Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Ghomari & al. / Mor. J. Chem. 8 N°2 (2020) 510-526

PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF NATURAL FERMENTING OLIVE BRINES.

Ghomari Imane [(a,*)], Elassri Soufiane[(a)], Rokni Yahya[(a)], Chihib Nour-Eddine[(b)], Ben Salah Riadh[(c)], Asehraou[(a)] Abdeslam.

[(a)]*Laboratory of Biochemistry and Biotechnology, Faculty of Sciences, Mohammed Premier University, B.P.717, Oujda, Morocco.*
[(b)]*INRA-UMR UMET 8207-Equipe PIHM, CNRS-INRA, université de Lille, 369 rue jules Guesde, BP 20039, 59651 ville neuve d'Ascq Cedex, France.*
[(c)]*Laboratory of Microorganisms and Biomolecules (LMB), Centre of Biotechnology of Sfax, University of Sfax, Road of Sidi Mansour Km 6, P.O. Box 1177, Sfax 3018, Tunisia*

* *Corresponding author: imaneghomari@gmail.com*
*Received 10 Feb 2020, Revised 28 Feb 2020, Accepted 02 A*pril 2020.

Abstract

The main objective of this work is to study the characterization of physico-chemical and microbiological of fermenting green olive brines at different times of fermentation. The technological and probiotic properties of lactic acid bacteria strains isolated from brines were also characterized, 24 brine samples were sampled from Triffa conserves entreprise, an industrial zone of Oujda city in eastern Morocco, during 2014-2015 and 2015-2016 campaigns, 240 isolates of lactic acid bacteria have been isolated, and they have undergone a series of tests to finally select the so-called probiotic bacteria. 41 strains were resistant to low pH (3, 2.5, 2), bile salts (0.3 and 1%, w/v) and 7 strains among the 41 were resistant to saline stress (8%, w/v) and endowed with acidification capacity and have a good enzymatic activity and a broad spectrum of antibacterial activity against pathogenic and non-pathogenic bacteria such as (*Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua*, *Pseudomonas aeruginosa* and *Micrococcus luteus*). These results show the ability of these strains to dominate and grow in the digestive tract.These potential probiotic strains will be used as concentrated ferments in the fermentation and bioconservation of foods.

Keywords: *Green olives, Lactic acid bacteria, Fermentation, Ferment.*

1. Introduction

Table olive production in Morocco was estimated about 120 000 Tones during 2015-2016 campaign (IOOC, 2018). Almost 88 000 Tones are exported mainly to European, American and Arab markets. The fermented green olive is the main type produced and exported in Morocco. The Spanish style is the main method used worldwide and in Morocco for the industrial preparation of Fermented green olive [1]. It is based on the alkali-treatment of the olive with sodium hydroxide (2-2.5%, w/v) to eliminate oleuropein, the main phenolic glucoside responsible for its bitterness. The olive is then washed in tap water and then brined at 10-12% (w/v) of Sodium Chloride and led to undergo a natural fermentation process for 5 to 12 months. During the brining, the olives undergo a slowly conducted lactic fermentation which ends up being rather important at the expense of the availability of fermentable substrates, salt content, pH, temperature, aeration and the presence of spoilage microorganisms [2], [3]. Mastery of the processes occurring during this development phase is fundamental to improve the preparation, storage and safety of the finished product. Various groups of microorganisms are involved in both the fermentation of table olives and their conditioning, determining the quality and flavor of the finished product. This heterogeneous flora is generally brought by the fruit, the brining water or by the fermentation equipment [4]. The lactic fermentation of green table olives occurs spontaneously by the natural microflora associated with olive fruits and containers, without addition of initial culture. In general, the fermentation process is carried out by lactic acid bacteria (LAB) and/or yeasts. LABS are highly desired for the success of the fermentation process. They are the main microorganisms involved in the acidification of brine by the production of lactic acid from fermentable substrates, resulting in lower pH and thus providing microbiological stability to the fermented olive, and therefore a successful of the fermentation process.

Thus, the strains of LAB involved in this process should display a wide biochemical activity, including production of enzymes, osmotic regulators and antimicrobials [5], [6], to dominate in this environment and to assure a success full lactic fermentation process of green olive. Furthermore, LAB strains encompassing probiotic properties, should lead to the production of fermented olives with probiotic properties. The main objective of this work is to characterize the microbiological and biochemical properties of natural fermenting industrial green olive brines, and the enzymatic properties of the LAB strains isolated from the olive brines.

2. Materials and methods

2.1. Sampling

Samples of natural fermenting Picholine green olive brines, fermented according to the Spanish style, were randomly taken at the Triffa Enterprise Oujda Morocco, during the 2014-2015 and 2015-2016 campaigns. These samples, were harvested at different times of brining in aseptic conditions, and transported at 4°C to the laboratory for physico-chemical and microbiological analyses. Samples were coded from B1 to B12 for those collected in 2014-2015; while those collected during 2015-2016 were coded from B13 to B24.

2.2. Physico-chemical analyses of brines

PH

The pH was measured using the pH meter type Symphony SB70P, calibrated with pH4 and pH7 buffer solutions. Three independent measurements were performed for each brine sample.

Titratable acidity

The titratable acidity was determined on a volume of 10 ml of brine sample using NaOH (0.1N) in the presence of phenolphthalein as indicator. This assay is based on the shift from colorless phenolphthalein to persistent pink. The

titratable acidity obtained is expressed in g/l of lactic acid. Three independent measurements were performed for each brine sample.

Chlorides

The chlorides contents were determined using the Mohr method. Chlorides, expressed as a percentage (%), were determined by silver nitrate ($AgNO_3$) 0.1N in the presence of Potassium Chromate (K_2CrO_4) 0.5% as indicator. The value is taken after the indicator turns from yellow to red. Three independent measurements were performed for each brine sample.

2.3. Microbiological analyses of brines

The brine samples were diluted successively to 1/10 in sterile physiological water, and seeding was carried out on the surface of 0.1 ml of each dilution. Enterobacteria were counted on the Mac Conkey Agar medium (BIOKAR, France) after 48 hours of incubation at 30°C. Bacillus spores were counted after heating brines to 70°C for 15 minutes and seeded on the Plate Count Agar (PCA) medium (BIOKAR, France). The incubation was carried out at 30°C for two days. Staphylococci were counted on the Chapman medium (BIOKAR, France) after incubation at 30°C for 48 hours. The leuconostocs were counted on MSE (MAYEUX, SANDINE and ELLIKER) agar medium (BIOKAR, France), and incubated at 30°C for 24 hours. Lactic acid bacteria were counted on De Man Rogosa and Sharpe medium (MRS) Agar (BIOKAR, France). After 3 days of incubation at 30°C, the colonies presumed to be lactic acid bacteria were confirmed by Gram staining and catalase was testing. Colonies that are Gram positive and catalase negative have been purified on MRS Agar and stored at 4°C for further studies. Yeasts and molds were counted on the Potato-Dextrose Agar (PDA) medium (BIOKAR France) after 3-4 days of incubation at 25°C. The test was repeated twice.

2.4. Characterization of LAB strains

Forty-one strains of lactic acid bacteria isolated from brines were used in this study. These strains were selected from 280 isolates obtained, from fermenting olive brine and selected for their tolerance to bile-salts (0.3 & 0.1%) and acid pH (3, 2.5 & 2) and their enzymatic activity.

Production of gas from glucose

Overnight cultures of the LAB strains were inoculated (0.1 ml) in tubes containing 5 mL of semi-solid MRS medium with agar at 7g/l. After inoculation, 1ml of sterile white agar (20g/L) is added to the surface of each tube. After 24 hours of incubation at 30°C, isolates showing cracks or gas pockets in the culture medium are considered as gas positive. The test was repeated twice for each strain.

Tolerance to acid pH and bile salts

The method of Tambekar and Buthada (2010) [7], was used in this work, with some modifications. A volume of 5 µl of overnight culture of each LAB strain is inoculated in tubes containing 5ml of MRS broth previously adjusted with HCl 1M to different pH values of 2, 2.5 and 3. The cultures were then incubated at 37°C for 24 hours. Positive results showed a turbidity indicating the tolerance of the strain to the pH of the medium. For each strain, a tube of MRS medium (pH 6.8) inoculated and incubated under the same conditions as the other tubes is used as a control. The test was repeated twice. At the same time, bile salts were added to 5ml of MRS broth at a concentration of 0.3% (w/v) and then inoculated with 5µl of an overnight culture of the LAB strains. The cultures were then incubated at 37°C for 24 hours. MRS medium, without bile salts addition, inoculated and incubated under the same conditions as the other

cultures was used as controls. The tests were repeated twice. LAB strains showing growth after 24 hours are considered acid or bile salts tolerant and are retained for further studies.

Enzymatic activity of LAB strains

Hydrolysis of arginine

For each strain, 0.2 ml of an overnight culture was inoculated in tubes containing 2 ml of Moêller arginine broth and a control tube (Moêller medium without arginine) [8]. The cultures were covered with 4 to 5 mm of sterilized paraffin oil (V/V). After 24 hours of incubation at 30°C, the culture in the control tube showed a yellow shift due to the acidification of the medium (glucose metabolism) [9], [10]. The degradation of arginine, leading to the formation of ammonia, is revealed by color change of the medium to purple, due to its alkalinisation. The test was repeated twice.

Glucosidase activity

Overnight cultures of LAB strains were spot-inoculated on the surface of the MRS agar containing 0.3% (w/v) X-Glu. MRS agar without X-Glu was used as control. After incubation at 30°C for 5 days, the activity is revealed by black zones obtained around developed colonies [11].The test was repeated twice.

Urease activity

Urease test was performed by culturing the isolates on a medium containing (g L-1) peptone 1, sodium chloride 5, mono- potassium phosphate 2, glucose 1, urea 20, agar 15 and phenol red 0.1, incubation for 3 days at 34°C. [12].

Proteolytic activity

The proteolytic activity of the LAB strains was evaluated, on the MRS agar supplemented with 2% (w/v) skim milk, according to the method of Van Den Berg et al. (1993) [13]. Overnight cultures of LAB strains were spot-inoculated on the medium and then incubated at 30°C for 24 to 48 hours. The proteolytic activity resulted in the appearance of a clear halo around colonies. The test was repeated twice.

Lipolytic activity

The lipolytic activity of the LAB strains was investigated on MRS agar buffered at pH 7 (Phosphate buffer Na_2HPO_4/NaH_2PO_4; 0.2M) and supplemented with 1% (v/v) olive oil as the sole lipid [14]. Overnight cultures of LAB strains were spot-inoculated on the medium and then incubated at 30°C for 7 days. The lipolytic activity resulted in the appearance of an opaque halo around colonies. The test was repeated twice.

Hemolytic activity

The hemolytic activity of the LAB strains was evaluated on Triptycase Soy Agar (TSA) supplemented with 5ml of fresh horse blood. Overnight cultures of the LAB strains were streaked on the medium and then incubated at 30°C for 24 hours. After incubation, the type of hemolysis was examined. The α-hemolysis "partial hemolysis" is indicated by a green color around colonies. The β-hemolysis "total hemolysis" is indicated by clear zone around colonies; while δ-hemolysis indicated non hemolysis. The test was repeated twice.

Amylolytic activity

The amylolytic activity of the LAB strains was studied on modified MRS medium, where glucose was replaced by 20 g of soluble starch [15]. Overnight cultures of the LAB strains were spot-inoculated on the medium and then incubated

at 30°C for 24 to 48hours. Amylolytic activity of the strains was assessed by the presence of clear area around colonies obtained after using iodine solution [16]. The test was repeated twice.

Cellulolytic activity

The medium used to demonstrate cellulase production by LAB strains contained mineral salts, 0.1% (w/v) of yeast extract, 0.5% (w/v) of (Carboxymethyl Cellulose) CMC and 1% (w/v) of agar, and adjusted to pH 7.0 [17]. In addition to trace elements and phosphate salts, the mineral solution contained ammonium sulphate (1g/l) as a source of nitrogen [18]. The agar medium was inoculated with overnight culture of the strains and then incubated at 30°C for 18h at 24h. Cellulase activity was determined by measuring clear areas around the colonies after using phenol red. The test was repeated twice for each strain.

2.5. Acidifying capacity of LAB strains

Culture assay

The acidification capacity of LAB strains was studied on MRS broth. For this purpose, 0.5ml of overnight cultures of the strains were inoculated in 50 ml of MRS broth and incubated at 37°C for 24 hours. The test was repeated twice for each LAB strain.

Determination of pH and acidity

The pH and acidity (A) were measured at initial time (pH_i and A_0) and after 24 hours of incubation (pH_f and A_f), using the same methods as described above. The results are expressed according to the following formula:

$\Delta pH = pH_f - pH_i$ and Δ Acidity (g/l) $= A_f - A_0$. No inoculated medium was used as negative control.

Microbial biomass measurement

The biomass measurement was performed by measuring the OD at 600nm, using a spectrophotometer, at initial time (OD_i) and after 24 hours of incubation (OD_f). The results of the OD measurement were calculated using the formula: $\Delta OD = OD_f - OD_i$.

2.6. Effect of NaCl on growth of LAB strains

The growth of the LAB strains was tested, in duplicate, at different concentrations of NaCl. 0.5ml of overnight cultures of LAB strains were inoculated in 50ml of MRS broth supplemented with different concentrations of NaCl (%, w/v) (2, 4, 6, 8 and 10%). The cultures were then incubated at 37°C for 24hours. The pH, acidity and the biomass were measured initially and after 24hours of incubation, using the methods described above.

2.7 Antibacterial activity of LAB strains

The antibacterial activity of the LAB strains was evaluated, using the agar diffusion method. For this, 60 µl of the overnight cultures were introduced in wells made in Mueller-Hinton agar, previously inoculated with target strains, and then incubated at 37°C for 24hours. The strains used as targets were Listeria innocua, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa and Staphylococcus aureus. After the incubation, the inhibition zones were measured (average of two perpendicular diameters). All the assays were made in triplicate.

2.8. API50CHL gallery identification of LAB strains

Among the LAB strains studied, 7 Strains showing good results in terms of tolerance to low pH (2, 2.5, 3) and bile salts (1%), acidification capacity and antibacterial activity, were identified using a gallery API50CHL Medium (BioMérieux, France). Overnight cultures of LAB strains were inoculated in each tube of the gallery and then incubated at 30 ° C for 48 hours. The biochemical profiles obtained were analyzed using identification software APIWEB to obtain the LAB species.

2.9. Statistical analysis of the data

The data obtained were initially subjected to an analysis of the association between variables (OD, pH and acidity) using Pearson correlation coefficients. The overall structure of the variability of the strains was described by Principal Component Analysis (PCA).

3. Results and Discussions

3.1. Physico-chemical parameter of the analyzed samples

Table 1: Physico-chemical properties of brine samples of fermenting green olives

Samples Brine (B)	Date of brining	Date of sampling	fermentation period (Days)	pH	Chlorides (%)	Titratable acidity(%)
2014 – 2015						
B.1	27/10/2014	07/01/2015	72	4.70±0.00	5.90±0.01	0.40±0.01
B.2	26/10/2014	07/01/2015	73	4.60±0.10	6.60±0.02	0.44±0.05
B.3	16/10/2014	07/01/2015	83	4.50±0.00	7.01±0.01	0.51±0.06
B.4	24/10/2014	16/01/2015	84	4.90±0.10	6.00±0.09	0.40±0.10
B.5	24/10/2014	16/01/2015	84	4.60±0.10	6.50±0.13	0.43±0.04
B.6	15/10/2014	07/01/2015	84	4.80±0.10	5.90±0.01	0.45±0.09
B.7	15/10/2014	07/01/2015	84	4.50±0.00	5.65±0.02	0.44±0.07
B.8	12/10/2014	07/01/2015	87	4.50±0.10	5.65±0.00	0.44±0.06
B.9	11/10/2014	07/01/2015	88	4.50±0.10	5.65±0.01	0.44±0.03
B.10	01/10/2014	07/01/2015	98	4.40±0.10	5.85±0.01	0.45±0.05
B.11	26/10/2014	11/02/2015	108	4.20±0.00	5.85±0.02	0.45±0.07
B.12	26/10/2014	11/02/2015	108	3.90±0,10	7.20±0.11	0.90±0.01
2015 – 2016						
B.13	02/11/2015	30/11/2015	28	4.60±0.00	6.73±0.03	0.45±0.03
B.14	31/11/2015	30/11/2015	30	4.60±0.00	7.31±0.09	0.53±0.05
B.15	28/10/2015	30/11/2015	33	4.60±0.00	7.60±0.11	0.44±0.06
B.16	26/10/2015	30/11/2015	35	5.00±0.10	6.73±0.13	0.37±0.05
B.17	10/11/2015	26/12/2015	43	5.25±0.01	5.85±0.030	0.18±0.02
B.18	09/11/2015	26/12/2015	44	5.00±0.00	5.85±0.10	0.18±0.03
B.19	08/11/2015	26/12/2015	45	5.14±0.01	7.605±0.10	0.19±0.01
B.20	08/11/2015	26/12/2015	45	5.19±0.01	5.85±0.02	0.19±0.08
B.21	18/10/2015	26/12/2015	66	4.43±0.01	9.36±0.15	0.56±0.05
B.22	12/10/2015	26/12/2015	72	4.27±0.01	5.85±0.07	0.64±0.05
B.23	10/10/2015	26/12/2015	74	4.22±0.03	5.85±0.14	0.56±0.07
B.24	09/10/2015	26/12/2015	75	4.5±0.00	7.02±0.10	0.51±0.08

(Legend: *B. Samples Brine).

The results of the physico-chemical analyses of the olive brines are indicated on Table 1. Brine samples (B1, B2, B3, B4, B6, B11, B12, B13, B14, B15 and B16) show pH values range of 3.9-4.5, acidity values range of 0.44-0.9%, and

chlorides range of 5.65-9.36%. The pH values obtained are lower than the pH limit (pH 4.5) of microbiological stability of fermented food recommended by FA/OMS. However, in the other brine samples, the values ranges obtained were 4.6-5.25, 0.18-0.40% and 5.90-7.60% in their pH, acidity and chloride contents, respectively. All these brine samples showing their microbiological stability (with pH<4.5) were subject to fermentation times range of 66 to 98 days. However, the brines of 28 to 45 days of fermentation were instable microbiologically (with pH > 4.5). These findings indicate that the natural lactic fermentation process of alkali-treated Moroccan Picholine green olive took at least 45 days of fermentation to pass the limit of spoilage of fermented olives. The acidification at pH lowers than 4.5 reduces the fermentation time by promoting the growth of lactic acid bacteria [19]. Similarly, the salt concentration of brine has a great effect on biomass growth as well as acidification rate [20], especially at the beginning of fermentation [21].

3.2. Microbiological analyses of brines

Table 2: Microbial profiles (cfu/ml) of brine samples of fermenting green olives

Samples Brine	Fermentation period(Days)	LAB	Leuc.	Y&M	Entero.	Staph.	Rods
		2014 - 2015					
B.1	**72**	$4\ 10^6 \pm 0$	<1	$9.9\ 10^6 \pm 0.17$	<1	<1	<1
B.2		$7\ 10^6 \pm 3.46$	<1	$12\ 10^6 \pm 0$	<1	<1	$5\ 10^4 \pm 0.02$
	73						
B.3	**83**	$3.4\ 10^6 \pm 0.4$	<1	$6.4\ 10^6 \pm 0.69$	<1	<1	4±0
B.4	**84**	$10\ 10^6 \pm 0$	<1	$8.6\ 10^6 \pm 0.52$	<1	<1	$3.6\ 10^3 \pm 0.01$
B.5	**84**	$11.2\ 10^6 \pm 0.52$	<1	$5.4\ 10^6 \pm 0.69$	<1	<1	2±0
B.6	**84**	$6\ 10^6 \pm 0$	<1	$7\ 10^6 \pm 0$	<1	<1	<1
B.7	**84**	$11.5\ 10^6 \pm 2.5$	<1	$10\ 10^6 \pm 0$	<1	<1	<1
B.8	**87**	$28\ 10^6 \pm 0.52$	<1	$3.8\ 10^6 \pm 2$	<1	<1	3±0
B.9	**88**	$3.8\ 10^6 \pm 0.52$	<1	$5.5\ 10^6 \pm 0.86$	<1	<1	2±0
B.10	**98**	$8\ 10^6 \pm 2$	<1	$6\ 10^6 \pm 1$	<1	<1	$1\ 10^5 \pm 0.01$
B.11	**108**	$4\ 10^6 \pm 0$	<1	$6.4\ 10^6 \pm 0.4$	<1	<1	<1
B.12	**108**	$4.5\ 10^6 \pm 0.5$	<1	$11\ 10^6 \pm 0$	<1	<1	1
		2015 – 2016					
B.13	**28**	$12\ 10^6 \pm 1.73$	<1	$18\ 10^6 \pm 0$	<1	<1	<1
B.14	**30**	$10\ 10^6 \pm 3.46$	<1	$12\ 10^6 \pm 0$	<1	<1	<1
B.15	**33**	$6\ 10^6 \pm 1.73$	$2\ 10^6 \pm 0$	$16\ 10^6 \pm 0$	<1	<1	$2.75\ 10^3 \pm 0.1$
B.16	**35**	$7\ 10^6 \pm 1.73$	<1	$16.6\ 10^6 \pm 2.94$	<1	<1	8±0
B.17	**43**	$5\ 10^6 \pm 1.73$	<1	$20\ 10^6 \pm 0$	<1	<1	<1
B.18	**44**	$8.2\ 10^6 \pm 0.72$	$2\ 10^6 \pm 1$	$7.1\ 10^6 \pm 0.17$	<1	<1	<1
B.19	**45**	$7.6\ 10^6 \pm 0.52$	$2\ 10^6 \pm 0$	$14\ 10^6 \pm 0$	<1	<1	$1.98\ 10^3 \pm 0.01$
B.20	**45**	$5\ 10^6 \pm 0$	$4\ 10^6 \pm 1.73$	$5.2\ 10^6 \pm 0.34$	<1	<1	2±0
B.21	**66**	$16\ 10^6 \pm 2$	<1	$5.1\ 10^6 \pm 0.17$	<1	<1	3±0
B.22	**72**	$15.5\ 10^6 \pm 0.72$	<1	$6.5\ 10^6 \pm 0.86$	<1	<1	1±0
B.23	**74**	$12\ 10^6 \pm 1.73$	<1	$5.8\ 10^6 \pm 0.34$	<1	<1	<1
B.24	**75**	$10\ 10^6 \pm 0$	<1	$6\ 10^6 \pm 0$	<1	<1	<1

(Legend: *LAB:** Lactic acid bacteria**; *Leuc:** Leuconostocs**; *Y&M:** Yeasts and moulds**; *Entero:** Enterobacteriaceae**; *Staph**:** Staphylococcus**)**.

Table 2 showed the results of microbial flora counts in the different samples analyzed. When the pH is in the order of 4, yeasts and molds are lower important than lactic acid bacteria, so when the pH reaches values in the order of 5,

yeasts become more important in brine. These two microbial groups remain interacting in the medium; lactic acid bacteria tend to acidify it, while yeast tends to neutralize the brine. The staph and enterobacterial load are always lower than 1 CFU/ml for the 24 samples of brine from the table olives studied, indicating that these samples are exempts of staph and enterobacteria. These low levels may be due to the inhibitory effects of salt, bacteriocins produce by lactic acid bacteria [22]; polyphenols [23], [24] and organic aci ds [25], Table 2. Hight acidification of fermented, green olives brines in storage and high concentrations of sodium chlorides inhibit the growth of Gram-negative bacteria [26], sporulating bacteria and certain lactic acid bacteria, particularly heterofermentative. Only yeasts and certain lactic bacteria can tolerate these conditions. Yeasts play a very important role in improving the acidification rate of the medium by lactic acid bacteria [27], [28]. This improvement could be due to the stimulation of the growth and fermentation power of lactic bacteria by carbon dioxide and other metabolites produced by yeasts [29], [30]. For the isolation of leuconostocs there was no problem with sodium azide. Given the pH of the samples, leuconostocs were not dominant in the environment (Table 2).

3.3. Examination of morphological characteristics of LAB strains

The LAB strains obtained from brine samples were examined for their Gram and catalase activity. The results showed that the strains were Gram positive and catalase negative, not sporulated and rod shaped. Concerning the fermentative type, the strains showed no production of gas (CO2) from glucose, so they were considered as homo-ferments, except leuconostocs. The results of the behaviour of the different strains subjected to the action of acidic pH (2, 2.5 and 3) show that the different isolates are resistant to these pH values. In addition, the bile juice resistance study revealed that all strains tested tolerated 0.3% and 1% of bile.(Table 3).

3.4. Enzymatic activities

Arginine hydrolysis

The search for the enzyme arginine decarboxylase is of great importance in the identification of bacteria. According to the evaluation of the results of the test performed on our strains, there was acidification of the medium related to the use of glucose but there was no realcalinization. The substrate (arginine) has therefore not been degraded (ADH-), except in a few strains: (S109, S82, S15, S3, S97, S167, S107, S1, S193), There has been acidification of the medium linked to the fermentation of glucose and then there has been realkalinization of the medium linked to the degradation of the substrate (arginine) by the bacterium, the bacteria has ADH (ADH+).

Urease

Urease, enzyme hydrolyzing urea, this activity is detected by the red coloration which reflects an alcalinization of the medium, following the hydrolysis of urea and formation of ammonium carbonate: urease+ and if the medium persists orange then no alkalizing test urease -. Our strains tested are all urease -.

ß-Glucosidase

Esculine is a glycoside consisting of a complex carbohydrate that can be degraded by esculinase. The reaction products are glucose and the esculitine formed can react with iron ions to form a black halo around the colonies. The hydrolysis characteristic of esculine appears to be variable in isolated strains, all of which are capable of degrading it except for 239 and 240 strains, which are unable to hydrolyze esculine.

Table 3: Morphological characteristics and physiological properties (activity)

Phenotypic properties		Strains Number	%
Form	Rod	39	95
	Cocci	2	5
Catalase	-	41	100
Gas	-	41	100
pH growth	2	41	100
	2.5	41	100
	3	41	100
Bile salt tolerance	0.3%	41	100
	1%	41	100
Enzymatic activity		**Strains Number**	**%**
Urease	-	41	100
Hemolysis	-	41	100
ADH	+	8	98
	-	33	20
β-Glucosidase	+	39	95
	-	2	5
Proteolysis	+	41	100
Lipolysis	+	12	30
	-	29	71
Amylolysis	-	41	100
Cellulolysis	-	41	100

(Legend: ***ADH:** Arginine hydrolysis**; *+:** positive reaction**; *-:** negative reaction**.**

Proteolysis

The proteolytic activity of the isolates is observed as around the colonies. Based on the results (Table 3), it was found that the positive control and all isolates tested expressed proteolytic activity. Thus, the uninoculated negative control does not give activity. The results obtained allow us to confirm the proteolytic character of lactic acid bacteria as reported by other researchers [31], [32].

Lipolysis

The lactic isolates tested on MRS agar added with 1% olive oil are devoid of this activity. This could be due to the fact that lactic acid bacteria are considered low lipolytic [33]. Lipases are widespread in bacteria, yeasts and molds. They are as well produced by Gram-positive bacteria as by Gram-negative bacteria [34]. Lactic acid bacteria are considered weakly lipolytic compared with other bacterial species.

Amylolysis

The hydrolysis of starch by bacterial amylases is revealed on MRS starch agar by the disappearance of the specific coloring of starch with iodine (dark brown coloring). None of our strains showed amylolytic activity.

Cellulolysis

Cellulolytic bacteria are detected by a clear area around colonies obtained the CMC agar. The cellulolytic index was calculated after the addition of 1% Congo red dye. None of our strains tested showed cellulolytic activity.

Hemolysis

The absence of haemolytic activity is a selection criterion for probiotic strains, as long as they indicate that these bacteria are not virulent [35]. None of the strains examined in our work revealed hemolytic activity when cultured on blood agar media; this finding confirms the results of Mami, Henni and Kihal (2008) and Kalui, Mathara, Kutima, Kiiukuk and Wongo (2009) [36].

3.5. Study of acidifying capacity

Acidity

At zero time (before incubation), the pH is stable at an average of 5.8±0.03. After 24h of incubation, the pH decreases towards an average of 4.2 ±0.025. After incubation, the LAB ensures the fermentation of carbohydrates in lactic acid, the medium becomes more acidic and therefore the pH value has decreased. All isolates had a good ability to decrease pH with an average Δ pH of 1.55 ± 0.4. Thus Δ pH ranged from 0.3 (isolates S71 and S97) to 2 (isolates S85 and S114). These results are consistent with the results found by: Lairini et al (2014) [37]. The majority of isolates have a higher acidifying capacity with an average of 13.3 g / l, due to the accumulation of lactic acid. This acidifying capacity ranged from 0.9g/l (case of isolate S96) to 38.55 g / l (isolate S76). Isolates S96, S71, S97 and S117 have very low acidifying properties. These results are based on the results by Anas (2013), and Latrech (2016) [38], [39].

Biomass measurement

After 24h of incubation, the cell concentration reaches higher values due to increased bacterial growth (biomass) in their favorable MRS medium. The results obtained are consistent with the results found by: (Naouel et al., 2010) [40].

3.6. Effect of NaCl on physiological activity of LAB strains

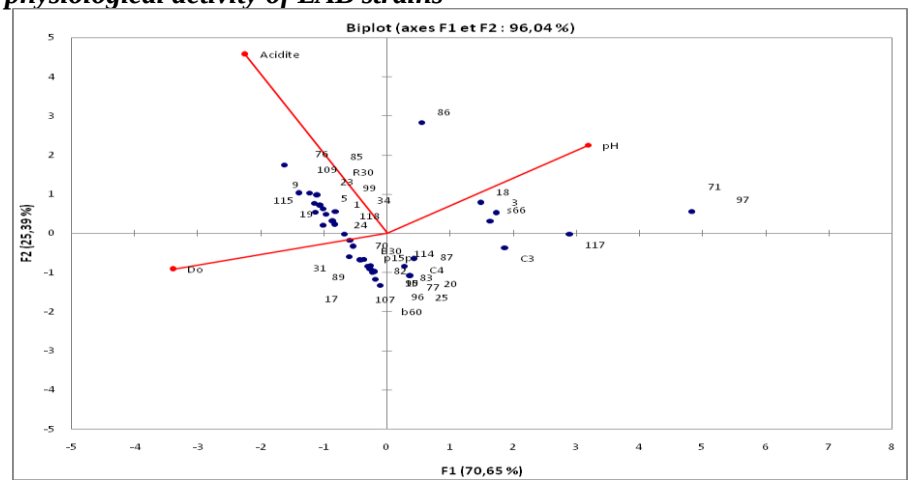

Figure 1: Analysis of data for the three variables: OD; pH and acidity for Nacl concentration of 8%.

Growth tests of LAB isolates in the absence and presence of NaCl was carried out at different concentrations (0%, 2%, 4%, 6%, 8% and 10%). The culture of the isolates in the presence of different concentrations of NaCl allowed us to see their ability to grow in the presence of osmotic stress. After incubation at 30°C for 24 hours, a bacterial disorder is

observed for certain salt concentrations. This indicates that growth is different from one isolate to another, even if they come from the same source of isolation. The results of the 41 isolates were subjected, like the analysis of the previous data, to a Principal Component Analysis (PCA), but this time at different concentrations of NaCl. This analysis allows an overall distribution of isolates (according to pH, acidity and OD) according to different NaCl concentrations. Based on the results obtained from the pH, acidity and OD measurements of the 41 strains at different concentrations of NaCl (0,2,4,6,8 and 10%) it can be inferred that the strains resisted a NaCl concentration of 8% are S240, S19, S77, S90, S24, S118, S167, S122, S117, S25, S3 and S70. The results obtained lead to the following observations (Figure 1).

3.7. Study of the antibacterial activity of BL strains

The results of the antibacterial activity of the strains are expressed in mm (inhibition zone).

Inhibition of gram positive bacteria (Escherichia coli, Pseudomonas aeruginosa).

The results of the antibacterial test of the 41 strains against *Escherichia coli* clearly show that the majority of strains had an inhibitory activity against *E. coli*. It is also noted that S240, S76, S1, S107, S122 and S239 have no inhibition. Lengths of inhibition zones ranged from 1 (strain S193) to 14 mm (strain S20). LAB inhibition of *E. coli* strains has already been described by several studies [41], [42], [43]. Whereas for inhibition results against pseudomonas *aeruginosa* clearly show that the majority of strains had an inhibitory activity. It is also noted that strains S76 and S122 have no inhibition. Thus, the lengths of the inhibition zones ranged from 2 (strain S117) to 13 mm (strain S82). The inhibition of *Pseudomonas aeruginosa* strains by LAB has already been described by several studies [38], [40].

Inhibition of gram negative bacteria (Micrococcus luteus, Listeria innocua and Staphylococcus aureus)

The results show that most strains have an inhibitory activity against *Micrococcus luteus* except strains S240, S167, S122 and S239. Thus, the lengths of the inhibition zones ranged from 1 (strain S86) to 15 mm (strain S118). The inhibition of *Micrococcus luteus* strains by LAB has already been described by several studies [44], [39]. For results of antibacterial activity against *Listeria innocua*, the majority of lactic strains show inhibitory activity, with inhibition zone diameters ranging from 3 mm for strains S167, S193 and S155 to 15 mm for strains S9, S99, S31 and S70. The absence of inhibition of S241, S83, S76, S25 and S107 strains was noted. The inhibition of *Listeria innocua* strains by LAB has already been described by several studies: (Naouel et al., 2010), (Hwanhlem et al., 2014). [40], [43]. Based on the results obtained of *Staphylococcus aureus*, it appears that there are lactic strains that have antibacterial activity against the latter; whose diameters of the inhibition zones vary from 1 mm for strain S115 to 7 mm for strains S3, S71, S97, and S186. The inhibition of *Staphylococcus aureus* strains by LAB has already been described by several studies: (Naouel et al., 2010), (Anas, 2013), (Hwanhlem et al., 2014). [40], [38], [43]. The results obtained from the ANOVA test for the relation between the inhibition diameter and the fermentation period did not show a good correlation so there is not strongly a related relation between the inhibition diameter and the fermentation period (Figure 2; 3;4 5). Olive brine samples that are fermented for more than 66 days are microbiologically stable because of their pH <4.5 (food codex) and titratable acidity which varies from 0.4 to 0.5 as well as good resistance to different concentrations of NaCl (from 0% up to 10%). On the other hand, samples that have a fermentation period of less than 45 days are microbiologically unstable, given their pH> 4.5 which shows a dominance of the alteration flora which therefore requires adequate control.

Table 4: Antibacterial activity (inhibition diameter, mm) of lactic bacteria strains

	Sample brine	Fermentation Period (Days)	LAB	*E. coli*	*L. innocua*	*Ucc.Luteus*	*P.aeruginosa*	*S.aureus*	[NaCL]
2014-2015									
	B.1	**72**	**S70**	4±0	15±0.3	4±0	11±0.17	0±0	8%
	B.3	**83**	**S76**	0±0	0±0	5±0	0±0	5±0.04	2%
	B.4	**84**	**S71**	3±0	5±0	3±0	6±0	7±0.02	4%
			S122	0±0	4±0	0±0	0±0	0±0	8%
	B.5	**84**	**S77**	8±0	13±0	5±0.04	11±0.17	0±0	8%
			S82	6±0	9±0.08	12±0.3	13±0.17	0±0	2%
			S85	7±0	7±0.3	6±0	7±0	5±0.02	6%
			S86	5±0	7±0	1±0	4±0.02	4±0	2%
			S87	4±0	5±0	3±0	8±0.04	3±0	6%
			S89	5±0.05	5±0	8±0	7±0	0±0	2%
			S96	3±0.03	5±0	6±0	5±0	0±0	8%
	B.8	**87**	**S23**	2±0.03	4±0.02	4±0	7±0.02	6±0.02	2%
			S25	7±0	0±0	8±0.05	9±0	0±0	4%
			S34	5±0	5±0	3±0	7±0	3±0	6%
	B.9	**88**	**S186**	7±0,03	7±0	7±0	8±0.017	7±0.02	0%
	B.10	**98**	**S1**	0±0	4±0	4±0.02	6±0	5±0.03	2%
			S20	14±0.17	9±0.3	8±0.04	5±0.01	0±0	6%
			S90	7±0	9±0	8±0	9±0	0±0	8%
			S24	8±0	5±0	8±0	7±0	0±0	8%
	B.11	**108**	**S118**	3±0	5±0.08	15±0	11±0	0±0	8%
			S114	7±0	3±0	7±0	7±0	4±0.02	2%
			S115	5±0	4±0	3±0	4±0	1±0	2%
	B.12	**108**	**S31**	5±0.02	15±0	8±0	5±0.02	0±0	0%
			S109	5±0	7±0	6±0	5±0	0±0	4%
			S155	5±0.3	3±0	3±0	5±0.02	5±0	4%
2015-2016									
	B.13	**28**	**S117**	3±0	0±0	3±0	2±0	4±0	0%
			S193	1±0	3±0	4±0	5±0	4±0.02	2%
	B.14	**30**	**S107**	0±0	0±0	4±0.03	3±0	0±0	6%
	B.15	**33**	**S167**	7±0.02	3±0	0±0	5±0.02	5±0	8%
	B.16	**35**	**S83**	3±0.02	0±0	5±0.02	5±0	4±0	2%
	B.18	**44**	**S239**	0±0	0±0	0±0	6±0.017	3±0	6%
	B.20	**45**	**S240**	0±0	6±0.03	0±0	5±0.03	5±0.08	10%
	B.22	**72**	**S15**	4±0	6±0	8±0.17	5±0	0±0	2%
			S17	6±0	5±0	5±0	6±0.02	0±0	8%
			S18	4±0	5±0	4±0	3±0	0±0	2%
			S19	4±0	5±0.03	6±0.02	9±0	0±0	10%
	B.23	**74**	**S5**	8±0	11±0.2	8±0	9±0	0±0	2%
			S3	4±0	7±0	4±0	8±0	7±0.03	8%
			S9	6±0.03	15±0.3	6±0.03	9±0.03	0±0	2%
	B24	**75**	**S97**	3±0	5±0.02	3±0.02	6±0	7±0	2%
			S99	5±0	15±0.04	7±0	5±0	0±0	6%
	C.		**Lactic acid**	27±0.2	35±0	28±0	33±0	24±0.3	

(**Legend: ****E. coli* **:** *Escherichia coli* **; ****L.innocua:** *Listeria innocua* **; ****Ucc.Luteus* **:** *Micrococcus luteus* **; ****P.aeruginosa* **:** *Pseudomonas aeruginosa* **; ****S.aureus* **:** *Staphylococcus aureus* **; *C. :** Control (lactic acid)**; *B**.: Sample Brine).

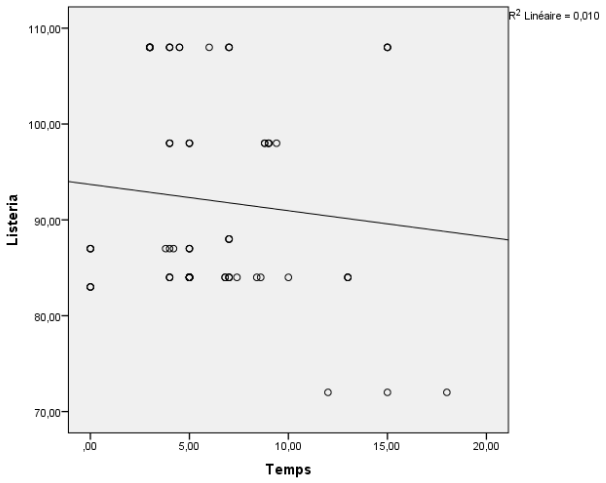

Figure 2: Inhibition diameter of LAB againt Listeria / fermentation time (2014-2015).

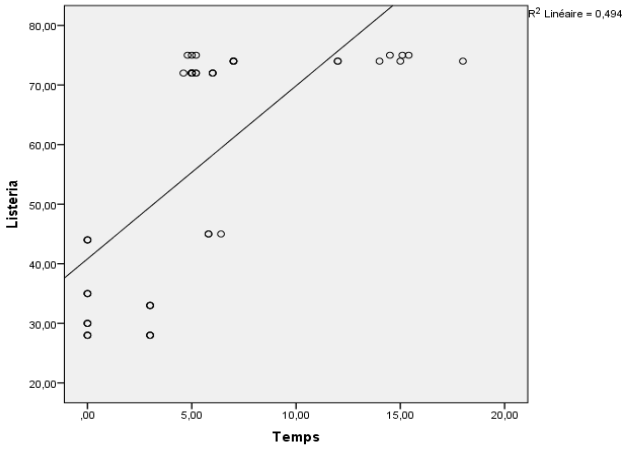

Figure 3: Inhibition diameter of LAB againt Listeria / fermentation time (2015-2016).

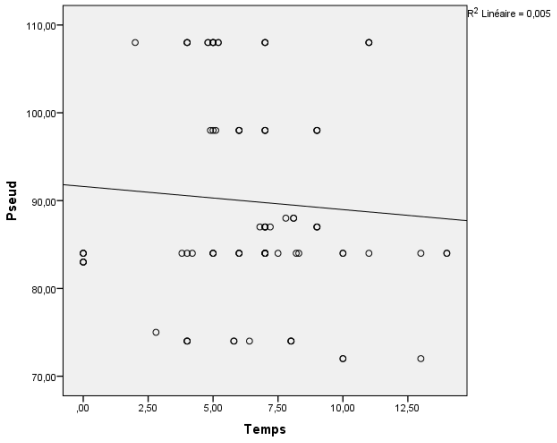

Figure 4: Inhibition diameter of LAB againt Pseudomonas/fermentation time(2014-2015).

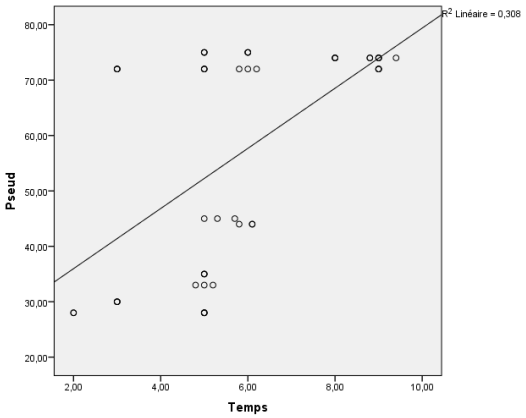

Figure 5: Inhibition diameter of LAB againt Pseudomonas/fermentation time(2015-2016).

3.8. Strain identification by API 50 CHL gallery

The fermentation of carbohydrates and derivatives of the gallery has identified Lactobacillus species and which are all *lactobacillus plantarum*. With percentages varying between 98.9% and 99.9%.

4. Conclusion

The physico-chemical study of the fermentation process showed that:

The isolation of lactic acid bacteria species from preserved Triffa samples from the Guercif region was interesting. The identification of the different strains showed a fairly large diversity of species. Lactic bacteria belong to a group of beneficial bacteria, whose virtues are no longer questionable and which produce lactic acid as the final product of the fermentation process. They are found throughout nature, and also in the digestive system of man. Among the kinds of lactic acid bacteria we have been able to isolate *lactobacillus*, *leuconostocs* and *lactococci*. The results of physico-chemical and microbiological tests carried out on the samples collected allowed us to isolate and purify different species of: lactic bacteria and yeast. The microbiological study of the fermentation process showed that the fermentation and stabilization of olives in brine are governed by the nature and strength of lactic bacteria that grow in brine together with the yeast. The balance between these two microbial groups is an important factor in the success of the table olive production process. Proper preservation of table olives and stabilization of brine requires a predominance of the biochemical activity of lactic acid bacteria over that of yeast. In this study, we tested the antibacterial activity of 41 strains of lactic acid bacteria. The results shown in Table 4 indicate that the strains had varied antibacterial activities. They showed active activity spectra over the entire collection of indicator strains tested both positive and negative grams, with inhibition zones up to 15 mm, showing good or high antagonistic potential.

According to the tests carried out we found that the 41 isolates of lactic bacteria have a good resistance to acidic pH (3, 2.5 and 2) and to a concentration of 0.3% of bile. The salt concentration has a very important role in the evolution of the fermentation process. As a result, at high concentrations (> 8%) the lactic acid bacteria responsible for the fermentation can be inhibited. The concentration found, which is of the order of 6%, can very strongly inhibit a part of the alteration flora, in particular coliforms, without affecting the lactic flora. This salt concentration is the same optimal concentration for a recommended fermentation. However, too high a concentration of salt (10 to 14%) inhibits the growth and metabolism of lactic acid bacteria, especially the production of lactic acid. So the degree or salt content of the brine is a very important factor in the fermentation process and the conservation of table olives. The identification by API 50 CHL of lactic acid bacteria, selected, has shown that it is: Lactobacillus plantarum (7 strains).

Acknowledgments:

The authors are grateful to the CNRST (PPR/19/2015), and Tunisian cooperation (17TM06) for their supports.

References

[1] Fernandez AG, Adams M. R. & Fernandez-Diez,M (1997). *Table Olives: Production and Processing*. Chapman & Hall, London. 134-206.

[2] Abriouel, H., Benomar, N., Lucas, R., and Gálvez, A. (2011). Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally fermented Aloreña green table olives. Int. J. Food Microbiol. 144, 487–496.

[3] Tassou, C. C., Panagou, E. Z., and Katsaboxakis, K.Z. (2002). Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines. Food Microbiol. 19, 605–615.

[4] Aponte, M., Ventorino, V., Blaiotta, G., Volpe, G., Farina, V., Avellone, G., Lanza, C. M., and Moschetti, G. (2010). Study of green Sicilian table olive fermentations through microbiological, chemical and sensory analyses. Food Microbiol. 27, 162–170.

[5] Glaasker, E; W.N. Konings, and B.Poolman. 1996. Osmotic regulation of intracellular solute pools in lacto-bacillus plantarum.J.Bacteriol. 178:575-582.

[6] Piuri M.Sanchez-Rivas, Ruzal SM (2003). Adaptation to High salt in Lactobacillus : rôle of peptides and proteolytic enzymes.J.Appl. Microbiol 95: 372-379

[7] Tambekar, D.H. and Bhutada, S.A. An evaluation of probiotic potential of lactobacillus sp. from milk of domestic animals. and commercial available probiotic preparations in prevention of enteric bacterial infections. Recent Research in Science and Technology.2010; 2: 82-88.

[8] Moêller V, 1955. Simplified tests of some amino acid decarboxylases and for the arginine dihydrolase system. Acta. Pathol. Microbiol. Scand. 36: 158-172.

[9] Larpent-Gourgaud Monique, Michaux odile, Larpent J.P, Desmasure Nathalie, Desamazeaud Michel, Mangin Irène, Masson Florence, Montel M.C. et Tailliez Patrick. Les ferments lactiques et bactéries apparentées In Microbiologie alimentaire Techniques de laboratoire. Larpent J-P. Tec&Doc, Lavoisier, pp 199-255-1997.

[10] Carr F.J., Chill D., Maida N., The lactic acid bacteria: a literature survey. Crit Rev Microbiol. 28 (2002) 281-370 H. Forouhandeh, S. Zununi Vahed, M.S. Hejazi, M.R. Nahaei and M Akbari Dibavar; Isolation and Phenotypic Characterization of Lactobacillus Species from Various Dairy Products. Current Research in Bacteriology 3 (2): 84-88, 2010 ISSN 1994-5426.

[11] Lelliot et stead, 1987 (LELLIOTT R.A ET STEAS D.E. 1987. Methods for diagnosis of bacterial diseases of plants. Blackwell Scientific Publications Volume 2. Oxford (GB).

[12] H. Forouhanden, H. Zununi Vahed, M.S. Hejazi, M.R. Nahei , M. Akbari Dibavar. Isolation and phenotypic characterization of *Lactobacillus* species from various dairy products. Curr. Res. In Bacteriol.3 (2): 84-88. 2010).

[13] Van Den Berg J.C., Smits A., Pot B., Ledeboer A.M., Kersters K., Verbakel J.M.A., Verrips C.T. 1993. Isolation, screening and identification of lactic acid bacteria from traditional food fermentations processes and culture collections. Food Biotechnology, 7, 189-205.

[14] Guiraud J.P. et Galzy P. 1980. L'analyse microbiologique dans les industries alimentaires. édition de l'usine nouvelle. P 239.

[15] Brabet C, Chuzer G, Oufour D, Raimbaultt ME, Giraudtt J (1996). Impoving cassava sour starch quality in Colombia, Progress in Research and Development, CIRAD chapter 27. ISBN 958-9439-88-8 pp. 242-246.
[16] Tapha N, Pal J, Tamang JP (2006). Phenotypic identification and technological properties of lactic acid bacteria isolated from traditionally processed fish products of the Eastern Himalayas. Int. J. Food Microbiol. 107: 33-38.
[17] ZUCKER, M. & HANKIN, L. (1970). Regulation of pectate lyase synthesis in Pseudomonas fluorescens. Journal of Bacteriology 104, 13-18.
[18] LESTER HANKIN AND SANDRA L. ANAGNOSTAKIS. Solid Media Containing Carboxymethylcellulose to Detect C, Cellulase Activity of Micro-organisms. Journal General Microbiology (1977), 98, 109-1 I 5.
[19] Panagou E.Z., Schillinger U., Franz C.M.A.P. and Nychas G.J.E. 2008. Microbiological and biochemical profile of cv. Conservolea naturally black olives during controlled fermentation with selected strains of lactic acid bacteria. Food Microbiol., 25, 348-358.
[20] Leal-Sanchez M.V., Ruiz-Barba J.L., Sanchez A.H., Rejano L., Jimenez-Diaz R. and Garrido A. 2003. Fermentation profile and optimization of grenn olive fermentation using *Lactobacillus plantarum* LPCO10 as a starter culture. Food Microbiol., 20, 421-430.
[21] Duran-Duintana M.C., Garcia Garcia P and Garido Fernandez A. 1999. Establishement of conditions for green table olive fermentation at low temperature. Int. J. Food Microbiol., 51, 133-143.
[22] Gibbs P.A 1987. Novel uses for lactic acid fermentation in food preservation. J. Appl. Bacteriol. Symp. Suppl. 63: 51S-58S.
[23] Juven B., Henis Y., 1970. Studies on the antimicrobial activity of olive phenolic compounds. J. Appl. Bacteriol. 33: 721-732.
[24] Rodriguez Ma.M., Perez J., Menzana M.C.A. et Maritnez J. 1988. Effect of extracts obtained from olive mill wast waters on *Bacillus megaterium* ATCC33085.J.Appl.Bacteriol., 64(3): 219-226.
[25] Wong H.C, Chen Y.L., 1988. Effect of lactic acid bacteria and organic acids on growth and germination of *Bacillus cereus*. Appl. Environ. Microbiol. 54(9): 2184.
[26] Gonzalez Cancho F., Nosti Vega M., Fernandez Diez M.J. et Buzcu N. 1970. Especies de Propionibacterium relacionadas connla «zapateria ». Factores que influyen en su desarrollo. Microbiologia Espanola, 21: 129-141.
[27] Duran Quintana M.C.; Romero Barranco C.; Garcia P. ; Brenes Balbuena M. et Garrido Fernandez A. 1997. Bacteria del acido lactico en la fermentacion de aceintunas de mesa. Grasas y Aceites, 48(5): 297-311.
[28] Leroi F., courcoux P. 1996. Influence of pH, temperature and initial yeast: bacteria ratio on the stimulation of *Lactobacillus hilgardii* by *Saccharomyces florentinus* isolated from sugary Kefir grains. J. Appl. Bacteriol., 80: 138-146.
[29] Leroi F., Pidoux M. 1993a. Detection of interactions between yeasts and lactic acid bacteria isolated from sugary Kefir grains. J. Appl. Bacteriol., 74: 48-53.
[30] Leroi F., Pidoux M. 1993b. Characterization of interactions between *Lactobacillus hilgardii* and *Saccharomyces florentinus* isolated from sugary Kefir grains. J. Appl. Bacteriol., 74: 54-60.
[31] Shirai K., Guerrero I., Huerta S., Saucedo G., Castillo A.O., Gonzalezr., George m. 2001. Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation Hall Enzyme and Microbial Technology, p. 446– 452.
[32] FRANCOIS Z.N. N., FLORANCE F.A., PAUL M.F., FELICITET M., EL SODA M., 2007. Biochemical properties of some thermophilic lactic acid bacteria strains from traditional fermented milk relevant to their technological performance as starters cultures. Biotechnol, vol.6, n°1, p. 14-21.

[33] De Roissart, H. et Luquet, F.M., 1994. Bactéries lactiques. Vol. I et II, Edition Lorica. Fickers, P., Destain, J. et Thonart, P. 2008. Les lipases sont des hydrolases atypiques: principales caractéristiques et applications. Biotechnol. Agron. Soc. Environ. 12: 119- 130.
[34] Fickers, P., Destain, J. et Thonart, P. 2008. Les lipases sont des hydrolases atypiques: principales caractéristiques et applications. Biotechnol. Agron. Soc. Environ. 12: 119-130.
[35] De Vuyst L, Foulquie MR, Revets H (2003). Screening for enterocins and detection of hemolysin and vancomycin resistance in Enterococci of different origins. Int. J. Food. Microbiol. 84: 299-318.
[36] Kalui C.M., Mathara J.M., Kutima P.M., Kiiyukia C., Wongo L. E. 2009. Functional characteristics of Lactobacillus plantarum and Lactobacillus rhamnosus from ikii, a Kenyan traditional fermented maize porridge. African Journal of Biotechnology. 8:4363- 4373.
[37] LAIRINI S. N. BEQQALI R. BOUSLAMTI R. BELKHOU F. ZERROUQ. Isolement des bactéries lactiques à partir des produits laitiers traditionnels Marocains et formulation d'un lait fermenté proche du Kéfir. Afrique SCIENCE 267-277-2014.
[38] Anas. M, 2013. Recherche des bactéries lactiques de bactériocines à large spectre d'action vis-à-vis des germes impliqués dans les toxi-infections alimentaires en Algérie, 25.
[39] Latrech Bilal. 2016. Caractérisation des bactéries lactiques isolées du beurre cru, évaluation de leurs aptitudes technologiques et leur utilisation dans la fabrication de la crème sure. Mémoire master de technologie alimentaire, Algérie.
[40] Naouel, F., Hellal, A., & Laraba, A. (2010). Etude de l'activité antimicrobienne des souches de lactobacilles thermophiles utilisées dans l ' industrie laitière . Nature, 13– 20.
[41] Pringsulaka, O., Thongngam, N., Suwannasai, N., Atthakor, W., Pothivejkul, K., & Rangsiruji, A. (2012). Partial characterisation of bacteriocins produced by lactic acid bacteria isolated from Thai fermented meat and fish products. Food Control, 23(2), 547–551. https://doi.org/10.1016/j.foodcont.2011.08.029
[42] Cálix-Lara, T. F., Rajendran, M., Talcott, S. T., Smith, S. B., Miller, R. K., Castillo, A.Taylor, T. M. (2014). Inhibition of Escherichia coli O157: H7 and Salmonella enterica on spinach and identification of antimicrobial substances produced by a commercial Lactic Acid Bacteria food safety intervention. Food Microbiology, 38, 192–200. https://doi.org/10.1016/j.fm.2013.09.006
[43] Hwanhlem, N., Chobert, J. M., & H-Kittikun, A. (2014). Bacteriocin-producing lactic acid bacteria isolated from mangrove forests in southern Thailand as potential biocontrol agents in food: Isolation, screening and optimization. Food Control, 41(1), 202–211. https://doi.org/10.1016/j.foodcont.2014.01.021.
[44] Burton, J. P., C. N. Chilcott, and J.R. Tagg. 2005. The rationale and potential for the reduction of oral malodour using Streptococcus salivaruis probiotics. Oral Diseases 11:29-31.