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SOME CHARACTERISTICS OF TURKISH-STYLE FERMENTED GARLIC PICKLES PRODUCED USING FOUR DIFFERENT POTENTIALLY PROBIOTIC STARTER CULTURES

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ABSTRACT

Mono-cultured or mixed-cultured fermentations have been preferred to spontaneous fermentation in pickle production. Therefore, five different groups of garlic pickles were produced adopting the Turkish-style fermentation using monocultures or mixed-cultures containing four different probiotic strains. The inhibitory effect of garlic on those strains, the antagonistic activities, the organic acid production abilities of the strains were examined. The chemical properties of the produced pickles, the viability of the strains, their relationship with yeasts in this process were investigated. At the end of the fermentation, there was a approximately 1-logarithmic-decrease in the microbial counts of all the tested strains used for the pickle groups, of which their initial counts was an average of 6.99 Log CFU/ml. All of the strains produced nine different organic acids. This result was associated with the hetero/homo-fermentative properties of the strains. The results showed that the production by probiotic bacteria supported the functional properties of the pickles.

Keywords: Garlic pickle, starter culture fermentation, food quality, antimicrobial property, lactic acid bacteria

DÖRT FARKLI POTANSİYEL PROBİYOTİK KÜLTÜR KULLANILARAK ÜRETİLEN TÜRK TİPİ SARIMSAK TURŞULARININ BAZI ÖZELLİKLERİ

ÖZ

Son zamanlarda turşu üretiminde tek veya karışık kültür fermentasyonları spontane fermantasyonlara tercih edilmektedir. Bu nedenle çalışmamızda Türk usulü fermente edilmiş, tek ve karışık formda beş farklı grup sarımsak turşusu, dört farklı probiyotik suş kullanılarak üretilmiştir. Sarımsakların bu suşlar üzerindeki inhibitör etkisinin olup olmadığı, suşların antagonistik aktiviteleri ve organik asit üretim yetenekleri belirlenmiş, üretilen turşuların kimyasal özellikleri, kullanılan suşların canlılıkları ve bu süreçte mayalarla olan ilişkileri araştırılmıştır. Fermantasyon sonunda turşu grupları için kullanılan probiyotik suşların canlılıklarında yaklaşık 1 logaritmik azalma olmuş ve ortalama 6.99 Log CFU/ml ile fermentasyonu tamamlamışlardır. Şuşların fermantasyon sonunda dokuz farklı organik asit

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ürettikleri belirlenmiş, bunun, suşların hetero/homo-fermentatif özellik göstermeleri ile ilgili olabileceği düşünülmüştür. Elde edilen sonuçlar probiyotik bakteriler tarafından üretilen turşunun fonksiyonel özelliklerinin desteklendiğini göstermiştir.
Anahtar kelimeler: Sarımsak turşusu, starter kültür fermantasyonu, gıda kalitesi, antimikrobiyal özellik, laktik asit bakterileri

INTRODUCTION

Fermentation is one of the oldest methods of food preservation and fermented foods generally have beneficial flora for the regularity and health of human digestive system. The flora in fermented foods mainly consists of lactic acid bacteria (LAB) and they produce lactic acid by the utilization of fermentable sugars. Thanks to the lactic acid fermentation, the ingredients of the herbs, and the quality, taste and aroma of the product are well preserved (Bamforth 2005; Çetin 2011; Tokatlı et al., 2015; Chaiyasut et al*.,* 2018). Today, food production methods are preferred in which microorganisms such as LAB with various extra characteristics (Herreros et al*.,* 2005). Pickles are one of the main fermented foods produced by using such microorganisms and preserving foodstuffs under high acid concentration, enabling their preservation for a long time without any need of refrigeration (Nurul and Asmah 2012; Chaiyasut et al*.,* 2018; Behera et al*.,* 2020). Especially *Lactiplantibacillus* strains have been known as preferred starter cultures for use in fermented pickles. This was associated with the fact that some lactobacilli species are facultative hetero-fermentative and they are able to transform hexoses into lactic acid and, subsequently, to acetic acid, also producing various metabolites with desirable properties. Also, when pickles are fermented by LAB, they will possibly have a distinctive flavor and exert positive health effects (Behera et al*.,* 2020). These positive effects can be be related to many compounds such as antimicrobials, lactic acid, acetic acid, hydrogen peroxide, diacetyl, ethanol, and bacteriocins produced by LAB. Those important propertied of LAB starter cultures also contribute to the assurance of food safety and technological quality of fermented products (Herreros et al*.,* 2005; Irkın and Songun 2012; Choi et al*.,* 2018). Release of inhibitory chemical compounds produced by LAB and high salt concentration in fermented foods may suppress growth of pathogenic or spoilage bacteria (Inatsu et al*.,* 2005). Other than the inhibitory substances of LAB, some vegetables contain various natural substances that have the capacity to inhibit the growth of pathogenic and spoilage microorganisms.

Garlic (*Allium sativum*) is a small bulbous plant belonging to the *Alliaceae* or *Liliaceae* family, and has been known for many years to contribute to various health benefits and cuisine flavor. (Sethi et al*.,* 2014; Sadeghi, 2016). Garlic is also rich in vast amounts of nutritional compounds including protein, calcium, magnesium, iron, potassium, zinc, arginine, saponins, polyphenols, and selenium and commonly contain abundant levels of Vitamin A, Vitamin B6 and B1, and vitamin C (Sethi et al*.,* 2014).

The present study mainly aimed to produce garlic pickles adopting the Turkish-style fermentation using bactierial strains isolated from natural sources, and known to have various probiotic properties. The second aim was determine the effects of the production made with these selected strains on garlic pickles. For this purpose, the study examined the inhibitory effect of garlic on these LAB, and investigated whether this effect was affected by temperature. In addition, the organic acids produced by the strains at the end of fermentation were determined and the relationship between the organic acids produced and the microbiological quality of the pickles was examined.

MATERIAL AND METHODS

Material

Isolation and identification of the lactic acid bacteria

The strains used in the study were isolated from naturally fermented cheeses, pickles, and fresh fruits obtained from farmers' markets and identified (Alp, 2018). In addition, their various cultural and biochemical features (fermentation of carbohydrates, salt tolerance, growth at different

temperatures, gas (CO_2) production from glucose, production of H_2S) were determined by the procedures described by Schillinger and Lücke (1987), and various probiotic properties (Alp, 2018; Alp and Kuleaşan 2020).

Methods

Determination of the inhibitory effect of garlic on lactic acid bacteria

Many reserach demonstrated that garlic has inhibitory/lethal effect on various microorganisms (Abiy and Berhe 2016). For this purpose, in our study, we determined whether it has an inhibitory effect on LAB. Two different groups of fresh garlic were used. The first group was kept in boiling water for 1 minut whereas the second group received no treatment. The aim here was to determine whether the heat application had an effect on this feature. The agar well diffusion method was used to determine the antibacterial activity of garlic on the LAB (Andersson et al*.,* 1988). All LAB were incubated at 30°C for 18h. At the end of the incubation, they were adjusted to 0.5 Mc Farland in phosphate-buffered saline solution (PBS). After they (100 µl) were homogeneously injected into the MRS medium, they were poured into sterilized Petri dishes. For the investigation of the antibacterial activity, 10- mm-diameter wells were cut into the media and then garlic was filled into these wells in the agar plates directly. At the end of the incubation period, inhibition zones formed on the media were evaluated (Herreros et al*.,* 2005; Strika et al*.,* 2017).

Antagonistic activity

Spot on lawn assay method was used to determine the antagonistic activity. The reference cultures used in the study were *Salmonella enterica* subspecies *enterica* serovar Enteritidis and *Escherichia coli* type 1*, Listeria monocytogenes* and *Staphylococcus aureus*, obtained from culture collections of Süleyman Demirel University, Department of Food Engineering. All microorganisms were incubated under their suitable conditions and media, then the optical density of each culture was adjusted to 0.5 McFarland standard in Phosphate-Buffered Saline (PBS). Then the pathogens were inoculated (1%) into 15 mL of Tryptic Soy Soft agar (Merck) medium, and the lactic acid bacteria were inoculated (1%) into 15 mL De Man Rogosa Sharpe (MRS). First, MRS agar was poured into the petri dishes, then Tryptic Soy soft agar medium was poured on to the MRS agar medium. After a 24-h incubation, zone diameters were measured (Sumathi and Reetha 2012; Fijan, 2016).

Preparation of garlic pickles

First, garlic bulbs were cleaned, and the cloves were peeled and placed in containers in equal grams under aseptic conditions. A total of 6 different groups of garlic pickles were prepared using 4 different LAB, 5 different groups, and a control group. The names of the groups and the lactic acid bacteria they contain and isolation source are given in Table 1. Each pickle group was inoculated with (0.5 MacFarland) $1.5x10^8$ Log CFU/mL of test bacteria. Also, each pickle group received 5% salt, 1% citric acid, and no preservatives were used in the process.

Determination of viability in garlic pickle of LAB and other microorganisms during fermentation

For the microbiological analysis of garlic pickle, samples were taken randomly during fermentation, and then the number of viable cells was determined by serial plating. For total bacterial counts, Plate Count Agar (PCA) was used while, for LAB counts MRS agar was used, Eosin Methylene Blue (EMB) agar was used for Enterobacteriaceae and Potato Dextrose (PD) agar was used for yeasts. All microbiological analyses were made in triplicate and the mean values and standard deviations were calculated (Beganovic et al*.,* 2011).

Determination of titratable acidity and pH value of garlic pickles during fermentation

The titratable acidity values were determined according to Tyl and Sadler (2017). Titratable acidity analyses were carried out by the titration of brines with 0.1 N NaOH and were expressed as percentile lactic acid (% w/v). The pH was determined by a digital pH meter (InoLab, pH Level 1).

Determination of organic acids produced by LAB

Organic acids were determined at Application and Research Center for Innovative Technologies Center (YETEM) of Süleyman Demirel University by Thermo Scientific Ultimate 3000 HPLC device equipped with Fortis MSMS Dedector.

Statistical analysis

Microbial enumerations were done in triplicates and the results are presented as mean±standard deviation. The Minitab 18 statistical software (Minitab, Inc, State College, PA, USA) was used. The differences were determined by one-way ANOVA with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and identification of the lactic acid bacteria

Fermented foods provide a healthy effect on our gastrointestinal system they make this generally by beneficial bacteria and their different natural property. Besides, these beneficial bacterias will improve the vegetable's sour taste that is rich in vitamins through various fermentations (Al-Shawi et al., 2019). Therefore, these starter cultures posess at least one strain-specific and functional property and they are used to improve the quality of the end product (Beganovic et al., 2011).

In the present study, pickles were produced from garlic that is known to be beneficial for health, by using 4 different LAB with various functional properties (Alp, 2018; Alp and Kuleaşan 2019). All isolates were Gram-positive, catalase-negative and non-spore-forming. In addition, various characteristics of isolates such as development in different pH environments, resistance to pepsin, pancreatin enzymes and EPS production were determined in our previous studies (Alp, 2018; Alp and Kuleaşan 2019). Garlic pickle was produced with these four well-known strains (Table 1). The selection of the species to be used in pickle production is based on a previous study (Karovičová and Kohajdová 2003).

Table 1. The names of the groups and the lactic acid bacteria and their isolation source

Group Name	Lactic acid bacteria content	Source
Control	Any strain wasn't added	
Group 1	*Lactobacillus plantarum* DA100	Pickle
Group 2	*Lactobacillus fermentum* DA134	White cheese
Group 3	*Lactobacillus coryniformis* DA256	Rosemary
Group 4	*Leuconostoc lactis* DA268	Eriobotrya japonica
Group 5	All of the total 4 different strains which is reside in each group were added	

Inhibitory effect of Garlic on lactic acid bacteria

Garlic is an antibacterial agent thanks to its main component, allicin, which is responsible for the pungent smell, and, by this means, has antibacterial activity against various Gram-negative and Gram-positive bacteria (Ankri and Mirelman 1999; Harris et al., 2001; Abiy and Behre 2016). However, the relationship of this effect with the heat applied to the garlic is not fully revealed. Therefore, the viability of the tested strains were tested against boiled and unboiled garlic. The purpose of this process was to determine how efficient the garlic's known antimicrobial effect is against the tested strains. Garlic which was boiled for 1 minute did not show any inhibition effect on the strains, whereas the fresh garlic showed various inhibition effects on LAB (Table 2).

Untreated garlic showed various inhibitory effects on the tested strains and the difference between the heat-treated and untreated garlic was statistically significant. The highest inhibitory effect was recorded for *Lactobacillus fermentum* DA134 (26.66±1.15 mm). Figure 1 shows that the results of tested strains in terms of the antimicrobial effect of boiled and unboiled garlic.

A study indicated that fresh garlic has bacteriostatic activity against the Gram-positive bacteria (Strika et al., 2017). Also, the reserachers compared the fresh and heat-treated garlic bacteriostatic activity and the results showed that the fresh garlic has a stronger antimicrobial activity than that of the heat treated.

Table 2. Results of the antimicrobial effects of fresh garlic on lactic acid bacteria

Microorganisms	Zone of inhibition (mm)
Lactobacillus plantarum DA100	24.33 ± 0.57[b]
Lactobacillus. fermentum DA134	26.66 ± 1.15[a]
Lactobacillus coryniformis DA256	20.00 ± 1.00[d]
Leuconostoc. lactis DA268	22.33 ± 0.56[c]

Values are means of three independent determinations and standard errors. In the same column, the letters from a to d represent the largest to smallest order of numeric values, respectively ($p < 0.05$)

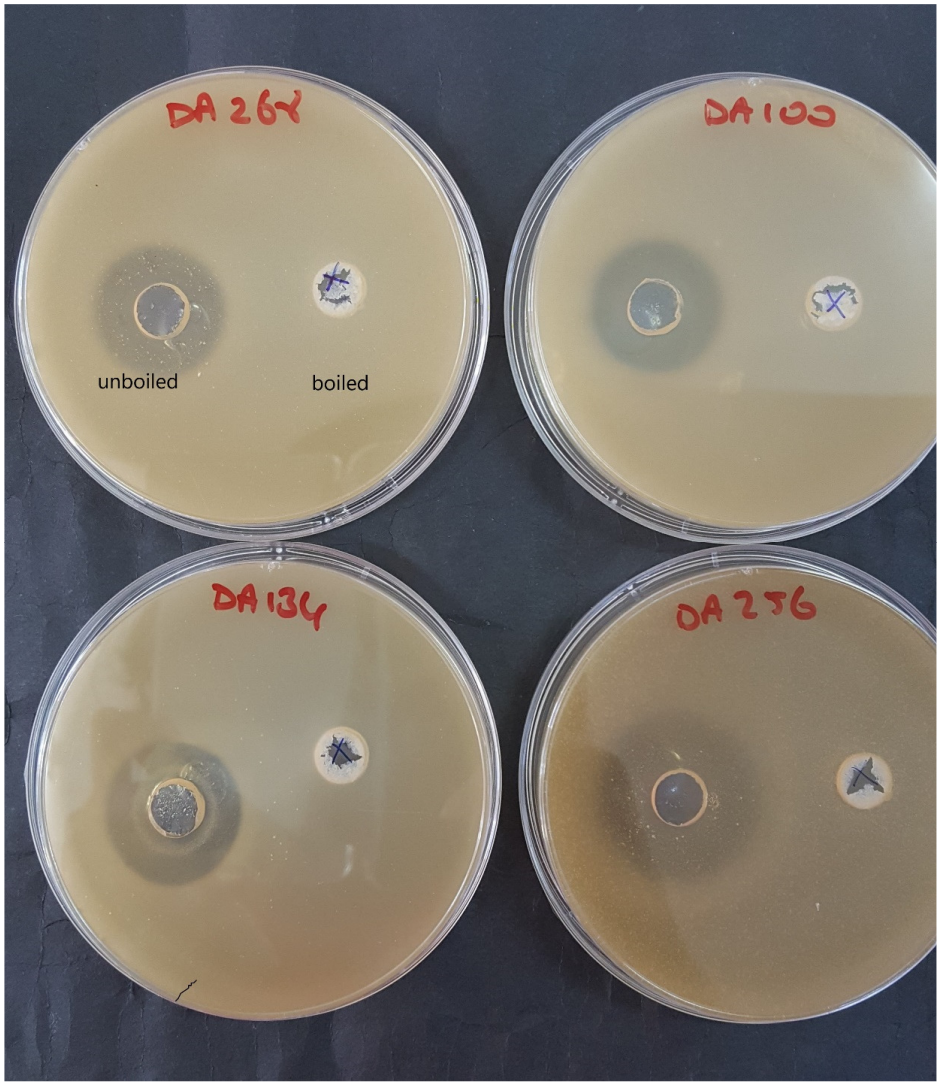

Figure 1. The results of tested strains against the antimicrobial effect of boiled and unboiled garlic

Antagonistic activity

The antagonism between various groups of microorganisms like lactic acid bacteria and

pathogens is widely known in nature and these abilities are based on their organic acids, carbon dioxide, and specific antibiotic substances synthesize (Tsugkiev et al., 2018). Some reserachers indicated that strains of the same species of a microorganism may be widely different from each other in terms of their antagonistic ability (Tsugkiev et al*.,* 2018). Moreover, the reserachers have stated that the antagonistic properties of combinations formed by the correct selection of various strains of the same microorganism species may be more effective.

In the present study, antagonistic activity of selected strains was determined by the Spot on lawn assay method against various pathogenic bacteria and the results are evaluated statistically (Table 3). It was observed that the same strain may have different antagonistic abilities against different pathogens. These differences in the antagonistic properties of lactic bacteria were thought to be due to their ability to synthesize organic acids, carbon dioxide, and certain antimicrobial substances.

Table 3. The inhibition zone diameters of lactic acid bacteria against different pathogen bacteria (mm)

Microorganisims	*E. coli*	*S.* Enteritidis	*L. monocytogenes*	*S. aureus*
L. plantarum DA100	25.66 ± 0.57^{cD}	34.00 ± 1.00^{cC}	35.66 ± 0.57^{cB}	44.00 ± 1.73^{aA}
L. fermentum DA134	40.33 ± 0.57^{aB}	34.33 ± 1.15^{cC}	44.33 ± 0.58^{aA}	44.33 ± 1.15^{aA}
L. coryniformis DA256	35.00 ± 1.00^{bD}	54.66 ± 0.57^{aA}	35.33 ± 0.58^{cC}	40.33 ± 0.05^{bB}
L. lactis DA268	33.00 ± 1.00^{bD}	44.66 ± 0.57^{bB}	41.00 ± 1.00^{bC}	45.00 ± 0.00^{aA}

Mean values and standard deviations of three replicates are presented.

*[a] Different letters in the same column give results of strains against a single pathogen, between groups significant differences ($p < 0.05$)

** [A] in the same row indicate result of same strain against all pathogens, between groups significant differences ($p < 0.05$).

Our results showed that the same strain may have different antagonistic abilities against different pathogens. For example, *L. corniformis* DA 256 showed the strongest antagonistic property against *S. enteritidis,* contrary to other pathogens. Against *E. coli*, the largest zone (40.33 mm) and the smallest zone (25.66 mm) were observed in *L. fermentum* DA134, and *L. plantarum* DA100, respectively. Compared to other pathogens' zone diameters, *S. aureus* had the largest zone among all tested bacteria, giving the largest zone by *L. lactis* DA268 (45.00 mm).

It was thought that these differences in the antagonistic properties of lactic bacteria may be due to their ability to synthesize various organic acids or antibiotic-like substances. In a study, similar to our results, four different LABs were found to have antagonistic activity against *S. aureus* and *E. coli* (Datta et al*.,* 2013). Strains produced a zone of 14mm against *E. coli*, 13mm against *S. aureus*. Similar results have been reported by numerous studies (Urapian and Hongpattarakere, 2015; Gutiérrez-Cortés et al., 2017; Bisht and Garg, 2019; Reuben et al., 2019). The similar results with our study results supports the hypothesis that the isolates may have different antagonistic activity against various pathogens.

Viability of LAB and other microorganisms in garlic pickle during fermentation

Some LAB are used as suitable starter cultures for the improvement of quality of fermentation. Also, it has been stated that the use of starter culture help standardize the fermentation by controlling the microbial flora (Xiong et al*.,* 2014). In the present study, a total of six different pickles were produced, a control group, four test groups fermented by using different strains individually and the final group fermented by mixture of all four strains of LAB. While there were almost 1 logarithmic lactic acid bacteria in the control group at the beginning of fermentation, each experimental pickle group (0.5 Mac Farland) had $1.5x10^8$ Log CFU/mL test bacteria initially.

In the control group, LAB were found below the detection limit (<1 Log CFU/g) on days 14 and 21, and no LAB remained at the end of fermentation. In other groups, approximately 1

logarithmic decrease was observed in LAB countst at the end of fermentation. Also, no coliform group bacteria and *E. coli* was determined in any of the groups during fermentation. Group 1 and Group 4 completed fermentation with approximately 6.15 logarithms while other groups showed that an average of 1 logarithmic decrease at the end of fermentation, and they completed fermentation with more than 7.00 logarithms. In the control group, the presence of LAB was determined below 2 logarithms at the beginning, and the end of the fermentation (Table 4).

Generally, yeasts are undesirable in fermentation as they facililtate the activities of oxidative and fermentative activities, CO_2-induced degradation and other degradation factors. But they can help the aroma development of the product by producing aroma substances such as diacetyl as a result of their metabolism. In addition, yeast and mold count is also one of the most important factors for the shelf life of pickle. Yeast counts of samples were found to be below the detection limit on day 21 (<1 Log CFU/g) except for the control group and Group 2. In the total microorganism counts, they completed fermentation with an average of approximately 5 logarithms, although there was no mold growth in any of the samples. All results of microorganisms of the pickle groups are shown in Table 4.

It was thought that there might be a few possibilities for the reason of these results. The first is fermentable sugar remained in the environment after the primary fermentation ended and as a result of this condition, the yeasts took part in the secondary fermentation stage by using up these sugars. The second is both positive and negative interactions between yeast and LAB species. Some LAB can secrete galactose, which can help lactose-negative yeasts grow. However, this can also be the opposite. For example, they can be inhibited by compounds such as phenyl-lactic acid, 4-hydroxy-phenyl-lactic produced by LAB (Alvarez-Martin et al*.,* 2008). In a study in which pickled cucumbers were produced usingh starter cultures, it was determined that there was 6.65 CFU/g yeast in the samples at the end of 6 days (Nilchian et al*.,* 2016). Another study reported similar results (Tokatlı et al*.,* 2019). In addition, the total number of microorganisms obtained in both studies is similar to those of our study.

Titratable acidity and pH value of garlic pickles during fermentation

It is known that when the pH value falls below 4.5 in fermented products, its provides a limiting factor for the growth of *Enterobacteriaceae* (Özer and Yıldırım, 2018). In our study, *Enterobacteriaceae* were not detected during or at the end of fermentation. We thought that this result may be related to the development of acidity and also the organic acid production.

The titration acidity of the samples on the first day of fermentation was found between average 0.65%, we thought that these results were releted to the citric acid added to the brine. Acidity development in the control sample was slower as was the case in pH values. In addition, acidity development was found to be slower in Group 2. Besides these results, pickle samples using starter culture produced acid faster in the first days of fermentation compared to the control sample except for Group 2. The highest titration acidity (0.90%) at the end of fermentation was determined in Group 5. This was attributed to the fact that they contained mixed culture and had good acid producing abilities in pickle fermentation. Also, in our previous studies (Alp, 2018), it was seen that these strains can highly survive in acidic environments. These results we obtained are in line with the results of the various studies (Tokatlı et al*.,* 2019).

Throughout fermentation, the mean pH value of pickle groups fermentations decreased approximately from 5.50 to 3.35 (Fig 2). Pickle group results showed a pH value drop during day 7, and the fastest decrease in pH was observed on day 14. A significant difference ($p < 0.05$) was observed in pH value decreases among pickle groups between the fermentation days. During fermentation, LAB decreased the pH, and this provided a key element for a successful fermentation process. Thanks to the rapid pH

decrease, most Gram (-) bacteria and spore-forming bacteria were inhibited. Figure 2 and Table 4 shows the changes in all samples changed in terms of pH (b) values and titratable acidity (a) during fermentation.

Table 4. All results of microorganisms and changed titraable acidity values and pH of the pickle groups during fermentation.

		Control	Group 1	Group 2	Group 3	Group 4	Group 5
Lactic Acid Bacteria (CFU/mL)	0	1.85±0.01^{aB}	8.17±0.00^{aA}	8.17±0.00^{aA}	8.17±0.00^{aA}	8.17±0.00^{aA}	8.17±0.00^{aA}
	7	1.70±0.01^{bF}	6.85±0.01^{bD}	7.17±0.01^{dA}	7.07±0.03^{dC}	7.13±0.03^{cB}	6.49±0.03^{dE}
	14	0.00±0.00^{cF}	6.45±0.02^{cE}	7.28±0.02^{cB}	7.25±0.05^{bC}	7.11±0.04^{bD}	7.75±0.02^{bA}
	21	0.00±0.00^{cF}	6.17±0.02^{dD}	7.90±0.02^{bA}	7.07±0.03^{cC}	6.11±0.02^{dE}	7.73±0.02^{cB}
Yeast (CFU/mL)	0	2.00±0.03^{dA}	0.99±0.02^{bF}	1.09±0.01^{dE}	1.19±0.02^{bB}	1.16±0.03^{bD}	1.17±0.03^{cC}
	7	3.61±0.01^{bB}	1.01±0.01^{aF}	3.27±0.03^{aC}	2.89±0.03^{aE}	2.95±0.03^{aD}	3.82±0.03^{aA}
	14	5.75±0.03^{aA}	0.00±0.00^{cD}	3.00±0.01^{bB}	0.00±0.00^{cF}	0.00±0.00^{cE}	2.09±0.01^{bC}
	21	3.46±0.02^{cA}	0.00±0.00^{cC}	2.13±0.02^{cB}	0.00±0.00^{cF}	0.00±0.00^{cD}	0.00±0.00^{dE}
Total count microorganism (CFU/mL)	0	2.03±0.01^{dF}	5.90±0.04^{bA}	3.00±0.02^{dE}	4.93±0.03^{dC}	5.13±0.02^{dB}	3.47±0.02^{dD}
	7	4.32±0.02^{cF}	5.67±0.04^{cD}	5.63±0.03^{bE}	5.89±0.03^{bB}	5.99±0.01^{aA}	5.77±0.02^{bC}
	14	6.05±0.03^{aB}	6.77±0.02^{aA}	5.90±0.02^{aC}	5.76±0.03^{cE}	5.67±0.03^{bF}	5.87±0.02^{aD}
	21	5.69±0.03^{bB}	5.33±0.03^{dE}	4.99±0.03^{cF}	5.99±0.01^{aA}	5.67±0.03^{cC}	5.48±0.02^{cD}
pH	0	3.50±0.01^{C}	3.61±0.01^{A}	3.48±0.02^{D}	3.57±0.02^{B}	3.38±0.02^{F}	3.46±0.03^{E}
	7	3.57±0.02^{A}	3.44±0.03^{D}	3.38±0.02^{F}	3.48±0.02^{B}	3.45±0.03^{C}	3.40±0.03^{E}
	14	3.50±0.02^{A}	3.41±0.03^{B}	3.37±0.04^{D}	3.40±0.04^{C}	3.40±0.02^{C}	3.36±0.03^{E}
	21	3.45±0.02^{A}	3.37±0.02^{C}	3.35±0.02^{D}	3.32±0.01^{E}	3.38±0.02^{B}	3.30±0.01^{F}
Titraable acidty (%)	0	0.75±0.02^{B}	0.69±0.03^{C}	0.59±0.02^{E}	0.54±0.03^{F}	0.60±0.02^{D}	0.78±0.02^{A}
	7	0.81±0.02^{A}	0.73±0.02^{C}	0.63±0.02^{E}	0.59±0.01^{F}	0.66±0.01^{D}	0.80±0.01^{B}
	14	0.84±0.02^{C}	0.81±0.01^{D}	0.65±0.02^{F}	0.80±0.02^{E}	0.88±0.01^{A}	0.86±0.02^{B}
	21	0.88±0.04^{B}	0.85±0.03^{C}	0.68±0.02^{E}	0.82±0.02^{D}	0.90±0.01^{A}	0.90±0.01^{A}

Values are means of three independent determinations and standard errors. In the same column, the letters from A to F represent the largest to smallest order of numeric values, respectively ($p < 0.05$)

Organic acids produced by lactic acid bacteria

Some LAB strains have antifungal or antimicrobial properties, and what gives them these properties are their organic acids such as lactic, acetic, formic, caproic, propionic, butyric and valeric acids (Zala´n et al*.,* 2010), proteinaceous compounds, fatty acids and bacteriocin-like substances production abilities. Most known antimicrobial activity of LAB is production of organic acids which are associated with the primary metabolism from carbohydrates fermentation (Guimarães et al*.,* 2018). Production of organic acids can help lower the pH of the media and thus, they prevent the growth of various pathogenic microorganisms in food products or human intestinal microflora (Ammor et al*.,* 2006). Figure 3 shows the organic acid production in pickle groups with lactic acid bacteria. Various studies showed that citric acid and its salts can inhibit the common pathogens growth. These inhibitory mechanisms may be accessing the microbial cell by ionization of undissociated acid molecules and cause the disruption of substrate transport by altering cell

membrane permeability or reduction of proton motive force (Shokri 2011).

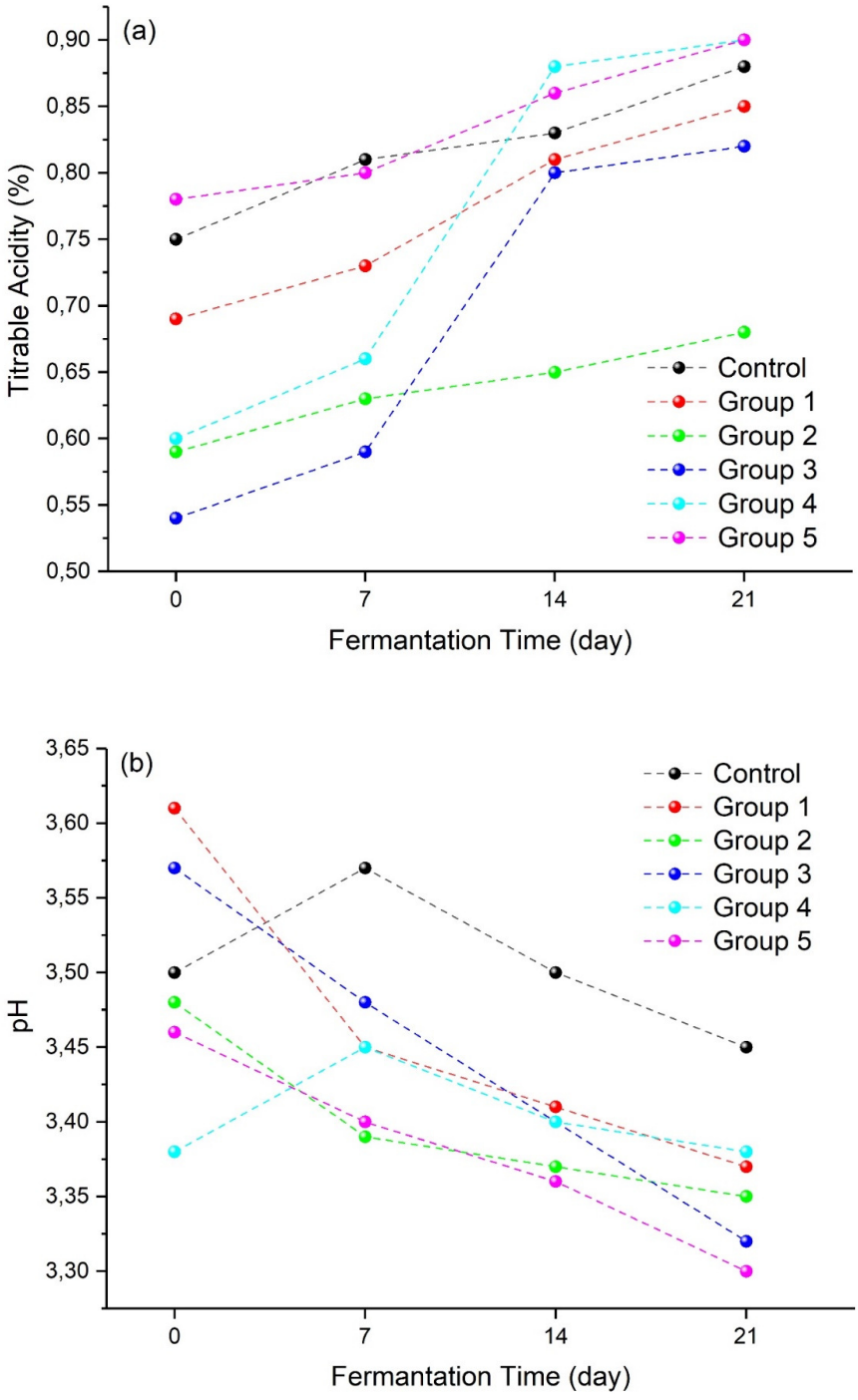

Figure 2. Changes in titratable acidity (a) and pH (b) during fermentation.

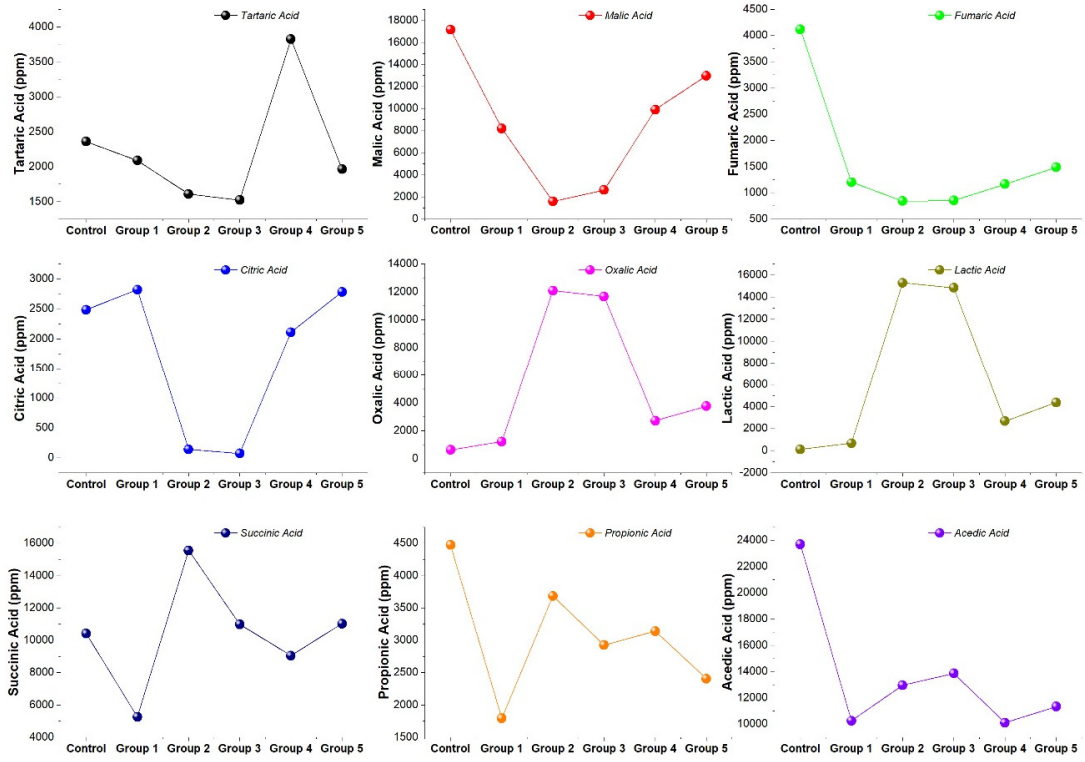

Figure 3. Organic acid production in pickle groups of lactic acid bacteria.

In the present study, a total of 9 in different quantity and variety organic acids were produced in pickle groups. There was a significant difference ($p<0.05$) between the groups in terms of organic acid production. Besides the lowest was in Group 2, malic acid production was observed in all pickle groups. In the control Group, acetic acid was the maximum. Organic acid production like lactic acid can vary between the different LAB species, as well as between the individual strains within a species (Peev et al., 2017). This variablity was also noteworthy in the present study. Lactic acid production wasthe highest in Groups 2 and 3. It was also noteworthy that there were succinic and oxalic acid productions in the groups. Also, these results may be related to the activities homo- and/or heterofermantative LAB. When the organic acid contents of the pickle groups were examined separately, the most common organic acids were found to be malic acid and succinic acid. While it was determined that there were high levels of lactic acid in pickle Groups 2 and 3, this value was low in groups 1 and 4. These results suggested that groups 1 and 4 could be heterofermantative whereas groups 2 and 3 could be homofermentative LAB. Besides, group 5 gave an average result of all in terms of organics. Because it contains both types of bacteria. Apart from lactic acid bacteria, it is known that also some yeasts produce various organic acids (Hesham et al., 2020). Although the test strain was not added to the control group, a low amount of lactic acid and different amounts of organic acid were detected.

In general, *Lactobacillus* strains produce acetic acid substantially late in fermentation and their contribution to inhibition is considered secondary as their final amount is low compared to lactic acid (Rossland et al., 2005). In the present study, contrary to this generalization, high levels of acetic acid was determined at the end of fermentation in the groups. Therefore, it was thought that some organic acid production especially acetic acid may have been produced by

yeasts, LAB, or the yeasts have produced some ethanol and then the acetic acid bacteria may have converted them into acetic acid. Also, it was thought that another reason for the high levels of lactic acid and low levels of citric acid in Groups 2 and 3 was that bacteria produced lactic and / or acetic acid using citric acid. Organic acid consumption and production by yeast, have been linked mostly to central carbon metabolism during fermentation. Mainly, organic acids are intermediates or by-products of glycolysis but they may also be derived from the glyoxylate pathway (Chidi et al*.,* 2015). In the present study, it was thought that some organic acids are produced this way by yeasts. In a study of citric acid production and optimization of various yeast strains, the strains produced an average of 25 g/L citric acid (Hesham et al*.,* 2020). In the present study, it was observed that there was succinic acid production in addition to citric acid.

It was thought that succinic acid might have been produced by yeasts as well as LAB, affected by changes in temperature and pH. Similarly, studies have shown that both yeasts and LAB produce succinic acid (Martinez et al*.,* 2019). The presence of tartaric acid in all groups, albeit in low amounts, suggested the possibility of its natural presence. Based on the results of the study conducted by Ho et al*.,* (2018). Various studies support similar organic acid productions (Martinez et al*.,* 2019; Nuryana et al*.,* 2018; Alp and Öner 2014; Rossland et al*.,* 2005). The fact that no coliform groups were encountered in the groups at the beginning and end of fermentation it was thought that this may be due to the fact that acetic acid is a better preservative compared to organic acids such as lactic acid due to its higher pKa value.

In conlcusion, the results of this research showed that the application of the beneficial isolates positively influenced the fermentations by improving the beneficial properties of the final product. Positive properties of these isolates are considerably affecting the prevention of spoilage and undesirable microorganism growth. The formulated pickles have been evaluated for the organic acids and microbiological quality.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects.

AUTHOR CONTRIBUTIONS

DA: Designed study, conducted all analyzes, and prepared pickle samples for HPLC analysis and wrote the publication. HA: Wrote the manuscript, analyzed the data by statistical program.

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