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Microbial, Chemical, Textural and Organoloptic Properties of Iranian Fermented Cucumbers by *L. plantarum*

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Cucumber fermentation is a natural process that lactic acid bacteria are effective in quality of product. In this assay we put the cucumbers in brine with 5% and 7% NaCl (w/v) that inoculated with different values of specific *L. plantarum* strain and analyzed for microbial and physicochemical characteristics. The maximum numbers of *L. plantarum* strain was 8.48-8.57 $\log_{10}$ cfu/ml in 5% NaCl solutions with $6x10^8$ cfu/ml bacterial inocula but the numbers of yeasts was 7.21-7.24 $\log_{10}$ cfu/ml in the 9th day. In high inoculated 7% NaCl solution the lower numbers of aerobic mesophilic was 6.84 $\log_{10}$ cfu/ml in this day. The value of pH 3.42 and titratable acidity 0.58% observed in high inoculated 5% NaCl solution in the 15th day. During the storage, the numbers of *L. plantarum* (4.85-6.61 $\log_{10}$ cfu/ml), yeasts (5.21-6.48 $\log_{10}$ cfu/ml) and aerobic mesophilic (5.03-6.19 $\log_{10}$ cfu/ml) at 25°C were higher than at 4°C. In the inoculated 7% NaCl solution, pH and titratable acidity was 4.08-4.33 and 0.19% in 30th day at 4°C. In the 15th day of fermentation, the samples in high inoculated 7% NaCl solution had a lower hardness and l*, a*, b* value and the highest rank of flavor. In this assay, the growth of pathogenic organisms was not observed in the samples.

Key words: *L. plantarum*; Iranian fermented cucumbers; microbial and physicochemical characteristics.

Fermentation is one of the oldest forms of food preservation that can increase the shelf-life, sensory and nutritional properties of foods. A kind of fermented vegetables is cucumber fermentation that is popular throughout the world[1]. Cucumbers are members of the cucurbit family that are related to gourds, gherkins, pumpkins, squash, and watermelon. The first type was selected in the 1700s, and later applied in Europe[2]. Cucumbers are an important commercial and garden vegetable that China, Turkey, Iran, Russia, and the United States are the important cucumber producing countries[3]. Fermented cucumber, like other natural fermented vegetables, is a traditional lactic acid fermentation, which correlate to microorganisms presence in the raw material[4]. Lactic acid bacteria, yeasts and a variety of contaminating microorganisms from different sources acted together in the cucumber fermentation[5]. When lactic acid bacteria were more than yeasts, lactic acid fermentation is favored for a product with a lower pH that is desirable in naturally vegetables fermentation[6]. LAB is very important because they have appropriate strains for using as starter culture[7]. In this regard, industrial countries used starter cultures, particularly for large scale of food fermentation[8]. In some vegetables fermentation, the produce of lactic acid is not adequate and so spoilage occurs by other microorganisms[4]. A kind of LAB strains is *Lactobacillus plantarum* that in the traditional process its growth in the fermented brines is

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essential to provide the amount of lactic acid that needed for preservation of products[9]. The use of suitable *L. plantarum* starter has the potential to improve the microbiological control of the process and increase the scale of lactic acid[4,10]. *L. plantarum* often becomes the predominant species therefore this is one of the choice starters for vegetables fermentation such as table olives[11] or sauerkraut and cucumber[12]. In this regard, it is difficult to achieve more uniform products in natural fermentations so in this study we survey the use of suitable *L. plantarum* starter culture that isolated from Iranian vegetable fermentation to improve the control of cucumber fermentation process with high quality.

MATERIALS AND METHODS

Cucumber samples

The samples of cucumber were supplied from different places of Iran. After washing, they were put in glass vessels (450 g) containing fresh brine that was prepared by following: The fresh brine was contained 5% and 7% (w/v) NaCl (99% purity) and food grade acetic acid (0.18 N) to reach the pH 4. All of the brine samples were heated at 73 ^{0}C for 10 min and chilled up to 30^{0}C. The cucumbers were put in glass vessels and filled with different prepared brines (300-350ml) in sterile condition.

Preparation of bacterial inocula for cucumber fermentation

We used *L. plantarum* strain that were isolated from Iranian cucumber fermentation according to PCR methods with primers LPL-1/LPL-2 and sequences of GAAACCTACACAC TCGTCGA/CCTGAACTGAGAGAATTTG that was used as PCR primers for the specific detection of *L. plantarum*[13]. PCR mixture and DNA were heated at 95 ^{0}C for 5 min by a thermal cycler that contained 35 PCR cycles. For each PCR cycle, denaturation, annealing, and extension were carried out at 94 ^{0}C for 1min, 58 ^{0}C for 1min and 20s, 72^{0}C for 1min and 20s, respectively. Final extension was carried out at 72^{0}C for 7min. To detect the amplified product, 14 µl of the PCR product was examined by electrophoresis through 1% agarose gel in 20ml of 1×TBE buffer (10×TBE: 27 g/250ml Tris, 13.91 g/ 250ml Buric acid, 1.86 g/250 ml EDTA) [Modified of 13]. This strain was kept in glycerol tubes at -80 ^{0}C. In the first, 2 tubes were activated in 20 ml sterile broth MRS and incubated at 25^{0}C for 24 h. The culture media was centrifuged at 3000 rpm, and after outpouring of the MRS broth, the sediment of culture media was resuspended in 150 ml sterile fresh saline 4.5% w/v NaCl, that adjusted with acetic acid for pH 4 and then incubated at 25^{0}C for 24 h that starter culture adapted to the saline environment of the brine[6].

Inoculation of bacterium to samples

We prepared four treatments: A) sample of natural fermentation, B) inoculated sample with 4×10^6 cfu/ml bacterial inocula, C) inoculated sample with 4×10^7 cfu/ml bacterial inocula and D) inoculated sample with $6'10^8$ cfu/ml bacterial inocula with two kinds of brine solutions. The inoculation was done immediately after that the brine added in sterile condition and all of the samples put at 25^{0}C for fermentation process. Samples were analyzed for their respective characteristics in 1, 5, 7, 9, 12 and 15 days during fermentation. When fermentation completed, 3% NaCl solution were added and the best samples analyzed during 30 days of storage at 25^{0}C and 4^{0}C.

Microbiological analyses

The brine of samples was analyzed for 1, 5, 7, 12 and 15 days of fermentation. 1 ml of them was aseptically transferred to 9ml sterile diluter solution. Decimal dilutions in diluter solution were prepared and surface spreading technique was performed by spreading 0.1 ml of the appropriate dilutions [14] on the following agar media (all of them are Merck, Darmstadt, Germany): Nutrient agar incubated at 37 ^{0}C for 48 h to determine the aerobic mesophilic bacteria counts; MRS agar for *L. plantarum* that incubated at 25^{0}C for 24- 48 h; Sabrose Dextrose agar (SDA) for yeasts and molds incubated at 25 ^{0}C for 48–72 h; for the assay of *Listeria monocytogenes* without dilution, 0.5 ml of the brine were added to Listeria Enrichment broth and incubated at 37^{0}C for 72 h then 0.1 ml of broth media spread on the surface of Listeria Selective agar and incubated at 37^{0}C for 24-48 h; *S. aureus* were determined by spreading 0.1 ml of the brine on Baird-Parker selective agar and incubation at 37 ^{0}C for 24–48 h; Vibrio spp. determined with 0.1 ml brine that spread on the surface of TCBS agar (Thiosulfate-Citrate-Bile-Salt-sucrose Agar) and incubated at 25^{0}C for 24–48 h [Modified of 6, 14].

Chemical analysis

The NaCl, pH, titratable acidity values

were carried out by the following methods[15]: pH by a digital pH meter (JENWAY, Bibby Scientific Ltd, UK), titratable acidity by titration of brines with 0.1 N NaOH that expressed as percent of acetic acid and lactic acid (% w/v), NaCl concentration by titration according to Mohr method with 0.1 N $AgNO_3$. The cucumbers of each sample were mixed with their brines and then the filtrated solution was used to measure pH, titratable acidity (2.5ml), and NaCl concentration (1ml) of fermented cucumbers.

HPLC analysis

Organic acids (lactic, acetic, citric, propionic, tartaric and malic) were measured by HPLC (High Performance Liquid Chromatography) (Organic acids analysis system, column; Shima-pack SCR-101H, Shimadzu, Kyoto, Japan). A 5 µl portion of a sample or standard solution was injected into a (300 × 7.9 mm) Shim-pack SCR-101H column coupled to a refractive index monitor (UV-vis spectrophotometry detector-SPD6AV; wavelength 214 nm). The column was operated at 75^0C. The used solvent was a 0.009 N sulfuric acid (pH 2.1-2.2) at a flow rate of 0.6 ml/min. Integration and calibration curves were analyzed with peak-ABC Software [Modified of 16].

Textural survey

Hardness and evolution of texture in cucumbers were determined by a texture analyzer (Brookfield. CT3, Cominerce BLVd, MA 02346) that equipped with a 33 mm cutting wire probe with aluminum frame. The samples were cut by the wire probe about 10 mm. The test speed was 0.5 mm/s and the trigger force was set at 5 g [Modified of 17].

Color analysis

Color of all samples were analyzed by CIE-*Lab* system that include l^*, a^* and b^* values that express the 'brightness', the 'green-red' and the 'blue-yellow' axis, respectively[18]. The CIE-*Lab* system is frequently used as a reliable method to assess the color of vegetables and its changes during storage and processing[19]. Color indexes (l^*, a^*, b^*) were determined by take pictures and analyzed with software photo shop 7.0 and mean values was calculated.

Sensory analysis

A sensorial evaluation of fermented samples was performed at the end of process by 10 persons. The cucumbers were tasted at random and separately. Score of 1-8 (number 1 refers to not acceptable and number 8 to excellent taste and crunchiness) was used in the evaluation and mean values was calculated [Modified of 20].

Statistical analysis

All statistical analyses were performed by statistical software SPSS 11.5 for windows. One-way ANOVA was used to evaluate significant differences (significance levels at $p<0.05$) and DUNCAN was used for differences between treatment means at $P<0.05$.

RESULTS AND DISCUSSION

Microbial changes during fermentation

The microbial changes of the different groups during of the brine fermentation were determined by plating count. Results of ANOVA analysis were shown high significant differences ($p<0.05$) for *L. plantarum* strain between samples during the fermentation. In all of the fermented samples of cucumber, L. *plantarum* strain and yeasts were the prevailing microorganisms throughout the process. The maximum population of the inoculated *L. plantarum* strain was in all of the samples with 5% NaCl solutions than 7% NaCl solutions. Apart from the brine concentration, the higher growth was observed in samples of D and C, respectively. The initial salt concentration of 5% (w/v) was favored for the rapid growth of *L. plantarum* which reached a maximum population of 8.57, 8.38, 8.32 and 7.93 $\log_{10}$ cfu/ml in samples of D, C, B and A, respectively in 9th day of fermentation. But in this day in the initial salt concentration of 7% (w/v), we observed the slower growth of *L. plantarum* with a maximum population of approximately 8.48, 8.32, 8.30 and 7.88 $\log_{10}$ cfu/ml in samples of D, C, B and A, respectively. The decrease of L. *plantarum* population were ranged approximately from 7.99 to 8.01 $\log_{10}$ cfu/ml in two kind of brine concentration in sample of D in 15^{th} day. The population of the inoculated *L. plantarum* strain was an important proportion of the total population that persisted in the end of the process. Changes in the population of the inoculated *L. plantarum LPCO10* strain followed a similar pattern that this strain rapidly proliferated in the brines[9]. The low concentration of NaCl can stimulate the growth of *L. plantarum* due to the small decrease in the water activity[21] and we also obtained the same result.

Yeasts coexisted with lactic acid bacteria and followed a similar growth profile. In this assay the yeasts increased rapidly in the first 5 days of fermentation. After that, their population in samples with salt concentration 7% (w/v) NaCl decreased in the range of 6.60- 6.72 $\log_{10}$ cfu/ml in 15th day of fermentation. This decline was lower than the samples with salt concentration 5% (w/v) NaCl. In samples with 5% NaCl (w/v) solution population of *L. plantarum* and yeasts were higher than other samples. But in the samples with high bacterial inocula, population of yeasts was lower than other fermented samples. Inoculation of *L. plantarum* strain and even different inoculum sizes resulted in difference of yeasts growth in the treatments. So, the yeasts population in the inoculated process was significantly lower than control samples. In a proper fermentation, the process is finally dominated by lactic acid bacteria and yeasts that coexist throughout fermentation[22]. In a process, yeasts are dominant when the concentration of salt in the brine stays at 10%. Lactic acid bacteria are more active to limit the negative effects of spoilage yeasts on the products quality, e.g. gas pocket formation and softening of the fruit[23]. But some of the yeast strains play a role in increasing of growth of *L. plantarum*. Some yeast strains are able to produce large amounts of nicotinic and pantothenic acids, biotin or vitamin B6, which are essential for growth of *L. plantarum*[10]. The *L. plantarum* population generally coexists with a yeast population until the end of the fermentation process and during storage[11]. Apart from the brine concentration, the used starter in this study rapidly decreased the pH to less than 4 and preserved high cell numbers (8.0–8.57 log cfu/ml) of *L. plantarum* throughout the fermentation process and storage. Almost the same changes of pH and microbial inhibition were found for other lactic acid bacteria when they used as starters for the fermentation of cucumber[24].

Aerobic mesophilic count were very significant ($p<0.05$) that in all samples decreased during the fermentation. Between the samples, the lowest aerobic mesophilic population were observed in 7% NaCl (w/v) solution in samples D, C and then for 5% NaCl (w/v) solution with the same bacterial inocula. The 7% NaCl (w/v) solution in sample B showed the highest decrease in aerobic mesophilic population than 5% NaCl (w/v) solution in sample C. The decrease of pH value and the proliferation of *L. plantarum* inhibit the presence of other microorganisms, as the decline of aerobic mesophilic count in other research was shown from 2th day of fermentation[25] and in this research from 5th day of fermentation. The inoculated *L. plantarum* strain inhibits the wild lactobacilli and controls the growth of cocci and remains as a marked proportion until the end of the fermentation[9]. In this study, any molds were not presented in fermented samples.

pH, acidity and NaCl concentration

The variations of pH during the different fermentations are determined. The pH and titratable acidity were used to show the fermentation process. The cucumbers of each sample were mixed with their brine and then the filtrated solutions were used. In the first day of fermentation, the solutions of fermented samples had pH value that ranged from 4.46-5.19 and the total titratable acidity value that ranged from 0.12-0.16 as % of acetic acid and from 0.18-0.24 as % of lactic acid. After 15 days of fermentation, the range of pH in the brine solutions decreased to 3.42-3.47 and the range of total titratable acidity increased to 0.55-0.58 as % of acetic acid and 0.83-0.87 as % of lactic acid. In ANOVA analysis of filtrated solutions, the variation of pH showed a high significant difference ($P < 0.05$) between all treatments during the days of fermentation. In the different days of fermentation, pH values decreased rapidly during the first 5 days, especially in samples D and C in two kinds of solution and then this change slowed down until the 7th day and after this moment, pH stabilized. The decrease of pH values in two kinds of solution in samples B and A was slower than D and C that their pH stabilized in the 9th and 12th day, respectively. In the 15th day the decrease of pH in 5% NaCl solution in samples of D, C, B were more than 7% NaCl solution in the same samples, respectively. In other researches, about 38% of LAB strains could show the strong acidification properties by reducing of pH to less than 5 [26]. The ability of some species of LAB particularly *L. plantarum* in acidification of the samples is significant for food preservation[27]. It was found that some isolated LAB strains from ethnic fermented vegetables of the Himalayas were able to lower the pH to 4.0 [28, 29], but in our study the isolated strain of *L. plantarum* from Iranian

fermented cucumbers were able to reduce pH to 3.42 in 7 and 9 days of fermentation. The lowest pH value (pH 3.42) was registered in the 5% NaCl solution in sample D. Inoculation with *L. plantarum* starter culture leads to a rapid decrease of pH which helps to reduce the risk of spoilage during the first days of fermentation[9]. The decrease of pH values in the samples A were slower than inoculated samples that high inoculum size of this starter culture leads to a rapid decrease of pH and increase of its positive effect, this results were the same as other authors[9]. The pH of the 5% NaCl (w/v) solution without inoculation was slightly higher than the 7% NaCl (w/v) solution without inoculation after 12 days that according to some of authors[30] this may be due to the spoilage of fungal microflora. In the study of the fermented pineapples, autochthonous lactic acid bacteria caused a further decrease of the pH to 2.73 that the spontaneous fermentation did not show the same extent of acidification[30].

The trend of titratable acidity as % of acetic acid during fermentation is determined. All of the samples with different treatments had a high significant difference. Since the beginning fermentation, differences in the acidification process were observed in among treatments. Changes in titratable acidity increased rapidly during the first 5 days and then a constant increase of acidity was observed during the 12-15th day of process. But titratable acidity as % of acetic acid (0.58%) and as % of lactic acid (0.86%) in the 5% NaCl solution in samples of D and C was higher than 7% NaCl solution in the same samples. In both of the solutions in samples of D and C, the titratable acidity was finally stabilized in 7th day of fermentation rather than other treatments. The concentrations of brine were effective in acidity values, because NaCl is more prone to solubilisation of organic matter and formation of combined acidity that brines with high combined acidity will show higher pH[32]. So the increase of NaCl concentration leads to increase in pH and decrease of acidification. In this regard the selection of appropriate initial brine and bacterial inocula is important to increase the titratable acidity[9]. This influence was also observed by others[33] for traditional fermented vegetables.

Salt concentrations in filtrated solutions gradually decreased throughout the process (no NaCl solution was added during the fermentation). The final decrease in the range of 2.8-4.15 was related to inoculated 5% and 7% NaCl solutions, respectively. In our study no brine solution added during the fermentation so it could help to decrease the brine concentration in presence of cucumbers.

The concentration of organic acids

The best samples with highest bacterial inocula in two kinds of brine were analyzed for their organic acids. The value of organic acids is shown in Table 1. In this assay, lactic acid was generated in large amounts during fermentation and its concentration in inoculated 5% NaCl solution was higher than inoculated 7% NaCl solution. In other researches, lactic acid was also the only organic acid in large amounts (64.4mM) in 4th day of fermentation[34]. The difference in acidity value can confirm the changes in the concentration of lactic acid. The difference of lactic acid concentration did not have more negative effects on the quality of cucumbers between two kinds of brine. Acetic acid concentration was lower than lactic and propionic acid in both of the brines and its concentration was lower than 5mM. The levels of lactic, acetic and propionic acid in inoculated 5% NaCl solution were higher than inoculated 7% NaCl solution. In other researches, this acids (lactic, acetic and propionic) were the major metabolic products in the brines that in this regard, the final value of lactic acid was about 66.6 mM in the inoculated samples whereas citric and malic acids had very low concentrations[6, 25, 35]. The production of acetic acid is most likely as a result of metabolism of citric acid, which had disappeared from inoculated brines as the same other results[36] and acetic acid might be the only major final metabolite of citric acid metabolism[37]. In this assay, citric, tartaric and malic acids were not observed in two kinds of samples after fermentation and in other research, citric acid also was not detected after 28 days of fermentation[38]. A significant reduction of citric acid and the increase of lactic acid and also the cause of decrease of pH can be the growth of lactic acid bacteria in the fermented samples. These bacteria can use glucose and citric acid as a carbon source to produce lactic acid faster than yeasts[39]. Malic acid can degrade to lactic acid and CO_2, by a malolactic enzyme[40]. Propionic acid value was higher than the acetic acid as the same other results in fermented vegetable[41]. This compound can

associate with the growth of microorganisms of the genus *Propioni bacterium*, which is related to zapatera spoilage[42]. This kind of spoilage causes the complete degradation of lactic acid and development of off-odor, a fact that was not observed in this research. In addition, degradation of lactic acid in zapatera spoilage results in increase of pH, which was also not observed in both of the brine as the same other results[6].

Texture analysis

In texture survey, there was a high significant difference between treatments during 1-15 days of fermentation. In all of the samples, the value of hardness decreased during fermentation. In the first day the highest hardness was observed in samples with 7% NaCl solution that in this regard the samples without inoculation were harder than the others. But in 5% NaCl solution the samples without inoculation had lower hardness than other samples. In the 15th day of fermentation, apart from of inoculation value, the samples with 7% NaCl solution had higher hardness than samples with 5% NaCl solution so the concentration of brine had the important role in hardness of texture [43]. In 7% NaCl solution, the samples of D and C had lower hardness and the samples B had higher hardness. In 5% NaCl solution, the samples A had lower hardness that in these samples the population of yeasts was higher than others. Hardness and sensory properties of inoculated vegetables were preferable rather than other samples that the other researches achieved the same results[44]. The application of starter cultures have positive effects on the reduction of fermentation time, reduction of spoilage risk, increase of self-life, improvement of process control, improvement of sensory quality and improvement of safety aspects[45].

Color analysis

Color is one of the first qualitative attributes that a customer can detect in fruits and vegetables. It is also the most important attribute used by the customer to evaluate the quality of a product and its possible taste without touching the commodity[18]. In this assay the results showed that all of the samples had dark green color. There is general agreement that the main cause of green vegetable discoloration during processing is the conversion of chlorophylls to pheophytins by the influence of pH[46]. The green color of vegetables turns to an olive green in acidic conditions[19]. The decrease of discoloration by increasing pH is indicating that the green color retains at higher pH conditions[46]. Some authors also studied the effect of pH on the rate of color change in broccoli at pH 3-8 and stated that production of pheophytin followed a first-order reaction and color degradation accelerated with decreasing pH, as expected[19]. During the storage, the colorless samples in both brines were not observed at 4 and 25 ^{0}C. Dechelatase of magnesium can affect directly on the chlorophyll molecule to produce pheophytin that is olive-brown and can be converted to pheophorbide via chlorophyllase activity[47]. The inoculated 7% NaCl solution had lower l^* value than inoculated 5% NaCl solution. In different brines without bacterial inocula l^* value was lower than others. In both of the brines, the samples without inoculation had lower a^* value than the inoculated samples. In inoculated 7% NaCl solution, a^* value was lower than inoculated 5% NaCl solution. The increase of a^* value may attribute to reactions of browning and enzyme-catalysed[17]. The inoculated 7% NaCl solution had lower b^* value than the inoculated 5% NaCl solution. The decrease of b^* value is due to some substances that are released during fermentation and then subject to browning. In samples without inoculation, b^* value was lower than the inoculated 5% NaCl solution. In inoculated samples, the higher increase of b^* was due to the lower browning[44]. In this assay the samples had acceptable appearance. This indicates

Table 1. Metabolic organic acids of sample D in two kinds of brine after 15 days of fermentation

Treatments	Products(mM)					
	Lactic acid	Acetic acid	Propionic acidacid	Citric acid	Tartaric acid	Malic acid
5%, D	76.67	4.83	39.68	ND[a]	ND	ND
7%, D	75.00	4.33	37.25	ND	ND	ND

[a] ND, not detected

that higher production of lactic acid by *L. plantarum* causes better appearance and coloration[48]. For inoculated Pineapple, the yellow (b^*) and bright (l^*) indexes were significantly higher than others. As compared with natural fermentation, inoculated samples also showed the better preservation of the natural colors[31].

Sensory test results

A sensory evaluation of cucumbers samples was performed at the end of fermentation by eight non-trained persons. In sensory test, for flavor, the panelists gave the higher rank to inoculated 7% NaCl solutions than other treatments. For crunchiness trait, the higher rank was for samples of C and D with 7% NaCl solution and samples of D with 5% NaCl solution, respectively. It appears that 5% NaCl solution was responsible for the softness of cucumbers. LAB contributes to the aroma and flavor of fermented products. They acidify food and cause a tangy lactic acid taste[49]. According to others the salt concentration had more effects on tissue hardness[43] and stated that too little or too much salt can lead to softer and poorer quality product[50]. The inoculated samples had suitable flavor and texture when optimally fermented in 7% NaCl solution with pH 3.44. Comparison of untreated and treated samples revealed that treated 5% NaCl solution had lower values of saltiness and hardness than the treated 7% NaCl solution. The inoculated samples with *L. plantarum* exhibited better crunchiness as other results of researchers[51]. The rapid increase of acidity minimizes the influence of spoilage bacteria. Reducing the influence of spoilage bacteria probably improves the microbiological and sensory quality of the fermented product and uniform products can probably obtain by application of starter cultures[52]. Other researchers stated that at the end of storage, the highest score of sensory attributes was significantly related to samples that inoculated by autochthonous lactic acid bacteria. Overall, fermentation by autochthonous lactic acid bacteria determines an agreeable quality[31].

Especially, autochthonous starters modified the profile of organic acids leading to the decrease of the concentration of malic acid, and synthesizing lactic and acetic acids. This modification might have direct (pH) or indirect repercussions (redox potential) on the activity of endogenous browning enzymes, oxidation and sensory properties (colour, flavour and aroma) of pineapples[53]. Indeed, started pineapples had the highest antioxidant activity throughout processing and storage[31].

Antimicrobial activity of L. plantarum

After the fermentation completed, all of the samples were examined for *Listeria monocytogenes, S. aureus* and *Vibrio spp*. in 10 plates with different medium. In all of the inoculated samples with *L. plantarum*, the levels of *Listeria monocytogenes, S. aureus and Vibrio spp.* on the surface of plates were undetected and there were no pathogenic bacteria in Iranian fermented cucumber samples. *Listeria monocytogenes* is a pathogenic organism in a non-acidified, refrigerated pickle product[54]. Green table olives could support survival of *Listeria*, despite its low pH and high salt concentration, but in this assay *Listeria spp.* was not observed[55]. The pathogenic bacteria such as Vibrio spp. only at the beginning of fermentation detected that the low pH and anaerobic conditions of fermentation do not favor for the survival of this contaminant[56]. *L. plantarum* AMA-K can produce bacteriocin against the cells number of *Listeria innocua F* and decrease to undetectable levels[57]. The antagonistic activities of LAB strains, from ethnic fermented vegetables have indicated bacteriocin activity[26]. The growth of *L. plantarum LPCO10* in the olive brines can produce plantaricin that contributes to an effective control of the indigenous lactobacilli and other LAB[9]. *L. plantarum* can decrease the pH in brines rapidly and it is an important step in the fermentation process because it limits the development of basophilic bacteria, and especially coliforms and other spoilage microorganisms[58].

Microbial and chemical changes during storage

For the survey of the shelf life during the storage (1-30 day), after completion of fermentation in 15th day, 3% NaCl solution were added to the best samples (the samples of D and C with 5% and 7% NaCl (w/v) solution) and surveyed at 25^{0}C and 4^{0}C during the storage. Apart from the value of bacterial inocula, the population of *L. plantarum*, yeasts and aerobic mesophilic in two kinds of brine at 25^{0}C were higher than their population at 4^{0}C. In compare to brine concentrations of samples at 4^{0}C, we could state that the numbers of *L. plantarum*,

yeasts and aerobic mesophilic in samples with 5% NaCl solution were higher than their numbers in 7% NaCl solution. In the 5% NaCl solution high numbers of yeasts and aerobic mesophilic caused the decrease of quality in the end of the storage. The sample that included high inoculation and 7% NaCl solution at 4^0C had the lower population of yeasts and aerobic mesophilic in 30th day that caused to remain the population of *L. plantarum* to 5.47 $\log_{10}$ cfu/ml rather than other treatments. In other studies, some of the yeast species decreased to the 6.0 $\log_{10}$cfu/ml and LAB decreased to the 10 cfu/ml at 4 °C during 90 days of storage[16]. According to others, the microbiological control by the *L. plantarum LPCO10* is completely effective during the first 20 days and after that the wild microflora increases[9]. In this regard, the inoculation can affect the population of the different microorganisms throughout the rest of the fermentation. In the study of fermented pineapples with autochthonous starters, although its starters decreased at the end of storage at 4 ^{0}C after 30 days, its number of lactic acid bacteria was higher than the other samples and the number of yeasts decreased[31]. In the inoculated 7% NaCl solutions, the average of pH was lower than the inoculated 5% NaCl solutions at 4 and 25 ^{0}C in 30th day. The mean of pH at 4 and 25 ^{0}C for inoculated 7% NaCl solution was 4.08, 4.33 and for inoculated 5% NaCl solution was 4.98, 4.74 in 30th day, respectively. Titratable acidity of samples D with 7% NaCl solution was higher than other treatment at 4 ^{0}C after 30 day. These changes can show that pH of the media is a main factor to control growth of yeasts and quality of products and temperature is a factor to enhance the effect of pH[41]. In this regard the high values of *L. plantarum* can produce an acidic condition, so some of the yeasts can grow at relatively low pH and in the presence of weak concentrations of lactic acid.

CONCLUSION

In conclusion, for control of fermented cucumbers, it is advisable to make the initial 5-7% NaCl concentration, a correction of slight initial pH with acetic acid, and a heavy inoculation with *L. plantarum* that produce uniform and safe products with high quality. Inoculation with *L. plantarum* and appropriate initial brine concentrations can decrease pH during the 15 days of the fermentation and yield higher titratable acidity than the traditional process. Inoculation also alters the microbial population of fermentation that the inoculated strain inhibits the pathogenic bacteria. In samples with 5% NaCl (w/v) solution the population of LAB, yeast and aerobic mesophilic were higher than other samples. The flavor of inoculated 7% NaCl solutions had the higher rank than other treatments. During the storage, apart from the value of inoculation, the high population of *L. plantarum*, yeasts and aerobic mesophilic in two kinds of brine were observed at 25^0C.

REFERENCES

1. Li, L., Feng, L., Yi, J., Hua, C., Chen, F., Liao, X., Wang, Z., Hu, X. High hydrostatic pressure inactivation of total aerobic bacteria, lactic acid bacteria, yeasts in sour Chinese cabbage. *International Journal of Food Microbiology*, 2010; **142**: 180-184.
2. Sargent, S.A., Maynard, D.N.: Postharvest biology and technology of cucurbits. In: *Horticultural reviews* (Janick J, ed). Hoboken, NJ: Wiley–Blackwell, 2009; pp 315-354.
3. FAOSTAT. Food and Agriculture Organization of the United Nations. 2009; Available on the Internet: http://faostat.fao.org/site/567/DesktopDefault.
4. Garrido Fernández, A., García García, P., Brenes Balbuena, M.: Olive fermentations. In: *Biotechnology: Enzymes, biomass, food and feed* (Rehm H-J, Reed G, eds). New York: VCH Publishers, 1995; pp 593-627.
5. Garrido-Fernández, A., Fernández Díez, M.J., Adams, M.R.: Table Olives: Production and Processing. London: Chapman and Hall, UK, 1997.
6. Panagou, E.Z, Schillinger, U., Franz, C.M.A.P., Nychas, G.J.E. Microbiological and biochemical profile of cv. Conservolea green olives processed by the Spanish-method. *Food Microbiology*, 2008; **23**: 199-204.
7. Badis, A., Guetarni, D., Moussa-Boudjemaa, B., Henni, D.E., Tornadijo, M.E., Kihal, M. Identification of cultivable lactic acid bacteria isolated from Algerian raw goat's milk and evaluation of their technological properties. *Food Microbiology*, 2004; **21**: 343-349.
8. Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U., Huis in't Veld, J.H.J. Over view of gut flora and probiotics. *International Journal of Food*

Microbiology, 1998; **41**: 85-101.

9. Leal-Sánchez, M.V., Ruiz-Barba, J.L., Sánchez, A.H., Rejano, L., Jiménez-Díaz, R., Garrido, A. Fermentation profile and optimization of green olive fermentation using *Lactobacillus plantarum* LPCO10 as a starter culture. *Food Microbiology*, 2003; **20**: 421-430.
10. Ruiz-Barba, J.L., Jiménez-Diaz, R. Availability of essential B group vitamins to *Lactobacillus plantarum* in green olive fermentation brines. *Applied and Environmental Microbiology*, 1995; **61**: 1294-1297.
11. Ruiz-Barba, J., Cathcart, D., Warner, P., Jiménez-Díaz, R. Use of *Lactobacillus plantarum* LPCO10, a bacteriocin producer, as a starter culture in spanishstyle green olive fermentations. *Applied and Environmental Microbiology*, 1994; **60**: 2059-2064.
12. Daeschel, M., Fleming, H. Selection of lactic acid bacteria for use in vegetable fermentations. *Food Microbiology*, 1984; **1**: 303-313.
13. Tsai, C.C., Lai, C.H., Yu, B., Tsen, H.Y. Use of PCR primers and probes based on the 23S rRNA and internal transcription spacer (ITS) gene sequence for the detection and enumerization of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in feed supplements. *Anaerobe*, 2010; **16**: 270-277.
14. Paramithiotis, S., Hondrodimou, O.L., Drosinos, E.H. Development of the microbial community during spontaneous cauliflower fermentation. *Food Research International*, 2010; **43**: 1098-1103.
15. Fernàndez-Diez, M.J., Castro, R., Fernàndez, A., Cancho, F.G., Pellissò, F.G., Vega, M.N., et al.: Biotecnologìa de la aceituna de mesa. In: (CSIC ed). Madrid, Spain: CSIC, 1985.
16. Gardner, J.N., Savard, T., Obermeier, P., Caldwell, G., Champagne, C.P. Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *International Journal of Food Microbiology*, 2001; **64**: 261-275.
17. Romeo, F.V., De Luca, S., Piscopo, A., Perri, E., Poiana, M. Effects of post-fermentation processing on the stabilisation of naturally fermented green table olives (cv Nocellara etnea). *Food Chemistry*, 2009; **116**: 873-878.
18. Tijskens, L.M.M., Barringer, S.A., Biekman, E.S.A. Modelling the effect of pH on the color degradation of blanched broccoli. *Innovative Food Science and Emerging Technologies,* 2001; **2**: 315-322.
19. Gunawan, M.I., Barringer, S.A. Green colour degradation of blanched broccoli (*Brassica oleracea*) due to acid and microbial growth. *Journal of Food Processing and Preservation*, 2000; **24**: 253-263.
20. Tuorila, H., Hellemann, U. (eds):. Elintarvikkeet aistien puntarissa (Sensory Evaluation of Foods). University of Helsinki, 1993; pp 37-39.
21. Tsapatsaris, S., Kotzekidou, P. Application of central composite design and response surface methodology to the fermentation of olive juice by *Lactobacillus plantarum* and *Debaryomyces hansenii*. *International Journal of Food Microbiology*, 2004; **95**: 157-168.
22. Sánchez Gómez, A.H., García García, P., Rejano Navarro, L. *Elaboration of table olives. Grasas Aceites*, 2006; **57**: 86-94.
23. Ozay, G., Borcakli, M. Effect of brine replacement and salt concentration on the fermentation of naturally black olives. *Food Research International*, 1996; **28**: 553- 559.
24. Desai, P., Sheth, T. Controlled fermentation of vegetables using mixed inoculum of lactic cultures. *Journal of Food Science and Technology*, 1997; **34**: 155-158.
25. Sánchez, A.H., de Castro, A., Rejano, L., Montanõ, A. Comparative study on chemical changes in olive juice and brine during green olive fermentation. *Journal of Agricaltural and Food Chemistry,* 2000; **48**: 5975-5980.
26. Tamang, J.P., Tamang, B., Schillinger, U., Guigas, C., Holzapfel, W.H. Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas. *International Journal of Food Microbiology*, 2009; **135**: 28-33.
27. Ammor, M.S., Mayo, B. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: an update. *Meat Science,* 2007; **76** (1): 138-146.
28. Tamang, J.P., Tamang, B., Schillinger, U., Franz, C.M.A.P., Gores, M., Holzapfel, W.H. Identification of predominant lactic acid bacteria isolated from traditional fermented vegetable products of the Eastern Himalayas. *International Journal of Food Microbiology*, 2005; **105**: 347-356.
29. Tamang, B., Tamang, J.P., Schillinger, U., Franz, C.M.A.P., Gores, M., Holzapfel, W.H. Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo tender shoots of North East India. *International Journal of Food Microbiology*, 2008; **121**: 35-40.
30. Zhao, D., Ding, C. Studies on the low-salt Chinese potherb mustard (Brassica juncea, Coss.) pickle. I—The effect of a

homofermentative L(+)-lactic acid producer *Bacillus coagulans* on starter culture in the low-salt Chinese potherb mustard pickle fermentation. *LWT*, 2008; **41**: 474-482.

31. Di Cagno, R., Cardinali, G., Minervini, G., Antonielli, L., Rizzello, C.G., Ricciuti, P., Gobbetti, M. Taxonomic structure of the yeasts and lactic acid bacteria microbiota of pineapple (Ananas comosus L. Merr.) and use of autochthonous starters for minimally processing. *Food Microbiology*, 2010; **27**: 381-389.
32. Bautista-Gallego, J., Arroyo-López, F.N., Durán-Quintana, M.C., Garrido-Fernández, A. Fermentation profiles of Manzanilla-Aloreña cracked green table olives in different chloride salt mixtures. *Food Microbiology*, 2010; **27**: 403-412.
33. Ballesteros, C., Palop, L., Sánchez, I. Influence of sodium chloride on the controlled lactic acid fermentation of 'Almagro' eggplants. *International Journal of Food Microbiology*, 1999; **53**: 13-20.
34. Chanprasartsuk, O., Prakitchaiwattana, C., Sanguandeekul, R., Fleet, G.H. Autochthonous yeasts associated with mature pineapple fruits, freshly crushed juice and their ferments; and the chemical changes during natural fermentation. *Bioresource Technology*, 2010; **101**: 7500-7509.
35. Tassou, C.C., Panagou, E.Z., Katsaboxakis, K.Z. Microbiological and physicochemical changes of naturally black olives fermented at different temperatures and NaCl levels in the brines. *Food Microbiology*, 2002; **19**: 605-615.
36. Sánchez, A. H., Rejano, L., Montanõ, A., de Castro, A. Utilization at high pH of starter cultures of lactobacilli for Spanish-style green olive fermentation. *International Journal of Food Microbiology*, 2001; **67**:115-122.
37. Hugenholtz, J. Citrate metabolism in lactic acid bacteria. *FEMS Microbiology Reviews*, 1993; 12: 165-178.
38. Panagou, E.Z., Katsaboxakis, C.Z. Effect of different brining treatments on the fermentation of cv. Conservolea green olives processed by the Spanish-method. *Food Microbiology,* 2006; **23**: 199-204.
39. Amerine, M.A., Kunkee, R.E. Microbiology of wine making. *Annual Review of Microbiology,* 1968; **22**: 323-358.
40. Montanõ, A., Sánchez, A.H., de Castro, A. Controlled fermentation of Spanish-type green olives. *Journal of Food Science*, 1993; **4:** 842-844.
41. Savard, T., Beaulieu, C., Gardner, N.J., Champagne, C.P. Characterization of spoilage yeasts isolated from fermented vegetables and Inhibition by lactic, acetic and propionic acids. *Food Microbiology*, 2002; **19**: 363-373.
42. González Cancho, F., Rejano Navarro, L., Borbolla y Alcalá, J.M.R. Formation of propionic acid during storage of green table olives. Responsible microorganisms. *Grasas Aceites*, 1980; **31**: 245-250.
43. Roberts, J.S., Kidd, D.R. Lactic acid fermentation of onions. *Lebensm.-Wiss. u.-Technology,* 2005; **38**: 185-190.
44. Di Cagno, R., Surico, R.F., Siragusa, S., De Angelis, M., Paradiso, A., Minervini, F., De Gara, L., Gobbetti, M. Selection and use of autochthonous mixed starter for lactic acid fermentation of carrots, French beans or marrows. *International Journal of Food Microbiology*, 2008; **127**: 220-228.
45. Holzapfel, W.H. Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*, 2002; **75**: 197-212.
46. Koca, N., Karadeniz, F., Burdurlu, H.S. Effect of pH on chlorophyll degradation and color loss in blanched green peas. *Food Chemistry*, 2006; **100**: 609-615.
47. Matile, P., Hörtensteiner, S., Thomas, H. Chlorophyll degradation. *Annual Review of Plant Physiology and Plant Molecular Biology*, 1999; **50**: 67-95.
48. Martínez-Castellanos, G., Shirai, K., Pelayo-Zaldívar, C., Pérez-Flores, L.J., Sepúlveda-Sánchez, J.D. Effect of *Lactobacillus plantarum* and chitosan in the reduction of browning of pericarp Rambutan (Nephelium lappaceum). *Food Microbiology*, 2009; **26**: 444-449.
49. Leroy, F., De Vuyst, L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*, 2004; **15**: 67-78.
50. Pederson, C.S. Improving methods for salting sauerkraut. *Food Packer*, 1946; 27: 53-57.
51. Randazzo, C.L., Fava, G., Tomaselli, F., Romeo, F.V., Pennino, G., Vitello, E., Caggia, C. Effect of kaolin and copper based products and of starter cultures on green table olive fermentation. *Food Microbiology*, 2011; **28**: 910-919.
52. Viander, B., aki, M.M., Palva, A. Impact of low salt concentration, salt quality on natural large-scale sauerkraut fermentation. *Food Microbiology*, 2003; **20**: 391-395.
53. Hernández, Y., Lobo, M.G., González, M. Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. *Food Chemistry*, 2009; **114**: 734-741.

54. Romick, T.L.: Biocontrol of Listeria monocytogenes, a psychrotrophic pathogen model, in low salt, non-acidified, refrigerated vegetable products. PhD Diss, Raleigh: NC State University, NC, 1994.
55. Caggia, C., Randazzo, C.L., Di Salvo, M., Romeo, F., Giudici, P. Occurrence of *Listeria monocytogenes* in green table olives. *Journal of Food Protection*, 2004; **67**: 2189-2194.
56. Abriouel, H., Benomar, N., Lucas, R., Gálvez, R. Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally-fermented Aloreña green table olives. *International Journal of Food Microbiology,* 2011; **144**: 487-496.
57. Todorov, S.D., Nyati, H., Meincken, M., Dicks, L.M.T. Partial characterization of bacteriocin AMA-K, produced by *Lactobacillus plantarum* AMA-K isolated from naturally fermented milk from Zimbabwe. *Food Control*, 2007; **18**: 656-664.
58. De Castro, A., Rejano, L., Sanchez, A.H., Montano, A. Fermentation of lye-treated carrots by *Lactobacillus plantarum*. *Journal of Food Science*, 1995; **60**(2): 316-319.