

Improvement of Fermented Cucumber Characteristics by Starter Culture of *Lactobacillus plantarum*, *L. bulgaricus* and *S. thermophiles*

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ABSTRACT: Fermented cucumber is a traditional lactic acid fermentation that is related to the presence of microorganisms. In this study, the use of suitable *L. plantarum* starter culture with *L. bulgaricus* and *S. thermophilus* to improve the cucumber fermentation process with high quality has been studied. Cucumbers in brine containing 4 to 6% NaCl and 0.2 to 0.4% Inulin (w/v) were inoculated with different LAB strains and analysed for microbial and physicochemical characteristics. The numbers of LAB strains were 7.46 log₁₀ cfu/ml in a solution containing 4% NaCl, 0.4% Inulin and a mixed starter culture on the sixth day. The initial salt concentration of 4% (w/v) and Inulin concentration of 0.4% was favored for the rapid growth of LAB. In the mixed starter culture, the value of titratable acidity and pH were 0.6% and 3, respectively, on the sixth day. The inoculated 6% NaCl and 0.4% Inulin solution with mixed starter culture had lower numbers of aerobic mesophilic and yeast (6.38 and 6.65 log₁₀ cfu/ml) on the sixth day. The inoculated 4% NaCl solution had lower hardness and I*, a*, b* values and the highest ranking in flavor. In order to control the fermentation of cucumbers, it is advisable to make heavy inoculation with the mentioned strains that produce high quality uniform and safe products.

Keywords: *Fermented Cucumbers, Microbial and Physicochemical Characteristics, Starter Co-cultures.*

Introduction

Functional products, i.e. foods or beverages offering specific health benefits beyond basic nutrition are increasingly valued. Today, most probiotics, mainly lactobacilli (FAO/WHO, 2002; Reid, 2006) are used in yogurts, fermented milks and pharmaceutical products for their anecdotal health effects (Holzapfel *et al.*, 1998; Mattila-Sandholm, 1999). Fermented cucumber, like other natural fermented vegetables is a traditional lactic acid fermentation, which is correlated to microorganisms presence in the raw material

(Garrido Fernández *et al.*, 1995). When lactic acid bacteria are more than yeasts, for a product with a lower pH, they are desirable in natural vegetables fermentation (Panagou *et al.*, 2008). A kind of LAB strains is *Lactobacillus plantarum* that in the traditional process, its growth in the fermented brines is essential to provide the amount of required lactic acid for preservation of products (Leal-Sánchez *et al.*, 2003). Other strains like *S. thermophilus* and *L. bulgaricus*, for example in the milk, each produces one or more substances that stimulate the growth of other. The symbiosis phenomenon was studied by several authors, who observed a positive effect of the co-

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culture compared to mono-culture in terms of growth, acidification and production of flavors (Béal *et al.*, 1994). Studies about the stimulating factors of each one of these bacteria showed that *L. bulgaricus* is stimulated by formic acid and by CO₂ produced by the *streptococcus*, while *S. thermophilus* is stimulated by the amino acids and small peptides from the metabolic activity of *L. bulgaricus* (Nakada *et al.*, 1996). CO₂ is a stimulating factor of *L. bulgaricus* and results from the decarboxylation of urea by urease that is produced by *S. thermophilus*. *L. plantarum* is often a predominant species that is chosen as a starter for vegetables fermentation such as table olives (Ruiz-Barba *et al.*, 1994).

Besides probiotics, functional foods usually contain prebiotics, i.e. non-digestible ingredients that stimulate the growth and/or activity of bacteria in the digestive system, which are beneficial to the health of the body (Gibson *et al.*, 2004). Among these, inulin is a natural component of several fruits and vegetables with beneficial effects on health and its technological properties is interesting.

Although the shift in metabolic pathways in response to environmental conditions is well documented for homofermentative and heterofermentative lactobacilli (Axelsson, 1998), metabolic changes are very important from a technological standpoint, since the amount of organic acids and volatile compounds influence the flavor and texture of the fermented product (Axelsson, 1998).

Lactic acid bacteria (LABs) used in fermented products help to provide and preserve sensorial and nutritional properties of food products. These bacteria in fact not only ferment lactose and citrate mainly to lactic acid, but synthesize short chain fatty acids (Kitazawa *et al.*, 1998), thus they influence organoleptic quality and texture development of the fermented product (de Vos & Hugenholtz, 2004). In this research work the application of suitable *L.*

plantarum starter culture isolated from Iranian vegetable fermentation, *L. bulgaricus* and *S. thermophilus* to improve the cucumber fermentation process with high quality is studied.

Materials and Methods

- Inoculum preparation

L. plantarum strain was isolated from Iranian cucumber fermentation according to the following procedure; a 2 µl tube of glycerin from *L. plantarum* was activated in 5ml sterile MRS broth that was incubated at 30 °C for 24-48 hours. 0.001g of yogurt starters (*L. bulgaricus*, *S. thermophiles*) were activated in 5ml sterile MRS broth that was incubated at 37 °C for 48 hours. Further to the observation of turbidity and activation of the said species, the culture media was centrifuged at 3000 rpm for 8 min, and after outpouring of the MRS broth, the sediment of culture media was suspended in 150ml sterile fresh saline containing 4.5% w/v NaCl and the pH was adjusted at 4 by acetic acid followed by incubation at 30 °C for 24 h and yogurt starters were incubated at 37°C for 48 hours and starter cultures were adapted to the saline environment of the brine (Panagou *et al.*, 2008).

- Cucumber Preparation

The cucumber samples were supplied from different location of Iran, and were washed and placed in glass vessels (450 g) containing fresh brine that was prepared according to the following procedure.

The first type was prepared using 4% (w/v) NaCl with 99% purity and the pH was adjusted at 4 by 2% normal acetic acid. The second and third types were made from previous solution with 0.2% and 0.4% inulin, respectively. The fourth type was prepared from 6% (w/v) NaCl and 0.2% inulin solutions at the pH of 4 and the fifth type contained 6% (w/v) NaCl and 0.4% inulin solutions at the pH of 4.

All of the brine samples were heated at 73°C for 10 min and chilled to 30°C. The cucumbers were put in glass vessels and filled by different prepared brines (300-350ml) in sterile condition.

- *Inoculation of samples*

The following treatments were prepared; fermentation without bacterial inocula, fermentation with 1ml *Lactobacillus plantarum*, fermentation with 1ml yogurt starter, fermentation with 1ml *Lactobacillus plantarum* and 1ml yogurt starter. After the addition of brine, the inoculation was carried out in sterile condition immediately and all of the samples (Table 1) were placed at 25°C for fermentation process. Samples were analyzed in terms of pH changes, titratable acidity, texture hardness, color and total number of aerobic mesophilic and lactic acid bacteria during first, sixth, thirteenth and sixteenth days of fermentation. When the fermentation was completed, 3% NaCl solution was added and the concentration of organic acid was studied.

- *Microbiological analysis*

The brines of the samples were analyzed during the period of fermentation. 1 ml of the sample was aseptically transferred to 9ml of sterile diluter solution. Decimal dilutions in diluter solution were prepared and surface spreading technique was performed by spreading 0.1 ml appropriate dilutions (Paramithiotis *et al.*, 2010) on the agar

media (Merck, Darmstadt, Germany). MRS agar for LAB was incubated at 25°C for 24-48 h and the nutrient agar was incubated at 37°C for 48 h to determine the aerobic mesophilic bacteria and sabrose Dextrose agar (SDA) was incubated at 25°C for 48–72 h to determine yeasts and molds (Panagou *et al.*, 2008; Paramithiotis *et al.*, 2010).

- *Chemical analysis*

Salt, pH, titratable acidity values were determined according to the following methods (Fernández-Diez *et al.*, 1985); pH by a digital pH meter (JENWAY, Bibby Scientific Ltd, UK), titratable acidity by titration of brines with 0.1 N NaOH and was expressed as percent of acetic acid (% w/v). The cucumbers of each sample was mixed with the brine and then the filtrated solution was used to measure the pH and titratable acidity (2.5ml) of fermented cucumbers.

- *The concentration of organic acids*

The organic acids (lactic, acetic, citric and botyric acid) were measured by the application of High Performance Liquid Chromatography. 5 µl of a sample or standard solution was injected into a (300 × 7.9 mm) Shimapack SCR-101H column. The column was operated at 75°C. The solvent used was 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 (Gardner *et al.*, 2001).

Table 1. Different samples with different brine solution and bacterial inocula

Samples	Kinds of brine and bacterial inocula
Control	Without bacterial inocula and with 4% (w/v) NaCl, pH 4
A	1ml <i>Lactobacillus plantarum</i> with 4%(w/v) NaCl, pH 4
B1	1ml yogurt starter with 4% (w/v)NaCl, 0.2% inulin, pH 4
B2	1ml yogurt starter with 4%(w/v) NaCl, 0.4% inulin, pH 4
C	1ml <i>Lactobacillus plantarum</i> , 1ml yogurt starter with 4%(w/v) NaCl, pH 4
D1	1ml <i>Lactobacillus plantarum</i> , 1ml yogurt starter with 4%(w/v) NaCl, 0.2% inulin, pH 4
D2	1ml <i>Lactobacillus plantarum</i> ,1ml yogurt starter with 4%(w/v) NaCl, 0.4% inulin, pH 4
E1	1ml <i>Lactobacillus plantarum</i> ,1ml yogurt starter with 6%(w/v) NaCl, 0.2% inulin, pH 4
E2	1ml <i>Lactobacillus plantarum</i> ,1ml yogurt starter with 6%(w/v) NaCl, 0.4% inulin, pH 4

- *Textural survey*

Evaluation of the texture and hardness of cucumbers were determined by a texture analyzer (Brookfield. CT3, Cominerce BLVd, MA 02346) that was 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 (Romeo *et al.*, 2009).

- *Color analysis*

Color of the samples were analyzed by CIE *Lab* system that included L^* , a^* and b^* values that express the 'brightness', the 'green-red' and the 'blue-yellow' axis, respectively (Tijskens *et al.*, 2001). The CIE-*Lab* system is frequently used as a reliable method to assess the color of vegetables and the changes of color (Gunawan & Barringer, 2000). Color indexes (L^* , a^* , b^*) were determined by taking pictures and analyzed with software photo shop 7.0 and then the mean values were calculated.

- *Sensory analysis*

Sensory evaluations of the fermented samples were carried out at the end of the process by a panel of 10 people. The cucumbers were tasted at random and separately. Score of 1 to 8 (number 1 refers to not acceptable and number 8 to excellent taste and crunchiness) was used in the evaluation and the mean values were calculated (Tuorila & Hellemann, 1993).

- *Statistical analysis*

All the 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 the means of treatments at $P < 0.05$.

Results and Discussion

- *Microbial changes during fermentation*

The microbial changes in different groups during the fermentation were determined by plating count. According to the results of ANOVA analysis, significant differences were not observed between the inoculated samples containing 4% salt but significant differences were obtained between these samples with the control and also inoculated samples containing 6% salt ($p < 0.05$) during fermentation. In all the fermented cucumber samples, LAB strains were the prevailing microorganisms throughout the process and the maximum population of the inoculated strain was in D₂ sample. The initial salt and inulin concentrations of 4% (w/v) and 0.4% respectively in the treated sample was favored for the rapid growth of LAB where the population was 7.46, 7.39, 7.36 and 7.38 log₁₀ cfu/ml in the samples of D₂, C, B₂ and A, respectively on the 6th day of fermentation and their growth improved up to the 13th day (Figure1). The initial salt concentration of 6% (w/v) resulted slower growth. The decrease of LAB population was 6.86 log₁₀ cfu/ml in the D₂ sample on the 16th day. The population of the inoculated LAB strains was an important proportion of the total population of starter that remained until the end of process. The low concentration of NaCl can stimulate the growth of LAB due to the little decrease in the water activity (Tsapatsaris & Kotzekidou, 2004).

Yeasts coexisted with lactic acid bacteria and followed a similar growth profile. In this assay, the numbers of yeasts in the samples with salt concentration of 6% (w/v) NaCl has decreased. In solution containing 6% of NaCl and 0.4% of Inulin that was inoculated by mixed starter culture, lower numbers of yeast (6.65 log₁₀ cfu/ml) were observed on the 6th day. In samples with 4% of NaCl (w/v) and 0.4% of Inulin solution, the

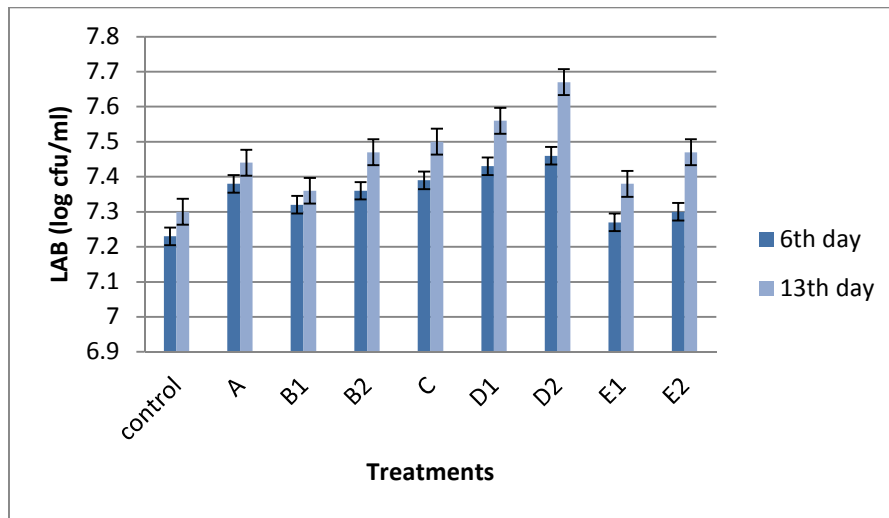


Fig. 1. The population of LAB in different samples on the 6th and 13th day

than other samples, but in the samples with mixed bacterial inocula, the population of yeasts was lower than other fermented samples. In a proper fermentation, lactic acid bacteria are more active to limit the negative effects of spoilage yeasts on the products quality (Ozay & Borcakli, 1996). In this study, apart from the brine concentration, the used starters decreased the pH to 3 rapidly and preserved high cell numbers (7log cfu/ml) of LAB throughout the fermentation process. The same changes of pH and microbial inhibition were found for other lactic acid bacteria when they were used as starters for the fermentation of cucumber (Desai & Sheth, 1997).

The number of aerobic mesophilic was very significant ($p < 0.05$) therefore in all the samples they were decreased during fermentation. Between the samples, the lowest aerobic mesophilic population was observed in E₂, E₁ samples containing 6% of NaCl (w/v) solution and followed by D₂, D₁, A, C, B₂ and B₁ samples containing 4% of NaCl (w/v) solution respectively. The decrease in pH value and the proliferation of LAB inhibit the presence of other microorganisms as the numbers of aerobic mesophilic decreased since the 6th 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 (Leal-Sánchez *et al.*, 2003).

- Evaluation of chemical and physicochemical changes

The filtrated solutions were used to determine the pH and titratable acidity in fermentation process. In the first day of fermentation, the pH value of fermented samples ranged from 4.20 to 6.0 and the total titratable acidity value (acetic acid) ranged from 0.048 to 0.096%. After 6 days of fermentation, the range of pH in the brine solutions decreased to 3.40 to 3.10 and the range of total titratable acidity increased to 0.45 to 0.63%. In ANOVA analysis of filtrated solutions, the variation of pH showed a high significant difference ($P < 0.05$) between the control sample and others but there were no significant differences between inoculated samples during the days of fermentation. In the different days of fermentation, the pH values decreased rapidly during the first 6 days, especially in D₂, B₂, A and C samples and then pH was stabilized. The decrease of pH values in B and E samples was slower than others and the control sample, respectively. The decrease of pH in 4% solution of NaCl was more than 6% solution of NaCl in the

same samples. Other researchers have indicated that about 38% of LAB strains could show a strong acidification properties by reducing the pH to less than 5 (Tamang *et al.*, 2009). The ability of some species of LAB, particularly *L.plantarum*, in acidification of the samples is significant for food preservation (Ammor & Mayo, 2007). In the first, the lowest pH value (pH 3) was registered for D₂ sample containing 4% of NaCl. Inoculation with starter culture leads to a rapid decrease of pH which helps to reduce the risk of spoilage during the first day of fermentation (Leal-Sánchez *et al.*, 2003). The decrease of pH values in the control samples were slower than inoculated samples. According to some authors, the spoilage of fungal microflora causes to reduce the pH in the control sample (Zhao & Ding, 2008).

Concerning the titratable acidity, inoculated samples with different treatments had no significant difference. Changes in titratable acidity increased rapidly during the first 6 days and then a constant increase of acidity was observed during 6th to 16th days of process. But titratable acidity (0.63%) in the sample of D₂ was higher than other samples (Figure 2). In the solutions of D samples, the titratable acidity was finally stabilized on the 6th day of fermentation. In

D₂ samples, high value of Inulin contributed to more action of starters but in A samples, *L. Plantarum* could have made better acidity without the presence of competitor microorganisms. The concentrations of brine were effective in acidity values because NaCl is more prone to solubilisation of organic matters and formation of combined acidity and brines with high combined acidity will show higher pH value (Bautista-Gallego *et al.*, 2010). Therefore the increase of NaCl concentration leads to an increase in pH and a decrease in acidification. In this regard the selection of appropriate initial brine and bacterial inocula is important to increase the titratable acidity (Leal-Sánchez *et al.*, 2003).

- The concentration of organic acids

The values of organic acids in different samples are shown in Table 2. In this assay, lactic acid was generated in large amounts during fermentation and its concentration in D₂ sample was higher than others. The difference in acidity value can confirm the changes in the concentration of lactic acid. Acetic acid concentration was lower than lactic and butyric acids in all of the brines and the concentration was 19.18 to 34.60 mg/100g. The levels of all organic acids in D₂ sample with 4% solution of NaCl were

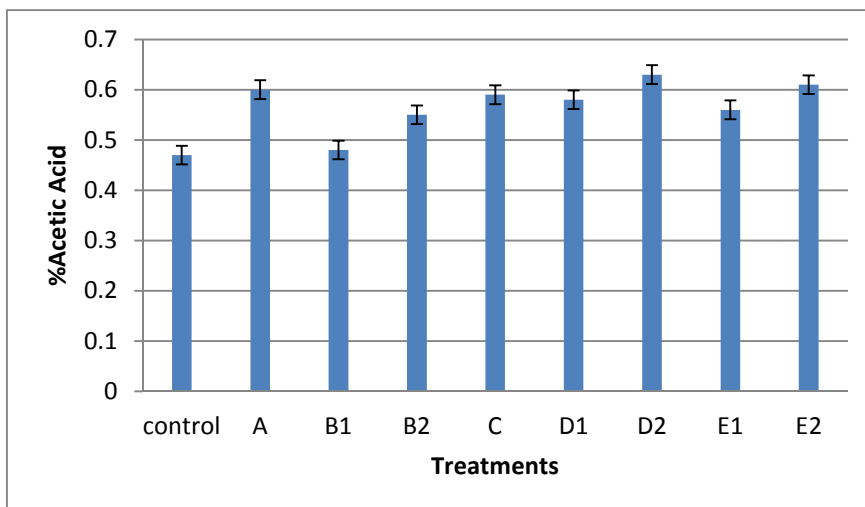


Fig. 2. The value of titratable acidity in different samples in 6th day

higher than others. Other researchers have shown that lactic acid was the major metabolic product in the brines and the concentrations of citric and malic acids were very low (Panagou *et al.*, 2008). The production of acetic acid is most likely as the result of metabolism of citric acid, which had disappeared from inoculated brines (Sánchez *et al.*, 2001). In this assay, citric, tartaric and malic acids were not observed, but value of butyric acid was 525.95 to 766.85 mg/100g. Lactic acid bacteria in the fermented samples can use glucose and citric acid as a carbon source to produce lactic acid faster than yeasts (Amerine & Kunkee, 1968). Malic acid can be degraded to lactic acid and CO₂ by a malolactic enzyme (Montañó *et al.*, 1993).

- Texture analysis

In this respect, there were high significant differences between the treatments during fermentation. In all of the samples, the value of hardness increased up to the 6th day of fermentation. In this regard, the samples without inoculation were harder than the others but in solution of 6% NaCl, the samples had higher hardness than 4% NaCl solution therefore the concentration of brine had an important role in hardness of texture (Roberts & Kidd, 2005). The E, C and A samples had higher hardness and B and D samples had lower hardness, respectively. The B samples had lower hardness among these samples where the population of yeasts was higher than others. Hardness and sensory properties of inoculated vegetables were preferable as compared to other

samples. The starter cultures in E, D, C and A samples have positive effects on the reduction of spoilage risk and increase of self-life.

- Color analysis

Color is the most important attribute used by the customer to evaluate the quality of product and its possible taste without touching the commodity (Tijsskens *et al.*, 2001). In this assay, 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 acidic conditions (Koca *et al.*, 2006). Some authors studied the effect of pH on the rate of color change in broccoli at pH of 3 to 8 and stated that the production of pheophytin followed a first-order reaction and color degradation accelerated with decreasing the pH (Gunawan & Barringer, 2000). The 6% solution of NaCl had lower l*, a* and b* values than the solution of 4% NaCl. In control sample, l* and a* values were lower than the other samples. The increase of a* value might attribute to the browning reactions (Romeo *et al.*, 2009). The decrease of b* value is due to some substances that are released during fermentation and subjected to browning. In control samples without inoculation, b* value was lower than the inoculated solution. In this assay the samples had acceptable appearance. This indicates that higher production of lactic acid by LAB causes better appearance and coloration (Martínez-Castellanos *et al.*, 2009).

Table 2. The values of organic acids in different samples (mg/100gsample)

Organic acids	A	B1	B2	C	D1	D2	E1	E2
Lactic acid	1134.50	907.90	1228.17	1084.34	1037.48	1228.78	1061.99	1069.91
Acetic acid	31.90	19.27	30.66	26.73	19.80	33.60	19.18	32.20
Butyric acid	683.91	525.95	723.36	593.84	715.49	766.85	755.86	695.54

- Sensory test results

Sensory evaluation of cucumber samples was performed at the end of fermentation by 10 non-trained persons. In sensory test, for flavor, the panelists gave the higher rank to E samples than other treatments. For crunchiness trait, the higher rank was given to samples of E, D₂, D₁, C, A, B₂, B₁ and D, respectively. LAB contributes to the aroma and flavor of fermented products. They acidify the food and cause lactic acid taste (Leroy & De Vuyst, 2004). The salt concentration had more effects on tissue hardness and showed that too little or too much salt can lead to softer and lower quality product (Pederson, 1946). The inoculated samples had suitable flavor and texture when were optimally fermented in solution with the pH of 3. The rapid increase in acidity minimizes the influence of spoilage bacteria and reduces the influence of spoilage bacteria and probably improves the microbiological and sensory quality of the fermented product and uniform products can probably be obtained by application of starter cultures (Viander *et al.*, 2003).

Conclusion

In order to control the fermentation of cucumbers, it is advisable to make a heavy inoculation with *L. plantarum*, *L. bolgaricuse* and *S. thermophyluse* to produce a uniform and a high quality product. Inoculation with LAB and appropriate initial brine concentrations can decrease the pH during the fermentation and yield higher titratable acidity than the traditional process. Inoculation also alters the microbial population of the fermentation where the population of the inoculated LAB strains is an important proportion of the total population that remains until the end of the process. LAB contributes to the aroma and flavor of the fermented products where flavor of inoculated solutions have better ranking. The inoculated samples have suitable flavor and texture when were

optimally fermented in solution with pH of 3. Reducing the influence of spoilage bacteria probably improves the microbiological and sensory qualities of the fermented products.

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