



Original research

## Antimicrobial effects of pomegranate peel extract on *Lactobacillus plantarum* and shelf life of Thousand Island dressing

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### ABSTRACT

Nowadays, consumers become interested to use the substitution of natural preservatives instead of chemical preservatives. It has been proven that pomegranate peel contains large amounts of antimicrobial and antioxidant compounds. In this study, the antimicrobial effect of ethanolic extract of pomegranate peel was evaluated on total microbial count, mold and yeast and *Lactobacillus plantarum* during 3 months storage of thousand islands dressing at 4°C. The treated sauce samples were containing different concentrations (0.05, 0.1 and 1%) of pomegranate peel extract and 0.07% sodium benzoate, potassium sorbate mixture (BS) as control. All samples were inoculated with bacterial suspension containing 10<sup>5</sup> cfu/mL of *L. plantarum*. MIC and MBC of pomegranate peel extract on *L. plantarum* were obtained 1 mg/mL. All concentrations of pomegranate peel extract significantly decreased total bacterial count ( $p < 0.05$ ). The results showed that adding 0.05% pomegranate peel extract reduced 2 Log cfu/g of total mold and yeast counts compared to the control sample in souse. There was no significant difference of adding different concentration of extract on the survival of *L. plantarum* ( $p > 0.05$ ). Adding 0.05% pomegranate peel extract did not cause undesirable sensory attributes. Pomegranate peel extract can increase the thousand islands dressing shelf life by its antimicrobial effects as a natural antimicrobial agent and preserving the probiotic properties of the product.

Keywords: Antibacterial, *Lactobacillus plantarum*, Pomegranate peel extract, Thousand Island dressing

Received 30 October 2019; Received 13 March 2020; Accepted 14 March 2020

## 1. Introduction

Food products with natural resources have interested both consumers and food producers. There has been a growing demand for processed foods with fewer synthetic additives (Barsegar et al., 2008). FDA authorized concentration of sodium benzoate as a food preservative is limited to less than 0.1% wt (Jafari Khattayloo et al., 2017). Sodium benzoate, in combination with vitamin C, becomes a carcinogen. Alone with DNA damage to the stem cell, it can cause diseases such as Parkinson's and neurological diseases (Jafari khatayloo et al., 2017). The in vivo effects of oral administration of varying concentrations of sodium benzoate (a commonly used food preservative) on Hemoglobin concentration, white blood cell (WBC) count, total plasma protein and some plasma electrolytes levels of wistar albino rats were investigated. The findings of significant decreases suggest the induction of anaemic condition in the rats (Eberechukwu et al., 2007).

Thousand Island dressing is an oil-in-water emulsion and one of the most well-known sauces that has favorable taste. In industrially made mayonnaises, the main microbiological risk is the

presence of yeasts (*Saccharomyces*, *Candida*, *Zygosaccharomyces*, *Debaryomyces*, *Pichia*), lactic acid bacteria and other bacteria (*Bacillus subtilis*, *B. pumilis*, *B. polymyxa* and *B. megaterium*), but the presence of pathogens (*Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp.) cannot be excluded (Kucerova et al., 2006). However, acetic acid and other organic acids are the primary preservative agents. Antimycotic agents such as sodium benzoate and/or potassium sorbate have been frequently used. Some dressings, because of their low acid content, must be refrigerated to retard spoilage (Smittle, 1981). One of the problem of these products is using chemical and synthetics preservatives like sodium benzoate and potassium sorbate that have been proven disadvantages. Acidic conditions in combination with other factors like reduced water activity, which exist in salad dressings and mayonnaise, prevent the growth of most microorganisms commonly associated with food spoilage. However, microbial spoilage of these products occasionally does occur as a result of the growth of a select group of microorganisms (Pederson, 1930).

Salad dressings and sauces range greatly in their flavors, chemical make-up and physical characteristics. However, acetic acid and other organic acids are the primary preservative agents.

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Benzoate as a chemical preservative is commonly used for microbial stability in sauces. There have been numerous studies regarding the side effects of sodium benzoate such as fetal mortality and morbidity, mutations, blood cell genetic abnormalities, neurological diseases such as Parkinson, Alzheimer, Huntington, skin problems and hyperactivity in children have been reported (Esfandiari, 2016). One study found that oregano essential oil had an antimicrobial effect on *Salmonella enteritis* in mayonnaise (Ghorbani et al., 2015). In another study, the effect of peppermint oil as a substitute for sodium benzoate in mayonnaise was investigated (Jafari khatayloo et al., 2017). The effect of chubak extract on rheological properties was investigated and it was found that chubak extract increases the rheological stability of mayonnaise (Ghahremani et al., 2015). In particular, fruit peels contain promising novel compounds having antimicrobial activity against *Lactobacillus* spp. and antioxidant activity that can pre-vent food-borne illnesses and food spoilage. These compounds are generally secondary metabolites (phenolic compounds, steroids and alkaloids in particular) exerting plenty of useful effects on human health (Singh et al., 2018).

The pomegranate (*Punica granatum* Punicaceae family), is natively from the Himalayas in northern India to Iran, nevertheless it has been cultivated and naturalized since ancient times over the entire Mediterranean region. Pomegranate extracts (PEs) were also reported to possess noteworthy antibacterial, antiviral and anti-inflammatory bioactivities thanks to the polyphenolic compounds content, which includes punicalagins, gallic acid, and ellagic acid derivatives. Furthermore, the pomegranate extracts act as natural inhibitors of pathogens, bacteria, and fungi (Tehraniifar et al., 2011). It has been reported that pomegranate by-products and punicalagins significantly are able both to inhibit the growth of pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridia*, and *Staphylococcus aureus* and to increase the growth on beneficial bacteria including *Bifidobacterium* spp. and *Lactobacillus* (Sorenti et al., 2019). In this study, we replaced pomegranate peel extract instead of synthetic preservative, then evaluate antimicrobial effects on total probable count bacteria, fungi flora in Thousand Island dressing for three months.

## 2. Material and Methods

### 2.1. Extraction of pomegranate peel

Pomegranate fruits (5 kg) were purchased from local grocery store in Tehran, Iran. Then pomegranate peels were manually separated and removed then was dried in the shade at room temperature for one week. It was then powdered by electric milling and kept at 4°C until the test. Extraction was done by maceration method (Rahnemoom et al., 2017). 20 g of pomegranate powder is mixed with 200 mL of 70% ethanol (1 to 10), the extract was filtered with Whitman 35 filter paper and separated into a solvent evaporator using a solvent extractor (BUCHI, Switzerland).

The solvent was evaporated from the extract and after concentrating the extract, it was kept in a glass container outside the light at 4°C. The extract was dissolved in sterile distilled water (DW) in desired concentrations (0.05, 0.1 and 1% w/w) before adding to sauce.

### 2.2. Total phenolic compounds analysis

Total phenolic content, flavonoids were determined by procedures that were adopted in author's previous studies. In the last decade there are numerous publications proving the antibacterial activity of many plant extract. Total phenolic content of the extracts was estimated by the HPLC method that was done by Rahnemoom et al. (2016) For being in similar situations, results showed ethanol/water ratio 60:40, 25°C and maximum amount of phenolic compound.

### 2.3. Preparing 0.5 McFarland suspension

The standard suspension 0.5 McFarland was prepared by adding 0.5 mL of the aqueous solution of 1.175 g of barium chloride, slowly by continuous mixing to 99.5 mL of sulfuric acid 1%. The turbidity by this suspension created a cell density of approximately  $3 \times 10^8$  cfu/mL and then the turbidity was measured using a spectrophotometer set (CECIL 2502- Instruments Cambridge England Serial No. 125-624) at a wavelength of 625 nm.

### 2.4. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of pomegranate extract

In this study, *Lactobacillus plantarum* was purchased from the Iranian Fungi and Bacteria Collection Center, culture media were provided by Merck German Company.

*L. plantarum* was cultured on MRS broth and incubated on MRS agar at 37°C (Shahrampour et al., 2017). Micro-dilution method was used to determine MIC Thus, a series of 8 test tubes consist of 6 tubes for test different dilutions of the extract and positive control tube and one negative control tube.

In order to evaluate the antimicrobial activity of the extract 0.05% and 0.1% and 1% pomegranate peel extract were added to the sauce samples.

### 2.5. Sauce preparation

In order to study the antimicrobial properties of pomegranate peel in Thousand Island dressing was prepared according to Table 1. First pasteurized for aqueous phase, water and paste at boiling temperature for 7 minutes then vinegar and other powdered compounds such as sugar, salt, citric acid, sodium benzoate, potassium sorbate (BS) and mustard and gums are blended together then sunflower oil and olive oil were added to the mixer in equal proportions to form oil phase. In the mixer the eggs were first added to the powder, then added about 1/3 of aqueous phase including ketchup, water, and vinegar. The oil was poured into the mixer gradually (over a period of 5 minutes). At the end of the work, a constant rate of flow of the aqueous and oil solution was poured into a homogenizer at rate 5000 rpm. The sauces were stored in a 20 g glass lid covered at 4°C. In each of the dishes a specific weight of the extract was dissolved. Microbial suspension was also added to finally the microbial population in each sample of the sauce reach  $10^5$  cfu/mL.

After preparing Thousand Island dressing, the samples were divided into 5 parts containing pomegranate peel extract (0.05, 0.1 and 1%), sample containing sodium benzoate-potassium sorbate and control sample (without preservative).

Table 1. Compounds of Thousand Islands dressing.

Raw materials	Quantity (%)
Oil	56
Vinegar 10%	10
Egg	5
Sugar	5
Citric acid	1
Ketchup sauce	5
Salt	2
Aromatic vegetable	2
Xanthan gum	0.3
Sodium benzoate	0.04
Potassium sorbate	0.03
Mustard	0.3
Pickle	3
Water	Total:100

### 2.6. pH analysis

5 g sauce sample were added to 4 mL of distilled water. Then pH was measured through pH meter (set with a buffer solution) (National Iranian Standard No. 5272).

### 2.7. Microbial analysis

#### 2.7.1. Total counts

Colony method of microorganism was used for counting microbes according to National Iranian Standard No. 5272.

#### 2.7.2. Mold and yeast count

Yeast extract glucose chloramphenicol agar culture medium was used to count the mold and yeast. So that 1 mL of the sample dissolved in ringer solution was poured into sterile plates and added to the sterile culture and cultured. Put at 25°C for 96 hours in an incubator (National Iranian Standard No. 10899-2).

#### 2.7.3. *Lactobacillus plantarum* count

Yeast In this study, MRS agar medium for counting *Lactobacillus plantarum* was used. All samples were examined at intervals immediately after production, 1, 2, and 3 months later (3 replicates) according to National Iranian Standard 8923-1, 2965 and 2454.

### 2.8. Sensory evaluation

Sensory evaluation of the selected samples, each of the 5 specimens (treated and control) were evaluated based on 5-point hedonic method for taste, color, odor, texture and general acceptance. The scoring method was that number 5 represented the highest score and number 1 represented the lowest score. The test was conducted by a group of 10 people including food industry experts and consumers.

### 2.9. Statistical analysis

The difference between the different treatments was determined based on the factorial experiment statistical design with ANOVA at 5% probability level. The data were compared by Duncan test with SPSS version 19 and Excel version 2013.

## 3. Results and Discussion

### 3.1. Determination of MIC and MBC of Pomegranate Extract

MIC and MBC of 20% pomegranate peel extract were determined for *Lactobacillus plantarum* 1 mg/mL. Pomegranate peel extract showed a strong broad-spectrum antimicrobial activity against Gram-positive (*Bacillus subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *Klebsiella pneumonia*) bacteria, with MIC ranging from 0.2 to 0.78 mg/mL (Fawole et al., 2012).

### 3.2. Total phenolic content

Results showed that maximum amount of phenolic compound, flavonoids and anthocyanin extracted in ethanol/water ratio 60:40, 25°C solution condition were 349.518 mg gallic acid/g, 250.124 mg routine/g and 252.047 mg cyanidin-3-O-glucoside/100 g, respectively.

### 3.3. Total microbial counts

The results of different concentrations of pomegranate peel extract showed decrease in bacterial count of Thousand Island dressing at 4°C (Fig. 1). the amount of colonies is lower than the standard limit of 10<sup>4</sup> microorganisms per gram of salad dressing during 3 months. The trend of changes in TPC in the present study was incremental in the control sample. It means the lowest value at day zero and the highest value at month 3.

There was no significant difference between 0.1% and 1% treatment ( $p > 0.05$ ). That depending on the amount of Phenolic Components of pomegranate peel extract and its comparison with their antibacterial properties against *Alicyclobacillus Acidoterrestriis* addition of pomegranate extract to chicken meat products enhanced its shelf life by 2–3 weeks during chilled storage. Pomegranate extract showed good antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus* having minimum inhibitory concentration of 0.01%. *Pseudomonas* could be inhibited at a higher concentration of 0.1% while it was ineffective against *Escherichia coli* and *S. typhimurium*. Phenolic compounds are responsible for the antimicrobial activity of the extract, according to research by Jooyandeh et al. (2017).

Basiri et al. (2015), showed that microbial growth of vacuum packed and PE treated (1 or 2%) shrimps were retarded during storage time in comparison with the control group ( $p < 0.05$ ).

In another study by Basiri et al. (2015), it was investigated the combined effect of vacuum packaging and methanolic extract of pomegranate peel on shelf life and quality of oceanic shrimp. They achieved similar results. Also, according to the results of Jafari Khattayloo et al. (2017), with the removal of chemical preservatives in peppermint sauce containing mayonnaise, while removing the chemical preservatives, it retains the product's shelf life and has special effects on exposure to Gram-positive and Gram-negative microorganisms (*E. coli*, *Bacillus subtilis*, *Salmonella*, mold and yeast).

Table 2. The effects of different treatments on mold and yeast counts in Thousand Islands dressing (Log cfu/g).

Sample	day	0	30	60	90
control		negative	Less than 10	2.07± 0.04 <sup>A</sup>	0.12 ±2.68 <sup>B</sup>
BS*		negative	Less than 10	less than 10	Less than 10
0.05%		negative	Less than 10	Less than 10	Less than 10
0.1%		negative	Less than 10	Less than 10	Less than 10
1%		negative	Less than 10	Less than 10	Less than 10

Different letters (A-B) within a row indicate significant difference (p < 0.05).

\*BS: Sodium benzoate and Potassium sorbate.

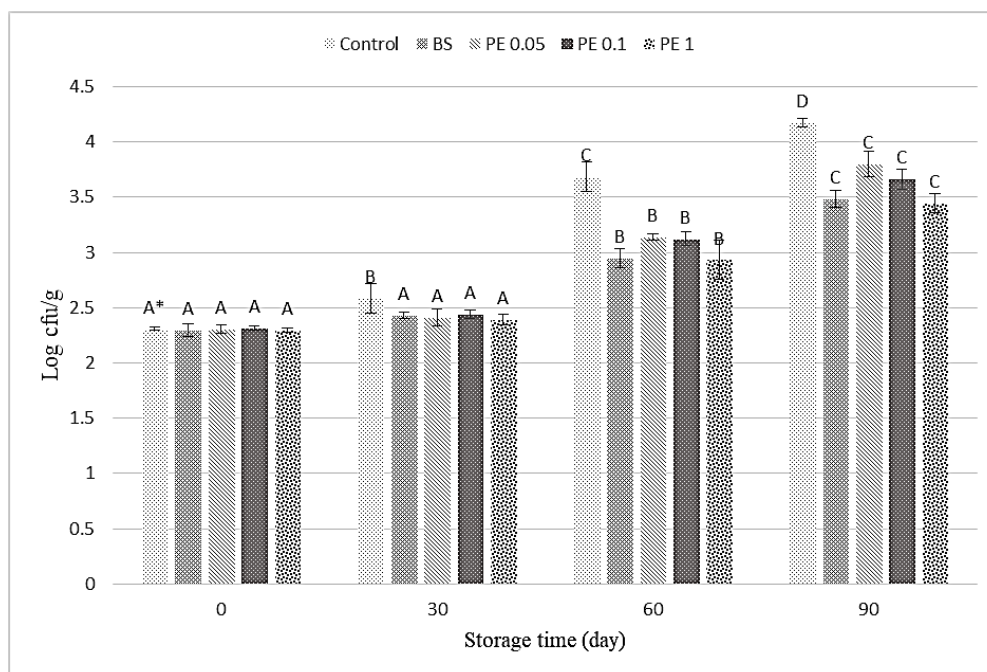


Fig. 1. The inhibitory effect of different treatments on total bacterial counts of sauce (BS = Sodium benzoate and Potassium sorbate, PE= Pomegranate extract). Capital letters means followed by the same letter are not significantly different (p < 0.05).

### 3.4. Mold and yeast evaluation

The number of molds and yeasts in the control sample increased during the storage period and in the other samples it was less than 10 per gram (Table 2). Mold growth was inhibited in all samples except the control after 3 months. These findings are in agreement with the results of Selahvarsy et al. (2010), which investigated the relationship between antioxidant and antifungal activity of different parts of pomegranate extract with its phenolic content and showed that methanolic extract with the mean of 47.6% and 37.7% had the highest inhibitory effect on mycelial growth (IMG) and spore germination of fungi. Studies carried out by manipulation on extracts of 4 plant extracts (wild pomegranate

peel, willow bark, belle fruit and betel) by disk diffusion method showed that pomegranate skin extract in addition to antimicrobial properties against Gram positive and negative bacteria, They had inhibitory effect on yeast and molds. High levels of polyphenolic compounds in pomegranate peel extract have been attributed to its powerful antifungal properties (Tehranifar et al., 2011). Numerous studies have demonstrated that pomegranate peel extract contains tannins that can control fungal growth and high level of punicalagin reported in pomegranate peel extract is responsible for antifungal activity (Endo et al., 2010). Water-methanol pomegranate peel extract was effective against *Aspergillus niger*, *Candida utilis* and *Saccharomyces cerevisiae* having inhibition zones of 12, 18 and 14 mm respectively (Al-Zoreky, 2009).



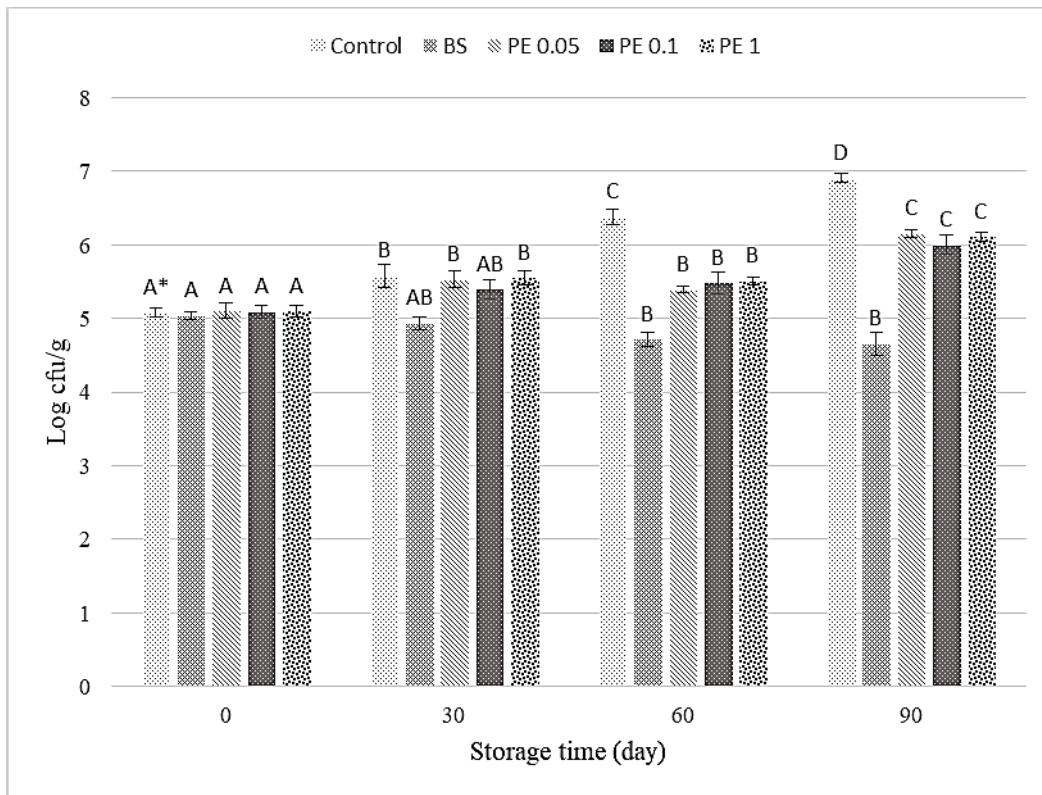


Fig. 2. The effect of different concentration of pomegranate peel extract on *Lactobacillus plantarum* during 3 months storage (BS = Sodium benzoate and Potassium sorbate, PE= Pomegranate extract). Capital letters means followed by the same letter are not significantly different ( $p < 0.05$ ).

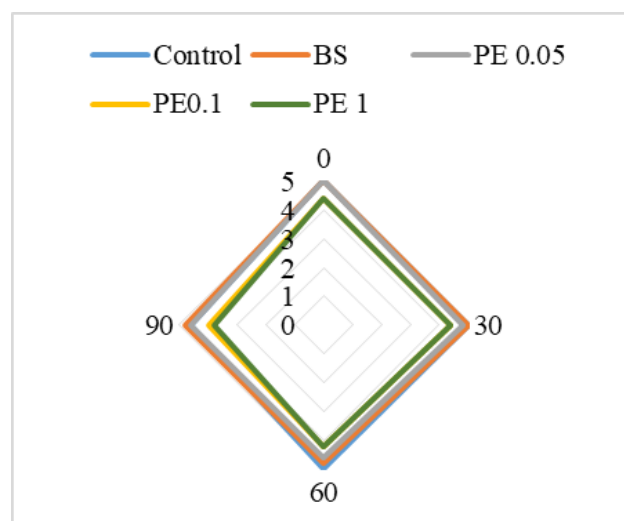


Fig. 3. Color score of Thousand Island dressing during storage time.

### 3.5. *Lactobacillus plantarum* enumeration

The number of *Lactobacillus plantarum* in samples containing pomegranate peel extract increased, while benzoate-sorbate sample

decreased (Fig. 2). In the first month of storage no significant difference was observed. In this study, we observed a marked increase in *L. plantarum* count in the control sample (approximately one logarithmic phase) which may be due to the lower pH of the control sample and corresponds to the results of the

Golbooni nejad under the probiotic enrichment of watermelon water using four Lactobacillus species. Four species of *Lactobacillus* after 42 days of refrigerated storage, number of bacteria was consistent with minimum number of probiotic ( $10^6$ ) bacteria. In a study by Hosseini et al. (1986) with the aim of investigating the effect of alginate / chitosan microencapsulation on the survival of *L. plantarum* in elephant fish, they concluded that according to standards, the number of probiotics needed to be healthy At least  $10^6$  cfu/gr of the probiotic product present. The highest bacterial count was observed in samples containing 0.5 mg/mL (E 0.5). The results of this study are in agreement with the results of Seifi Mohammadzadeh et al. (2016), who investigated the effect of pomegranate and inulin extract on the viability of *Bifidobacterium bifidium* and with increasing percentage of pomegranate extract in soy sauce, the number of probiotic decreased. There have been many reports on the tolerance and acid resistance of *L. Plantarum*, for example Shahrampour et al. (2017), who investigated the antibacterial and antifungal activity of *L. Plantarum* strains isolated from various foods and reported that the antimicrobial metabolites derived from this against heat stroke, proteolytic enzymes retained their antibacterial activity, which is consistent with the results of this study. These results are in agreement with studies of (Sorrenti et al., 2019) that the synergistic properties of combining foods such as pomegranate and probiotics may exert combined health benefits. These data demonstrate that, even if in our experimental conditions it was not observed a prebiotic effect, filtered SB obtained from LGG incubated with PE (LGG-T1), might contain, besides the beneficial bacterial secreted bioactive compounds, also small amounts of PE-derived bioactive compounds.

3.6. pH analysis

The pH of all treatments and control samples were between 3.5 and 3.8 which are in accordance with national standard 2954 (less than 4.1). All treatments containing pomegranate extract showed a slight decrease in pH over time whereas in the control sample after 3 months the lowest pH was observed. This may be due to a decrease in pH during storage, possibly by the breakdown of some ester groups and their conversion to acidic groups. This was appropriate with the results of Jafari Khattayloo et al. (2017) findings.

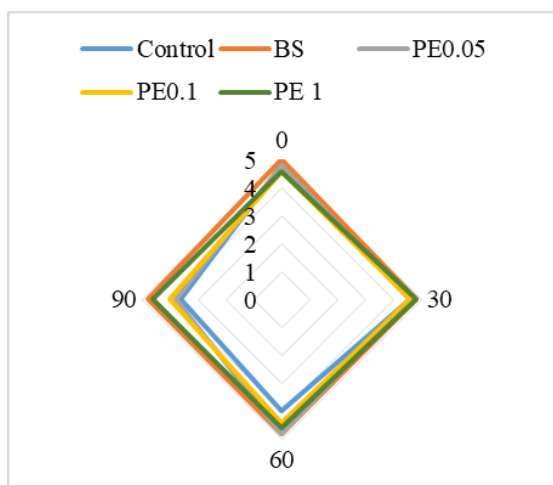


Fig. 4. Odor score of Thousand Island dressing during storage time.

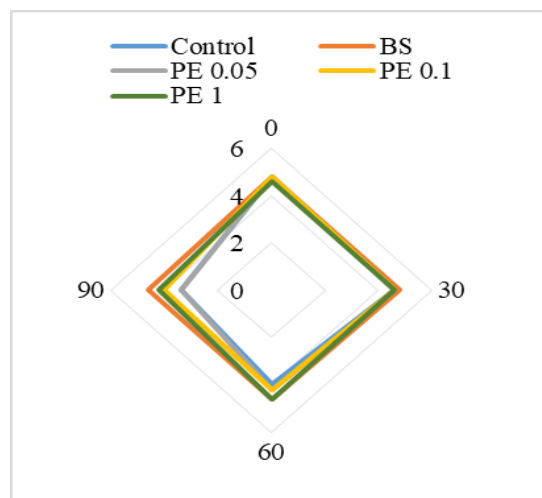


Fig. 5. Flavor score of Thousand Island dressing during storage time.

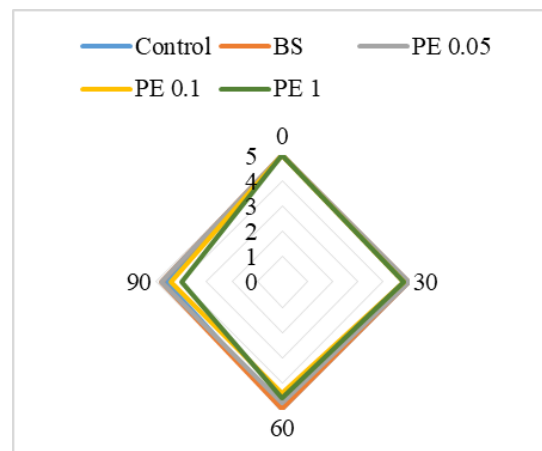


Fig. 6. Texture score of Thousand Island dressing during storage time.

3.7. Sensory evaluation

The results of statistical analysis showed highest color, taste and flavor score for the sample contained benzoate-sorbate after 90 storage days (Fig. 3-7). In terms of flavor and taste, control had lowest score ( $p < 0.05$ ).

Increasing the percentage of pomegranate peel extract, decreased the color and texture score of the sauce. Therefore, when adding pomegranate peel extract to the sauce, it should pay attention to its adverse effects on the texture and color properties, and must be add in appropriate amounts that do not have an undesirable effect on mentioned attributes. The results of sensory evaluation agree with Jooyandeh et al. (2016) findings. Basiri et al. (2015) also showed that the combination of pomegranate peel extract and vacuum packing has no significant effect on the sensory properties.

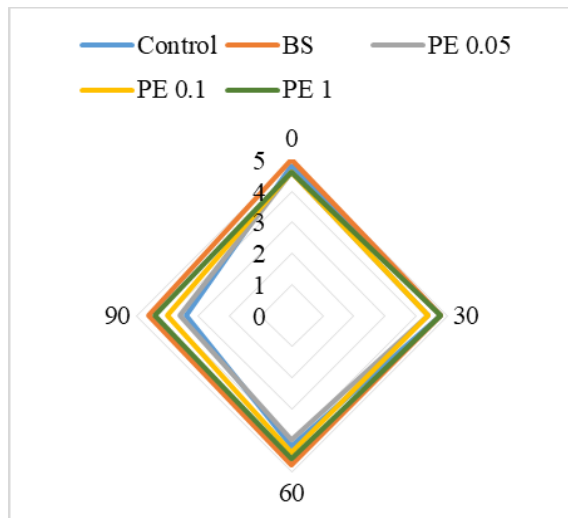


Fig. 7. Overall acceptance of Thousand Island dressing during storage time.

#### 4. Conclusion

Pomegranate peel extract as an antimicrobial agents improve Thousand Island sauce and applying 10% extract (w/w) in sauce reduced 0.72 and 1.68 log cfu/g the total microbial and mold and yeast counts, respectively, compared to the control sample, which was equal to the inhibitory effects of benzoate and sorbate as normal synthetic antimicrobial agent. Also the sensory attributes analysis showed that adding pomegranate peel extract had no significant inappropriate effect (except color) considering the taste, flavor and overall acceptability. On the other hand, the results of this study indicate the least inhibitory effects of the extract on *Lactobacillus plantarum* strain compared to the added synthetic antimicrobial agent and it can satisfy consumers' demand for production the natural foods with probiotic properties. Therefore, pomegranate peel extract with appropriate concentration can be a good substitute for chemical preservatives such as benzoate and sorbate in food processing and shelf life.

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