al.*, 1999).

Previous studies of some food products offer the best choices as starter cultures for those same products, because they would be more competitive than LAB from other sources (Jeppesen & Huss, 1993; Vignola *et al.*, 1993). The initial quality of raw materials, in terms of their freshness, microbiological loads and physical damage, is an important factor influencing the quality of the final product (Fuselli *et al.*, 2003). LAB-fermented food products may also be healthy, because of probiotic effects. In recent years, great interest has been devoted to the use of LAB and their metabolic products as potential probiotics in aquaculture (Ringo & Gatesoupe, 1998; Gatesoupe, 1999).

The aim of the present study was to evaluate the microbiological and chemical qualities of fermented minced fish of the sardine "*Sardina pilchardus*" breed with LAB strains isolated from lightly fermented fish and to determine the potential of LAB used as starters and biopreservative cultures in different fish based products.

## MATERIALS AND METHODS

**Isolation of lactic acid bacteria strains.** A 5 kg of fresh sardine were purchased from the local market in Rabat (Morocco) and transported to the laboratory using a refrigerated box (4°C) and characterized immediately microbiologically and chemically. LAB strains were isolated from samples of raw fish sardine and from lightly fermented fish (from natural fermented fish, brined sardine & minced sardines with sucrose & salt) by homogenizing a 10\_g sub-sample of each sample for 2 Min in 90 mL of saline water (0.85% NaCl w/v) and plating appropriate serial dilutions into MRS agar (De Man, Rogosa & Sharpe). Triplicate plates were incubated at 30°C for 48 h. The isolates were purified on MRS agar plates at 30°C and freeze-dried at -20°C in MRS broth (Difco, USA) containing glycerol 20% v/v.

**In vitro antibacterial activity.** LAB strains isolated from sardines were selected on the basis of their inhibitory activities on various Gram-positive and Gram-negative bacteria to select the most efficient strain for use in fish preservation. The antimicrobial activity of the isolated LAB strains was tested on *Escherichia coli* (ATCC 25921), *Pseudomonas aeruginosa* (one strains), *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (1 strain), by the well-diffusion assay (Pidcock, 1990). All tested strains were from collection of Department of Food Engineering and Technology, IAV Hassan II, Rabat. For the detection of antibacterial activity of the LAB strains, 10 mL of MRS broth was inoculated with each LAB strains using an inoculum rate of 1% and were incubated at 30°C for 48 h. After incubation, a cell-free solution from MRS broth culture was obtained by centrifugation at 6000 x g for 10

min, followed by filtration of the supernatant, through a Millipore membrane filter (0.45 µm). The pathogenic test bacteria were incubated in brain heart infusion (BHI) broth (Difco, USA) at appropriate temperature for 24 h. Petri dishes with 20 mL of Mueller Hinton agar were prepared, previously inoculated with 0.1 mL of a 24 h broth culture of bacteria tested. Four wells of 6 mm of diameter were punched after solidification of Mueller Hinton agar and 100 µL of cell-free filtrate were added to the well. After initial incubation for 2 h at 4°C, plates were finally incubated for another 24 h at 37°C. The inhibitory activity of the culture filtrate against pathogenic bacteria was determined by measuring the clear zone around the wells.

**LAB selection and characterization.** One the most efficient LAB strains were chosen for this study. The selection of suitable strains was based on inhibition and fermentation proprieties. The isolate was initially tested for colony morphology phase, using phase contrast microscopy, motility, Gram staining and catalase reaction (with 10% H<sub>2</sub>O<sub>2</sub>). Gas production from glucose was determined in MRS-broth supplemented with 1% glucose containing inverted Durham tubes at 30°C for 48 h. Arginine dihydrolase was determined in MRS broth supplemented with 0.3% arginine, which was incubated for 3 days (Jeppesen & Huss, 1993). In addition, the LAB isolate was tested for the ability to grow in MRS-broth containing 2, 5, 7, 10 and 15% NaCl (w/v) at 30°C for 3 days. The ability of LAB strain to grow at different pH (2, 3, 4 & 6.5) and temperature values (15, 37, 40 & 45°C) was also checked using MRS broth. Carbohydrate fermentation profiles of the selected isolate were investigated using API 50 CH strips (API system, Bio-Merieux, France).

**Inoculation of minced fish with LAB strain.** Amounts of 6 kg of fresh sardine were purchased from the local market in Rabat (Morocco) and transported to the laboratory using a refrigerated box (4°C) and were immediately characterized microbiologically and chemically. Samples of flesh sardines were cleaned, sliced and minced and divided into two batches (3 kg each) and prepared for fermentation. The pH was measured before inoculation.

The selected strain identified as *Lactobacillus delbrueckii subsp. delbrueckii* was grown on MRS for 24 h at 30°C. From this culture, dilutions up to 10<sup>6</sup> were plated on MRS to determine the cell concentration. The amount used to inoculate was approximately around 10<sup>6</sup> to 10<sup>7</sup> cfu g<sup>-1</sup>, with the initial count made 2 h after inoculation (initial). Inoculated minced fish fillet was thoroughly mixed, packed in plastic bags and left to ferment for 2 weeks at 30°C. The fermented fish product contain a carbohydrate source (glucose: 2 to 4% w/w) is assumed to be the substrate for fermentation by LAB, supplemented with salt (NaCl: 3 to 5% w/w). Uninoculated portion served as control (minced fish with added salt). Samples were drawn for analysis at predetermined intervals with pH measurements, chemical and microbiological analyses.

**Chemical determinations.** To determine the pH of

samples, 5 G of sample were homogenized in 10 mL of purified water for 1 min in a stomacher and the pH measured with a glass electrode. Dry-matter content was determined by drying a 10 G of sample homogenate at 102 - 105°C for 12 - 14 h until a constant weight was obtained. Water content was calculated from the dry matter content. Appropriate weights were analyzed for protein (total N\_x 6.25), moisture, fat and ash according to AOAC procedures (AOAC, 1990). Total volatile basic nitrogen (TVB-N, mg N 100 g<sup>-1</sup> fish) was determined in a distillatory system (UDK 130A), as described by Antonacopoulos and Vyncke (1989). Trimethylamine (TMA, mg N 100 g<sup>-1</sup> fish) was determined by FAO (1986). Thiobarbituric acid (TBA, mg malonaldehyde kg<sup>-1</sup>) assay, as in index for lipid oxidation, was carried out according to the procedure of Schmedes and Holmer (1989). TBA value expressed as mg malonaldehyde (MA) kg<sup>-1</sup> of fish sample. All analyses were done in triplicate.

**Microbiological determinations.** Ten (10) G of sample were thoroughly homogenized using 90 mL of saline water (NaCl, 0.85% w/v). From the 10<sup>-1</sup> dilution, other decimal dilutions up to 10<sup>-7</sup> were prepared. Appropriate media were used for enumeration and identification of microflora. LAB and standard plate count (SPC) were determined respectively by plating appropriate dilutions on MRS-agar and on plate count agar (Difco, USA). Plates were incubated for 48 h at 30°C. Staphylococci were determined on Mannitol Salt Agar (Merck, Germany). Plates were incubated at 37°C for 24 h. Yellow colonies on the medium were checked for Gram and catalase reactions. Coliforms were determined using Mac Conkey agar (Biokar France), the plates were incubated for 24 h at 37°C for total coliforms and at 44°C for fecal coliforms. *Salmonella* was determined in the raw material and in the final product by transferring 25 g portions into 250 mL flasks containing 100 mL of peptone water. The flasks were incubated at 37°C for 18 h and then 1 mL of the culture was transferred to selenite broth (Merk, Germany) and tetrathionate broth (Merk, Germany). Positive tubes were isolated using Shigella-Salmonella agar (SS) and Hektoen agar (Merck, Germany). Non-colored colonies with and without a dark center were purified and streaked on trypticase soya agar (Biokar, France) slants and stored at 4°C. Yeasts, determined by spread-plating on Potato Dextrose Agar (PDA, Difco) were incubated for 48 h at 30°C. Spores-forming bacteria (*Clostridium sp.*) were enumerated in the raw material and in the final product on sulphite polymyxin sulfadiazine (SPS). The initial dilution was heat-activated at 80°C for 10 min and immediately cooled in iced water plates were incubated at 37°C for 24 h. Microbiological data were transformed into logarithms to assess the number of colony forming units (cfu g<sup>-1</sup>). All experiments were conducted in triplicate.

**Statistical analysis.** Statistical analysis of microbiological and chemical determinations was carried out using Microsoft Excel 7.0 and SAS 6.12 Programmes (SAS

Institute Inc, 1990). Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test the differences between means at 5% significance level.

## RESULTS

**Characterization of LAB strain.** The strain isolated from fermented fish was characterized as rod, Gram-positive, nonsporing, nonmotile and showed negative catalase reaction. It was identified as *Lactobacillus delbrueckii subsp. delbrueckii* using API 50 CHL identification kit (Biomérieux, France) This strain does not produce CO<sub>2</sub> from glucose after incubation at 30°C for 48 h and produces an arginine dihydrolase. *Lactobacillus delbrueckii subsp. Delbrueckii* can grow at variable temperatures from 30°C to 45°C, but not at 15°C and can tolerate salt concentrations up to 7% (NaCl, w/v) and it is resistant to low pH values from 2 to 6.5.

**Antibacterial activity.** The strain of *L. delbrueckii subsp. Delbrueckii* used was tested for its antibacterial activity against variable strains of Gram-positive and Gram-negative bacteria. Results showed that all used pathogenic strains were sensitive to the LAB strain with diameters of inhibition zones (mm).

**Chemical analyses.** Chemical composition of raw material and fermented fish showed variations during the fermentation process and the raw material for moisture, proteins fat matter and ash contents (Table II). There were changes of moisture, protein, fat and ash, TBA (thiobarbituric acid) and titratable acidity contents of raw material and fermented sardine. The data, moisture, protein, fat and ash contents of final fermented fish samples were also significantly ( $p < 0.05$ ) different from the initial values. Titratable acidity content reached  $2.37 \pm 0.24\%$  of final product. TBA is a good indicator of the quality of the fish. TBA value is a widely used indicator for the assessment of degree of lipid oxidation. It has been proposed that a maximum TBA value, indicating the good quality of the fish, is 5 mg MA kg<sup>-1</sup>, while fish may be consumed TBA up to a TBA value of 8 mg MA kg<sup>-1</sup> (Schormiiller, 1969). TBA value of raw sardine was 1.01 mg MA kg<sup>-1</sup>. In the fermentation process, value increases significantly ( $p < 0.05$ ) in the initial TBA value of 3.87 mg MA kg<sup>-1</sup>. The results indicated that oxidative rancidity in fermented sardine; suggest that TBA values are within the quality limits after 25 days of processing.

The chemical changes including pH, TBVN and TMA of fermented fish by LAB and control indicates that pH of inoculated fish has decreased from  $6.08 \pm 0.20$  to  $4.30 \pm 0.05$ , while for control assay has it increased from  $6.20 \pm 0.11$  to  $7.16 \pm 0.06$ . Both TVB-N and TMA are the traditional chemical means most widely used for evaluation of the degree of spoilage in seafood. The initial TVB-N in raw sardine was  $11.25 \pm 1.05$  mg N 100<sup>-1</sup> G fish and changed to  $36.31 \pm 0.83$  mg N 100<sup>-1</sup> G fish for fermented fish after 3 weeks of storage. During storage, a slight

**Table I. Inhibition of same pathogens by *L. delbrueckii* subsp. *Delbrueckii* supernatant at pH 4.4**

|             | Pathogens                   |                      |                               |                  |
|-------------|-----------------------------|----------------------|-------------------------------|------------------|
|             | <i>E. coli</i> (ATCC 25921) | <i>P. aeruginosa</i> | <i>S. aureus</i> (ATCC 25923) | <i>B. cereus</i> |
| Lactic acid | -                           | -                    | -                             | -                |
| LAB strain  | 20.60* ± 0.41               | 15.93* ± 0.97        | 9.10* ± 0.13                  | 12.13* ± 0.16    |

- : No inhibition detected. LAB strain: *L. delbrueckii* subsp. *delbrueckii*, All tests were performed in triplicate.

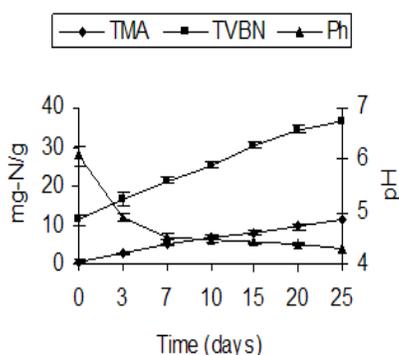
\* Inhibition zone shown in mm (mean ± SD)

**Table II. Changes in chemical composition (% on a wet weight basis), TBA and Titratable acidity content of raw material and fermented sardine for final product of storage**

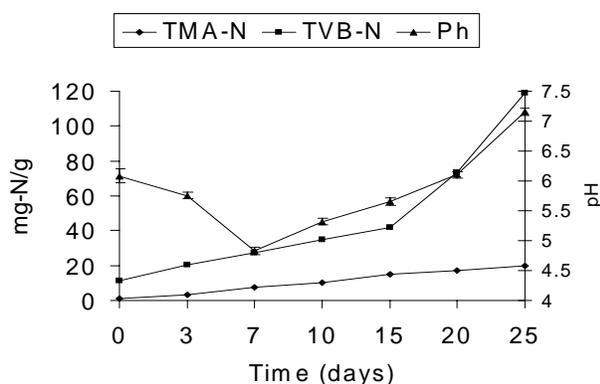
| Samples       | Moisture(%)   | Protein (%)  | Fat (%)     | Ash (%)     | TBA (mg MA kg <sup>-1</sup> ) | TA (%)      |
|---------------|---------------|--------------|-------------|-------------|-------------------------------|-------------|
| Raw material  | 71.92* ± 1.47 | 14.91 ± 0.22 | 3.70 ± 0.14 | 1.02 ± 0.05 | 1.01 ± 0.03                   | 0.42 ± 0.10 |
| Final product | 65.55 ± 0.89  | 16.20 ± 0.23 | 4.54 ± 0.10 | 3.94 ± 0.35 | 4.02 ± 0.12                   | 2.37 ± 0.24 |

\* Means ± SD (number of replicate = 3)

**Fig. 1. Evolution of pH, total volatile basic nitrogen (TVBN) and trimethylamine (TMA) in fermented fish (n=3, mean ± SD)**



**Fig. 2. Evolution of pH, total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) during storage of control assay (n=3, mean ± SD)**



increase in the TVB-N value was noticed at the end of experiment. Although the fermented sardine almost exceeded the permissible level of 35 mg TVB-N 100<sup>-1</sup> G fish flesh specified by the EC guidelines (Commission Decision 95/149/EC, 1995). However, for the control samples, TVB-N increased gradually to reach a concentration of 118.74 ± 1.15 mg N 100<sup>-1</sup> G fish and exceeded largely the maximal permissible level.

Fresh fish naturally contain trimethylamine oxide (TMAO). TMAO is a tasteless non-protein nitrogen

compound, which has an osmoregulating function and its content varies with the fish species, environment season, size and age of fish (Huss, 1995; Ozogul *et al.*, 2006). In seafood spoilage, TMA particularly contributes to the characteristic ammonia-like off-odour and “fishy” off-flavours (Gram & Huss, 1996). The initial TMA level in raw sardine was 0.81 ± 0.23 mg-N 100<sup>-1</sup> G fish. After the fermentation process, the initial TMA value increased slowly until 11.26 ± 0.78 mg-N 100<sup>-1</sup> G fish in inoculated assay. For control assay, TMA value increased significantly (p < 0.05) and exceeded the level of 19.56 ± 0.42 mg-N 100<sup>-1</sup> G fish at the end of storage. A TMA value of 5 - 10 sample was reported as the limit for the acceptability of fish (Sikorski *et al.*, 1990). In this study, a TMA value of 11.26 ± 0.78 mg-N 100<sup>-1</sup> G fish coincided with the onset of spoilage (day 25) for the inoculated samples, while control samples exceeded widely.

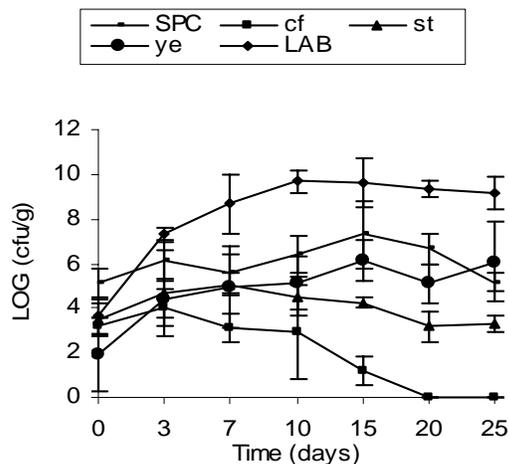
**Microbiological analyses.** Results of the evolution of different microbial groups during the fermentation are reported in Fig. 3. As shown for fermented fish, standard plate counts (SPC) and yeasts grew and reached high number (1.8 10<sup>6</sup> cfu g<sup>-1</sup>). Total coliforms reached 10<sup>4</sup> cfu g<sup>-1</sup> after 3 days before falling to less than 1.0 cfu g<sup>-1</sup> in the fermented product and faecal coliforms were not detected. Presumptive Staphylococci decreased and reached less than 10<sup>3</sup> cfu g<sup>-1</sup> at the end of the fermentation process; while in the control assay (Fig. 4), LAB numbers reached 2.3 10<sup>2</sup> cfu g<sup>-1</sup> at the end of storage, the yeasts flora increased progressively to reach 1.6 10<sup>7</sup> cfu g<sup>-1</sup> after 2 weeks.

## DISCUSSION

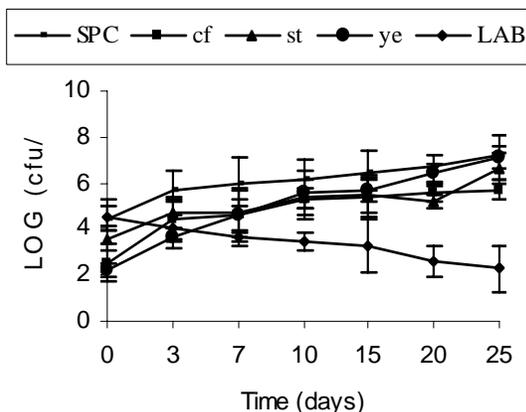
The inoculated and control samples revealed significant (P < 0.05) differences in TVB-N and TMA values. Gelman *et al.* (2001) obtained qualitative changes of fermented fish inoculated with *Lactobacillus plantarum*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* and stored at 10°C during 51 days. The amount of TMA found in fish was used as an index of spoilage. Generally, in fresh fish, the TMA-N value is about 1 mg N 100<sup>-1</sup> G fish (FAO, 1986). The increase in TVB-N is generally caused by autolytic enzymes and desamination and is not related to microbiological activity. Such increases in TVB-N can be

**Fig. 3. Microbial profiles of fermented fish (n = 3, mean ± SD)**

SPC: standard plate count; cf: coliforms; st: staphylococci; ye: yeasts; LAB: lactic acid bacteria

**Fig. 4. Microbial profiles of non inoculated control assay (n=3, mean ± SD)**

SPC: standard plate count; cf: coliforms; st: staphylococci; ye: yeasts; LAB: lactic acid bacteria



explained easily by the volatile basis production ( $\text{NH}_3$ , TMA, DMA, hypoxanthine) and non-volatiles (histamine) and those compensatory of free fatty acids resulting from lipids deterioration (Shenouda, 1980). Trimethylamine oxide (TMAO) is part of the non-protein-nitrogen (NPN) fraction and its presence in all marine species is well-established (Hebard *et al.*, 1982). The spoilage of fresh fish is influenced by the presence of TMAO, particularly under anoxic conditions. A number of well-defined spoilage bacteria (*Shewanella putrefaciens*, *Photobacterium phosphoreum*, Vibrionaceae) are able to utilize TMAO as the terminal electron acceptor in an anaerobic respiration resulting in off-odours and flavours due to formation of TMA (Dalgaard *et al.*, 1993).

Generally, it is difficult to establish the limits of acceptability especially for the TVB-N, because of the big variability between species and regions, particularly for fat fish (Civera *et al.*, 1993). However, Ababouch *et al.* (1996)

proposed values that ranged from 25 to 35 mg N  $100^{-1}$  G for Moroccan sardines. El Marrakchi *et al.* (1990) reported that the TVB-N value was more useful for assessing the degree of sardine deterioration than for evaluating the changes occurring during the first storage stages. The TVB-N value is affected by fish species, age and sex; the catching season and the region of fishing.

The pH decrease in the final product provides evidence of good acidification through lactic acid fermentation through starter culture. This decrease in pH in the inoculated fish was closely related to the production of lactic acid by *L. delbrueckii subsp. Delbrueckii* during the fermentation process, although the suppression of growth of putrefactive and food poisoning bacteria was mainly due to the fall in pH during lactic acid fermentation. Recent studies show that various inhibitory substances (i.e., organic acids, diacetyl, bacteriocins, hydrogen peroxide) generated from lactic acid bacteria would also suppress the growth of these putrefactive bacteria (Callewaert & De Vuyst, 2000). The use of lactic acid bacteria in the preservation of organic materials is based not only on the production of lactic acid, but also on the production of bacteriocins (proteins of low molecular weight), which show a wide range of antibacterial effects on Gram-positive cells in putrefactive bacteria, such as *Clostridium* and *Staphylococcus* (Wang *et al.*, 2001).

The LAB strain used for the inoculation of sardine would constitute a suitable starter culture for the fermentation. The high population of LAB found at the end of the fermentation pointed out the suitable conditions for growth of the culture and also the efficiency of the used strain.

The safety of fermented product primarily depends on rapid decrease in pH. Adams *et al.* (1987), using a minced fish-salt-glucose model, suggested that a pH of 4.5 should be reached within the first 48 h of fermentation. The most important factor in controlling the fermentation is to decrease pH (Faid *et al.*, 1997), which must be achieved as quickly as possible in order to inhibit the growth of spoilage microorganisms in the final product. Gelman *et al.* (2001) obtained best organoleptic and chemical results from inoculated fish-based food products using *Leuconostoc mesenteroides*, supplemented with salt, sugar, spices and chemical preservatives, when starter cultures reached  $10^9$  cfu  $\text{g}^{-1}$  at the end of fermentation.

Microbiological examination of fermented fish products showed that LAB predominated (Fig. 3 & 4). Preservation of fermented fish products obviously depends on lactic acid and possibly on bacteriocin production. However, other factors may also contribute to the overall keeping of the quality of low-salt fermented fish products. These products are often produced using freshwater fish, where salt addition may have a significant inhibitory effect on mesophilic spoilage flora (Gram, 1991).

Yeasts counts were also determined during the fermentation period and profiles showed a net increase (around  $10^6$  cfu  $\text{g}^{-1}$  in the fermented product). Growth of the

two populations of yeasts and LAB was without antagonism and in good logarithmic phases (Fig. 3), which indicated suitable conditions for growth during fermentation. It should be emphasized here that low pH values encourage yeasts growth in the medium. A high yeasts count in the final product may not be problem. Alcohol production can take place and carbohydrates, such as sucrose are reduced during fermentation. Faid *et al.* (1994) demonstrated that fish silage in a controlled fermentation by LAB can be improved by yeasts addition for a combined alcohol/lactate fermentation. The phenomenon by which yeasts can encourage LAB is still being studied using LAB including dairy products and vegetables. The elimination indicator microorganisms such as coliforms in the fermented product at the end of fermentation process could be due to the acidification and/or to some inhibitory compounds formed by LAB. S P C and Staphylococci gradually decreased to  $10^5$  and less than  $10$  cfu  $g^{-1}$ , respectively at the end for inoculated fish. Coliforms showed a net decrease during fermentation to reach a minimum of  $<10$  cfu  $g^{-1}$  after 12 days. The reduction of coliforms number may ensure a good biopreservation against undesirable and/or hazardous microorganisms. This could be due to the acidification by lactic acid formed by the LAB. The population of Staphylococci was found in high counts in stored fresh fish, their presence is not unexpected, because of their resistance to salting. Counts of Staphylococci showed a net decrease to reach a minimum of  $10$  cfu  $g^{-1}$ . The suppression of growth *Clostridium* is very important, because these bacteria produce organic acids with offensive odours such as butyric acid (Wang *et al.*, 2001). *Salmonella* was not isolated in the final product and this may give evidence about the efficiency of the process in inhibiting hazardous microorganisms.

## CONCLUSION

Biotechnological process used here may be very convenient process for biological conservation of the huge amounts of Sardines produced in different food units in Morocco. Occurrence of fermentation by pure culture of LAB could be involved in both preservation and improvement of the organoleptic quality of the final product. This process would provide a safe product because of the inhibition of pathogenic microorganisms, the transformation of some compounds and the production of aroma in fermented fish.

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