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Biological conservation of food products

M.Sobh[1]*, A.Fadli[3], A.Foullous[2], N.Rhaiem[1], A.Hammoumi[2], R.Bengueddour[4], M.Ouhssine[1]

[1]Laboratory of Biotechnology, Environment and Quality, Faculty of Science, Ibn Tofail University, PO Box 133, 14000, Kenitra, (MOROCCO)

[2]Laboratory of Microbiology, Pharmacology, Toxicology and Environmental Ainchok, Faculty of Science, University Hassan II, BP: 5366, Maarif. Casablanca, (MOROCCO)

[3]Laboratory of Botany, Biotechnology and Plant Protection, Faculty of Science, Ibn Tofail University, P 133, 14000, Kenitra, (MOROCCO)

[4]Laboratory of Health and Nutrition, Faculty of Science, Ibn Tofail University P 133, 14000, Kenitra, (MOROCCO)

E-mail: sobh72@yahoo.fr

ABSTRACT

Two strains of lactic acid bacteria (lactobacillus SMLB2 SMLB1 and gender) and a yeast strain (SMLV1) are used in fermentation and bioconservation certain foods. They provided a perfect fermentation and thus life, a safety and integrity unparalleled.

Positive interactions between yeasts and lactic acid bacteria have shown a timing fermentative metabolism of all strains ferment component selected. Indeed, after 16 hours of incubation with the ferment S6 was able to stabilize the pH and acidity 3.57 to 3.57 and 1.22% acidity and that from the 30th day of controlled fermentation.

The pH is obtained according to the packaging of food products.

KEYWORDS

Consumers;
Food;
Vegetables;
Organic products;
Lactic fermentation;
Microorganisms;
Ferment;
Conservation.

INTRODUCTION

In response to concerns of consumers towards certain foods, there is a growing craze for organic products based on lactic fermentation.

The agribusiness sector in Morocco is booming, it represents around 2,000 companies, which provide almost 20% of total employment and contributes to 30 % of the value added, which is an essential element in the food security, however, the use of industrial product begins to be reluctant to follow successive uses of chemical products such as food additives

and have shown their impact on several levels on the health of the consumer as the case of citric acid, which causes high-dose erosion of teeth, skin allergy in the form of awareness, especially the oral and genital mucosa and causing disease (ulceration)[7], which damage a negative impact on the future of the industry and consequently on the economy

The country. The challenge therefore is to find alternative and it is in this sense that we will try to find a technique for biological conservation artichokes. This work has the following objectives:

- The realization of spontaneous Fermentation.
- Isolation, purification, identification of appropriate strains for the conduct of aproper fermentation.

- Selection of a suitable close.
- Preparation of a liquid.
- Creation of a controlled fermentation.
- Testing of biological conservation artichokes.

MATERIALS AND METHODS

The samples collected from the artichokes Gharb region. And especially from Kenitra, and SidiSlimaneSidiKacem.are returned to the laboratory, washed in running water, peeled and cut manually with a sterilized knife, then carefully deposited in glass jars.

core samples are prepared in parallel and olives.

A handful of salt (about nearly 150 g) is placed in a jar. Adding water at a rate of 200 ml for agitation, redissolution and homogenization. Salt water is completed up to the mark of the gauge 1-liter beaker. After the second homogenization, the brine is poured into the receptacles of artichoke cut into pieces of 20 to 30 g each. It should be noted that five pieces of lemon 10g each have already been integrated into the pre- marinated jar.

The fermentation is started right from the first contact of the sample with brine.

Periodic samples were taken. The time is stopped five days. The aliquots were subjected to physicochemical and microbiological analyzes.

The temperature of the sample is measured using a thermocouple (temperature, pH) Type Micro - pH Crison 2000. Values are read directly on the device display.

After calibration, the electrode of the pH meter is soaked in 20 ml of brine (salt water more) artichoke, olives and carrots contained in a 100 ml Erlenmeyer flask. The pH value is taken directly from the screen of the same apparatus mentioned above (thermocouple : Crison Micro pH 2000). The standards used are pH 4 and 7.

About 10 ml of brine contained in a 100ml Erlenmeyer flask are added 4-5 drops of phenolphthalein indicator solution 1%. The titration is carried out with a solution of NaOH (N / 9) until the indicator changes to pink cnoloré. The titratable acidity is expressed as a percentage of lactic acid (MW = 90.08 g) in 100 ml of brine. It is given by the following formula:

$$\% \text{ Lactic acid} = \frac{\text{Vol (NaOH)} \times \text{N (NaOH)} \times (90.08) \times (100)}{1000 \times (\text{weight of sample})}$$

A microbiological analysis

Microbiological analyzes were made on the fermented product concerned the flora of hygienic (the FMAT, fecal coliforms) and that of biotechnological interest (lactic acid bacteria and yeasts).

Flora hygienic interest

Enumeration of total aerobic mesophilic flora : FMAT

Counting FMAT provides information on the overall bacterial load biotope artichoke marinade. Strong support is an indicator of the degree of infection or putrefaction of the raw material (artichoke). The TSA [15g tryptone, 5g Soytone, 5g of sodium chloride,15g Agar, 1000ml of distilled water and the pH = 7.3 ± 0.2] (is our medium of choice for the enumeration of the FMAT. The culture media were inoculated and incubated at 30°C for 48 h.

The seedings are made from dilute solutions of brine. These are prepared using a methodology dilution ranging from 10 to 10.And, by including 1ml of the solution diluted in 9 ml of saline. From the chosen dilution, 1 ml was seeded at the bottom of a sterile box, where 20ml middle ASD, previously sterilized at 120 ° C for 20 min and cooled to 45-50 ° C, were poured aseptically. Just boxes with the number of colonies is between 30 and 300 are maintained for the enumeration.

Enumeration of fecal coliforms

The total and fecal coliform bacteria are common in the digestive tracts of humans and / or animals. Their existence in a medium is evidence of the degree of contamination of the sample analyzed and lack of hygiene compliance in the sample medium. The culture medium we used to accomplish this activity is the DLA (Desoxycholate Lactose Agar).

[10g peptone, 10g Lactose Desoxycholate 1g sodium, 5g of sodium chloride, 2 g K2HPO4, 1 g of iron citrate, 1 g of sodium citrate, 15g Agar, 0.03 g Neutral red, 1000ml distilled Water and pH = 7.3 ± 0.2]

Seeding coliform is carried out thoroughly. The incubation was performed at 37 ° C for total coliforms and 44.5 ° C for fecal coliforms. The reading is made after 3 days. Similarly, only the boxes where the number of colonies is between 30 and 300 are used for counting.

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Flora of biotechnological interest

Enumeration of yeasts

Enumeration of yeasts was performed on PDA (potato dextrose agar) medium. Serial dilutions are made. Seeding is done as before. Incubation is carried out at 30 ° C for 48 hours. The colony count is limited to clubs whose number is between 30 and 300.

Enumeration of lactic acid bacteria

The count of lactic acid bacteria is performed on MRS medium (ManRogosa and Scharp). 1 ml of the dilution chosen is deposited at the bottom of the Petri dish. It is covered by the following 20ml of medium previously sterilized and cooled to 45 ° C. After homogenization, the cultures were incubated at 30 ° C. The counting is done after 3 days of incubation and just boxes with the number of colonies is between 30 and 300 are used for counting.

Isolation, purification and identification of lactic acid bacteria

Isolation and purification

Isolated colonies, clear, simple and individualized are transplanted to the surface of new culture media MRS. A series of transplanting is subsequently triggered until the strain is macroscopically and microscopically homogeneous and identical. Generally, after four successive passages, the colony is presumed pure.

Preliminary identification

a. Macroscopic examination

Milky white or brown colonies, who regularly appear on the MRS are marked at the bottom of the box and retained for further identification.

b. Microscopic examination

Microscopic observation is made in the fresh state and set state. The two complementary observations allow us to determine the shape, size, grouping, mobility and the type of Gram characteristic of each strain to identify.

c. Gram stain

Gram staining allows differentiation between strains Gram-positive and Gram- negative ones. The aliquots are examined by the conventional technique of using gram crystal violet,Lugol, alcohol and acetone fushine. In some cases, it was difficult to distinguish between gram- positive and gram-negative bacteria us. We remedied by suspending colonies in drops of 5% KOH. The test is positive when the drop of KOH becomes viscous, otherwise it is negative.

d. Search catalase

Catalase test is made by emulsifying a bacterial culture in a drop of oxygen peroxide (H2O2) at 30 g / l placed on a slide object. The reaction is positive when it results in the release of gas bubbles (O2). Lactic acid bacteria are generally catalase -negative.

e. Gas production (CO2)

Gas production is highlighted by the following method : a central seed strain considered along the solid culture medium MRS tube. This is covered with a layer of sterile water agar. The presence of gas in the medium results in the appearance of gas bubbles, or gas pockets by a detachment of the agar.

The test gas production allow us to know the type of fermentative metabolism borrowed by the strain studied. The homofermentative pathway results in the unique production of lactic acid. Otherwise next to lactic acid is other organic acids, alcohols and gases such as CO2, SH2.

f. Production of the bacteriocin

Bacteriocin production was noticed by the inhibitory ability of the filtrate

Microorganism tested on the development of the target organism[3] (Benkerroum et al., 1993). The six strains of lactic acid bacteria were tested for antibacterial activity by the method of dissemination[4] (Barefoot and Klaenhammer 1983), agar TSA (Tryptic Soy Agar, Difco, Detroit, USA).

Isolation, purification and identification of yeast

Isolation and purification

Isolated colonies, clear, simple and individualized are transplanted to the new surface environments PDA (potato dextrose agar) cultures. A series of transplanting is subsequently triggered until the strain is macroscopically and microscopically homogeneous and identical. Generally, after four successive passages, the colony is presumed pure.

Preliminary identification

a. Macroscopic examination

The pasty white or brown colonies, which appear regularly in surface culture on PDA medium, or colonies in the shape of an eye fish culture depth, are marked at the bottom of the box and retained for further identification.

b. Microscopic examination :

Microscopic observation is made in the fresh state and set state. The two complementary observations allow us to determine the shape, size, grouping, and mobility. The presence or absence of mycelium and sporulation ability by staining with methylene blue after incubation at 30 ° C for 2 weeks on acetate medium

B. Technical and analytical tests conducted controlled lactic fermentation

1. Composition close

The choice of enzyme is mainly based on the following criteria:

- strain preferably homofermentary ;
- Strain major acidifying power ;
- Strain pronounced antibacterial activity ;
- strain accompanied by an intrinsic or extrinsic production of dietary supplements;
- strain accompanied by intrinsic or extrinsic growth factor production.

Selecting strains SMLB1 stopped on lactic acid bacteria and which are isolated SMLB2 brine carrot. The yeast is selected from the olive brine. Multiple subcultures were performed. The result is the production of three finely pure strains. Different combinations thereof, are used to get the most appropriate combination of our fermentation.

2. Choice close

Six solutions have been proposed to define the close fit with our mission. The solutions are semi-synthetic liquid media culture, which are added unique strains or combined (TABLE 1). Incubation of each solution is carried out at 30 ° C for 24 hours. On each periodic samples an hour, three analyzes were performed : DO, pH and acidity. The solution or combination having given the lowest pH is close adequate for us.

TABLE1 : The différent combinations of the ferment

Solution 1	Synthetic medium+strainlacticSMLB1
Solution 2	Synthetic medium+strainlacticSMLB2
Solution 3	Synthetic medium+strainlacticSMLB1+SMLV1
Solution 4	Synthetic medium+strainlacticSMLB1 + SMLV1
Solution 5	Synthetic medium+strainlacticSMLB2 + SMLB1
Solution 6	Synthetic medium+strainlacticSMLB2 + SMLB1+ SMLV1

3. Preparation of closing

The preparation of enzyme is the determination of the amount of the starter to start the fermentation properly controlled. To do this, different percentages of the solution 6 were prepared: 0.25%, 0.50 %, 0.75 %, 1%, 1.25%, 1.50%, 1.75 % and 2 %.

Different percentages were made in the middle of semi- synthetic culture and a final volume of 10ml. This will be the preculture culture 100ml of semi- synthetic medium.Then, cultures cascade will be made until the final volume of 1 liter. All incubations were done at 30 ° C for 24 h. On the final culture, only the pH was monitored. The percentage making us the lowest final pH will be selected for further testing.

Effect of adding sugar to the proliferation of cloe

The same medium described above is added yet different concentration of glucose will 1à7g / l. All solutions were incubated at 30 ° C for 24 h, the addition of sugar is made to promote the growth of the ferment.

Effect of adding extracted starch on the proliferation of close

The same medium described above is added yet different concentration of extract of starch will 1à7g / l. All solutions were incubated at 30 ° C for 24 h, uploading extract starch is carried out to favor propagation of the ferment.

4. Controlled fermentation

To pass the test controlled fermentation artichokes in three buckets of 1kg each, we will work with the lowest amount of enzyme that gives the lowest pH. These conditions have been met with a combination 1%. The latter is used in our midst to lead the fermentation trials artichokes in 3 buckets of 1kg each. The tests are applied to laboratory scale.

a. Effect of brine on the proliferation of close

The medium was supplemented with 15 % salt. It

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is sterilized at 120 ° C for 15 minutes. Seeding the ferment was made at 1 %. The goal is to verify the behavior of our face close to industrial salt concentration.

5. Application tests

Application tests were performed to verify and validate the degree of involvement of our close in biological conservation artichokes. The evaluations will be on semi pilot and test pilot.

Semi pilot and pilot testing

a. Preparing brine

salt 15%, glucose 4 g / l, the starch content to 4 g / l and close to 1 %. The volume of brine produced is equal to 1/3 of the final volume of the product to be preserved.

b. Semi pilot

About 10 kg of artichokes carefully placed in a bucket of 25 liters, are cast corresponding liters of brine. Monitoring of physical, chemical and microbiological analyzes will be performed on the mixture.

c.pilot

About 180 kg of artichokes was carefully placed in a 250-liter are cast corresponding liters of brine. Monitoring of physical, chemical and microbiological analyzes will be performed on mixture.

d. Physico chemical and microbiological analyzes

Are performed in the same way that précédament.

Test in jars

a. training

Artichokes are peeled (picture 3), cut into pieces (picture 4), washed and placed in jars, 21 cl glass (Picture 6). Their deposition is carried out so as to occupy two thirds of the total volume. They are then covered with a liquid called coverage (Picture7). The latter is hot cast (60-70°C) to fill one third of the remaining volume. The liquid medium is an organic liquid plus the close selected in the previous chapter. Artichokes are immersed in the solution coverage. The space between the liquid medium and the cover is equal to 1cm. And this is done to create the vacuum. The jars are closed manually by new metal capsules. The filled jars are pasteurized at a temperature of 100°C for 10 min (picture 8). They are heat shock through rapid cooling. Finally, a test of safety and stability is achieved through incubation jars in an oven at 30°C for 48 hours.

picture 1: cuting external leaves

piture 2 : cutting bases artichoke

picture 3 : artichoke heart

picture 4 : cutting heart artichoke

picture 5 : empty jar

picture 6 : artichoke pieces in a jar

picture 7 : artichoke pieces immersed in the coverage solution

picture 8 : artichoke pieces in closed jar and pasteurized

b. Evaluation of the hygienic quality of the product

Liquid packing

After stabilization of osmotic phenomena in contact with three biological elements (water, salt and artichoke), physico- chemical and microbiological analyzes were performed on the liquid medium. They are conducted on the third day succeeding the sterilization operation.

In addition to the sensory analyzes, analyzes are cited as previously.

Organoleptic analysis

The sensory analysis (texture, taste and smell) are treated as one based on a Likert scale of 5 levels survey (1 : not at all, 5: quite) given to 20 tasters to judge the acceptability of our product.

RESULTS AND DISCUSS

1. Flora of biotechnological interest

The results recorded (TABLE 2) show that the concentration of 5 % salt, lactic acid bacteria are represented with a population of 6103 cfu / ml on the 5th day of fermentation. The abundance increases to 7.106 cfu / ml on day 10 of fermentation.

Regarding yeast, it should be noted that the dominance of yeast was observed at the 25th day of fermentation. The abundance of the population reached 3107 cfu / ml for yeasts against 6.106 cfu / ml for lactic acid bacteria.

The results indicate that in the case of a 10% brine of salt, lactic acid bacteria appear the 5th day of fermentation with a rate of 5.103 cfu / ml and a rate of 4.103 cfu / ml after 35 days.

A 15 % salt, lactic acid bacteria are present in the brine artichoke with an abundance of 5.10^3 cfu / ml at the fifth day of fermentation. She has hardly changed even at the 35th day of fermentation. (The abundance of 5103 cfu / ml).

A 30 % salt, we noticed a total absence of lactic acid bacteria along the fermentation period. Yeasts begin to appear soon on the 10th day of fermentation with a population of 2102 cfu / ml. She rose to 2.104 cfu / ml on the 20th day thereafter down to 3.102 cfu / ml on the 35th day of fermentation.

TABLE 2 : Monitoring of changes in parameters: pH, acidity, temperature and changes in MTAF crops grown on TSA at 30°C for 48 h, cultures of coliform performed on DLA medium at 37°C for 48 h, yeast cultures performed on middle PDA at 30°C for 48 h and lactic cultures performed on MRS medium at 30°C for 48 hours during the spontaneous fermentation of artichokes. Culture performed at room temperature (the unit of count for FMAT,coliform, yeast is lactic acid is cfu / ml).

Sampling time	temperature		5%	10%	15%	30%
5days	25°C	Ph	6,31	6,22	6,52	6,85
		Acidity	0,28	0,32	0,20	0,08
		FMAT	3.10^3	3.10^3	2.10^2	2.10^2
		Coliforms	0	0	0	0
		yeast	3.10^4	3.10^3	2.10^3	2.10^2
		lactic	6.10^3	5.10^3	5.10^3	0
10days	18°C	Ph	5,76	5,75	5,83	5,86
		Acidity	0,48	0,48	0,44	0,44
		FMAT	3.10^6	3.10^6	2.10^6	3.10^2
		Coliforms	0	0	0	0
		yeast	2.10^5	2.10^4	2.10^3	2.10^2
		lactic	7.10^6	6.10^6	7.10^6	0
15days	19°C	Ph	5,54	5,50	5,63	5,72
		Acidity	0,60	0,56	0,52	0,48
		FMAT	3.10^6	3.10^6	3.10^6	2.10^2
		Coliforms	0	0	0	0
		yeast	4.10^5	2.10^4	2.10^3	2.10^3
		lactic	7.10^6	5.10^6	6.10^6	0
20days	19°C	Ph	5,46	5,58	5,66	5,71
		Acidity	0,60	0,54	0,52	0,48
		FMAT	3.10^7	3.10^7	2.10^5	2.10^3
		Coliforms	0	0	0	0
		yeast	3.10^7	2.10^7	2.10^7	2.10^4
		lactic	7.10^6	5.10^6	6.10^6	0
25days	20°C	Ph	5,24	5,33	5,32	5,45
		Acidity	0,68	0,64	0,64	0,60
		FMAT	2.10^6	2.10^5	2.10^5	2.10^2
		Coliforms	0	0	0	0
		yeast	3.10^6	4.10^6	3.10^6	2.10^3
		lactic	6.10^5	4.10^5	5.10^5	0
30days	19°C	Ph	4,55	4,57	4,57	4,78
		Acidity	0,92	0,92	0,92	0,80
		FMAT	9.10^4	8.10^4	2.10^4	1.10^2
		Coliforms	0	0	0	0
		yeast	4.10^5	3.10^5	3.10^5	2.10^3
		lactic	2.10^4	4.10^4	2.10^4	0
35days	24°C	Ph	4,58	4,57	4,58	4,78
		Acidity	0,92	0,92	0,92	0,80
		FMAT	8.10^3	4.10^2	2.10^2	3.10^1
		Coliforms	0	0	0	0
		yeast	4.10^3	3.10^3	4.10^3	3.10^2
		lactic	5.10^3	4.10^3	5.10^3	0

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Isolation, purification and storage of lactic acid bacteria

a. Isolation and purification

We have completed the isolation of lactic acid bacteria from spontaneous fermentations artichoke described above. Isolation took place as from other natural fermentation product. In particular, we include carrots and olives.

12 strains were isolated : 6 yeasts and lactic acid bacteria. The set will be subjected to screening tests to finally find a close with those made with interesting individual characters. To do this, we began by testing purification. The strain is presumed pure after four successive subcultures.

For lactic acid bacteria, each strain through a test three criteria. And to confirm his membership in the group of lactic acid bacteria (macroscopic aspect, type of gram and catalase).

A second selection criterion was adopted to further reduce the number of lactic acid bacteria ferment the component: the acidifying power, fermentation and antimicrobial. Two strains (SMLB1 and SMLB2) showed a strong acidifying and antibacterial. Their individualized by these characters, we were able to detain them for further work.

The other remaining lactic acid bacteria are low power and low acidifying antibacterial. These include the SMLB3,

SMLB4, SMLB5 and SMLB6.The six strains were selected as a result of a screening pushed. But for technical reasons, we decided to reduce the number of strains used in the fermentation controlled trials. The decision was made for SMLB1 and SMLB2.

b. Preliminary identification

SMLB1 the lactic acid bacteria are isolated and SMLB2 brine carrots. They are chosen for their high acidifying power. The color of the colony is whitish milky macroscopic observation. Their microscopic observation after Gram stain, allowed us to identify them as gram positive bacteria.

Similarly, microscopic observation

showed that the two strains of bacteria bacilli grouped by 2 or 4 and still. Other tests have shown that the said bacteria are catalase negative and homofermentative metabolism (no gassing in anaerobic test). To differentiate between gender Carnobacterium as Bacillus and Lactobacillus also being shaped bacillus, a certain number of characters has been verified.

SMLB1 the lactic acid bacteria are Lactobacillus and SMLB2. They are not part of Carnobacterium group as they grow at pH 9. This last character is missing groups belonging to Carnobacterium. Similarly, members of the genus Carnobacterium, grow poorly on MR medium containing acetate, while Lactobacillus species are multiplying easily.

The strains and SMLB1 SMLB2 provedthermophilic since grown to 50°C. The strains of the genus Carnobacterium not have this feature. Rather they are psychrophilic.

The SMLB1 SMLB2 and grow in the absence of oxygen (anaerobic test). They do not produce gas. They are therefore homofermentary is to say use carbohydrates to produce most of the lactic acid only. Lactobacillus sp. are of considerable importance in the food industry (close, aroma producers, probiotics...) and in the pharmaceutical industry (includes disorders météorisations, bloated bellies, digestive disorders...)

During fermentation, lactic acid bacteria succession according to their acidotolérance order, genus Lactobacillus is known for its greater adaptation compared to other types of lactic acid bacteria (Pediococcus, Leuconstoc)

c. Identification of lactic acid bacteria

We used the API 20C gallery (Bio Merieux) for further identification of the strain : SMLB1. Identification is carried out according to manufacturer's recommendations. This is Lactobacillus plantarum.

d. Production of bacteriocin and acidifying power

The strains and SMLB1 SMLB2 are chosen for their large acidifying and bactericidal vis-à -vis pathogens power.

Of the six strains of lactic acid bacteria isolated, the two most effective strains (SMLB1 and SMLB2) from brine carrots.

Pathogenic Gram + bacteria are generally more sensitive to the bactericidal effect of lactic acid bacteria[10]. Bacteriocins act by forming pores in the cytoplasmic membrane that cause disturbances of cellular functions[9]

The bactericidal activity of the strain SMLB1 is found exclusively in the culture medium. The bacteriocin is extracellular. Technological point of view, such production is so interesting that it is possible to make industrial production. The energy and cost for its extraction are very economical compared to the membrane or intracellular bacteriocin.

1. Isolation, purification and identification of yeast

Isolation and purification

We have completed the isolation of the yeast from spontaneous fermentations described above. Isolation took place as from other natural product fermentation. In particular, we include carrots and olives.

Strains isolated as mentioned previouslywere subjected to screening tests to finally find a closed compound with those with interesting individual characters.

To do this, we began by testing purification. The strain is presumed pure after four successive subcultures.

Yeast requires a test two criteria. And to confirm his membership in the group yeast candida (The acidifying power and potential high fermentative).

The six yeast strains were isolated and divided into two groups

Group yeast high SMLV1 acidifying power, used for further work

Group yeast low power acidifying SMLV2, SMLV3, SMLV4, SMLV5 and SMLV6

The two groups were kept as a result of a sorting pushed. But for technical logic, we decided to decrease the number of strains used in the fermentation controlled trials. The decision was made for SMLV1.

Preliminary identification

The colonies are pearly white. Microscopic observation showed that the strain is SMLV1 unicellular, ovoid, and shows no mycelium culture on acetate medium revealed that the strain is asporulante. SMLV1 the strain proved it is thermophilic since it stinks grow at 50°C.

2. Composition and selection of close

The two strains of lactic acid bacteria and yeast strain showed a very important acidifying power. The results of analyzes of different combinations (S1, S2, S3, S4, S5 and S6) are shown in (TABLE 3). For each combination, we followed the DO, pH and acidity. It emerges that the most correct combination is the S6 (TABLE 3). Indeed, after 16 h of incubation at 37°C, the test results showed for the S6 a pH of 3.50, an

TABLE 3 : Studies of changing parameters DO , pH and acidity solutions (S1, S2, S3, S4 , S5 and S6) on semi-synthetic liquid medium incubated at 37 ° C for 24 h.

times		kineticsofsixsolutions					
		S1	S2	S3	S4	S5	S6
	OD	0,20	0,20	0,32	0,22	0,22	0,24
2h	pH	6,60	6,64	6,58	0,60	0,60	6,59
	acidity	0,20	0,20	0,25	0,20	0,20	0,20
	OD	0,34	0,35	0,34	0,40	0,40	0,42
4h	pH	6,55	6,56	6,55	6,50	6,50	6,43
	acidity	0,25	0,25	0,25	0,25	0,25	0,30
	OD	0,42	0,55	0,61	0,68	0,67	0,64
6h	pH	6,43	6,34	6,27	6,25	6,25	6,26
	acidity	0,30	0,35	0,40	0,40	0,40	0,40
	OD	0,58	0,57	0,82	1,10	1,20	0,62
8h	pH	6,32	6,32	5,92	5,60	5,50	6,26
	acidity	0,35	0,35	0,50	0,65	0,70	0,40
	OD	1,15	1,22	0,96	1,60	1,72	1,45
10h	pH	5,74	5,52	5,71	5,00	4,83	5,30
	acidity	0,60	0,70	0,60	0,90	1,00	0,80
	OD	1,61	1,60	2,15	2,20	2,32	1,82
12h	pH	5,04	5,05	3,94	3,90	3,84	4,34
	acidity	0,95	0,95	1,21	1,21	1,21	1,16
	OD	2,22	2,23	2,86	2,60	2,60	2,62
14h	pH	4,43	4,42	3,63	3,75	3,75	3,74
	acidity	1,14	1,14	1,22	1,22	1,22	1,22
	OD	2,70	2,80	2,84	2,60	2,60	3,20
16h	pH	3,78	3,72	3,65	3,75	3,75	3,50
	acidity	1,22	1,22	1,23	1,22	1,22	1,24
	OD	2,65	2,75	2,82	2,60	2,60	3,13
18h	pH	3,78	3,74	3,66	3,75	3,75	3,55
	acidity	1,22	1,22	1,23	1,22	1,22	1,24
	OD	2,61	2,61	2,80	2,58	2,62	3,00
20h	pH	3,75	3,76	3,70	3,76	3,74	3,62
	acidity	1,22	1,22	1,22	1,22	1,22	1,23
	OD	2,60	2,58	2,72	2,59	2,61	2,93
22h	pH	3,75	3,77	3,74	3,76	3,75	3,72
	acidity	1,22	1,22	1,22	1,22	1,22	1,22
	OD	2,56	2,42	2,70	2,58	2,59	2,84
24h	pH	3,78	3,82	3,78	3,76	3,75	3,65
	acidity	1,22	1,21	1,21	1,22	1,22	1,23

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optical density of 3.20 and 1.24% acidity.

This can be explained by the timing of the fermentative metabolism of all strains ferment component S6. Positive interactions between yeasts and lactic acid bacteria have been reported in various fermentations[5,6](LeroiRivoire and Hallet, 1993; Halle Drouet and Hallet, 1992).

Preparation ofclosing

It appearsthatthe presence of 1% inoculum S6,thepHdecreases tothe valueof 3.57in the 15thday of fermentation. The acidityin the presence ofthis enzyme(S6) islarge comparedwith thatrecordedwith an inoculum of0.25% and 0.50%. The results obtainedin thisworkare closeto those ofperfectly[2] Bousmaha(2006).The variationsin the values ofmonitored parametersare the result ofthe proliferation ofstrainsadded asstarter. We found thatthe rate of conversionof the raw materialis fasterwhenitis inoculatedwitha concentration of 1%. Theconcentrationsgreater than 1% (1.25% 1.50% 1.75% 2%) were not givenvalues tomake a difference.Statisticalexamination showedthat therewas no significantdifferencebetween 1% andhigher percentages. (TABLE4)

TABLE 4 : Effect ofconcentrationof enzymeon theevolution of the parameterspHandacidityincubated at 37°Cfor 24h.

Times		inoculum concentration in %							
		0,25	0,50	0,75	1,00	1,25	1,50	1,75	2,00
T0 = 0day	pH	6,80	6,80	6,80	6,80	6,80	6,80	6,80	6,80
	Acidity	0,10	0,10	0,10	0,10	0,10	0,10	0,10	0,10
T1 = 5days	pH	5,95	5,92	5,89	5,87	5,76	5,45	5,48	5,46
	Acidity	0,50	0,50	0,55	0,55	0,60	0,75	0,70	0,70
T2 = 10days	pH	4,87	4,76	4,67	4,58	4,37	4,25	4,26	4,28
	Acidity	1,00	1,05	1,10	1,12	1,16	1,18	1,18	1,18
T3 = 15days	pH	4,23	4,20	3,58	3,57	3,58	3,57	3,58	3,58
	Acidity	1,18	1,18	1,24	1,24	1,23	1,25	1,24	1,24
T4 = 20days	pH	4,26	4,26	4,26	3,98	3,98	3,58	3,58	3,58
	Acidity	1,18	1,18	1,18	1,20	1,20	1,24	1,24	1,54

Effect ofthe addition ofsugaron the proliferationofclose.

The evolution of the growtho fourre presentatives close vary depending on theglucose concentration.The results ofall developmentsareinTABLE (5). The expression of thegrowthof starterisdetermined by the changeof pH andacid production. Indeed,it is clear thatthe pH decreasesto very lowvalues with a highacid productionprosglucose concentrationsof 4g/l.He emergesasthe preferred concentrationiscloseby our4g/l.

TABLE 5 : Effect ofthe concentration of thecarbon sourceon the development ofacidityandpHat 37 °C for 24 hoursand1% inoculum

Times		Glucose concentrationin g/L						
		1,00	2,00	3,00	4,00	5,00	6,00	7,00
T0 = 0day	pH	6,80	6,80	6,80	6,80	6,80	6,80	6,80
	Acidity	0,10	0,10	0,10	0,10	0,10	0,10	0,10
T1 = 5days	pH	5,82	5,73	5,97	5,26	5,32	5,35	5,26
	Acidity	0,55	0,60	0,50	0,85	0,80	0,80	0,85
T2 = 10days	pH	4,47	4,27	4,48	4,48	4,36	4,45	4,37
	Acidity	1,14	1,18	1,12	1,12	1,16	1,14	1,16
T3 = 15days	pH	4,24	4,27	3,78	3,58	3,58	3,58	3,58
	Acidity	1,18	1,18	1,22	1,24	1,24	1,24	1,24
T4 = 20days	pH	5,26	5,24	3,76	3,57	3,58	3,59	3,59
	Acidity	0,85	0,85	1,22	1,22	1,22	1,22	1,22

Effect of addingstarchtoextractthe proliferationof enzyme

The evolution of the growth of our components close change depending on the concentration of extracted starch. The consequences of all developments to locate in TABLE 6. The expression of the growth of starter is supplied by the change of pH and acid production. Indeed, it is clearly distinguishable as the pH drops to very low values with a serious acid production concentrations extracted starch 4g / l. He graduated as distinguished by our close concentration is 4 g / l.

TABLE 6 : Effect of extract concentration of starch on the evolution of pH and acidity Incubated at 37 for 24 h and with 1% inoculum and 4 g / l of glucose

Temps		Concentration d'extrait d'amidon en g/L						
		1,00	2,00	3,00	4,00	5,00	6,00	7,00
T0 = 0J	pH	6,80	6,80	6,80	6,80	6,80	6,80	6,80
	Acidité	0,10	0,10	0,10	0,10	0,10	0,10	0,10
T1 = 5J	pH	5,81	5,72	5,96	5,25	5,31	5,34	5,27
	Acidité	0,56	0,61	0,51	0,86	0,82	0,82	0,86
T2 = 10J	pH	4,46	4,26	4,47	4,47	4,35	4,44	4,37
	Acidité	1,13	1,17	1,13	1,13	1,15	1,13	1,16
T3 = 15J	pH	4,25	4,28	3,78	3,58	3,58	3,58	3,58
	Acidité	1,17	1,18	1,22	1,24	1,24	1,24	1,24
T4 = 20J	pH	5,27	5,25	3,75	3,57	3,58	3,58	3,58
	Acidité	0,85	0,85	1,22	1,22	1,22	1,22	1,22

2.controlled fermentation

To initiate our tests controlled artichoke fermentations, we inoculated three buckets each containing 1kg of artichokes and brine with 1 % of the ferment (S6) already selected. The driving conditions cultures are those required by the ferment.

Effect of brine on the proliferation of close

The brine used in our fermentation is 15%. This value is found adequate during spontaneous fermentation we drive. The same concentration was found in the work done[2] Bousmaha(2006). Similarly, other authors have shown through testing that they have done, that the concentration of 15% is most suitable for the conservation of biological products. Concentration adopted by the Technical Standards Committee and approved by the interdepartmental committee Moroccan stop the use of a concentration between 12 and 15 % salt for any purpose of conservation of biological products in unsealed containers.

Of the lower concentrations of these values can give rise to alterations in the biological product and higher concentrations can cause death of the ferment about preservation of our product.

Study of physico- chemical stability of artichokes mixed culture

After mastering the optimum growth conditions of our close, we considered conducting a test called a mixed culture. This is to gather all the preferred settings for the ferment in the same medium. We launched the start of the fermentation monitoring of key parameters. The monitoring results are shown in (TABLE 7). He emerges as the pH decreases rapidly to its minimum value of 3.57 after 1 month of fermentation. This latter value is then kept stable even after 6 months of stabilization. The same is met with the acidity, the value of 1.22% is obtained die in the first month to remain stable until the sixth month of stabilization.

TABLE 7 : Study of the physicochemical stability of artichokes with time

Temperature	Times	pH	Acidity
24°C	T_0	5,24	0,68
24°C	1 month	3,57	1,22
25°C	3 months	3,56	1,23
24°C	6 months	3,57	1,22

The stabilization of key fermentation parameters are indicators of the stabilization of the conservation of the product used for these purposes. Weincludeartichokes.

La stabilisation des paramètres clés de la fermentation sont des indicateurs de la stabilisation de la conservation du produit utilisé à ces fins. Nous citons les artichautsWe note that with the use of enzyme selected, the pH of the mash fermentation reached 3.57 and 1.22% acidity, and from the 30th day of fermentation. The pH is obtained according to the packaging of food products since according[1] ALINORM (07/30/27), it requires that the pH of the solution coverage should not exceed 4.5.

The pH and acidity remain stable even after 6 months of stabilization. For food, the two parameters with the corresponding values obtained in our experiments show a good hygienic quality. Bacterial growth is inhibited under these conditions that the spores and cannot germinate when the pH is below 4. This result confirms the careful selection of strains that can therefore replace the traditional process.

Many authors have shown that an increase in the acidity of the medium, the pH value decreases. Microorganisms when they try to maintain the stable and above the internal pH of the external medium. Homeostatic mechanisms prevent the protons pass through the cell membrane and enter the cytoplasm, as they expel the protons actually entering the cell.

The attempt to repair the disruption of pH homeostasis and energy demand growth rate decreases. In addition, the demand for energy increases, so there is none left for other cellular functions. If homeostasis can no longer occur, the pH of the cell cytoplasm fall and die. The ability of microorganisms to grow at a low pH depends on their ability to prevent the protons enter the cytoplasm. The pH optimum for the development of many bacteria is between 6.5-7.5[8] (Stella Maris Alzamora et al., 2004).

Test in jars

The tests were conducted bioconservation jars. The solution and double pasteurization were performed as reported in Materials and Methods. (TABLE 8) highlights the results of microbiological and physicochemical analyzes control the course of the bioconservation.

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TABLE 8 : Monitoring of changes in parameters: pH, acidity, and temperature changes the FMAT, coliforms, yeasts and lactic acid in the bioconservation artichokes pasteurized, and a salt concentration equal to 1% in jar.

Jar		
Sampling time	Parameter	Value
	Salt	1%
5 days	pH	3,7
	Acidity	1,20%
	FMAT	3.10
	Coliforms	0
	Yeast	$3,5.10^1$
	Lactic	5.10^1
12 months	Salt	1%
	pH	3,7
	Acidity	1,20%
	FMAT	3.10
	Coliforms	0
	Yeast	$3,5.10^1$
	Lactic	5.10^1

Haut du formulaire

Physicochemical and microbiological analyze

a. Change in pH

The pH values of parameter are both 3.7 times control, the 5th day and the 12th months of storage of our product in jars have not changed, and this can be explained by the stability of our product.

b. Variation of acidity

Similarly, the two values of acidity parameter is 1.2, for both controls the 5th day and the 12th month of storage jar our product have not changed, this can be explained by the concentration remains stable the acid released in the middle of our close.

c. lactic acid bacteria and yeasts

Similarly, the results of two tests of the fifth day of the 12th month at the solution to hedge our product in a jar, have shown weak presence of lactic acid bacteria and yeast, this fall in the rate may be due to the same the high temperature pasteurization 100 ° C, which prevented the development and growth of microorganisms.

d. organoleptic analysis

The sensory analysis (texture, taste and smell) are treated as one based on a Likert scale of 5 levels survey (1: not at all, 5: quite) given to 20 tasters to judge the acceptability our product with respect to the consumer, the results are represented as a (TABLE 9)

As shown in TABLE (9), the percentage of tasters who are quite willing to texture is 80 % compared to those who agree. Regarding taste, we found a percentage of 90% of tasters who all agree and enjoyed our preservative.

TABLE 9 : Evaluation of parameters texture, taste andsmell to the artichoke

	Not at all agree	disagree	Neither agree nor disagree	Agree	Strongly agree
texture				4	16
tast				2	18
odor				6	14

As for the smell, 70% of tasters are quite agree and enjoyed the smell of our product. Note that wholes results converge to a single issue to decide that our work is successful, however, the results of which are tasters agreements can be exploited to work on continuous improvement in future work.

We arrive at much more favorable values with the application of our close chose the same product artichoke, at the semi-pilot and pilot testing, the pH reached 3.55 and 1.23% acidity. And from the 30th day of the controlled fermentation. For the jars, the pH reached 3.70 and acidity 1.20%, against the values found by the level of spontaneous fermentation are: pH 4.57 0.92 acidity and for the same period.

CONCLUSION

Strains of lactic acid bacteria were isolated, the two most effective strains (SMLB1 and SMLB2) have mounted a major bactericidal activity.

The bactericidal activity of SMLB1 (Lactobacillus plantarum) is found exclusively in the culture medium, the bacteriocin is extracellular.

Substances released by SMLB1 have been shown to be inhibitory substance of the growth of certain pathogenic strains.

These substances are not bacteriocins and organic acids or oxygen peroxide.

Neutralizing the effect of lactic acid and the elimination of the effect of oxygen peroxide promoted the

activity of antibacterial substances.

The choice of yeast :

A group of yeast high acidifying power and high potential fermentative) SMLV1 was chosen following a triage pushed.

Selecting the close :

The two strains of lactic acid bacteria (SMLB1 and SMLB2) and the yeast strain (SMLV1) have shown a very important acidifying power. Positive interactions between yeasts and lactic acid bacteria showed synchronization fermentative metabolism of all strains ferment component selected. Indeed, after 16h incubation with the ferment S6 was able to stabilize the pH and acidity 3.57 to 1.22 %

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