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ANTIOXIDANT AND ANTIMICROBIAL EFFECT OF SUMAC (*RHUS CORIARIA L.*) POWDER ON *E.COLI* AND *PENICILlIM NOTATUM* IN PREBIOTICS LOW FAT YOGHURT

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ABSTRACT

Yoghurt is one of the popular food diets with refreshing tasteand health benefits. The purpose of this study identify sumac powder with antioxidant and antimicrobial efficacy for controlling some food borne pathogens such as *Escherichia coli* and *Penicillium notatum.*The antioxidant and antimicrobial effects of sumac *(Rhus coriaria L.)* powder (0%, 1%, 1.5%and 5%) in prebiotic (Resistant starch type 2) low fat yoghurt, were performed respectively to the DPPH and surface cultivation during storage at 4C˚.The data were expressed as mean ± SD and were tested by one-way ANOVA at α=0.05. Titratable acidity of yoghurt containing sumac powder increased whereas the pH decreased with increasing amounts of added sumac powder. Also, the antioxidant activity of yoghurt samples were significantly increased with the increasing of sumac powder from 0 to 5% ($p<0.05$). Different concentration of sumac powder reduced the number of *Escherichia coli* and *Penicillium notatum* in prebiotic low fat yoghurt during 28 days of cold storage. Addition of 5% sumac resulted the best attributes in prebiotic low fat yoghurt.

1. Introduction

Yoghurt is the most popular fermented milk products all over the world because of its high nutritive ,therapeutic values and sensory properties (Srivastava et al, 2015). The properties of yoghurt are due to the presence of lactic acid bacteria, which ferment lactose to lactic acid, so improve the nutritional values of yoghurt (Adolfsson et al, 2004).

This product is made with different fat percentages, but nowadays it is preferred to use low-fat and non-fatty dairy foods, especially those with high blood lipids and cardiovascular disease (Aghazadeh et al, 2010). But considering that the amount of total solids in the milk will greatly affect the physical properties and the texture of the manufactured yoghurt, reducing the fat content of the solids will decrease, so the produced yoghurt, of these compounds have a weak tissue. Studies have been carried out to improve rheological and the physico-chemical properties of low-fat and non-fat yoghurts using various additives. Therefore, to create such properties in low-fat products, appropriate additives such as resistant starches type 2 should be used for texture improvement which is a prebiotic ingredient (Heshmati et al, 2016). Prebiotics are nonviable food ingredients that exert a benefit on the health of the host, linked with modulation of the intestinal flora (Cruz et al, 2010).

In spite of the growth of the food industry and the observance of health standards in the production and processing of food, a significant percentage of industrialized and non-industrialized countries are still affected by diseases caused by harmful microbes. Yoghurt as a fermented milk product is a favorite dairy food, the microorganisms cannot be survival in yoghurt. Given that most microorganisms, in particular intestinal bacteria, cannot tolerate a pH of less than 4.5, but there is evidence that some of the available yoghurts in the market may be infected with coliforms, including *E.coli* (Soomro et al, 2002). The reason for the presence of the coliform in yoghurt implies that it is contaminated with animal or human stools and indicates secondary contamination or violation in the production, storage and distribution stages (Faramarzi et al, 2012; Rad et al, 2019). Also the activity of lactic acid bacteria and high acidity as well as a relatively long duration of storage, they provide the conditions for the growth of molds, especially with the presence of *Penicillium notatum* in yoghurt. *Penicillium* notatum leading to unpleasant changes in the taste, smell and appearance of yoghurt, will remove it from the consumption cycle and bring heavy economic losses to yoghurt producer (Green & Ibe, 1987). Therefore, yoghurt can infect with bacteria and molds, cause digestive infections and food poisoning, so finding new compounds and methods to minimize growths and activity of harmful bacteria and mold in yoghurt is necessary.

Oxidative stress is caused by an imbalance between the production of free radicals within the body and the mechanisms of defense of biochemical antioxidants. In living organisms, peroxidation of lipids in the wall of living cells is one of the main objectives of free radicals. In this situation, cell wall structure and its function are affected. Therefore, the high presence of free radicals, especially peroxides, plays a key role in the pathogenesis of a number of diseases, such as aging, cancer, cardiovascular disease, various degenerative diseases of the lungs, and also plays an important role in the pathogenesis and progression of diabetes (Ejtahed et al, 2012; Thanonkaew et al, 2008).

So, the use of antioxidants is important to increase the shelf-life and safety of foods (McCarthy et al, 2001; Sadighara & Barin, 2010). According to previous studies, some of the synthetic antioxidants used in the food industry as preservatives have side effects. Therefore, the addition of natural antioxidants in order to confidence the quality and safety of the food is essential (Sadighara & Barin, 2010).

Recently, the use of various natural flavors in yoghurt manufacturing has been searched increasingly. Spices are a new source of functional flavoring agents. There is now mounting scientific evidence of health benefits of plant, including antibacterial, antifungal, antioxidant, as well as anti-carcinogenic properties (Azhdarzadeh & Hojjati, 2016). Sumac (*Rhus coriaria L.*) is used in the Mediterranean region and Middle East as a spice. The fruits have been reported to possess antimicrobial and antioxidant properties (Kossah et al, 2009).

Literature review implies that sumac powder was nor used for improvement of antioxidant and antimicrobial activity in yoghurt so far. The aim of this research was to manufacture functionally prebiotic low fat yoghurt containing sumac powder with increased antioxidant and antimicrobial activity.

2. Materials and methods

2.1. Preparation of sumac powder

Aerial parts of *Rhus coriaria L.* were planted in full flowering state of East Azarbaijan in summer (Kalībar, Iran) and confirmed by the Herbarium of the Faculty of Pharmacy of Tabriz University scientifically. Separate parts of the plant were cleaned and dried at room temperature for one week. The dried plant was then turned into powder well using the mill. In order to prepare the sumac powder, the dried parts of the sumac were completely milled and separated using a mesh No 335 nm evaluated.

Then they were stored in dark glass containers and refrigerated until to evaluation.

Preparation of yoghurt containing sumac powder

In order to produce low-fat yoghurt containing sumac powder, fresh cow milk is used accordance with Fig (1).

Bacterial and fungal strains

The *E. coli* (ATCC® 25922™) and one *p. nutatum* (ATCC® 9179™) were obtained from the Persian type culture collection (PTCC). They were recognize using conventional morphological as well as biochemical tests. Stock cultures of the bacteria were kept in 20% glycerol PBS (phosphate buffered saline) at -70°C. Active cultures were generated by inoculating 100 μl of the thawed microbial stock suspensions into 5 ml of nutrient broth (Merck, Germany), followed by overnight incubation at 37°C. The mold was cultured overnight at 35°C in Sabouraud Dextrose Agar (SDA) (Merck, Germany). Each microorganism was suspended in sterile saline and diluted at ca. 10^7 colony-forming unit (CFU/ml).

Preparation of McFarland solution

In this study, McFarland solution was used to be 0.5 and 1 respectively concentrations to 1.5×10^8 cfu/g and 3×10^8cfu/g, to prepare the 0.5 McFarland solution, first, 9.95 mm of acid sulfur 1% with 0.05 mm barium chloride% 1 and mixed with vortex was mixed (Mahon et al, 2011), Also to prepare one McFarland solution First, 9.9 ml of sulfuric acid 1% with 1.1 barium chloride 1% Mix and mix well with vortex.

2.2. Analysis of pH and titratable acidity

The pH of homogenized yoghurt was determined using a digital pH meter. Titratable acidity (TA) was determined by titration with 0.1N NaOH. Yoghurt sample (3 mL) was transferred into an Erlenmeyer flask containing 27 mL of dH2O. Three to five drops of 0.1% phenolphthalein as pH indicator were added. The yoghurt mixture was then titrated with 0.1N NaOH with continuous stirring until a stable pink color was achieved. The amount of acid produced during fermentation was calculated as follows:

TA (% Lactic acid)=Dilution factor×V NaOH×0.1N×0.009×100% (Eq.1)

Where VNaOH was the volume of NaOH required to neutralize the acid. A dilution factor of 10 was used.

2.3. Antimicrobial activity

The pathogens used for antimicrobial activity were provided by the persian type culture collection (PTCC).The pathogens cultured in EMB (Eosin methylene blue) and YGC (Yeast Extract Glucose Chloramphenicol Agar). Then 100 µl of each sample was spread by pasteurized pipette to each plate. The plates were incubated at 37℃. The experiment was repeated three times.

2.4. Determination of antioxidant activity using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

The antioxidant activity of different percentage of sumac powder in prebiotic low fat yoghurt was evaluated by monitoring their ability to quench the stable free radical DPPH (2, 2 diphenyl-1-picrylhydrazyl) using the method, described by Choi et al, (2002) with brief modifications.

Briefly, various concentrations of the samples (12.5, 250, 500, 625, 1250, 2500 µg/mL) were mixed with 6 ml of methanolic solution of DPPH (0.2mM). Absorbance was determined at 517 nm after 30 min of reaction time at room temperature. The various concentrations of sumac powder in prebiotic low fat yoghurt providing 50% inhibition (IC50) was calculated from the graph-plotting inhibition effect. Inhibition of DPPH oxidation (%) was calculated as conforms (Apostolidis et al, 2007):

% Inhibition= (Acontrol – Aextract) /Acontrol×100

Where A was absorbance at 517 nm

2.5. Sensory analysis

Sensory analyses were carried out by 8 trained no smoking panels of aged 24 and 30 years. Samples were coded with three-digit numbers and randomly served at 7 to 10℃ in plastic cups (9 mL). Assessors completed a test assessment form to compare four sensory attributes (texcher, color, taste, and overall acceptability) using a five-point hedonic scale (1, extremely poor; 2, poor; 3, fair; 4, good; 5, excellent). Sensory evaluation was accomplished at 1, 7, 14, 21 and 28 day of refrigerated storage at 4C˚.

2.6. Statistical analysis

The results were analyzed mean ± SD and Parameters were compared among groups by one way analysis of variance (ANOVA) followed by Tukey post hoc test. All statistical analyses were performed using the SPSS version 25 and Minitab 18 .$P<0.05$ were considered statistically significant.

3.Results and discussions

3.1. Changes in pH, titratable acidity

The pH and TTA of yoghurt prepared with (0%,1%, 1.5%,5%) sumac powder are shown in Fig. 2A and B, respectively. Yoghurt with 5% sumac powder showed a slightly lower pH than yoghurt with 0% sumac powder (Fig. 2A). These results were in agreement with that obtained by mahmoudi et al. (2013), who reported that the TA increased and pH decreased gradually during cold storage period of yoghurt treated with different concentration of the T. polium EO(Mahmoudi et al, 2014).Finally, reported that the composition of concentration of sumac powder, fermentation temperature, storage duration, contamination, could influence the overall level of acidity and pH of stored yoghurt samples(Singh et al, 2011).The constantly higher TTA in yoghurt added with (0%,1%, 1.5%,5%) sumac powder (Fig. 2B) could be attributed to higher acid production due to the addition of sumac powder to yoghurt. Yoghurt added with 5% showed TTA contents in the range of 3.68. TTA is generally high depending on the decrease of pH. However, the addition of sumac powder showed a different tendency with some unknown reason.

3.2. Antimicrobial activity

3.2.1. E.coli

The antibacterial activity of *R.* coriaria was the most effective against bacteria and this could be linked to the chemical constitutes of the plant including the phytochemical components and the rate of these substances in screened extracts, where most of these groups have the antibacterial properties. Plants have formed the natural products make excellent lead for new drug development. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs (AL-Mizraqch et al, 2010).

Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo et al, 2004). *R. coriaria* contains phenols, tannins, and as in many research explained the action of hydrophobic property of phenolic compounds (Seyyednejad et al, 2008).

The results show that, inhibitory effect was affected by increasing of sumac powder concentration to 5 present. In many reports, growth or survival of *E. coli* in yoghurt (pH 4.0 to 4.5) even the 30 to 40 days has been stated (Massa et al, 1997; Simsek et al, 2007). In this research, *E. coli* survived during 1days of cold storage in control sample. According to our results, Sean et al; (1998) reported that *E. coli* has been suggested not to survive during fermentation process of yoghurt also presence of this organism in ready to eat yoghurt would showed the post processing contamination (Dineen et al, 1998). The population of *E.coli* inoculated in plain live yoghurt constantly decreased from an initial inoculums level of 9.6 $\times 10^7$ cfu/g (Control sample) to 0 cfu/g (Sample containing 5% sumac powder) after storage for 1 day at 4°C. Farrag (1992) reported that population of the pathogenic microorganisms in yoghurt samples decreased at various levels during the cold storage (Farag et al, 1992).

3.2.2. P.notatoum

Fungal spoilage of food and feed is a common and global phenomenon. It has been estimated that 5–10% of the world's food production is lost as a result of fungal spoilage (Alves et al, 2000). In addition to the negative financial consequences, fungal spoilage of food and feed also poses a serious health concern. Fungal growth on foodstuffs can result in the production of mycotoxins which are known to be toxic to humans and animals (Sweeney & Dobson, 1998). The ability of fungi to grow in food and feed depends on a variety of factors including water activity (a_w), pH and nutrient availability. In addition, storage conditions as well as the presence of other microbes dictate which types of fungi will grow in a given food system (Montville & Matthews, 1997).Moulds have the ability to grow in a wide variety of foods, with different genera showing affinity for particular food types.

The occurrence of genera as Penicillium is a serious and frequent problem in the dairy industry, because such species can grow satisfactorily at the yoghurt/air interface (Ndagijimana et al, 2008). Sources of microbial contamination during yoghurt production include contaminated starters, poorly cleaned filters, contaminated cups and lids, overall hygiene in the manufacturing process, contaminated flavoring materials and air quality in packaging areas (Vedamuthu, 1991). The significance of fungal contamination in foodstuffs does not only refer to the potential of fungi as spoilers but also to the ability of many of them to produce a great variety of mycotoxins to which humans are susceptible (Lopez et al, 1998).

The results of the antimicrobial effect of different concentration of sumac powder (0%, 1%, 1.5% and 5%) after 28 days showed that there was a significant difference between the samples ($P< 0.05$) (Table 1). Maximum antimicrobial effect of sumac powder against *P. notatum* related to sample yoghurt containing 5% sumac powder. Interaction between storage time and sumac concentration showed in Fig3.

3.3. The antioxidant activity during the storage of yoghurt

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. These free radicals are the major points in lipid peroxidation. The antioxidants may mediate their effect by directly reacting with Reactive oxygen species (ROS), quenching them and/or chelating the catalytic metal ions. Several synthetic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commercially available but are quite unsafe and their toxicity is a problem of concern (Rashid et al, 2012). Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive which are capable of absorb and neutralize free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Recently focus has been concentrated on identification of plants components with antioxidant ability that may be used for human diet (Rashid et al, 2012).

In order to evaluate the antioxidant effect of sumac in (0%, 1%, 1.5% and 5%) during 1, 7, 14, 21 and 28 days. According to the results, there is a significant difference between the different concentrations of sumac powder ($P<0.05$). Analysis of variance in Table (2).

The level of antioxidant activity in yoghurt samples containing different percentages of sumac powder was significantly higher than that of control samples ($P<0.05$). The sample containing 5% of the sumac powder had the higher antioxidant activity. At 1% concentration, it showed the lowest antioxidant activity in all times (Fig 4).

3.4. Sensory evaluation

The sensory properties of the yoghurt prepared with sumac powder at concentrations of 0%-5% were evaluated by 8 trained no smoking panels of aged 24 and 30 years, and the results are summarized in Table 3. The flavor score of yoghurt containing sumac powder ranged from 2.475to 3.675. The color value of yoghurt containing sumac powder ranged from 3.625to 4.275.The texture value of yoghurt containing sumac powder ranged from 3.750 to 3.625. The mean acceptance scores ranged from 2.750 to 3.650 (Table3).

The overall acceptability increased with increasing amounts of added sumac powder. High scores was received by yoghurt with 5% sumac powder (Fig 5). Therefore, the addition of sumac powder in yoghurt improved the functional properties such as antioxidant activity as well as the sensory characteristics of yoghurt. These results indicate that the addition of sumac powder at a concentration of 5% would be good for the production of acceptable color and functionally enriched yoghurt fortified with antioxidant, anti-obesity, and anti-inflammatory components. In conclusion, the addition of sumac powder to yoghurt resulted in increased titratable acidity and water activity (aw). In sensory evaluation, yoghurt containing 5% sumac powder received higher scores for, flavor, color, overall acceptability.

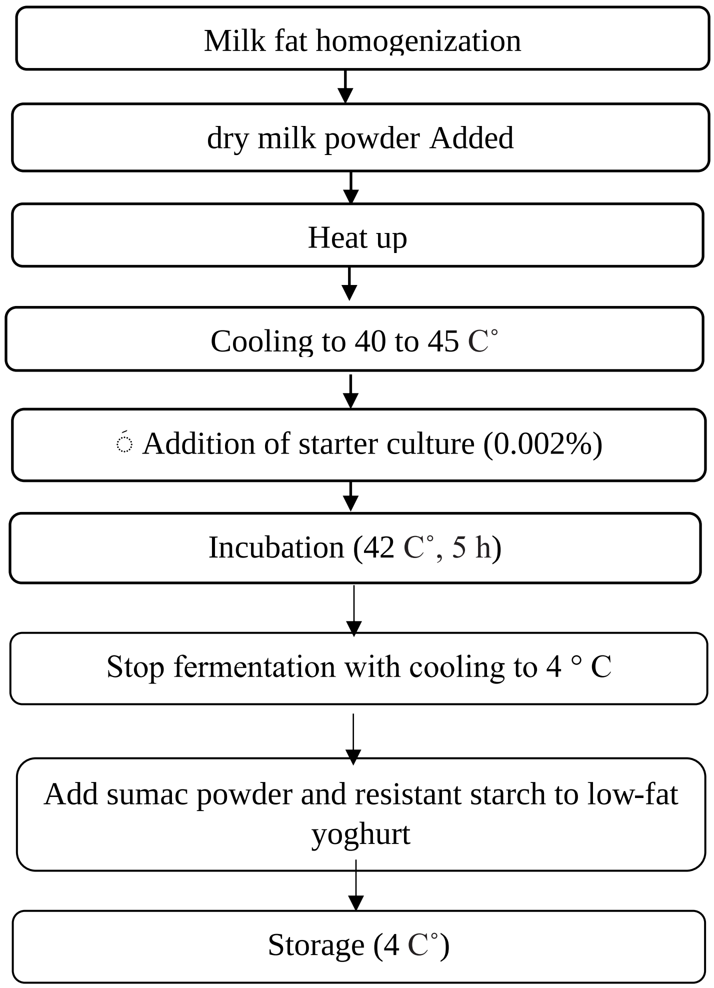

Figure 1. Procedure for the manufacture of low fat yoghurt with added sumac powder

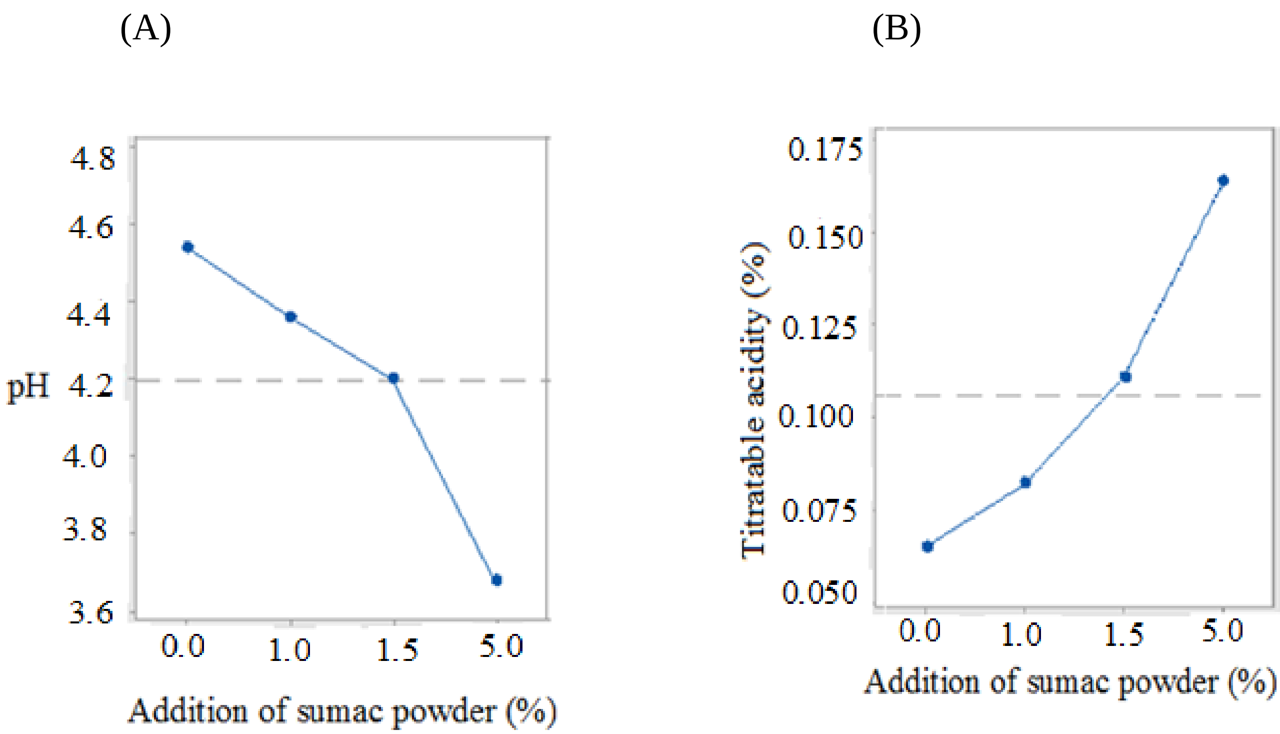

Figure 2. Changes in pH and titratable acidity (A) pH; (B) Titratable acidity

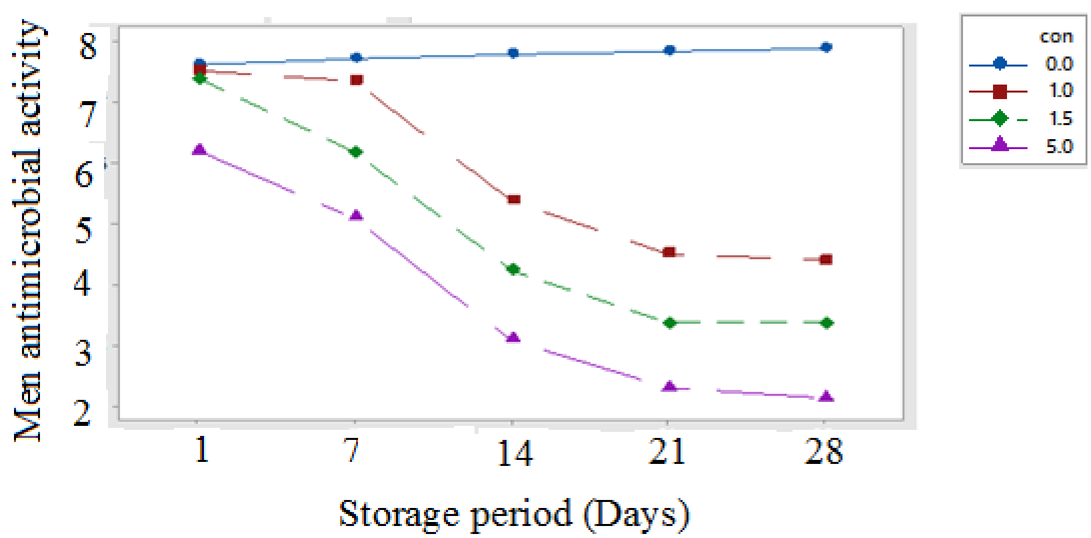

Figure 3. Change in antimicrobial activity versus storage time and sumac concentration

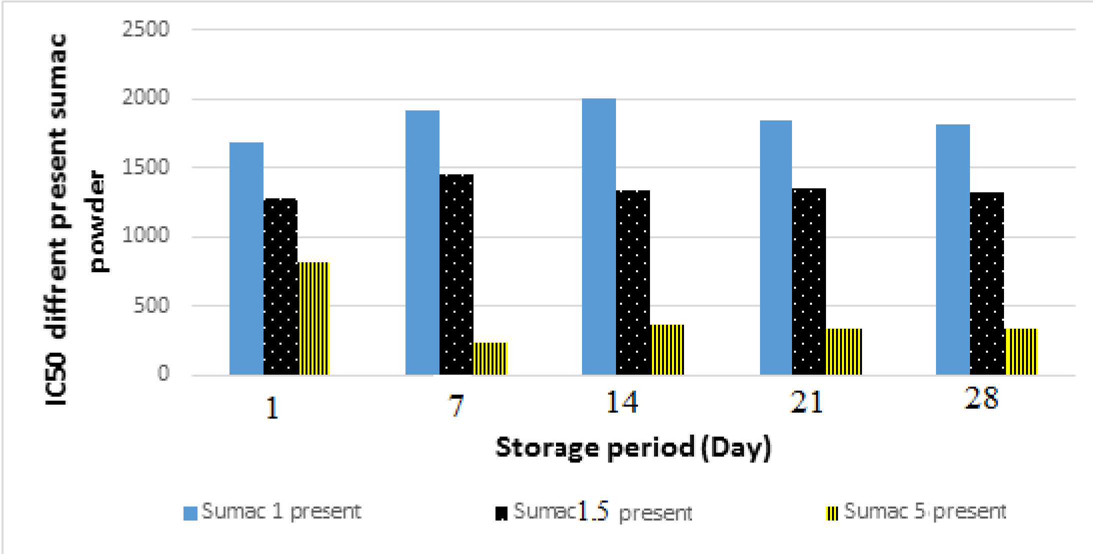

Figure 4. Antioxidant activity of different present sumac powder

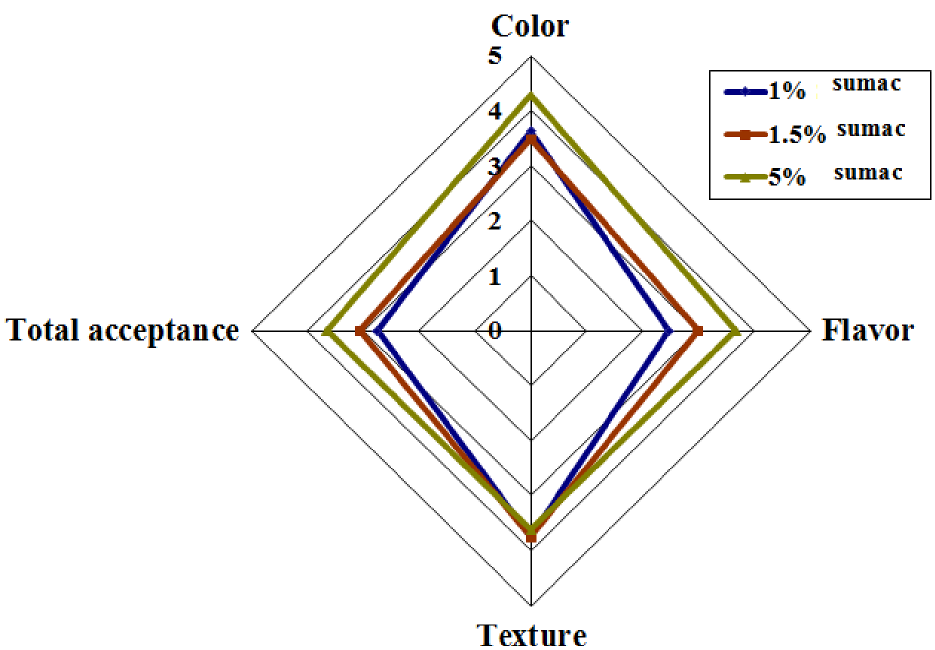

Figure 5. Spider chart representing sensory properties of yoghurt with sumac powder.

Table 1. Variation trend in the viable cell counts of *Penicillin notatum* during 28 days of refrigerated storage period, at 5-day intervals (log CFU/mL)

Day	0%	1%	1.5%	5%	P-Value
1	$4.3\times10^5\pm1\times10^{6\ cb}$	$3.4\times10^5\pm2\times10^{6\ cb}$	$2.5\times10^5\pm2.6\times10^{5\ d}$	$1.6\times10^4\pm2\times10^{5\ e}$	<0.001
7	$3.7\times10^5\pm2.7\times10^{6\ ab}$	$2.3\times10^5\pm2\times10^{6\ d}$	$1.5\times10^5\pm2.5\times10^{4\ e}$	$1.2\times10^4\pm1.5\times10^{3\ g}$	<0.001
14	$6.3\times10^5\pm 1.5\times10^{5\ ab}$	$2.6\times10^3\pm1\times10^{4\ f}$	$1.7\times10^3\pm1.5\times10^{2\ i}$	$1.2\times10^2\pm1.5\times10^{\ k}$	<0.001
21	$7.6\times10^5\pm1.5\times10^{5\ a}$	$2.6\times10^3\pm4.5\times10^{4\ h}$	$1.4\times10^3\pm2.5\times10^{2\ j}$	$2.3\times10\pm2\times10^{\ l}$	<0.001
28	$5.2\times10^6\pm4.5\times10^{5\ a}$	$2.7\times10^4\pm4.6\times10^{4\ ih}$	$2.4\times10\pm10^{2\ j}$	$1.3\times10\pm2.5\times10^{l}$	<0.001

The means shown with different letters are significantly different (P < 0.05).

Table 2. Variation trend antioxidant activity during 28 days of refrigerated storage period, at 5-day intervals

Day	0%	1%	1.5%	5%	P-Value
1	$7.633^{\ cb}\pm0.14$	$7.5309^{\ cb}\pm0.23$	$7.3963^{\ d}\pm0.29$	$6.2018^{\ e}\pm0.28$	0.000
7	$7.724^{\ ab}\pm0.11$	$7.3606^{\ d}\pm0.02$	$6.1817^{\ e}\pm0.25$	$5.889^{\ g}\pm0.01$	0.000
14	$7.8038^{\ ab}\pm0.24$	$5.3891^{\ f}\pm0.03$	$4.2460^{\ i}\pm0.27$	$3.0889^{\ k}\pm0.2$	0.000
21	$7.8491^{\ a}\pm0.53$	$4.5111^{\ h}\pm0.87$	$3.3792^{\ j}\pm0.28$	$2.30644^{\ l}\pm0.22$	0.000
28	$7.8994^{\ a}\pm0.01$	$4.394^{\ ih}\pm0.22$	$3.379^{\ j}\pm0.28$	$2.1305^{\ l}\pm0.20$	0.000

The means shown with different letters are significantly different (P < 0.05).

Table3. Sensory properties of yoghurt with sumac powder

Attributes		**Addition of sumac powder**		
	1%	1.5%	5%	**P-Value**
color	3.625±0.806	3.475±0.678	4.275±0.784	0.01
Flavor	2.475±1.280	3±1.339	3.675±1.071	0.04
Texture	3.750±1.103	3.750±0.980	3.625±0.952	0.06
Overall acceptability	2.750±0.980	3.050±0.845	3.650±1.001	0.03

Data are means±SD.

4. Conclusions

The results of the present study indicate that addition of different percentage sumac powder in prebiotic low fat yoghurt, not only minimized lipid and protein oxidation but also increased the microbial safety of the product. Sumac powder high levels of bioactive phenolic compounds that can help control foodborne pathogens and prevent lipid and protein oxidation. So, it is suggested that sumac powder, as a natural additive, could be used to increase the shelf life of industrial yoghurt, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic source. There was no limitation in this research. In this study functional prebiotic low fat yoghurt containing sumac powder was manufactured and antioxidant and antimicrobial activity were evaluated. However, more studies are needed to evaluate the clinical health effect of sumac containing yoghurt on metabolic and food born disease.

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