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RESEARCH ARTICLE OPEN ACCESS

Production of Probiotic (Turshi) Pickled Vegetables

Sarmad Ghazi Al-Shawi*, Wael Ali Swadi and Alaa Abdul Hussein

Department of Food Science, Agriculture College, University of Basrah, Iraq.

Abstract

In Iraq, people prepare turshi (fermented Iraqi vegetable pickles) from different vegetables such as cucumber, turnip, eggplants, cabbage, carrot and pepper. This study investigated the effect of adding probiotic bacteria (*Lactobacillus acidophilus*), synbiotic (*Lactobacillus acidophilus* + inulin) to Iraqi turshi product, on lactic acid bacteria counts, total count, yeasts and molds, pH values, organoleptic characteristics and the antagonistic activity of turshi against the pathogenic bacteria. Lactic acid bacteria counts were higher in synbiotic turshi log 9.68 cfu/ml comparing with log 9.54 cfu/ml and log 3.97 cfu/ml for probiotic and control turshi samples at the end of study period, respectively. Total count for control sample was higher (log 6.99 cfu/ml) comparing with probiotic and synbiotic samples (log 6.90 cfu/ml and log 6.52 cfu/ml) respectively after 30 days. It was observed that yeasts and molds counts for control sample were higher (log 2.39 cfu/ml) after 30 days, while probiotic and synbiotic samples were log 1.21 cfu/ml and log 0.71 cfu/ml respectively. pH values were close for both synbiotic (3.36) and probiotic (3.73) samples, while it was higher for control sample (4.53) after 30 days. The organoleptic characteristics were more acceptable for synbiotic sample followed by probiotic and control samples, respectively. The antagonistic activities of turshi samples against *Escherichia coli* and *Staphylococcus aureus* were higher for synbiotic sample followed by probiotic and control samples, respectively. It was clear that synbiotic turshi was more desirable in all studied characteristics comparing with probiotic and control turshi and this finding reveals that synbiotic turshi could be used as a potential healthy product.

Keywords: Turshi, pickles, synbiotic, probiotic, *L. acidophilus,* inulin.

***Correspondence:** sarmadghazi@yahoo.com

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INTRODUCTION

Fermentation, one of the oldest and safest ways to conserve food (Chavan and Kadam 1989). Fermented foods provide beneficial bacteria to our digestive tract to maintain the integrity and health of the digestive system. In addition, it allows the naturally beneficial bacteria to do the fermentation through which the vegetables will develop sour taste that is acceptable and rich in vitamins. The fermentation of lactic acid is the only way to preserve all natural plant components and improve the quality, smell and taste (Bamforth, 2005).

Fermented food have different kinds such as dairy, meat and vegetables. Vegetables fermentation is not familiar commercially as the other Fermented foods since it doesn't have standard ingredients and its composition varied depending on geographic conditions and climate. Different fermented vegetable products are produced in different places in the world such as sauerkraut. The wide spread fermented vegetables in Turkey are cucumber, olive, beet, pepper, celery root, eggplant, garlic, apple and cabbage. Turshi can be produced by using one kind of vegetables or a mixture of vegetables (Aktan *et al.,* 1998; Erten and Tang ler, 2010)

Vegetables are a good source of antioxidants like flavonoids, vitamins, phenolic compounds, carotenoids, dietary fibres and minerals (Sun *et al.,* 2009; Kusznierewicz *et al*., 2010). Lactic acid bacteria existed in pickled garlic have good inhibition ability against some food-borne pathogens (Sadeghi, 2016).

Lactic acid fermentations have widely known for years and used in various food industries such as fruit and vegetable processing, production of rye bread and fermented milk beverages (McMurtrie, 2016; Gorzelany *et al*., 2018; McMurtrie and Johanningsmeier, 2018).

USA regulations define pickles as a kind of low-acid food to which acid is added, water activity above 0.85 and the final equilibrium pH is 4.6 or below (Acosta *et al.,* 2015).

Probiotic came from (Pro Bios) the Greek word that means "for life". In ancient time the people knew the positive effect of fermented food on host health such as fermented dairy which known as an excellent medication without knowing the mechanism of its action (Neish, 2009).

Moreover, probiotic properties could be added to the product by microorganisms used in fermentation. There have been many studies found that the consumption of probiotic is useful in the treatment of lactose intolerance, immune function, cholesterol, diarrhoea, blood pressure, colon cancer, Irritable bowel syndrome, colitis, inflammation and absorption of minerals (Montalto *et. al*., 2006; Upadrasta and Madempudi, 2016; Levri *et. al.*, 2005; Simons *et. al*., 2006; Billoo *et. al*., 2006; Geier *et*. *al*., 2006; Bengmark, 2007; De Preter *et. al*., 2011)

Many antimicrobials produced by lactic acid bacteria such as (formic, acetic, lactic acids), hydrogen peroxide, ethanol, hydrogen peroxide, reuterin and diacetyl, have the ability to stop molds spoilage. Sorbic acid, propionic acid and benzoic acid are regarded as chemical food additives and are applied in pickles preservation, so the using of probiotic vegetable fermentation could be serve as an alternative to the chemical additives preservation (Leroy and Vuyst, 2004).

Prebiotics are carbohydrates which pass through the small intestine without any digestion, after that they reach the colon and stimulate the function and the growth of beneficial bacteria, especially bifidobacteria and lactobacilli. Inulin is a chicory extract prebiotic consists of long-chain fructooligosaccharides (Vandenplas, 2002)

Inulin, fructooligosaccharides, Galactooligosaccharides and β-glucans are the main types of prebiotics, and all are nutrients for probiotics (Roberfroid, 1993).

Adding probiotics, prebiotics or synbiotics into food is desirable for intestinal microbiota and they may be consumed as raw fruit and vegetables, vegetable pickles or dairy products (Markowiak and Slizewska, 2017)

Synbiotic consists of probiotic and prebiotic and created to overcome on some difficulties in probiotics survival in gastrointestinal tract (Rioux *et. al*., 2005). Therefore, a suitable mixture of both probiotic and prebiotic in a single product should ensure an appropriate effect comparing to the activity of probiotic or prebiotic alone (Bengmark, 2005; Panesar *et al*., 2009)

Study aims to investigate the effect of adding probiotic and synbiotic properties to Iraqi turshi during storage period and improving the organoleptic characteristics of the product.

MATERIAL AND METHODS

Probiotic Bacteria

L. acidophilus strain was purchased from LGG™ (Finland).

Prebiotic

Inulin was purchased from NOW Foods products (USA).

Production of Iraqi Turshi

Cucumber, green pepper, carrot and cabbage, garlic, black cumin, coriander and vinegar were purchased from Basrah local market. The Iraqi traditional method was used in turshi production as shown in Fig. 1. After completing the production of Iraqi turshi, it was divided into 3 jars. First jar is a control, *L. acidophilus* (10^9 cfu/g) 1% w/v was added into the second jar (probiotic), and *L. acidophilus* (10^9 cfu/g) 1% w/v and inulin 1% w/v was added into the third jar (synbiotic).

Microbiological analysis

Lactic acid bacteria (LAB), total count, yeasts and molds counts were evaluated during storage periods (0, 15 and 30 days). Ten milliliters of turshi sample transferred aseptically into 90 ml of 0.1% peptone plus 0.85% sodium chloride solution and homogenized. Serial dilutions were made. The enumeration of LAB (MRS agar) at 37°C for 24-48 hrs. anaerobically (Harrigan, 1998), total count (Nutrient agar) at 37°C for 24-48 hrs., yeast and mold (PDA agar) at 25°C for 5 days (Tournas *et. al.,* 1998).

Determination of pH

The pH of turshi samples were measured by using pH meter (Pye Unicam-Model 292MK. England).

Organoleptic Characteristics

For Organoleptic Characteristics, ten specialists from food science department evaluated turshi products according to Rajablou *et al.* (2012) for taste, color, texture and over all acceptability.

Antagonistic activity of turshi (pickled vegetables)

The antagonistic activity of turshi samples against *Escherichia coli* and *Staphylococcus aureus* was done by using the agar well diffusion assay on Mueller Hinton agar (Herreros *et al*., 2005). Each indicator bacteria were spread and then turshi sample (vinegar) were added to 5-mm diameter well on these Petri dishes. The indicator bacteria counts were the same (100µl, 10^8 cfu/ml) prepared from broth culture of bacteria according to 0.5 McFarland standard. The inhibition zone diameter of turshi samples was measured after 48 hrs. of an aerobic incubation at 37°C.

Statistical analysis

Triplicate Complete Randomized Design was used in data analysis of the studied characteristics by using SPSS (2009) version17. The averages were compared using less significant difference test rate within the program.

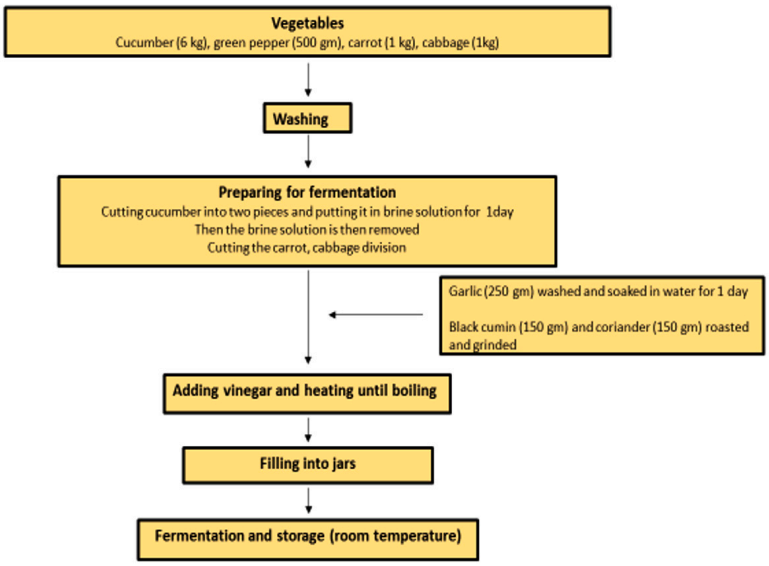

Fig. 1. Traditional Iraqi Turshi Preparation

RESULTS AND DISCUSSION

Microbiological content of turshi vegetables pickled

Results obtained in Table 1 show that LAB counts in control sample were low during the periods of the study which ranged between log (3.97-4.58) cfu/ml in contrast with LAB counts in probiotic and synbiotic samples which were higher at zero-time log (9.82 and 9.87) cfu/ml respectively, decreased after 15 days, and reduced a little after 30 days of the study period to reach log (9.54 and 9.68) cfu/ml respectively. Synbiotic sample was higher after 30 days in LAB counts over probiotic and control samples respectively and this may be attributed to the availability of inulin which provided a suitable environment to the growth of LAB on the account of other bacteria.

Total count as shown in Table 1 indicates that probiotic sample at zero-time was higher log (7.45) cfu/ml followed by synbiotic sample log (7.44) cfu/ml and control sample log (7.27) cfu/ml respectively. After 15 days, a high reduction happened in synbiotic and probiotic samples. Synbiotic sample was the higher in its total count reduction followed by probiotic sample and control sample respectively. At the end of the study period (30 days) there was a sharp reduction in synbiotic sample total count log (6.52) cfu/ml and probiotic sample log (6.90) cfu/ml, and there was a slight reduction in control sample log (6.99) cfu/ml, this may be attributed to the activity of LAB in synbiotic and probiotic samples and the support of inulin in synbiotic samples to produce acids and by-products which inhibited the growth of undesirable microorganisms. Yeast and molds counts as shown in table (1) were close in all study samples at zero-time. The counts increased gradually in control sample to reach log 2.39 cfu/ml at the end of the study period, while it decreased in probiotic and synbiotic samples to reach log (1.21 and 0.71) cfu/ml respectively at the end of the study period. The growing of LAB and producing acids reduced the pH and affected negatively on the growth of yeasts and molds.

The viability and activity of probiotic bacteria during preparation and storage are very important for their industrial applications. They can be added to probiotic products as fresh or lyophilized cells. High population levels, between 10^6-10^8 microbial cells/ml should be present in probiotic products. It should be also explained that during food processing, bacteria subjected to stress conditions such as freezing, drying and concentration stress and these are also decreasing the viability of LAB (Kos *et al*., 2008).

Both probiotic and synbiotic turshi samples of this study are considered as probiotic products as the probiotic levels were log (9.54 -

Table 1. microbiological content of Control, Probiotic and Synbiotic turshi samples

	LAB			Total Count			Yeast & Mold		
Day	0	15	30	0	15	30	0	15	30
Control turshi	4.58	4.31	3.97	7.27	7.07	6.99	1.75	2.28	2.39
Probiotic turshi	9.82	9.74	9.54	7.45	7.24	6.90	1.79	1.52	1.21
Synbiotic turshi	9.87	9.81	9.68	7.44	7.14	6.52	1.77	1.44	0.71
LSD	5.24	0.07	0.15	0.01667*	.07333*	.08667*	NS	0.073	0.49333*

Table 2. pH of Control, Probiotic and Synbiotic turshi samples

	pH		
Day Sample	0	15	30
Control turshi	5.533	4.833	4.533
Probiotic turshi	5.433	4.633	3.73
Synbiotic turshi	5.467	4.267	3.367
LSD	NS	0.2000*	0.3667*

Table 3. Organoleptic Characteristics of Control, Probiotic and Synbiotic turshi samples

	Taste	Colour	Texture	Over all acceptability
Control turshi	7.50	6.90	7.00	7.80
Probiotic turshi	8.70	7.50	7.90	8.40
Synbiotic turshi	9.10	7.80	8.30	8.70
LSD	1.20	NS	0.89	NS

Table 4. Antagonistic activity (mm) of Control turshi, Probiotic turshi and Synbiotic turshi samples against *E. coli* and *S. aureus*

	E. coli	*S. aureus*
Control turshi	12.40	11.06
Probiotic turshi	12.90	11.36
Synbiotic turshi	13.33	11.60
LSD	0.50	0.30

9.68) cfu/ml respectively, and this agreed with Beganovic *et. al*. (2011) who showed that *L. plantarum* L4 and *L. mesenteroides* LMG 7954 strains in sauerkraut pickles are considered as probiotic products because probiotic cells in final product was determined higher than 10^6 cfu/g. Same results reported by etin (2011) who found that the product kept its probiotic level during the 60-day storage period.

The addition of probiotic bacteria affected positively on the LAB counts and decreased the counts of total bacteria, yeasts and molds and these results agreed with etin (2011) who reported that *L. plantarum* had a positive effect on LAB counts in turshi samples .

Turshi (Pickled vegetables) pH value

The fermentation process' success depends on the pH which gives an indicator to the fermentation. pH values in Table 2 show that all samples were close in pH values at zero-time. There was a clear decrease in synbiotic and probiotic samples after 15 and 30 days while there was a slight decrease in control sample pH values. The study results agreed with Blanc (1996) who found that the pH was high at the beginning of the study period and because of acid formation, dropped quickly. The results came close with Pundir and Jain (2010) who reported that sauerkraut brine pH ranged between (3-4) and the pH showed a decreasing trend from the day of preparation till the end of preservation period.

pH values of this study were in contrast with etin (2011) who noticed that there was a similar change in pH values between probiotic and control turshi samples during the storage period, he noticed at the end of storage period that the pH levels of both samples increased. Susilowati *et al.* (2018) found that the lowest pH value was observed in pickled ginger prepared using 2.5% salt for 10 days was (3.33) and this result was close to our study result for synbiotic turshi after 30 days.

The rapid decrease in turshi pH for probiotic and synbiotic samples was because of LAB (probiotic) throughout the production of lactic acid as a main catabolite (Giraffa *et al*., 2010).

Organoleptic Characteristics

Results of organoleptic characteristics illustrated in table (3) show that synbiotic sample was more acceptable followed by probiotic and control samples respectively. This may be attributed to the taste, flavor and odor produced by probiotic bacteria (*L. acidophilus*) added to the probiotic and synbiotic turshi samples, and inulin which provided a suitable substrate to LAB. Results came in agree with etin (2011) who observed that the organoleptic value of the samples had been improved by adding of *L. plantarum* which increased desired flavour, taste and odour of turshi.

Antagonistic activity of turshi

All turshi samples showed antimicrobial activity against potentially pathogenic gram-negative and gram-positive bacteria (*E. coli* and *S. aureus*). This activity may be attributed to bacteriocin production by LAB (Giraffa *et al*., 2010).

The highest antimicrobial activity was for synbiotic turshi sample followed by probiotic and control turshi samples respectively as shown in Table 4.

Fermented pickled vegetables have a high microbial load since it could be a vector in transporting pathogenic bacteria from the farm and can't be pasteurized. So, LAB could reduce the number of undesirable microorganisms in these vegetables (Tamang *et. al*., 2009).

Adding a protective culture is considered as a safety factor guaranties the stability of food microorganisms and reduces the risk of growing pathogenic and food spoilage microoganisms (Holzapfel *et al.,* 1995; Inatsu *et al.,* 2005; Rabie *et. al.,* 2011).

CONCLUSION

The results of this study showed that probiotic and synbiotic turshi samples have probiotic product properties in regard of LAB counts, more desirable organoleptic characteristics and good antagonistic activity against the pathogenic bacteria, These data lead to potential probiotic and synbiotic products that have the

ability to be a good environment and carrier for the beneficial probiotic bacteria and keeping them for a long time comparing with dairy or other products which suffer from deterioration because the bad preservation methods.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR'S CONTRIBUTION

SGA and WAS conceived and designed the analysis, collected the data, Contributed data or analysis tools. SGA and AAH performed the analysis. SGA wrote the manuscript. All authors read and approved the manuscript for publication.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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