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Full Length Research Paper

Production of probiotic mixed pickles (Turşu) and microbiological properties

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Turşu is a traditional fermented Turkish pickle made of vegetables such as cabbage, cucumber, carrot, beet, green tomato, pepper, turnip, eggplant and beans. In this study, development of *Lactobacillus plantarum* was investigated in Turşu samples during storage periods (60 days). Changes in counts of lactic acid bacteria (LAB), yeast and mold, Enterobacteriaceae, *Staphylococcus aureus*, *Staphylococcus*/*Micrococcus* counts and pH values were monitored in these samples. Total averages of LAB, yeast and mold, Enterobacteriaceae, *S. aureus*, *Staphylococcus*/*Micrococcus* and pH level for probiotic (supplemented with *Lactobacillus plantarum*, Lp) and control (C) were found as 7.92, 3.69; 1.78, 1.73; <1, <1; <1, <1; 1.70, 1.72 (log cfu/ml) and 3.57, 3.61, respectively. In the study, it was observed that addition of vinegar provided a low pH level for initial fermentation conditions, thereby encouraging development of starter culture and restricting competitive microflora. Yeast development in the Turşu also restricted the shelf life of the product.

Key words: Turşu, fermented vegetables, probiotic, *L. plantarum*.

INTRODUCTION

Fermentation is one of the oldest and healthiest methods of food preservation. Fermented foods bring the beneficial flora to our intestines to keep our digestive system regular and healthy. In addition, it allows natural, beneficial bacteria to perform a fermentation process in which vegetables develop a pleasantly sour taste and remain rich in vitamins. Lactic acid fermentation is the only method of preservation that retains all the natural plant ingredients while improving the quality, taste and aroma (Bamforth, 2005). Furthermore, microorganisms used for fermentation can add probiotic properties to product. A number of studies have found probiotic consumption to be useful in the treatment of diarrhea, lactose intolerance, colon cancer, cholesterol, blood pressure, immune function and infections, mineral absorption, irritable bowel syndrome and colitis. Important probiotic bacteria can be listed as *Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Bifidobacterium longum, Pediococcus acidilactici* and *Saccharomyces boulardii* (Holzapfel et al., 1998; O'Brien et al., 1999).

There are many kinds of fermented meats, dairy and vegetable products in the world, but fermented vegetables are not widespread commercial products as fermented meat and dairy products. Because fermented vegetables do not have standard ingredient and its ingredients varied based on climatic and geographic conditions. Various fermented vegetable products are produced in different location of the world. Most known fermented vegetable products are sauerkraut, kimchi, safur, asin, etc. General name of fermented vegetables in Turkey is "Turşu". The most commonly fermented vegetable in Turkey is cabbage, cucumber, carrot, beet, green tomato, pepper and turnip, although, radish, bean, onion, unripe melon, okra, leaf of celery root, eggplant, parsley and garlic (to give flavor) are also preserved in this way. Turşu can be produced either plain or mixed. In general, mixed turşu formulation is composed by adding cabbage, cucumber, carrot, green tomato, pepper and garlic in Turkey (Aktan et al., 1998; Erten and Tangüler, 2010).

Microorganisms involved in the fermentation of turşu are *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Pediococcus pentocaceus* and *Lactobacillus plantarum*. The main microorganism in turşu fermentation is *L. plantarum*. In general though, Turşu is produced by small

family businesses that do not use starter cultures in Turkey (Aktan et al., 1998). The aim of this research was to improve probiotic properties of mixed turşu, investigate behavior of *L. plantarum* during storage period in the turşu and improve quality, taste and aroma characteristics of the product.

MATERIAL AND METHODS

Starter culture

L. plantarum BC 7321 strain was provided by the Food Microbiology Laboratory, Department of Food Engineering, Faculty of Agriculture, Atatürk University, Erzurum, Turkey. The identification of the microorganism used in the study was confirmed by the Sherlock microbial identification system version 4.0 (MIDI Inc., Newark) and the API 50 CHL (BioMerieux, France) (Çetin, 2006).

Acid and bile resistance of the *L. plantarum*

L. plantarum BC 7321 was stored in sterile glycerol at -80°C and revived in de Man, Rogosa and Sharpe (MRS) broth medium (Merck, Darmstadt, Germany) overnight at 37°C under aerobic conditions. The cells in 0.1 ml of the culture (10^8 to 10^9 CFU ml^{-1}) were collected by centrifugation and transferred to 1 ml of phosphate-buffered saline (PBS) with pH adjusted to 2.0, followed by incubation at 37°C for 3 h. Controls were performed at pH 7.2 under the same condition. To evaluate the acid tolerance for LAB strains, after the acid treatment for 3 h, *L. plantarum* cells were collected by centrifugation and resuspended in pH 7.2 phosphate-buffered saline (PBS). Serial dilutions of the cells were then spread on MRS (Merck, Darmstadt, Germany) agar and incubated at 37°C for 48 h anaerobically. Afterwards, viable *L. plantarum* cells were counted.

On the other hand, to evaluate the bile tolerance of *L. plantarum* BC 7321, the cells obtained from 1 ml of the culture and survived from the acid treatment (pH 2, 3 h) were centrifuged (5000 g, 5 min), washed and resuspended in 1 ml of PBS (pH 7.2) and mixed with 9 ml of MRS broth with and without 0.3% (w/v) oxgall bile (Merck, Darmstadt, Germany). The cultures were incubated at 37°C for 24 h, and the growth rates monitored hourly by taking 0.1 ml of the culture followed by counting the viable bacteria counts. Viable bacteria counts were determined by spreading the 0.1 ml dilutions of the strain on MRS agar followed by incubation at 37°C for 48 h anaerobically. The bile tolerance was then estimated by comparing the LAB growth rates (Chiu et al., 2008).

Culture preparation

L. plantarum BC 7321 was maintained as frozen stock at -80°C. Prior to experimental use, the cultures were propagated twice in MRS broth and incubated overnight at 30°C. The cells were harvested by centrifugation (4000 *g*, 10 min) (Hermle Z 383 K, Germany), then washed and resuspended in sterile water. Cultures were diluted so that each ml of inoculums would produce a concentration of approximately 10^8 CFU/ml (Halász et al., 1999).

Production of turşu

Turşu sample was produced using traditional method. Vegetables were obtained from the local market in Erzurum. Washed and sliced vegetables were put into the screw-capped glass jars and tamped. NaCl and vinegar (grape vinegar, Tariş, Turkey) concentrations in turşu brine were 4 and 2.5%, respectively. Turşu contained cabbage 25%, cucumber 25%, green tomato 20%, green pepper 15%, carrot 5%, and others 10% (garlic, red pepper, parsley). The fermentations were carried out with (LP) and without (c) starter culture (*L. plantarum*). Closed jars were held at 20 ± 2°C during the storage period (Figure 1) (Anonymous, 2007).

Microbiological analysis

Lactic acid bacteria (LAB), Enterobacteriaceae, *Staphylococcus aureus*, yeast and mold counts were monitored during storage (0, 15, 30, 45 and 60 days). For microbiological analyses, 10 ml of turşu sample was dispersed aseptically in 90 ml of 0.1% peptone plus 0.85% sodium chloride solution and homogenized. Serial dilutions were made in 0.1% peptone and 0.85% sodium chloride solution (Harrigan, 1998). The enumeration of LAB (de Man, Rogosa and Sharpe, Merck, Darmstadt, Germany) at 30°C for 3 to 5 days (Harrigan, 1998), total Enterobacteriaceae (violet red bile dextrose agar, Merck, Darmstadt, Germany) at 35°C for 24 h, yeasts and moulds (potato dextrose agar, Merck, Darmstadt, Germany) at 25°C for 5 days (Tournas et al., 1998), *S. aureus* (Baird-Parker Agar, Merck, Darmstadt, Germany) at 37°C for 24 h (Harrigan, 1998) and *Staphylococcus*/*Micrococcus* (mannitol salt agar, Oxoid, England) at 37°C for 48 h (Yıldız, 2011) were performed.

Determination of pH

The pH changes of turşu samples were measured by using a pH meter at pH 211 (Hanna Ins., Portugal). Free acidity of brine samples was determined according to Tassou et al. (2002) and Yıldız (2011). Three independent measurements were performed on each sample at room temperature and means were calculated.

Statistical analysis

In order to determine whether there is a statistically significant difference among the obtained results, variance analyses were carried out using SPSS version 10.0 software package (SPSS, Chicago, IL, USA). Differences between means were tested by the Duncan test and values with $P<0.05$ were considered significantly different. In the study, three independent measurements were performed.

RESULTS

Several LAB species are involved in the production of various fermented vegetable around of the different areas of the world, with *L. plantarum* being the most important species (Hutkins, 2006; Abriouel et al., 2008). Similarly, the bacterium also have main role in fermentation of turşu, traditional Turkish fermented vegetable product (Aktan et al., 1998; Özçelik et al., 1998; Erten and Tangüler, 2010). Acid and bile tolerance are two important criteria for probiotic strains because of restriction factors for gastro intestinal track. Resistant to stomach acid and bile are two important probiotic properties of microorganisms. By this aim, detection of

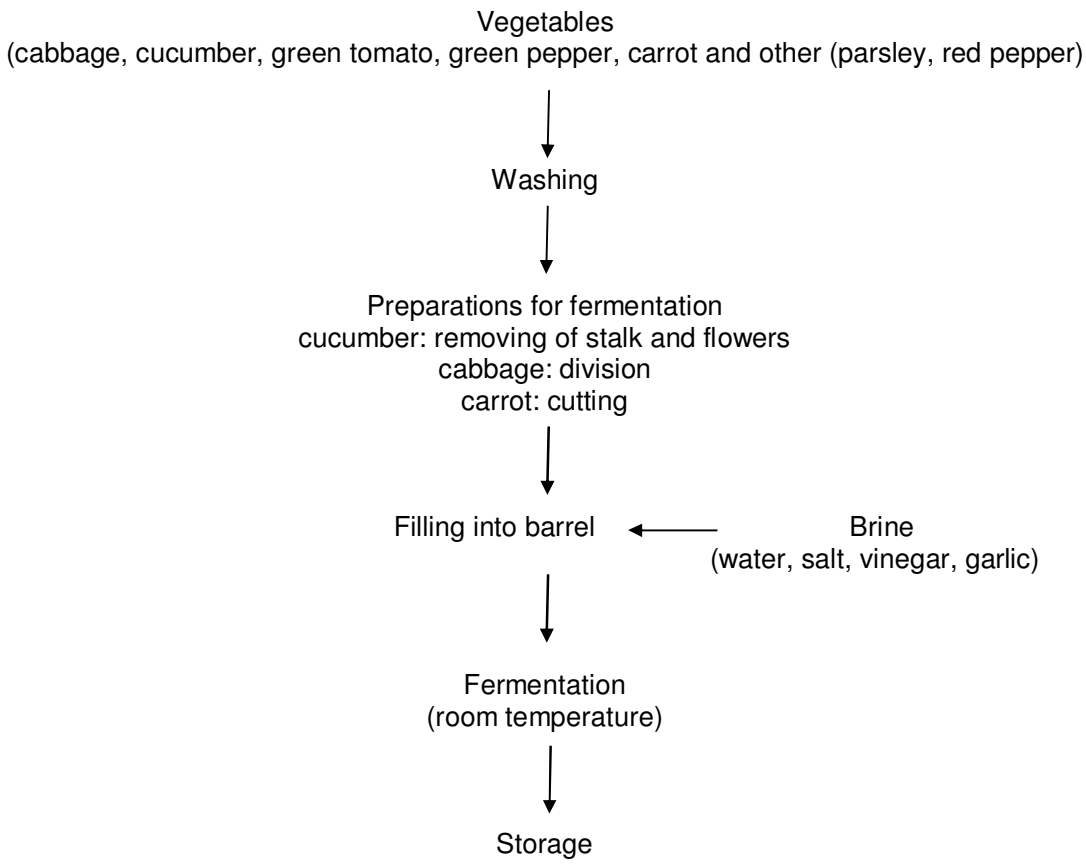

Figure 1. Flow chart for traditional preparation of mixed turşu (Anon, 2007).

Table 1. Detection of probiotic properties of *L. plantarum* BC 7321.

Time (h)	**0**	**3**	**3**		**12**		**24**	
Media	*MRS*	*MRS, pH 2*	*MRS*	*MRS bile*	*MRS*	*MRS bile*	*MRS*	*MRS bile*
Average	8.46	8.31	8.35	7.91	9.76	8.13	9.11	8.00
±SD	0.11	0.18	0.04	0.19	0.08	0.21	0.13	0.05

SD, Standard deviation; h, hour.

probiotic properties of *L. plantarum* BC 7321 were carried out by acid and bile tolerance tests. According to the results obtained (Table 1), it was observed that *L. plantarum* BC 7321 is a probiotic strain.

The change in counts of LAB, yeast and mold, Enterobacteriaceae, *S. aureus*, *Staphylococcus*/ *Micrococcus* counts and pH values in Turşu samples during the storage period are shown in Table 2. First Day counts of Probiotic (supplemented with *L. plantarum*, Lp) and Control (C) Turşu samples LAB, Yeast, Enterobacteriaceae, *S. aureus*, *Staphylococcus*/ *Micrococcus* and pH level were found as 8.60, <1; <1, <1; <1, <1; <1, <1; 3.27, 3.36 (log CFU/ml) and 3.58, 3.58, respectively. Total averages of the same parameters were found as 7.92, 3.69; 1.78, 1.73; <1, <1; <1, <1; 1.70, 1.72 (log CFU/ml) and 3.57, 3.61, respectively. Also Enterobacteriaceae bacteria and *S. aureus* were found to be below the detection limit for all turşu samples (<1 log CFU/g). As shown in Table 2, Enterobacteriaceae and *S. aureus* were found to be below the detection limit for all Turşu samples. Although, LAB counts of turşu samples with probiotic culture were significantly decreased, the product kept its probiotic level during the 60-day storage period ($p<0.01$). Moreover, counts of LAB decreased gradually during storage time and statically significant decrease was observed on the 30th day (Table 2). Addition of *L. plantarum* had important effect on LAB count of turşu samples ($p<0.01$). However, it has also been observed that addition of *L. plantarum* improved organoleptic value of the sample and also increased

Table 2. Microflora of turşu samples during storage period (60 days).

Storage (day)	LAB		Yeast and mold		Enterobacteriaceae		*S. aureus*		*Staphylococcus*/ *Micrococcus*		pH	
	Lp	C	Lp	C	Lp	C	Lp	C	Lp	c	Lp	C
0	8.60 ± 0.42^{a}	<1	<1	<1	<1	<1	<1	<1	$3.27^{aA} \pm 0.23$	3.27 ± 0.23^{aA}	3.58 ± 0.01^{abA}	3.58 ± 0.01^{bA}
15	8.61 ± 0.21^{aA}	1.88 ± 0.04^{cB}	<1	<1	<1	<1	<1	<1	$2.95^{aA} \pm 0.13$	2.91 ± 0.18^{aA}	3.53 ± 0.02^{bA}	3.57 ± 0.04^{bA}
30	7.41 ± 0.27^{bA}	4.22 ± 0.13^{bB}	1	<1	<1	<1	<1	<1	$2.30^{bA} \pm 0.19$	2.40 ± 0.21^{bA}	3.53 ± 0.04^{bA}	3.61 ± 0.13^{abA}
45	7.41 ± 0.07^{bA}	6.14 ± 0.19^{aB}	3.64 ± 0.14^{bA}	3.90 ± 0.08^{bA}	<1	<1	<1	<1	<1	<1	3.59 ± 0.02^{abA}	3.65 ± 0.07^{aA}
60	7.49 ± 0.01^{bA}	6.23 ± 0.11^{aB}	5.27 ± 0.06^{aB}	5.78 ± 0.06^{aA}	<1	<1	<1	<1	<1	<1	3.62 ± 0.03^{aA}	3.66 ± 0.04^{aA}
Total average	7.92 ± 0.60	3.69 ± 2.41	1.78 ± 2.36	1.73 ± 2.57	<1	<1	<1	<1	1.70 ± 1.50	1.72 ± 1.51	3.57 ± 0.04	3.61 ± 0.03

Lp, *Lactobacillus plantarum* added samples; C, control; SD, standard deviation; $^{A–B}$Means ± SD, horizontal differences for same microbiological group; $^{a–c}$Means ± SD, vertical differences for during the storage period (60 days) ($p<0,05$).

desired flavor, taste and odor of turşu.

Yeast count is also one of the most important factors for shelf life of turşu. In the study, despite the use of PDA agar, only yeast cells were grown on the media. Yeast growth on the media was confirmed by microscopic and macroscopic methods. Yeast counts of probiotic and control Turşu samples were found to be below the detection limit for 30th day (<1 log CFU/ml). Yeast counts were observed 45 and 60th day of storage, although, no significant difference was detected between probiotic and control samples on the 45th day. Because there was a thick layer of yeast at the surface of the turşu samples (Lp and control), the study was terminated at the 60th day. Yeast count of the control sample was higher than probiotic sample at 60th day.

Enterobacteriaceae and *S. aureus* were not determined any turşu samples during the storage period of 60 day. Enterobacteriaceae and *S. aureus* counts were found as <1 and <1 log CFU ml^{-1}, respectively. Initial level of *Staphylococcus*/*Micrococcus* count was 3.27 log log CFU ml^{-1}, but it was not detected after 45th days of storage period. *Staphylococcus*/*Micrococcus* counts of probiotic and control samples were therefore not statistically difference ($p<0.01$) during the storage period. More also, pH change of samples were similar during the storage period ($p<0.01$); the pH level of both samples increased at the end of storage.

DISCUSSION

The results obtained indicated that vinegar addition preserve Turşu against putrefactive and pathogenic microorganisms. In the study, it was observed that Enterobacteriaceae and S. *aureus* were not determined any turşu samples. *Staphylococcus*/*Micrococcus* count decreased during the storage period and was inhibited after 45th day. Similarly, Yıldız (2011) did not reported *Staphylococcus*/*Micrococcus* count in 11 homemade turşu samples collected from Turkey market. Also, low pH level improved LAB growth and fermentation of vegetables in the product, while on the other hand; high acidity encouraged the growth of yeast count, spoilage of the product and formation of film layer of the surface of Turşu samples.

Fleming et al. (2001) reported that 3.6% acetic acid is critic limit for preservation of pickles and sauces. Osmotolerant yeasts are the principal spoilage organisms in such products. Mold and film yeast may grow on the surface of the liquid chiefly as the result of faulty jar closure. LAB, propionibacteria and butyric acid bacteria also may cause spoilage in unpasteurized fermented vegetables that do not contain adequate concentrations of acetic acid and other preservatives. Usage of *L plantarum* in fermentation of vegetable products is most widely spread (Olsen and Perez-diaz, 2009; Halász et al., 1999; Zeng et al., 2009) because of the main bacterium in production of fermented vegetable (Abriouel et al., 2008; Hutkins, 2006; Çon and Karasu, 2009). Change in *L. plantarum* count and pH values during the storage period in our study was similar to results of the other studies (Özçelik et al., 1998; Özçelik and İç, 2000; Olsen and Perez-diaz, 2009; Halász et al., 1999; Erdoğan et al., 2006; Uylaşer and Erdem, 2004). Özçelik et al. (1998) stored cucumber turşu samples fermented with *L plantarum* at 22 ± 2°C for 12 days. They reported 1 log/ml

reduction of *L plantarum* counts at the end of storage period. In the other study (Özçelik and Ic , 2000), similar reduction was observed after 3 month storage at 20 ± 2°C. They similarly reported that yeast count was increased to 5.08 log CFU/ml. Similar results were also reported by Olsen and Perez-diaz (2009) for alteration of *L. plantarum* and pH in commercial cucumber fermentation.

L plantarum can be also use to develop functional foods in fermentation of vegetables. For this aim, probiotic *L plantarum* strain was used in production of turşu in the study and it was observed that probiotic bacterial level was maintained in turşu samples during the storage period (Table 2). Although, *Pediococcus pentosaceus* and *Leuconostoc mesenteroides* can also be used for the production of fermented vegetables (Jonganurakkun et al., 2008; Chiu, et al., 2007; Eom et al., 2008), *L plantarum* is more useful and adaptive. In addition, some *L plantarum* strains also have other beneficial characteristic; for example, conjugated linoleic acid producing capacity of *L. plantarum* NCULI005 is nearly double that of other lactic acid bacterial strains (Zeng et al., 2009). McFeeters et al. (2005) stated that LAB starter culture addition to cabbage fermentations ensured that texture and flavor were maintained, while allowing a 50% reduction in NaCl.

This study is the first report for *S. aureus*, Enterobacteriaceae and *Staphylococcus*/*Micrococcus* counts throughout the storage period of turşu. In the study, yeast development was observed to be a restriction factor for shelf life of turşu. Storage ended at 60th day for observation of yeast growth on surface of the turşu samples and similar result was also reported by Erdoğan et al. (2006). Biogenic amine production by some yeast strains is another important factor for pickle storage. Kung et al. (2006) identified six histamine producing yeast strains from mustard pickle products. On the other hand, Halász et al. (1999) reported that *L. plantarum* inoculated cabbage samples had lower values for total amine content than spontaneous sauerkraut samples during the storage period (71 days). From Table 2, it was shown that pH was weakly increasing at the end of the storage period, and according to Ogunshe et al. (2006), the increase in pH of the fermenting mash of *aisa* may be due to the abundant increase of NH during the later 3 stages of fermentation.

In conclusion therefore, addition of *L. plantarum* as a starter culture into the turşu improved taste and beneficial properties of the product. In traditional turşu production, addition of vinegar provides a low pH level for initial fermentation conditions. This condition encourages development of starter culture and thus restrict competitive microflora. However, acid resistant yeast grows in the turşu and forms a biomass at the top of the product, thereby restricting the shelf life. Hence, isolating and using antifungal *L. plantarum* strains can help to produce healthier and long life products.

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