Changes of Microbial Population and Some Components in Carrot Juice During Fermentation with Selected Autochthonous Lactobacillus Plantarum Strains

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Abstract: Since the great age, lactic acid bacteria (LAB) have taken a part in the human feeding. They participate in many food fermentations. The fermented vegetable products are the base of our study. The use of three starters of *Lactobacillus plantarum* locally isolated for lacto fermented carrot juices elaboration, allowed us to stir up the following results: the lactic acid content of juices is varying, pH values decrease from 6,5 to 3,55 – 3,57. We got a solution to the jellification problem thanks to the lacto fermentation of carrot juice as it was confirmed by the viscosity results. After three days of fermentation, microbiological analysis of juice revealed an average number of *Lb plantarum,* which reach 3 to 6× 10^4 cfu / mL. Finally, the sensory quality of the juices is acceptable.

Keywords: *Lactic acid bacteria, Lb. plantarum, Carrot juice, Lactic fermentation*

Introduction

The single most important development permitting the formation of civilization was the ability to produce and store large quantities of food. A solution of this dilemma is the use of fermentative microorganisms. In fermentation, the raw materials are converted by microorganisms to products that have acceptable qualities of food. In fermented products, lactic acid is produced by the starter culture bacteria to prevent the growth of undesirable microorganisms (Ray and Daeschel, 1993)**.** Microorganisms of genera *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus* and *Pediococcus* are involved in these fermentations (Daly and Davis, 1998)**.** Among, vegetables of industrial interest, the carrot can provide juices that could be preserved by lactic acid fermentation (Salih and Drilleau, 1992)**.** They present advantages of order dietary since the final juice contains little sugar, its content in carotene is identical to the one of the final juice and its content in nitrate is decreased (Andersson, 1985)**.** *Lb. plantarum* is probably the most advantageous of the more commonly used bacteria for the conversion of lactose, sucrose, glucose and fructose to lactic acid because it not only utilizes the sugars with high conversion rates but also utilizes other compounds such as pectin present in plant products. *Lb.plantarum* is often used as a starter culture in the production of fermented commodities such as sausage, cucumber pickles and silage (Fu and Mathews, 1999).

Factors affecting microbial growth are mainly temperature, degree of exposure to air, properties of carrots such as fermentable sugar level, buffer capacity, pH, acidity, natural inhibitory compounds and produced lactic acid amount. These factors affect maximum specific growth (mm) of *Lb. plantarum* in carrot mash fermentation process (Passos et al., 1993). The growth yield is directly related to kinetic parameters of the product formation in the fermentation process. There are very few kinetic studies related to complex natural vegetable medium.

Many lacto fermented products have the reputation to be beneficial for health, because the lactic acid bacteria play a primordial role in the digestive microbial ecosystem.

The aims of this research were in the first; to include tests of manufacture of the fermented carrot juices using the locally strains of *Lactobacillus plantarum* BJ0021, BJ041 and BJ052. In second, it consists in testing pectinolytic activity of the strains in the carrot juices and lastly, to study the effect of the lactofermentation on the stability and the composition of the carrot juices.

Materials and Methods

Carrot

Carrots of variety Muscad of Algeria used in our experimentation were from the region of Mostaganem located in western Algeria. The production of carrot juice was performed according to the method described by Salih and Drilleau (1992)**.** The carrots were washed, peeled, ground and pressed. Carrot juice is pasteurized to 85 °C during 5minutes then cooled and is centrifuged at 30 000 g for 5 minutes. After filtration and distribution into bottles the carrot juice was heated to 80 °C during 2 minutes and stored at -2 °C.

Preparation of inocula and development of fermentation

Strains of *Lactobacillus plantarum* BJ0021, BJ041 and BJ052 used for the controlled fermentation of carrot juice were isolated from traditional Jijelian butter at the Laboratory of Molecular Biology and Genetics, University of Oran, Algeria (Idoui and Karam, 2008).

Mother cultures were applied for the fermentation experiments. By putting the dried starter culture in carrot juice and using the acidified juice as an inoculum, pH 4.6 was reached in approximately 18 hours. The carrots juices were sowed by their inoculums starters and incubated at 35 °C. The lactofermented juices were processed according to the flow diagram shown in Figure 1.

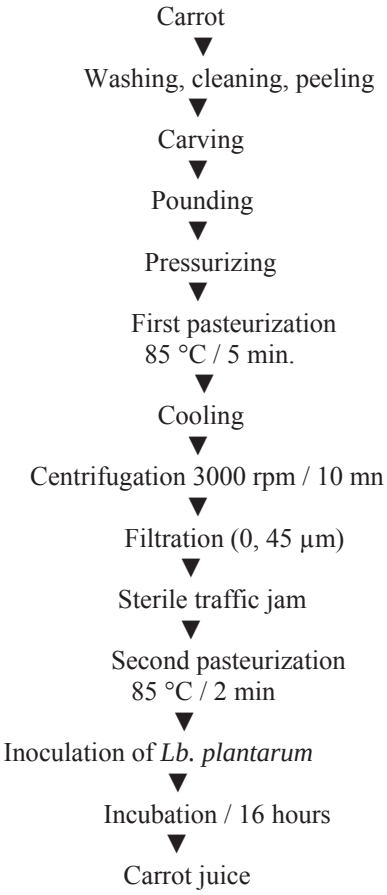

Figure1. Flow diagram of carrot juice production

Analytical methods

The gotten products were submitted to an analysis physicochemical at the first day, the third day and the sixth day. The pH measurement was obtained with a pH meter (HANNA), calibrated with two standard solutions buffered at pH 4.00 and pH 7.00. The total acid values were obtained by titration a volume of juice with a 0.02 N NaOH to pH 8.3. Total dry matter weight was evaluated after evaporation and desiccation of carrot juice for 04 hours at least under steams at 105 °C. Determination of minerals Na^+ K^+ and Ca^{2+} was done as follows. The samples were lyophilized separately. Then 0.8 g of lyophilized samples was mineralized in a microwave oven with concentrated HNO_3. The concentrations of all elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer using the flame method (Gorinstein et al., 2001).

The determination of the viscosity was obtained as follows: a glass ball of density 2.2 was introduced in a small cylinder filled of carrot juice. This ball browses a distance (x) during the time (t); the hold of time (t) is done with the help of a chronometer. This operation takes place in adequate conditions (20 to 25 °C - Pressure 1 atm). The viscosity is determined by the following formula:

$$\eta = K(\tau_1 - \tau_2)t$$

[K is a constant according to the density of the ball (mpa.s. cm^3 / g. s), τ_1 and τ_2 are respectively the ratio mass: volume (g /cm^3) of the glass ball and of the carrot juice. t: The time browsed by the ball between two points].

Microbiological Quality of carrot juice

The numbering of the lactic acid bacteria and the yeasts-moulds has been achieved. *Lb. plantarum* was enumerated on MRS agar. Plates were incubated under anaerobic conditions at 35 °C for 24 Hours (Idoui and Karam, 2008). Total yeasts and moulds were counted on oxytetracycline glucose agar (OGA); the plates were incubated at 25 °C for 3 days to 5 days.

Sensorial evaluation

The carrot juices have been submitted to the appreciation of a jury composed of eight judges. Sensorial evaluation was performed by describing the odor, flavor and appearance of samples. Taste, odor and total effect were evaluated out of 5 points, and color was evaluated out of 3 points. Results were classified as preferable (total points: 15–18), barely acceptable (total points: 11–14), needs modification (total points: 8–10) and not acceptable (total points <7) (Schobinger, 1985).

Statistical Analysis

The results were summed to variance analysis (Newman Keuils at 5 % and 1 %).

Results and Discussion

Physicochemical parameters evolution

According to results of the table1, the total dry matter of the lactofermented carrot juices increases from 61.43 ± 0.12 % to 68.90 ± 0.09 %, 67.50 ± 0.18 % and 65.50 ± 0.09 % with +7.47 %, + 6.07 % and + 4.07 % to the profit of juice fermented by the strains BJ052, BJ0021 and BJ041 respectively, in comparison to the witness juice ($P < 0.05$). With regard to the pH, there is a reduction of this last of pH6.5 to about pH3.55 in juices fermented, whereas it reaches pH 3.86 in the witness's case during period of conservation. Otherwise, the total acidity initially oscillate between 5.85 g / L to 15.50 g / L with *Lb. plantarum* BJ052, 5.95 to 13.70 g / L with BJ0021strain and 6.65 to 13.90 g / L with the BJ041 strain during the manufacture and the conservation. The raw juice knew a fluctuation with a maximum of 6.06 g / L at the third day. There is heterogeneity within the same species ($P < 0.05$), of the point of view of the acidification of the medium. Indeed *Lactobacillus* is a heterogeneous kind in which one can note very appreciable differences of acidifying properties between strains. BJ0021 and BJ052 are good acidifying strains compared to BJ041. The sowed juices presented some superior quantities in lactic acid in comparison to the raw juice. We noticed a decrease of the acidity from the sixth day that can be explained by a contamination by oxidative yeasts that consume the lactic acid, leading to a better growth of contamination micro organisms.

The lactic acid fermentation permitted to have some edible products but with an acidic flavour. On the other hand, after 24 hours of manufacture, the raw juice showed a viscous aspect.

The use of the three *Lb. plantarum* strains permitted to solve the problem of jellification observed with raw juice. The thus gotten fermented products, rich enough in lactic acid, can present several nutritional and therapeutic interests (Aubert, 1981). Within the nutritional aspect, the deterioration of the pectin's carrot juice due to versatile activities led to the enrichment of the carrot juice in simple sugars that may easily been absorbable. In the production of vegetable juices, lactofermentation may also facilitate juice yield. Lactic starters such as *Lb. plantarum* can produce pectolytic enzymes such as polygalacturonase, pectinlyase and pectinesterase (Karam and Belarbi, 1995).

Otherwise, *Lb. plantarum* BJ0021 lowered the pH to 3.81 at the end of three days, ideal pH permitting to offer juice a pleasant, acidic and flawless flavour of odour .Our observations were also about the speed of lowering of the pH, *Lb plantarum* BJ0021 manages to lower the pH of the carrot juice to pH 3.81 at the end of three days. This value is very interesting on the technological plan because according to Salih and Drilleau (1992) the ideal pH that permits to offer the carrot juice a pleasant acidic and flawless flavour of odour is pH3.7. To improve the flavour of juice fermented it is necessary to search for other heterofermentative strains (Salih and Drilleau, 1992), in the same way the addition of salts or spices improves the flavour of vegetables lactofermented juice.

The table 2 shows that the viscosity of the raw juice increases to reach 1848 Cps. In industry of fruit juice and vegetables this kind of defect prevents the commercialization of the product. On the other hand, the inverse effect ($P < 0.01$) is observed with juices fermented with –30.8, -23.1 and-3.85 Cp to the profit of products fermented respectively by *Lb.plantarum* BJ052, BJ0021 and BJ041 and compared to the juice witness. The cellular partition of carrot understands more of 80 % of polysaccharides composed of pectin (45 %) highly methyl to fast jellification. The final phase of the jellification is characterized by a system biphasic. This result is in agreement with the works of Salih and Drilleau (1992). In the other although pectin of carrot become a jell in presence of

sucrose, and in acidic environment, the results show an absence of the jellification in small bottles of juice fermented it is related probably to a pectinolytic activity of these bacteria.

Table 1. Evolution of some physicochemical parameters of the lactofermented carrot juice and witness

Carrot juice		Dry matter (%)	pH	Lactic acid (g/ L)
Raw Juice	-	61.43 ± 0.12	6.50 ± 0.00	0.30 ± 0.00
	16 hours	61.60 ± 0.12	5.10 ± 0.00	0.71 ± 0.07
	1 day	62.43± 0.20	5.04 ± 0.00	1.31 ± 0.07
Witness	3 days	62.30± 0.10	4.24 ± 0.00	6.06 ± 0.02
	6 days	62.13± 0.20	3.86 ± 0.01	2.90 ± 0.00
	16 hours	63.80 ± 0.24	4.42 ± 0.00	5.75 ± 0.01
	1 day	64.90 ± 0.24	4.32 ± 0.00	5.80 ± 0.01
Lb.plantarum	3 days	67.90 ± 0.23	3.70 ± 0.00	14.00 ± 0.05
BJ052	6 days	68.90 ± 0.09	3.60 ± 0.00	15.50 ± 0.04
	16 hours	62.80 ± 0.24	3.91 ± 0.02	5.85 ± 0.02
	1 day	62.90 ± 0.15	3.81 ± 0.02	5.95 ± 0.02
Lb.plantarum	3 days	63.20 ± 0.08	3.52 ± 0.00	13.57 ± 0.04
BJ0021	6 days	67.50 ± 0.18	3.50 ± 0.01	13.70 ± 0.00
	16 hours	61.80 ± 0.24	4.65 ± 0.00	5.65 ± 0.00
	1 day	61.90 ± 0.21	4.35 ± 0.00	6.65 ± 0.00
Lb.plantarum	3 days	65.80 ± 0.23	3.76 ± 0.00	7.40 ± 0.00
BJ041	6 days	65.50 ± 0.09	3.57± 0.00	13.90 ± 0.04

Table 2. Evolution of viscosity, some mineral content in carrot juice samples

Carrot juice		Viscosity (Centipoises)	Elements (mg/ 100 mL) Na^+	K^+	Ca^{++}
Raw Juice	/	192.50	100 ± 1.21	180 ± 1.21	46 ± 1.21
	1 day	192.50	ND	ND	ND
Witness	3 days	261.03	ND	ND	ND
	6 days	1848.00	ND	ND	ND
	1 day	161.70	93 ± 1.21	103 ± 1.21	32 ± 1.21
Lb.plantarum	3 days	145.53	92 ± 1.21	103 ± 1.21	30 ± 1.21
BJ052	6 days	134.75	84 ± 1.21	98 ± 1.21	10 ± 1.21
	1day	169.40	100 ± 1.21	114 ± 1.21	32 ± 1.21
Lb.plantarum	3 days	147.84	95 ± 1.21	104 ± 1.21	30 ± 1.21
BJ0021	6 days	146.30	63 ± 1.21	84 ± 1.21	10 ± 00
	1 day	188.65	96 ± 1.21	111 ± 1.21	36 ± 1.21
Lb.plantarum	3 days	154.00	92 ± 1.21	103 ± 1.21	34 ± 1.21
BJ041	6 days	134.75	68 ± 1.21	85 ± 1.21	10 ± 00

ND: not determined

Evolution of microbial parameters

The middle number of cells *Lb plantarum* BJ0021, BJ041 and BJ052 reaches $3.7 \cdot 10^6$ CFU / mL of juice at the third day (table 3). This number is lower than the one found by Salih and Drilleau *(3)* with species of *Lb. plantarum* after 48 hours of incubation of carrot juice ($1.2 \cdot 10^9$ to $8 \cdot 10^8$ CFU/ mL) and it is also lower than reported by Demir et al. (2006). At the sixth day, the number of cells for the strains decreased, due to an inhibitory effect of the acidity on bacterial growth. This growth inhibition was primarily attributed to the protonated lactic acid form. Yabannavar and Wang (1991) showed that the growth inhibitory effect by lactate is small when compared with that by

uncharged lactic acid. Mc Donald et al**.** (1990) showed that the internal pH of *Lb. plantarum* is lowered when the cells are exposed to acid conditions.

The research of yeasts and moulds in the juices of fruits and the juices of vegetables proved to be necessary, because they may cause accidents of manufacture, deterioration of taste, inflation and bad presentation. The juices from raw or lactofermented carrot were unscathed of all fungal contamination at the beginning of manufacture. Nevertheless a contamination by reddish colored yeasts appeared in the juice witness after 24 hours. The use of lactic acid bacteria or « lactic starter » is interesting because it assures the fast and complete conversion of sugars in lactic acid and therefore prevents increase of bacterial contamination that often produce some undesirable substances (Salih and Drilleau, 1992)**.**

The sensory quality

The colour of the raw juice begins to deteriorate after the second day of fermentation. It may be explained by the formation of biphasic system related to the apparition of two layers, the serum and the frost. The three fermented juices took the same pace, and therefore the fermentation process permitted to offer juices a good colour. Really the colour of the carrot juice fermented by *Lb. plantarum* BJ0021 was more appreciated. A bad odour was recorded of the raw juice from the second day; it gets worse at the third day where it reaches a middle value of answers of 2 (table 4). The carrot juices fermented gave a better odour; the one fermented by the BJ052 got the best score. It can be explained by the synthesis of aromas by this bacterium.

Table 3. Growth of *Lb. plantarum* strains and numeration of yeasts and moulds in carrot juice samples

Carrot Juice		Lactic acid bacteria (cfu / mL)	Yeasts and Moulds(cfu/ mL)
Witness	-	00	00
Raw Juice	1 day	00	6.0×10^{2} (Red colour yeast)
	3 days	00	7.8×10^{3} (Red colour yeast)
	6 days	00	9.0×10^{4} (Red colour yeast)
	0 hours	2.0×10^{4}	00
	16 hours	3.0×10^{5}	00
	1 day	6.0×10^{5}	00
Lb.plantarum	3 days	6.1×10^{6}	00
BJ052	6 days	3.9×10^{6}	00
	0 hours	2.1×10^{4}	00
	16 hours	2.0×10^{5}	00
Lb.plantarum	1 day	3.0×10^{5}	00
BJ0021	3 days	5.5×10^{6}	00
	6 days	4.2×10^{6}	00
	0 hours	2.4×10^{4}	00
	16 hours	3.0×10^{5}	00
Lb.plantarum	1 day	4.0×10^{5}	00
BJ 041	3 days	5.4×10^{6}	00
	6 days	2.7×10^{6}	00

Table 4. Evolution of organoleptic quality of the carrot juice samples

Carrot juice		Organoleptic aspect
Raw Juice	-	Odour of carrot, sweetened flavour, good colour.
	1 day	Odour of carrot, sweetened flavour, good colour.
Witness	3 days	Viscous aspect, deterioration of these qualities after days with formation of frost.
	6 days	Viscous aspect. deterioration of the quality

	1 day	Pleasant odour, lightly acidic flavour, good colour.
Lb.plantarum	3 days	Acceptable flavour.
BJ052	6 days	Unpleasant odour, too acidic flavour, altered colour.
	1 day	Odour with bottom milkman, lightly acidic flavour
Lb.plantarum	3 days	Pleasant odour, flavour little acidic, change of colour
BJ0021	6 days	Change of the three aspects.
	1 day	Good odour and flavour, acceptable colour.
Lb.plantarum	3 days	Good odour, flavour lightly acidic, colour acceptable.
BJ041	6 days	Acidic odour, acidic flavour, altered colour.

Conclusions

The use of the lactofermentation process permitted to solve the problem of the jellification observed in raw carrot juice. The use of lactic acid bacteria as starter culture has also been found to be effective on yield of lactofermented carrot juice. *Lb. plantarum* as a starter culture produces pectolytic enzymes, and these can cause the softening of vegetable tissues and can increase the juice yield.
Under the experimental condition chosen, it was assumed that a fermentation time of 16 h can be recommended for a pH value under 4.5. From the point of total acidity, soluble solids and viscosity, the produced carrot juice was acceptable.

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References

Andersson, R. 1985. Nitrate reduction during fermentation by gram negative bacterial activity in carrot. Journal of Food Microbiology, 2, 219-225.
Aubert, S. 1981. La carotte (Daucus carota L), Revue de quelques facteurs d'intérêt diététique. Cahier de Nutrition et Diétetique, 16, 173-186
Daly, C. Davis, R. 1998. The biotechnology of lactic acid bacteria with emphasis on applications in food safety and human health. Agriculture and Food Science, 7, 251-264.
Demir, N. Savas Bahçeci, K. Acar, J. 2006. The effects of different initial *Lactobacillus plantarum* concentrations on some properties of fermented carrot juice. Journal of Food Processing and Preservation, 30, 352-363.
Fu, W. Mathews, A.P. 1999. Lactic acid production from lactose by *Lactobacillus plantarum*: Kinetic model and effects of pH, substrate,and oxygen. Biochemistry Engineering Journal, 3, 163–170.
Gorinstein, S. Zachwieja, Z. Folta, M. Barton, H. Piotrowicz, J. Zemser, M. Weisz, M. Trakhtenberg, S. Martin-Bellosso, O. 2001. Comparative Contents of Dietary Fiber, Total Phenolics, and Minerals in Persimmons and Apples. Journal of Agriculture and Food Chemistry, 49, 952-957.
Idoui,T. Karam, N.E. 2008. Lactic acid bacteria from Jijel's traditional butter: Isolation, identification and major technological traits. International Journal of Fats and Oils, 59, 361-367.
Karam, N.E. Belarbi, A. 1995. Detection of polygalacturonases and pectin esterases in lactic acid bacteria. World Journal of Microbiology and Biotechnology, 11, 559-563.
Mc Donald, L.C. Fleming, H.P. Hassan, H.M. 1990. Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. Applied Environment Microbiology, 56, 2120-2124.
Passos, F.V. Fleming, H.P. Ollis, D.F. Hassan, H.M. Felder, R.M. 1993. Modelling the specific growth rate of *Lactobacillus plantarum* in cucumber extracts. Applied of Microbiology and Biotechnology, 40, 143-150.
Ray, B. Daeschel, M. 1992. Food biopreservatives of microbial. In: Boca Raton, CRC Press Florida.
Salih, A.G.Drilleau, J.F.1992. Jus de carotte lactofermenté Quel avenir ?. Cahier Scientifique et Technique, 109, 7-9.
Schobinger, U. 1987. Frucht-und Gemuesesaefte, 2. Auflage. Verlag Eugen Ulmer, Stuttgart, Germany.
Yabannavar, V.M. Wang, D. I. 1991. Analysis of mass transfer for immobilized cells in an extractive lactic acid fermentation. Biotechnology Bioengineering, 37, 544-550.