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Production of Traditional Grape Pickle Using *Lactobacillus acidophilus* and Investigation of the Inhibitory Effect of the Product on *Bacillus cereus* and *Escherichia coli*

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ABSTRACT

Grape pickle is a traditional food that is made with grapes (*Vitis vinifera*), horseradish (*Armoracia rusticana*) and grape syrup. In this study, the survival of *Bacillus cereus* and *Escherichia coli* in grape pickles produced with or without using probiotic *Lactobacillus acidophilus* La-5 as well as microbiological, chemical and sensory properties of each group were examined during 35 days of ripening at 25°C and 5 months at refrigerated storage period. Molds and lactic acid bacteria (LAB) counts remained below the limit of detection (<1.3 log cfu/g). Yeast number of grape pickles with horseradish remained under the detection limit at the end of the ripening period, while the numbers in horseradish-free groups were found to be 4.95-6.59 log values. *L. acidophilus* did not retain viability at level of >6 log in samples to be considered a probiotic product. *E. coli* counts rapidly declined to undetectable level within 7 days, while *B. cereus* numbers was found 1.56-1.72 log cfu/g at the end of the storage period. As a result, it was established that traditional grape pickle is not suitable food matrix for probiotication. High total soluble solid content (63 °Brix) and presence of horseradish in grape pickles ensure the microbiological stability as well as the safety of product.

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Introduction

Grape pickle is a traditional food that produced and consumed in Thrace and Cappadocia region of Turkey. Grape pickle is a grape based non-fermented food, produced using Müşküle grape (*Vitis vinifera*), horseradish (*Armoracia rusticana*) and boiled grape must. Grape has many benefits to health, it contains several vitamins, minerals, and bioactive compounds such as isoquercetin, catechin and procyanidin B1 (Meng et al., 2017; Toscano et al, 2017).

Horseradish has a long tradition of use as a spice and medicinal plant (Bladh and Olsson, 2011). Antimicrobial activity of horseradish roots have been observed for many foodborne pathogens and fungi (Choi et al, 2017; Dekić et al., 2017; Petrovic et al., 2017). Horseradish roots contain mainly allyl isothiocyanates (AITC) (>65%) and 2-phenyl ethyl isothiocyanates (>30%). Allyl isothiocyanates have antimicrobial effects against pathogens even at low concentrations (Kim et al., 2015). Choi et al. (2015) reported that packaging of Jeotgal, a traditional fermented food, with polypropylene patches containing isothiocyanates obtained from horseradish roots can prolong the product shelf life and maintain sensory properties.

Probiotics are defined as "live microorganisms that when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Probiotics must be at minimum 10^6 cfu/mL in foods and must be consumed at least 10^8-10^{10} cfu to show the health benefits (Kechagia et al., 2013). The studies on the production of fruit and vegetable-based products containing probiotics are increasing due to increasing awareness of health benefits of fruits and vegetables. In many studies, it is reported that fruit juices and fruit-based foods are suitable for the growth and survival of probiotics (Champagne and Gardner, 2008; Rodrigues et al., 2012; Mohan et al., 2013).

Traditional foods are regarded by consumers as safe due to the long history of use. On the other hand, there are limited studies on the ability of pathogens to survive in some traditional foods. There is a need for challenge testing to

ensure the safety of traditional foods. To the best of our knowledge, the present study is the first that investigate the traditional grape pickles. The aims of the present study were to evaluate a) the microbiological and physicochemical properties of traditional grape pickles, b) the survival of *Bacillus cereus* and *Escherichia coli* in traditional grape pickle samples produced in laboratory conditions during the production and storage period of 5 months and c) the survival of probiotic *Lactobacillus acidophilus* in grape pickles for the determination of probiotication potential of traditional grape pickles.

Materials and Methods

Grapes

Two different white grape (*Vitis vinifera*) varieties, namely Müşküle and Yapıncak were obtained from local producers in Sakarya and Tekirdağ, respectively. Müşküle grapes known as late-maturing table variety were used in clusters to produce grape pickles. Yapıncak grapes were used to prepare grape must syrup by using traditional method as described below.

Horseradish Roots

Horseradish roots (*Armoracia rusticana*) were collected from a local producer in Tekirdağ, Turkey. Fresh horseradish roots were cut in size of 0.5 x 0.5 x 8.0 cm sticks to produce traditional grape pickles.

Bacterial Strains and Inoculum Preparation

Lyophilized culture of *Lactobacillus acidophilus* La-5 was provided from Chr. Hansen® (Chr. Hansen A/S, Istanbul, Turkey). *E. coli* ATCC 25922 and *B. cereus* ATCC 10876 strains were kindly obtained from Assoc. Prof. Dr. Şeniz Karabıyıklı in Gaziosmanpaşa University Food Engineering Department (Tokat, Turkey). *B. cereus* No:8 Holland strain was obtained from Microbiology Laboratory of Ege University Food Engineering Department (İzmir, Turkey). Each strain was stored at -20°C in Tryptic Soy Broth (TSB; pH 7.3±0.2, Merck, Darmstadt, Germany) containing 30% glycerol.

Lyophilized culture of *L. acidophilus* La-5 about 0.1 g was directly added into the jar to produce grape pickles containing *L. acidophilus* La-5. *E. coli* ATCC 25922 was activated twice in TSB at 37°C for 16-18 hours. *B. cereus* ATCC 10876 and *B. cereus* No:8 Holland strains were activated twice in TSB at 30°C for 12 h to supply the culture at the end of logarithmic growth phase. After the incubation period, two strains of *B. cereus* cultures mixed in 1:1 ratio and cells were harvested through centrifugation (2000xg for 10 min at 4°C), washed twice and resuspended in sterile peptone water to obtain culture suspensions of containing about 10^6 cfu/mL. The concentrations of bacteria were adjusted using MacFarland densitometer (Grant-bio®, United Kingdom). In order to confirm the numbers of bacteria in culture suspensions, plate count techniques were applied using Tryptone Soya Agar (TSA; pH 7.3±0.2, Oxoid, Hampshire, UK) and plates were incubated for 24 hours at 37°C for *E. coli* and 30°C for *B. cereus*. de Man Rogoso Sharpe Agar (MRS Agar, pH 5.5±0.2, Merck, Darmstadt, Germany) with double layer was used for *L. acidophilus* La-5 count and the plates were incubated at 37°C for 48 h.

Production of Grape Pickles with and without L. acidophilus

Preparation of Grape Must Syrup

Grape must syrup was produced from Yapıncak variety of grapes by using traditional production method. Yapıncak grapes were squeezed by using a food processor to produce grape must. Pekmez earth (1 kg) was added per 47 L of grape must and waited for 3 h for the separation of sediments and other debris on the surface. Aqueous phase was removed to a vessel and boiled for about 30 min. After this step, it was strained by using cheese cloth to obtain a clear liquid phase. Strained phase was boiled about 1 h and 45 min to concentrate. After the boiling process, the product was cooled to 25°C to get ready for producing grape pickles (Figure 1A).

Preparation of Grape Pickles

Müşküle grapes (100 g) and horseradish roots (20 g) were placed into a jar (200 cc) as one layer of grape with its cluster and one layer of horseradish root sticks. After the placement of grapes and horseradish roots, the jar was filled with grape must syrup (175 mL). Grape pickles without the addition of horseradish roots were also produced with the same amount of grape and grape must syrup for the determination of the effect of horseradish roots on grape pickles. For the production of grape pickles containing probiotic strain *L. acidophilus* La-5, grape pickles in glass jars were prepared as described above and 0.1 g of lyophilized culture was added to each jar. Four different group of grape pickles were prepared as follows: traditional grape pickle (TGP); grape pickle made without horseradish root (GPWH); traditional grape pickle containing *L. acidophilus* La-5 (TGPL); grape pickle made without horseradish root containing *L. acidophilus* La-5 (GPWHL) (Figure 1B). Grape pickles produced in four different group were ripened at 25 °C for 35 days and then stored at 4°C for 5 months. Grape pickles were analyzed once a week during the ripening period of five weeks and once a month during the cold storage period for 5 months for microbiological and physicochemical analysis. All experiments were repeated two times with duplicate samples.

Microbiological Analysis of Grape Pickles During the Ripening and Storage Period

Yeast and Mold Count

Food homogenate was prepared by mixing aliquots of 5 g grape pickle sample and 5 g of peptone water (0.1%, w/v). After that, serial dilutions were performed and appropriate dilutions were plated on Potato Dextrose Agar (PDA, pH 5.6±0.2 Oxoid) plates by using spread plate method. Inoculated plates were incubated at 25°C for 5 days (Tournas et al., 2001). After the incubation period, the numbers of mold and yeast colonies were counted separately, and the numbers were expressed as log cfu/g.

LAB Count

The numbers of LAB were determined on grape pickle samples that were not inoculated with *L. acidophilus* La-5. LAB were enumerated using MRS Agar. Appropriate dilutions were inoculated to MRS Agar plates as double layer to create microaerophilic conditions (ISO 15214:1998). After the incubation period of 3 days at 30°C, colonies were enumerated, and the results were calculated as log cfu/g.

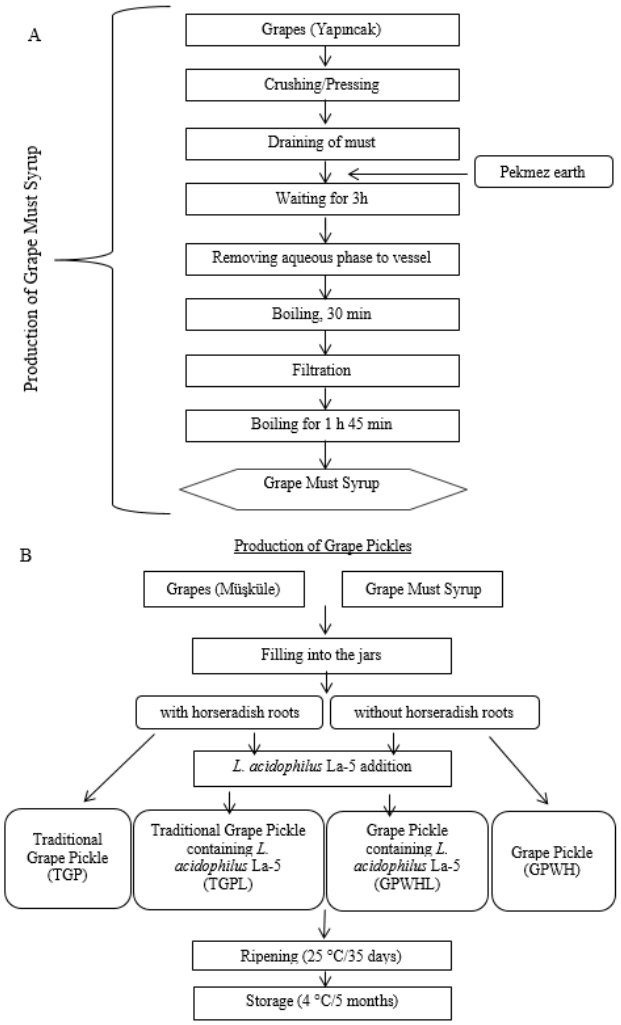

Figure 1. Production of grape must syrup (A) and grape pickle samples (B).

Lactobacillus acidophilus La-5 Count

Viability of *L. acidophilus* La-5 were determined in grape pickles (TGPL and GPWHL) inoculated with *L. acidophilus* La-5 during the ripening and storage period. Uninoculated grape pickles with probiotic strain also analyzed to ensure that there was no *L. acidophilus* before the inoculation. Enumeration of *L. acidophilus* La-5 in grape pickles was carried out using MRS-Bile Agar containing sterile 1.5% bile solution (10%, w/v) with double layered pour plate method and incubated at 37°C for 72 h (Gebara et al., 2015). After the incubation period, the numbers of *L. acidophilus* La-5 were calculated as log cfu/g.

Physicochemical Properties of Grape Pickles During the Ripening and Storage Period

The pH and titratable acidity values of grape pickles were determined on the day of production, during the ripening and the storage period. The pH values were measured by immersing the pH probe (Mod 821, NEL, Ankara, Turkey) into food homogenate directly. Titratable acidity was determined by using potentiometric method by titrating the food homogenate with 0.1 N NaOH to pH 8.1 and the results were expressed as g tartaric acid per 100 mL (Dorey et al., 2016). Total soluble solids (°Brix) values of traditional grape pickles were determined on the day of production, during the ripening and the storage period. Total soluble solids were measured using a refractometer at 25°C and expressed as °Brix (Esteban et al., 2002).

Determining the Sensory Properties of Grape Pickles

Sensory properties of grape pickles produced in four group were evaluated by 15 panelists after the storage period of 5 months at 4 ºC. Panelists were asked to score the products using a hedonic scale (1: dislike very much, 2: dislike slightly, 3: neither like nor dislike, 4: like slightly and 5: like very much) for colour, taste, flavour and overall acceptability (Hayoglu et al., 2009).

Survival of B. cereus and E. coli During the Ripening and Storage Periods of Grape Pickles

Survival of *E. coli* and *B. cereus* inoculated at two different doses of 10^3 cfu/g and 10^6 cfu/g in the grape pickles were investigated. The numbers of *E. coli* and *B. cereus* during the ripening and storage periods of grape pickles were analyzed once in 7 days during the ripening, once in a month during the storage period. In addition, uninoculated grape pickle samples were examined for *E. coli* or *B. cereus*. Survival of *B. cereus* cells were determined by using spread plate method on Mannitol Egg Yolk Polymyxin Agar (MYP, pH 7.2±0.2, Merck). Plates were incubated at 30°C for 18-24 hours (Morsy et al., 2018). In order to determine *E. coli* counts, appropriate dilutions were inoculated to Tryptone Bile X-Glucuronide Agar (TBX, pH 7.2±0.2, Oxoid, Hampshire, UK) plates using spread plate method and the plates were incubated at 37ºC for 18-24 hours (ISO 16649-2:2001). Colonies were enumerated and the results were expressed as log cfu/g.

Statistical Analysis

All experiments were replicated twice, and the analysis were conducted in duplicate. Statistical analysis were performed using SPSS 25 software (SPSS Inc., Chicago, Il, USA). Data examined using a Kruskal Wallis Test, for pair-wise comparisons Mann Whitney-U Test were used. Differences between means are considered significant at $P<0.05$.

Results and Discussion

Microbiological Properties of Grape Pickles with and without L. acidophilus During the Ripening and Storage Periods

Mold and Yeast Populations

The number of molds in four different types of grape pickle samples were in the range of 1.77 and 2.74 log cfu/mL at the beginning of the ripening period, and decreased by 0.5-1.0 log at the 7th day of ripening period. After the ripening period of 14 days and during the storage period of 5 months, the number of molds were below the detection limit (<1.30 log cfu/g) in all grape pickle samples (Table 1). Similar to our observations, significant reduction of *B. cinerea* was observed in white grape juice concentrate after 35 days of storage at refrigerated temperature. The growth inhibition and destruction of conidia explained as due to high sugar concentration (63.8 °Brix) and low water activity (0.769) of white grape juice concentrate (Torres-Ossandón et al., 2020). Choi et al. (2017) reported the

antifungal effects isothiocyanates extracted from horseradish root inhibited growth of the four pathogenic dermal fungi (*Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum canis*, and *Epidermophyton floccosum*) with the minimum inhibitory concentrations of 200, 200, 100, and 100 µg/mL, respectively.

The initial yeast counts of four different types of grape pickles were in the range of 2.17 and 2.38 log cfu/g. The population of yeast were significantly reduced on grape pickle samples containing horseradish with or without *L. acidophilus* on the 7th day of ripening period. On the 21st day of ripening, the number of yeasts in horseradish-free samples significantly increased to 5.88 and 6.31 log values ($P<0.05$), while the other two samples containing horseradish were found to be below the limit of detection (<1.30 log cfu/g). Yeast counts were below the detection limit in samples containing horseradish through the storage period, on the other hand, significantly higher numbers were observed for that of samples without horseradish (Table 2). The number of yeasts at the end of the 5th month was found to be 4.9 log cfu/g at grape pickle samples without horseradish. The number of yeasts in the samples with *L. acidophilus* was significantly lower ($P<0.05$) compared to the samples without *L. acidophilus* due to antagonistic interaction of *L. acidophilus*. In the present study, horseradish in grape pickles inhibited yeast growth significantly. Kyung and Fleming (1997) also reported sensitivity of yeasts to AITCs with minimum inhibitory concentration (MIC) values of AITCs for LAB and some pathogen bacteria were in the range of 50-500 ppm while MIC values of yeast were in the range of 1-4 ppm. Petrovic et al. (2017) also observed lower MIC values of horseradish root volatiles about 48-192 µg/mL for *Candida albicans*, while higher MIC values in the range of 265-795 µg/mL were obtained for bacterial test cultures. Shin et al. (2010) examined the effect of isothiocyanates obtained from horseradish root on the shelf life of tofu. The initial total viable count of 2.50-3.15 log cfu/g were increased to 8.14 log cfu/g in the control samples, while only 4.40 log units in the sample containing 300 ppm isothiocyanate after 10 days of storage at 10°C.

Total LAB Counts

The numbers of total LAB found in the grape pickle samples are seen in Table 3. There was no growth of LAB in grape pickle samples (TGP and TGPWH), the numbers were reduced significantly at 7th day of the ripening. After the 14th day of ripening period as well as the storage period, the numbers were below the detection limit (<1.3 cfu/g). No significant difference was found between the traditional grape pickle samples and grape pickle without horseradish in terms of changes in the number of LAB ($P> 0.05$). Similar to our observations, LAB count of syrup (65 °Brix sucrose solution at 40 °C) used for the osmotic dehydration of blueberries was 2.54 log at the beginning and steadily decreased to below detection level (<10 cfu/mL) (Kucner et al., 2013). High soluble solid content values of grape pickles are important growth inhibitors for LAB.

Table 1. Mould counts of grape pickles during the ripening period

Ripening period (days)	Mould Counts of Grape Pickles (log cfu/g)[1]			
	Traditional grape pickle	Grape pickle without horseradish	Traditional grape pickle containing *L. acidophilus*	Grape pickle without horseradish containing *L. acidophilus*
0	$2.74\pm0.70^{a,A}$	$2.09\pm0.07^{a,A}$	$2.04\pm0.73^{a,A}$	$1.77\pm0.55^{a,A}$
7	$1.60\pm0.33^{b,A}$	$1.77\pm0.49^{b,A}$	$1.39\pm0.12^{b,A}$	$1.39\pm0.28^{b,A}$
14	$2.30\pm0.00^{c,A}$	$<1.3\pm0.00^{c,A}$	$<1.3\pm0.00^{b,A}$	$<1.3\pm0.00^{b,A}$
21	$<1.30\pm0.00^{d,A}$	$<1.3\pm0.00^{c,A}$	$<1.3\pm0.00^{b,A}$	$<1.3\pm0.00^{b,A}$
28	$<1.30\pm0.00^{d,A}$	$<1.3\pm0.00^{c,A}$	$<1.3\pm0.00^{b,A}$	$<1.3\pm0.00^{b,A}$
35	$<1.30\pm0.00^{d,A}$	$<1.3\pm0.00^{c,A}$	$<1.3\pm0.00^{b,A}$	$<1.3\pm0.00^{b,A}$

[1]Data are expressed as mean ± standard deviation of two independent experiments with duplicate samples. Means with different small letters are significantly different within the same column ($P < 0.05$). Means with different capital letters are significantly different within the same row ($P < 0.05$).

Table 2. Yeast counts of grape pickles during the ripening and storage periods

	Yeast Counts of Grape Pickles (log cfu/g)[1]			
	Traditional grape pickle	Grape pickle without horseradish	Traditional grape pickle containing *L. acidophilus*	Grape pickle without horseradish containing *L. acidophilus*
Ripening period (days)				
0	$2.17\pm0.21^{a,A}$	$2.34\pm0.08^{a,A}$	$2.38\pm0.00^{a,A}$	$2.35\pm0.00^{a,A}$
7	$1.47\pm0.00^{b,A}$	$1.92\pm1.30^{b,A}$	$1.39\pm0.12^{b,A}$	$3.72\pm0.83^{b,B}$
14	$1.30\pm0.00^{c,A}$	$3.82\pm0.35^{c,B}$	$1.39\pm0.28^{b,A}$	$4.10\pm0.16^{c,C}$
21	$<1.3\pm0.00^{c,A}$	$6.31\pm0.17^{d,B}$	$<1.3\pm0.00^{d,A}$	$5.88\pm0.28^{d,D}$
28	$<1.3\pm0.00^{c,A}$	$6.85\pm0.23^{e,B}$	$<1.3\pm0.00^{d,A}$	$4.95\pm0.26^{d,C}$
35	$<1.3\pm0.00^{c,A}$	$6.59\pm0.20^{f,B}$	$<1.3\pm0.00^{d,A}$	$4.95\pm0.68^{d,C}$
Storage period (months)				
1	$<1.3\pm0.00^{a,A}$	$5.95\pm0.30^{b,B}$	$<1.30\pm0.00^{a,A}$	$3.98\pm1.43^{b,B}$
2	$<1.3\pm0.00^{a,C}$	$6.13\pm0.23^{b,A}$	$<1.3\pm0.00^{a,C}$	$3.26\pm0.30^{b,B}$
3	$<1.3\pm0.00^{a,C}$	$6.43\pm0.88^{b,A}$	$<1.3\pm0.00^{a,C}$	$3.30\pm0.42^{b,B}$
4	$<1.3\pm0.00^{a,B}$	$5.75\pm0.14^{b,A}$	$<1.3\pm0.00^{a,B}$	$2.63\pm0.29^{c,B}$
5	$<1.3\pm0.00^{a,B}$	$4.90\pm0.13^{c,A}$	$<1.3\pm0.00^{a,B}$	$1.95\pm0.63^{c,B}$

[1]Data are expressed as mean ± standard deviation of two independent experiments with duplicate samples. Means with different small letters are significantly different within the same column for each period ($P < 0.05$). Means with different capital letters are significantly different within the same row ($P < 0.05$).

Table 3. LAB counts of grape pickles during the ripening period

Ripening period (days)	LAB counts (log cfu/g)[1]	
	Traditional grape pickle	Grape pickle without horseradish
0	$1.81\pm0.10^{a,A}$	$2.25\pm0.50^{a,A}$
7	$1.38\pm0.41^{b,A}$	$1.48\pm0.42^{b,A}$
14	$<1.30\pm0.00^{c,A}$	$<1.3\pm0.00^{c,A}$
21	$<1.30\pm0.00^{c,A}$	$<1.3\pm0.00^{c,A}$
28	$<1.30\pm0.00^{c,A}$	$<1.30\pm0.00^{c,A}$
35	$<1.30\pm0.00^{c,A}$	$<1.30\pm0.00^{c,A}$

[1]Data are expressed as mean ± standard deviation of two independent experiments with duplicate samples. Means with different small letters are significantly different within the same column ($P < 0.05$). Means with different capital letters are significantly different within the same row ($P < 0.05$).

Table 4. Survival of *L. acidophilus* La-5 in grape pickles during the ripening and storage periods

	L. acidophilus La-5 Counts of Grape Pickles (log cfu/g)[1]	
	Traditional grape pickle	Grape pickle without horseradish
Ripening period (days)		
0	$7.92\pm0.00^{a,A}$	$7.90\pm0.00^{a,A}$
14	$5.55\pm2.33^{b,A}$	$2.77\pm0.75^{b,B}$
21	$2.35\pm0.18^{c,A}$	$2.54\pm0.28^{b,A}$
28	$2.51\pm0.10^{d,A}$	$2.77\pm0.40^{b,A}$
35	$2.71\pm0.50^{e,A}$	$4.37\pm0.51^{c,B}$
Storage period (month)		
1	$2.11\pm0.19^{b,A}$	$2.12\pm0.10^{c,A}$
2	$2.44\pm0.03^{a,A}$	$2.38\pm0.10^{b,A}$
3	$2.06\pm0.05^{b,A}$	$2.31\pm0.04^{b,A}$
4	$2.33\pm0.07^{a,A}$	$2.06\pm0.06^{c,A}$
5	$1.94\pm0.01^{c,A}$	$2.06\pm0.10^{c,A}$

[1]Data are expressed as mean ± standard deviation of two independent experiments with duplicate samples. Means with different small letters are significantly different within the same column for each period ($P < 0.05$). Means with different capital letters are significantly different within the same row ($P < 0.05$).

Survival of L. acidophilus During the Ripening and the Storage Periods

The number of *L. acidophilus* inoculated to traditional grape pickle samples with or without horseradish was about 7.9 log cfu/g. On the other hand, during the ripening and storage periods, the numbers were reduced significantly (Table 4). There was no significant difference between the two groups (TGPL and GPWHL) in terms of *L. acidophilus* La-5 numbers ($P>0.05$).

In order for a food to exhibit probiotic properties, it should contain at least 10^6 cfu/mL probiotic microorganisms (Nualkaekul et al., 2012; Kechagia et al., 2013). On the other hand, *L. acidophilus* did not survive well in grape pickles in the present study. Survival of *L. acidophilus* in traditional grape pickle is thought to be affected by the high sugar content of the product. High brix values in food (about 60 °Brix) were found to be unsuitable for the growth or/and survival of *L. acidophilus* La-5. Osmotic stress affects the probiotic viability in food matrices. Hutkins et al. (1987) indicated that growth of *L. acidophilus* was observed at 3 M concentrations of fructose or glucose, on the other hand there was no growth at sugar concentrations of 4 M. Sunny-Roberts et al. (2007) also reported that *Lactobacillus rhamnosus* cells treated at sugar concentrations of 1.5 M experienced membrane perturbation due to osmotic stress. In contrast to results obtained in this study, Nagpal et al. (2012) reported that *L. acidophilus* was able to maintain viability in grape juice and fermented the fruit sugar for their cell synthesis and metabolism. Pasteurized grape juice (15 °Brix) was suggested as a good alternative to dairy products for the delivery of probiotics (Afzaal et al., 2020).

Similar to our observations, Nematollahi et al. (2016) indicated that the populations of *L. rhamnosus* and *L. plantarum* inoculated to cornelian cherry juice decreased from the initial number of 8 log cfu/mL to 4.24 and 4.20 log cfu/mL, respectively after 7 days of storage and *L. rhamnosus* was not detected after 21 days of storage period at 4°C. Non-fermented food matrices have negative effects on the survival of probiotic bacteria (Nguyen et al., 2013). The populations of *L. acidophilus* decreased in dairy dessert throughout the shelf life of 28 days (Fernandes et al., 2013). Zeashan et al. (2020) also reported that *L. acidophilus* populations in ice cream decreased from 8.9 log to 3.5 log during the storage period of 120 days.

Development of non-dairy probiotic food is a challenge due to many factors effecting the probiotic survival. In apple juice supplemented with *L. acidophilus* the populations were decreased about 3.59-4.61 log units during the storage period of 28 days (Espirito-Santo et al., 2015). On the other hand, Randazzo et al. (2013) reported that *L. rhamnosus* strains showed better viability in commercial peach jam and remained viable at the value of 6 log until the end of storage period of 78 days. Maple concentrate inoculated with probiotic *B. lactis* and *L. rhamnosus* at level of 8 log, the surviving probiotic numbers remained constant during the storage period of 28 days at 4 °C (Khalf et al., 2010). Champagne and Gardner (2008) investigated the survival of *L. acidophilus*, *L. rhamnosus*, *L. fermentum*, *L. reuteri* and *L. plantarum* in commercially produced mixed fruit juices at 4 °C for 80 days. The numbers of *L. rhamnosus*, *L. fermentum*, *L. reuteri* and *L. plantarum* were 10^7 cfu/mL after 80 days of storage, on the other hand, the numbers of *L. acidophilus*

decreased to 10^6 cfu/mL at 40th days and 10^2 cfu/mL at 80th days of storage. Ribeiro et al. (2020) also reported higher decrease of *L. acidophilus* La-5 strain was observed in non-fermented blend of banana, strawberry and juçara beverage when compared with *Bifidobacterium* spp. which demonstrated that the survival and growth of probiotic bacteria in non-fermented beverages are strain dependent.

Physicochemical Properties of Grape Pickles with and without L. acidophilus During the Ripening and the Storage Periods

The pH value of the grape pickle samples with and without probiotics during the ripening and storage periods were depicted at Figure 2. At the end of ripening and storage period, the difference between the pH values of the samples was not found to be statistically significant (P>0.05). pH values remained at the level of 5.10-5.26 during the ripening period, and a decreasing graph was drawn during the storage period. At the end of the storage period of 5 months, the pH values of all samples were determined between 4.80-4.82. Nazzaro et al. (2009) reported that *L. acidophilus* in carrot juice reduced the pH from 5.79 to 3.37 after 8 weeks. The activity of the strain in food appears to be directly related to the food matrix.

The total titration acidity values of pickled grapes during the ripening and storage periods were given in Figure 3. The initial acidity value of 0.30 varies between 0.30 and 0.45 at the end of storage period. Since no growth of LAB and *L. acidophilus* were obtained in any of the samples, a slight decrease in pH and increase in acidity could not be attributed to microbial metabolism. The reason for this can be explained by diffusion of grape juice to the surrounding grape must. The decrease in total soluble solid contents (°Brix) of traditional grape pickle sample also confirms this opinion (Table 5). The initial brix value significantly decreased from 63 to 52.5 at the end of storage period due to the release of grape juice and the increase of water content of grape pickles. Porto et al. (2018) reported that the brix value of the orange and beet juice mixture, which was initially 12, decreased to 8.9-9.2 after 28 days.

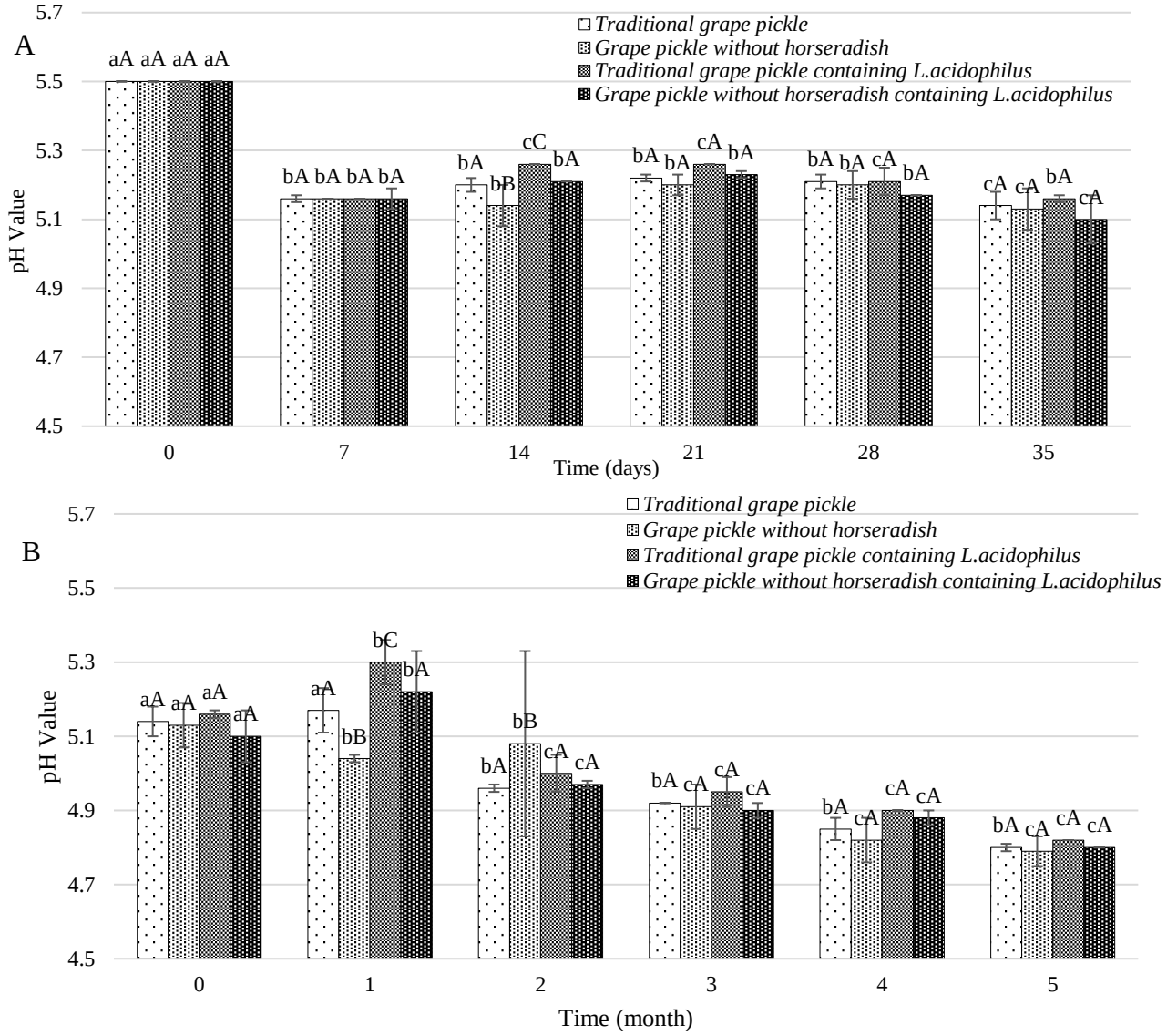

Figure 2. Changes in the pH values of grape pickle samples during the ripening period (A), and the storage period (B). Means with different uppercase letters at the same time period are significantly different (P < 0.05). Means with different lowercase letters in the same grape pickle group are significantly different (P < 0.05).

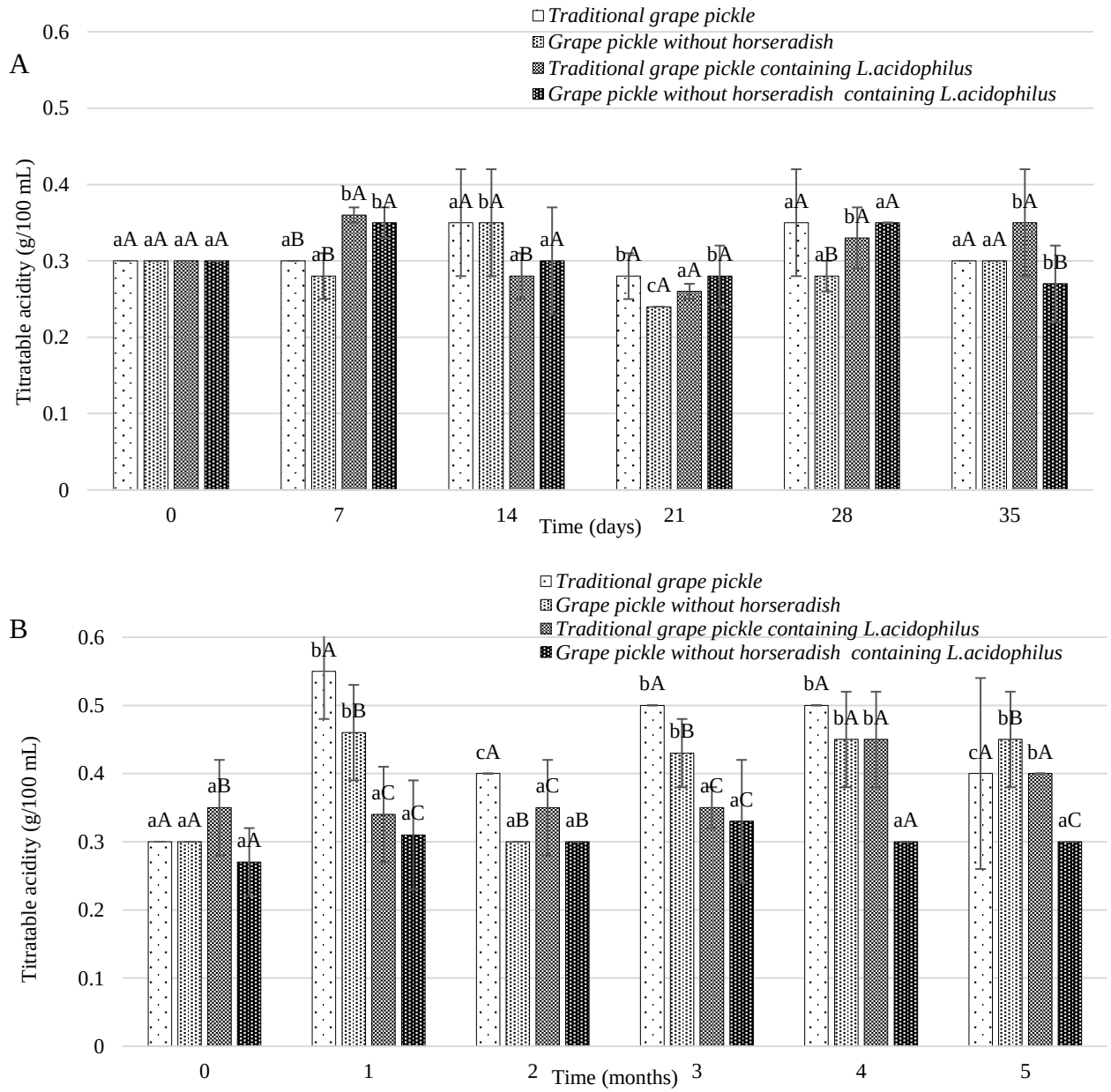

Figure 3. Changes in the titratable acidity values of grape pickle samples during the ripening period (A), and the storage period (B). Means with different uppercase letters at the same time period are significantly different ($P < 0.05$). Means with different lowercase letters in the same grape pickle group are significantly different ($P < 0.05$).

Table 5. Total soluble solid content (°Brix) of traditional grape pickle samples during the ripening and storage periods

	Total soluble solid content (°Brix) [1]
Ripening period (days)	
0	63.0±1.41^{a}
7	63.0±1.41^{a}
14	58.8±0.84^{b}
21	60.0±0.00^{b}
28	60.0±0.00^{b}
35	51.0±0.00^{c}
Storage period (month)	
1	58.5±2.12^{b}
2	58.5±0.70^{b}
3	55.5±2.12^{c}
4	55.5±3.53^{c}
5	52.5±0.70^{c}

[1]Data are expressed as mean ± standard deviation of two independent experiments with duplicate samples. Means in the same column for each period with different letters are significantly different ($P < 0.05$).

Sensory Properties of Grape Pickles with and without Probiotics

Sensory attributes of grape pickles after the storage period of 5 months were shown in Table 6. No significant differences in colour, taste, flavour and overall acceptance values were observed between grape pickles produced with and without horseradish (P>0.05). Addition of horseradish roots did not affect the sensory attributes of grape pickle samples.

The grape pickle samples with or without horseradish containing *L. acidophilus* had significantly higher sensory scores than the other samples (P<0.05). Mean scores of overall acceptances of all samples were in the range of 3.00 and 3.87 in 5-hedonic scale. The highest overall acceptance score was given to the grape pickles without horseradish containing *L. acidophilus*, on the other hand it was not significantly different from the samples with horseradish and *L. acidophilus* (P>0.05). Sensory scores significantly increased regardless the presence of horseradish in grape pickle samples containing *L. acidophilus*. Addition of probiotics seems to have a positive effect on sensory evaluation. Similar to our observations, Heenan et al. (2004) also reported that sensory properties of non-fermented frozen soy dessert containing *L. acidophilus* was better than the control samples during storage period of 7 months. Oliveira et al (2017) also reported that non-fermented milk with *L. acidophilus* showed a better sensory performance without altering the sensory characteristics. In another study, statistical differences were not observed in the sensory properties of the non-fermented probiotic beverage of banana, strawberry and juçara blend containing *L. acidophilus* when compared with the control samples (Ribeiro et al., 2020). In contrast to our study, Talebzadeh and Sharifan (2017) reported that the overall scores of flavour, odour, colour, mouth texture and overall acceptance of fruity jelly desserts with *L. acidophilus* were significantly lower than the control samples.

Survival of B. cereus and E. coli During Ripening and Storage of Grape Pickles

Survival of *B. cereus* in four groups of grape pickle samples inoculated at two different doses of 10^6 cfu/g (high inoculum) and 10^3 cfu/g (low inoculum) were given in Table 7. Before the inoculation, the number of *B. cereus* was analysed in grape pickles, and no *B. cereus* colony was detected before the inoculation. For low inoculation level (10^3 cfu/g), no growth of *B. cereus* was observed during the production and storage of grape pickles with or without horseradish (data are not shown). The numbers of *B. cereus* in grape pickle samples inoculated with high doses of *B. cereus* were significantly decreased about 3.84 - 4.23 log units during the ripening period of 35 days. *B. cereus* inoculated at high levels show long term survival and the populations of *B. cereus* remained in the range of 1.59-1.71 log cfu/g after the storage period of 5 months at 4 °C. In contrast to the present study, Mpofu et al. (2016) reported that in traditional mutandabota, non-fermented dairy food (pH 3.4), no viable cells of *B. cereus* were detected after 3 h of inoculation at the level of 5.5 log cfu/mL. In the study conducted by Güven and Benlikaya (2005), *B. cereus* was inoculated at 10^3 cfu/ mL and the survival status of *B. cereus* was examined during the 72-hour fermentation period of boza. It was found that the number of LAB and yeast increased, and the number of *B. cereus* decreased rapidly.

Table 6. Sensory analysis scores for grape pickles

Samples	Colour	Taste	Flavour	Overall acceptance
Traditional grape pickle	3.27 ± 0.96^{a}	2.73 ± 0.46^{a}	2.73 ± 1.10^{a}	3.00 ± 0.85^{a}
Grape pickle without horseradish	3.27 ± 1.22^{a}	2.93 ± 0.88^{a}	2.80 ± 1.15^{a}	3.00 ± 1.00^{a}
Traditional grape pickle containing *L. acidophilus*	4.13 ± 0.83^{b}	3.47 ± 1.01^{b}	4.07 ± 0.88^{b}	3.73 ± 1.00^{b}
Grape pickle without horseradish containing *L. acidophilus*	$3.93\pm0.88^{a,b}$	3.67 ± 0.98^{b}	3.87 ± 0.92^{b}	3.87 ± 0.92^{b}

Data are expressed as mean ± standard deviation of two independent experiments with duplicate samples. Means in the same column with different letters are significantly different (P < 0.05).

Table 7. Survival of *B. cereus* inoculated at high inoculation level in grape pickle samples during the ripening and storage periods

	B. cereus Counts of Grape Pickles (log cfu/g) [1]			
	Traditional grape pickle	Grape pickle without horseradish	Traditional grape pickle containing *L. acidophilus*	Grape pickle without horseradish containing *L. acidophilus*
Ripening period (days)				
0	$5.53\pm0.0^{a,A}$	$5.53\pm0.00^{a,A}$	$5.53\pm0.00^{a,A}$	$5.53\pm0.00^{a,A}$
7	$1.84\pm0.08^{b,A}$	$1.60\pm0.42^{b,B}$	$2.00\pm0.00^{b,C}$	$2.00\pm0.00^{b,C}$
14	$1.89\pm0.26^{c,A}$	$1.90\pm0.07^{c,A}$	$1.84\pm0.08^{c,A}$	$1.87\pm0.03^{c,A}$
21	$1.69\pm0.29^{d,A}$	$1.72\pm0.17^{d,B}$	$1.30\pm0.00^{d,C}$	$1.89\pm0.83^{c,D}$
28	$1.90\pm0.00^{c,A}$	$1.69\pm0.29^{e,B}$	$1.99\pm0.12^{e,C}$	$1.50\pm0.28^{d,D}$
35	$1.69\pm0.12^{d,A}$	$1.57\pm0.38^{f,B}$	$1.59\pm0.15^{f,C}$	$1.30\pm0.21^{e,D}$
Storage period (month)				
1	$1.30\pm0.00^{a,A}$	$1.45\pm0.21^{a,A}$	$1.92\pm0.03^{a,A}$	$1.54\pm0.33^{a,A}$
2	$1.75\pm0.00^{b,A}$	$1.54\pm0.02^{b,B}$	$1.72\pm0.05^{b,A}$	$1.59\pm0.01^{b,B}$
3	$1.52\pm0.10^{c,A}$	$<1.3\pm0.28^{c,B}$	$1.63\pm0.04^{c,A}$	$1.57\pm0.01^{b,A}$
4	$<1.3\pm0.28^{a,A}$	$1.30\pm0.00^{c,A}$	$1.68\pm0.00^{d,B}$	$1.72\pm0.00^{c,C}$
5	$1.59\pm0.04^{d,A}$	$1.57\pm0.01^{b,A}$	$1.71\pm0.09^{b,B}$	$1.59\pm0.15^{b,A}$

[1]Data are expressed as mean ± standard deviation of two independent experiments with duplicate samples. Means with different small letters are significantly different within the same column for each period ($P < 0.05$). Means with different capital letters are significantly different within the same row ($P < 0.05$).

E. coli culture suspension was inoculated into grape pickle samples at two different doses of 10^6 cfu/g and 10^3 cfu/g. *E. coli* was not observed in grape pickle samples before the inoculation. *E. coli* was more susceptible in that no surviving bacteria was detected (detection limit, 1.3 log cfu/g) at day 7 of ripening period and stayed below the detection level during the ripening period regardless of the inoculation dose. Bai et al. (2020) reported that the number of *E. coli* inoculated to blueberry samples reduced about 1.5 log cfu/g during osmotic dehydration in 43 °Brix sucrose solution for 15 h at 23 °C.

In order to determine whether the inhibitory effect was caused by high sugar concentration of the samples, the grape pickle samples (20 mL) produced in four group were diluted with equal amount of sterile distilled water. Diluted samples were inoculated with *E. coli* at two different levels of 10^6 cfu/g and 10^3 cfu/g, and the time-dependent changes in the number of *E. coli* samples were examined. *E. coli* in samples inoculated at high inoculation dose remained viable for up to 40 days, while in the low-dose inoculated samples were found to below detection level of 1.3 log cfu/g on the 20^{th} day (data not given). Enache and Chen (2007) also reported reductions of *E. coli* O157:H7 at least 5 log after 6 h of incubation in cranberry juice concentrate at high Brix level (46 °Brix), on the other hand lower reduction of 1.53 log was obtained at 18 °Brix level. In contrast to these studies, Oyarzábal et al. (2003) indicated that *E. coli* O157:H7 survived through 12 weeks at detectable levels in white grape juice concentrate (66.2 °Brix) at -23 °C. High soluble solid concentrations of traditional grape pickles have bactericidal effects on *E. coli* during the ripening and storage periods.

Conclusion

This is the first study conducted in the laboratory environment related to traditional grape pickles. The results of this study indicate that grape pickles are not a suitable food matrix to support the growth of *L. acidophilus*. High total soluble solid content and the presence of horseradish in grape pickles ensure the microbiological stability during the shelf life. Horseradish inhibits yeast growth in the product. The potential use of this plant as a food preservative in different types of foods should be investigated. Traditional foods are gaining wide consumer acceptance, more studies are needed to standardize their commercial production.

Conflict of interest

The authors of the paper declare that there is no conflict of interest.

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