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# The effect of propolis extract on physicochemical, sensory and microbial characteristics of synbiotic yogurt inoculated with *Lactobacillus casei*

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#### **ARTICLE INFO** ABSTRACT Dairy products such as yogurt are considered as one of the most popular **Article History:** foods in the world. Beside the probiotics, functional ingredients such as Received:2024/1/17 prebiotics are also used in various products. One of the functional Accepted:2024/3/10 ingredients is propolis which has antioxidant, anti-inflammatory, antifungal, anti-viral and anti-tumor properties, and in addition to this properties it can be a prebiotic which can have beneficial effects on the **Keywords:** human digestive system. This study was conducted to investigate the effect of propolis extract on the physicochemical, sensory and microbial propolis, characteristics of synbiotic yogurt inoculated with Lactobacillus casei. yogurt, This study was conducted in five treatments (control (A), 1% (B), 2% (C), synbiotic, prebiotic, 3% (D), 4% (E)) and three replications. Physical and chemical properties such as antioxidant activity and total polyphenol, acidity and pH, syneresis, probiotic, texture (hardness, adhesiveness, springiness and chewiness), probiotics Lactobacillus casei survival as well as sensory properties (taste, odor, texture, color and general acceptance) of yogurt samples in days 1, 7, 14 and 21 were measured. DOI: 10.22034/FSCT.21.152.77. According to the results of the sensory evaluation team, the highest level \*Corresponding Author E-Mail: of sensorial favorability was related to the control treatment. The lowest rezakarimi@guilan.ac.ir, pH value in the control sample was 1% and showed a significant difference rzakarimi@gmail.com with the 2%, 3% and 4% treatments. The results of the survival of L. casei on different days showed that the effect of time and propolis percentage on the survival of probiotic was significant. The highest count of L. casei was observed in 4% treatment. It can be concluded that propolis can be used as a prebiotic in yogurt, which can improve the functional and textural

properties of yogurt.

# **1-Introduction**

Over the past few decades, with the increase in the level of awareness of the general public regarding the high consumption of fat and diseases such as vasoconstriction. cardiovascular diseases. high blood pressure, and cancer, the demand for the production of healthy food products such as low-fat dairy products and probiotics has increased. For this reason, nowadays, most consumers pay attention not only to the healthiness of food and its nutritional value, but also to its health benefits [1, 2]. Functional foods are the foods that contain one or more special compounds that have a practical effect on improving the health and well-being of the consumer. These useful components may be naturally increased in the food or may be intentionally added to it in the production process and cause health effects such as regulation of metabolic activities, physical fitness, improvement of the digestive system, heart and blood vessels and etc [1-3]. According to the reports presented, yogurt is one of the most popular dairy products and also one of the most accepted and widely consumed probiotic products in the world, which is widely consumed all over the world. Due to its high nutritional value and the presence of useful bacteria, it has received a lot of attention [4, 5]. According to the researchers' report, the most common means used to deliver live cells of Lactobacillus acidophilus and Bifidobacterium bifidum to humans is yogurt [6-8]. Today, yogurt production methods have changed due to advances in technologies, although there is not so much changes in lactic acid bacteria [9-11]. The yogurts produced in the industry are very diverse, including low-fat yogurts, probiotic yogurts, drinking yogurts, and frozen yogurts [9, 12, 13]. Probiotic products are one of the most common types of beneficial foods, and in recent years, increasing efforts have been made to use probiotic microorganisms in the production of various foods [14]. A new group of foods

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called synbiotics contain probiotics and prebiotics [15]. Probiotics are live microorganisms that settle in the intestine after consumption and have positive effects on human health [15]. Also, several investigations have proven the effective role of prebiotic compounds in the sensory characteristics of fermented milk products such as yogurt, and it has also been determined that prebiotic compounds improve the structure and texture of synbiotic yogurt [16]. One of the compounds that has not been investigated so far is propolis, which is one of the most useful bee products and is a colloidal and gummy substance in terms of its physical structure [7, 17, 18]. Also, propolis has antioxidant and antimicrobial properties and can be used instead of chemical preservatives [19, 20]. Most importantly, oligosaccharides prebiotic in honey increase the viability of probiotic bacteria. For this reason, honey components can be used as a food matrix for the formulation of synbiotics [21-23], because propolis can play a prebiotic role [24]. The chemical composition of propolis has a direct relationship with changes in weather conditions and environmental conditions. Propolis has a fat-like structure, hard and brittle, and becomes soft, flexible, gummy and very sticky when heated. It has an aromatic and pleasant smell and its color varies depending on the source and age of the resins (green, red, yellow and brown) [25]. Propolis is recognized as generally safe (GRAS), it is considered in the category of green products [26], and it is also a functional material [27]. Due to the prebiotic nature of propolis, simultaneous consumption of prebiotic and probiotic products has a synergistic effect and causes an increase in the population of beneficial bacteria and also a decrease in the population of harmful bacteria in the intestine [28]. According to the mentioned issues, it seems necessary to pay attention to the beneficial probiotic dairy products

due to their high beneficial properties and the importance of preserving probiotic bacteria in synbiotic products. The purpose of this research is to investigate the viability of probiotics in yogurt by using propolis and to evaluate the physical, microbial and sensory characteristics of the product.

# 2- Materials and Methods

# 2.1. Samples preparation

This study was conducted in the Faculty of Agricultural Sciences, Gilan University, Gilan province, and Pegah Guilan Pasteurized Milk Company, with five treatments and in triplicate for each treatment. The factors of interest were examined on days 1, 7, 14 and 21. Propolis was obtained from Ardebil city, (Aihal Bal sales center). Sterilized milk (1.5% fat) was obtained from Pegah dairy company. The microbial strains used included the combined culture of yogurt (YoFlex®-L904) containing Lactobacillus delbrueckii bulgaricus and Streptococcus subsp. thermophilus as well as the probiotic single strain culture of Lactobacillus paracasei (L. CASEI 431®) LC-01, in freeze-dried form and of DVS type were obtained from Christian Hansen (Chr. Hansen), Denmark.

Large pieces of propolis were divided into smaller pieces, and then the soaking method was used for extraction. The required amount of propolis was mixed with 100 ml of distilled water at a temperature of 65 °C, preserved for 2 hours and ocassionally shaken until the end of the period. After cooling, the extract was centrifuged for 55 minutes. Then it passed through a Whatman 42 filter (size 12.5 cm). Before extracting and after extracting, propolis was kept in the freezer until the time of testing. The extract was filtered and sterilized before adding to the sample [20]. To produce the samples, milk was heated to 40°C and then propolis was added with specific concentrations. This study consisted of five treatments (control without propolis (A), 1% propolis (B), 2% propolis (C), 3% propolis (D), 4% propolis (E)) and three replications for each treatment were performed. In order to better hydration of propolis, it was used before thermal processing of milk. Next, the milk was heated for 3 minutes at a temperature of 90 °C [29]. The amount of one-tenth (0.1) percent of the starter and probiotic strain in the amount of five-tenths (0.5)percent was equivalent to the initial inoculation of  $10^7$  CFU/g based on the specified instructions from the factory packaging and calculating the ratio of inoculation. The mentioned proportion of the propolis was added to milk (this mixing was done at 44-45 °C). Then, it was placed in an incubator with a temperature of 42 °C for 4.5 hours until the pH reached 4.6. Then, it was removed from the incubator and transferred to the cold room (4-5 °C).

# 2.2. Probiotic viability

The viability of probiotic bacteria in yogurt samples was assessed using MRS agar medium containing bile salts by pour plate method. To do so, appropriate dilutions of the sample were prepared in sterile physiological serum solution and the cultured plates were incubated at about 37°C. Colonies were counted after 72-h incubation using an anaerobic jar and their number was reported in log of colonyforming units per gram (log cfu/g) [30].

# **2.3.** Physicochemical properties

# pH and acidity

To measure the pH value, after preparing the sample in a bain-marie, the pH values of the control and propolis treatments were measured using a pH meter (National Standard No. 4404). The sample of each treatment was poured into the beaker separately with 3 repetitions and the electrode of the pH meter was completely placed inside the sample after adjustment. After the pH meter was fixed, the pH value was determined [31]. The acidity of yogurt was measured using Iranian National standard No. 2852. To do this, the sample was titrated with n/9 normal sodium hydroxide in the presence of phenolphthalein reagent. Finally, the acidity was expressed in Dornic degrees [32].

## **2.4.** Texture measurement

The texture was measured by Micro Texture measuring device (stable system. England), and the penetration force of the cylindrical probe was recorded up to a depth of 10 mm at a speed of 1 mm/s. The probe used had a diameter of 36 mm and a height of 3.5 mm, and the speed of the probe before and during the test was 1 mm/s and after the test was 10 mm/s [33]. The measured properties included hardness (the height of the main peak in the first curve in the forward stage in N), cohesiveness (the area under the second curve in the forward stage to the area under the first curve in the forward stage), springiness (the distance between the beginning of the second curve and the peak), the gumminess (the product of the hardness and cohesiveness values), chewiness (the product the of the and springiness gumminess values), adhesiveness (the area under the curve in the negative area) and adhesive force (the maximum force in negative area) [34].

## 2.5. Sensory evaluation

Sensory properties of the yogurt samples such as flavor (aroma and taste), appearance, mouthfeel, and total acceptance were evaluated using a 5-point hedonic test. Flavor, texture and color of yogurt samples were evaluated by 9 panelists on the specified days [34]. They were asked to consider color, aroma flavor

were asked to consider color, aroma, flavor, hardness, apparent transparency and total acceptance and give scores on a five-point scale with descriptive terms included in a pre-designed table with a maximum of 5 points (1= unusable, 2= usable, 3= good, 4= very good, 5= excellent).

#### 2.6. Statistical analysis

Data were analyzed using SPSS software. The normality of the data was checked using the Kolmogorov-Smirnov test and the homogeneity of the data was tested using the Levene's test. Then, the presence or absence of significant difference between the treatments was evaluated by the one-way ANOVA and Duncan multi-range test. The significance level was considered as p<0.05.

### **3-Results**

## **3.1.** Physicochemical properties

#### 3.1.1. pH and acidity value

The effect of different percentages of propolis on the pH and acidity of the samples is shown in Table 1. As shown in the Table 1, the pH increased with increasing propolis percentage over time. The pH of the samples ranged from 4.22 to 4.53. The effect of time on all samples was significant (p<0.05). The lowest pH was observed for control sample, followed by sample B (1%) on day 21. The highest pH was found for samples D (3%) and E (4%) on the first day. The current results showed that generally sample D (3%) had the highest acidity.

Table 1- Results of measurement of pH and acidity of different synbiotic yogurt treatments inoculated with *L. casei* 

Treatments	Day 1	Day 7	Day 14	Day 21
Treatments		pН		
А	$4.5 \pm 0.007$ °	$4.41\pm0.005^{b}$	$4.33\pm0.000^{\text{e}}$	$4.22\pm0.011^{\text{b}}$
В	$4.51\pm0.005^{bc}$	$4.41{\pm}0.005^{c}$	$4.35\pm0.005^{d}$	$4.23\pm0.011^{\text{b}}$
С	$4.52\pm0.005^{ab}$	$4.44\pm0.001^{bc}$	$4.37\pm0.000^{\rm c}$	$4.25\pm0.005^a$

D E	$\begin{array}{c} 4.53 \pm 0.001^a \\ 4.53 \pm 0.005^a \end{array}$	$\begin{array}{l} 4.45 \pm 0.015^{b} \\ 4.48 \pm 0.005^{a} \end{array}$	$\begin{array}{c} 4.40 \pm 0.001^{a} \\ 4.38 \pm 0.005^{b} \end{array}$	$\begin{array}{l} 4.26 \pm 0.011^a \\ 4.25 \pm 0.011^a \end{array}$
		Acidity		
А	$53.75 \pm 0.23^{\circ}$	$77.86\pm0.11^{\rm d}$	$81.20\pm0.34^{d}$	$82.60\pm0.34^{e}$
В	$76.06\pm0.30^{\rm c}$	$78.06\pm0.30^{cd}$	$81.53\pm0.23^{\text{d}}$	$83.53\pm0.46^d$
С	$76.86\pm0.50^b$	$78.66\pm0.23^{bc}$	$82.13\pm0.23^{\rm c}$	$84.46\pm0.50^{\rm c}$
D	$78.60\pm0.34^{\rm a}$	$79.20\pm0.69^{b}$	$83.93\pm0.11^{a}$	$86.86\pm0.23^a$
E	$79.29\pm0.34^{\mathrm{a}}$	$80.53\pm0.23^{\rm a}$	$83.46\pm0.23^{b}$	$85.66\pm0.57^{b}$

Different letters in each column represent significant differences (p<0.05).

Treatments (percentage of propolis) are A: Control, B: 1%, C: 2%, D: 3% and E: 4%.

In the study Gunes-Bayir et al. (2022) on yogurt samples containing propolis and cinnamon, the samples containing the highest concentration of cinnamon (2.5%) and propolis (0.03%) had the lowest titratable acidity and the highest pH value. This trend could be seen with lower concentration of cinnamon [35]. The findings of the present study depended on increasing titratable acidity by propolis, which also led to a decrease in the pH value. Our results are in agreement with the results obtained by Korkmaz et al. (2021) who examined homemade yogurt containing propolis powder and extract and showed that the pH value of different yogurts decreased significantly from day 1 to day 7. The change in pH value was insignificant. The presence of propolis affected the absorption of water and the mobility of hydrogen ions and caused a decrease in pH and an increase in acidity. Santos et al. (2019) studied the quality parameters of probiotic yogurt containing Brazilian red propolis as a replacement for potassium sorbate in regular yogurt and suggested that the addition of red propolis at a of 0.05% concentration instead of potassium sorbate did not change the acidity of the yogurt. In Korkmaz et al., (2021) research, in a similar study examined homemade yogurt prepared with propolis extract, the acidity levels of different yogurts increased from day 1 to day 7 [36]. Also, our findings are consistent with the results obtained by Gheibi et al. (2021) who investigated the pH and acidity of milk samples containing aqueous propolis extract and found a decrease in pH

and an increase in titratable acidity during storage at two temperatures compared to control, although the changes were insignificant [20]. In a recent study, the results showed that the pH level in all treatments decreased and acidity increased with increasing time. Esfandiari and Moslehishad (2019) stated that the cause of this phenomenon is mostly related to the production of lactic acid by lactic acid bacteria, which can produce four molecules of lactic acid from two molecules of lactose [37]. Also, the results of studies have shown that the process of decreasing pH and increasing acidity during storage is normal [37, 38]. The results related to pH values and acidity of yogurt showed that by adding propolis extract, pH values decreased and acidity values increased (p<0.05). It seems that the addition of propolis extract has increased the metabolic activity of bacteria in yogurt [37]. So that in the initial times of the incubation, with the increase of the substrate for the growth of microorganisms, the metabolic activities of the bacteria increase and cause a decrease in pH and an increase in acidity in treatments containing the extracts [38].

# **3.1.3.** Examining the properties of aqueous extract of propolis

Table 2- Results of measurement of totalpolyphenols and DPPH of aqueous propolis extract

Parameters	Content	Unit
Total	8.7	100 ml.mg
polyphenols		
DPPH radical	38.62	100 ml.mg
scavenging		

The results of the examination of total polyphenols and DPPH compounds are presented in Table 2. In the current study, the total polyphenol and DPPH compounds of aqua propolis were evaluated. The results showed that the amounts of total polyphenols and DPPH were 8.7 and 38.62, respectively. The most important pharmacologically active components in propolis are flavones, flavonols, and flavanones, which are called flavonoids. As a result, propolis has various therapeutic biological properties and activities. including antimicrobial. antifungal, antiviral, wound healing, boosting the immune system, and stopping the growth of cancer cells [39]. Propolis is commercially used as а dietary and therapeutic supplement. Also, the antioxidant, antimicrobial and antifungal activities of propolis make it a good product to be used in food technology [40]. The total phenolic compounds is an important parameter for quantitative evaluation and measuring the biological capacity of the product [41]. Tosic et al. (2017) investigated total phenolic compounds of the ethanolic extract of propolis collected from different parts of Argentina, which was prepared by maceration method for one week and reported that its amount ranged from 41.8 to 33.32 w/w % [42]. Esfandiarifard (2021) studied the antioxidant activity of ethanolic, methanolic and aqueous extracts of propolis and stated that the highest antioxidant activity (80.62) was observed for the methanolic extract followed by the aqueous extract (48.41). The ethanolic extract had the lowest antioxidant activity. They also stated that propolis extract is a good barrier to the formation of free radicals and all reactive oxygen species and

this is one of the reasons for the health benefits of propolis [19].

## 3.2. Evaluation of textural properties

# 3.2.1. Hardness, Adhesiveness, Springiness, Chewiness

The results of the investigation of the effect of aqueous propolis extract on the hardness adhesiveness, springiness and chewiness of synbiotic yogurt texture inoculated with L. casei are presented in Table 3. The highest hardness on days 1 and 7 was found for sample A, and on days 14 and 21 for sample B. Sample E (4%) had the lowest hardness. The highest adhesiveness on days 1, 7, 14 and 21 was found for sample B (1%). The lowest adhesiveness was observed for sample E (4%). The results of the present study showed that during the days of the experiment, the amount of adhesiveness in different treatments initially increased and then decreased. The highest springiness on days 1, 7, 14 and 21 was found for sample D (3%). Also, the lowest springiness on day 1 was found for sample B (1%), on day 7 for sample A, and on day 14 and 21 for sample E (4%) (p < 0.05). The highest value of the chewiness parameter is related to sample A (0%) and the lowest chewiness is related to sample E (4%) (p<0.05). The results of this study show that during the test days, with the increase of storage days in different treatments, the amount of ability) chewing (chewing initially increased and then decreased (p < 0.05). The results of this study showed that during the test period, with the increase in the storage period, the hardness and stickiness of the samples decreased in different treatments.

Table 3- Results of measurement of hardness, adhesiveness, springiness and chewiness of different synbiotic yogurt treatments inoculated with *L. casei* 

Treatments	Day 1	Day 7	Day 14	Day 21
Treatments		Hardness		
A	$19.99\pm0.02^{\mathrm{a}}$	$21.05\pm0.02^{\rm a}$	$21.02\pm0.01^{\circ}$	$21.10\pm0.15^{\text{b}}$
В	$19.73\pm0.15^{\mathrm{b}}$	$20.29\pm0.02^{b}$	$22.61\pm0.02^a$	$22.04\pm0.02^{a}$
С	$18.38\pm0.25^{\rm c}$	$19.94\pm0.01^{c}$	$21.15\pm0.04^{\text{b}}$	$20.07\pm0.09^{\rm c}$
D	$16.98\pm0.10^{\rm d}$	$19.36\pm0.04^{d}$	$20.55\pm0.05^{\rm d}$	$19.12\pm0.55^{\text{d}}$

E	$16.10\pm0.02^{\text{e}}$	$18.79\pm0.15^{\text{e}}$	$19.89\pm0.03^{\text{e}}$	$18.28\pm0.03^{\text{e}}$
Adhesiveness				
А	$2.13\pm0.02^{\rm d}$	$3.97\pm0.03^{b}$	$4.11\pm0.01^{b}$	$4.01 \pm 0.02^{b}$
В	$3.95\pm0.03^{\rm a}$	$4.79\pm0.01^{\rm a}$	$4.56\pm0.03^{\rm a}$	$4.50\pm0.01^{\rm a}$
С	$2.63\pm0.02^{\rm b}$	$3.69\pm0.02^{\text{d}}$	$3.88\pm0.02^{\rm c}$	$3.83\pm0.04^{\rm c}$
D	$2.58\pm0.03^{b}$	$3.49\pm0.01^{\text{d}}$	$3.61\pm0.05^{\text{d}}$	$3.04\pm0.04^{d}$
Е	$2.48\pm0.02^{\rm c}$	$2.20\pm0.01^{\text{e}}$	$3.15\pm0.04^{e}$	$2.02 \pm 0.05^{e}$
		Springiness		
А	$14.23\pm0.02^{ab}$	$14.03\pm0.02^{\text{d}}$	$14.12\pm0.02^{a}$	$14.24\pm0.01^{a}$
В	$14.20\pm0.17^{b}$	$14.75\pm0.01^{\rm a}$	$13.79\pm0.04^{\rm c}$	$13.44\pm0.03^{b}$
С	$14.42\pm0.08^{\rm a}$	$14.34\pm0.01^{\text{b}}$	$13.96\pm0.07^{b}$	$13.05 \pm 0.02^{\circ}$
D	$14.36\pm0.01^{ab}$	14.30 ±0.02°	$14.35\pm0.05^{\text{d}}$	$14.39\pm0.04^{a}$
Е	$14.24\pm0.12^{ab}$	$14.27\pm0.02^{\rm c}$	$13.15\pm0.05^{e}$	$12.99\pm0.04^{\circ}$
		Chewiness		
А	$9.88\pm0.02^{\rm a}$	$10.39\pm0.01^{\circ}$	$11.39\pm0.03^{a}$	$11.38\pm0.01^{a}$
В	$9.82\pm0.03^{a}$	$11.50\pm0.02^{\text{b}}$	$10.89\pm0.04^{b}$	$10.74\pm0.05^{b}$
С	$9.42\pm0.37^{ab}$	$10.40\pm0.01^{\circ}$	$10.46\pm0.06^{c}$	$10.62\pm0.03^{\rm c}$
D	$9.01\pm0.45^{b}$	$11.89\pm0.01^{\rm a}$	$9.79\pm0.03^{\rm d}$	$9.04\pm0.04^{\rm d}$
Е	$803 \pm 0.42^{\circ}$	$9.80\pm0.05^{\rm d}$	$9.30\pm0.04^{e}$	$8.30\pm0.02^{\rm e}$

Different letters in each column represent significant differences (p<0.05).

Treatments (percentage of propolis) are A: Control, B: 1%, C: 2%, D: 3% and E: 4%.

The feeling caused by touching foods is often considered as one of their important characteristics. The important properties include firmness, softness, juiciness, chewiness, fibrousness, grittiness, oiliness and tenderness.

A concept which has not been defined well texture. Texture is related is to adhesiveness, elasticity and other physical properties of food. Texture characteristics of food are divided into geometrical, mechanical (particle size, shape and position) and properties related to moisture and fat content. The primary indicators related to mechanical properties are hardness, cohesiveness, viscosity, elasticity and adhesiveness. Secondary indicators include tenderness, chewiness and gumminess. Different types of texture have been identified, which are graded based on compression and toughness. The hardness degree can be found by cutting and separating different parts of food without any deformation of each separated section. Standard measurement scales for hardness, tenderness, chewiness, adhesiveness and viscosity have been developed. These also used in sensorial scales are measurement of texture [43]. Hardness is defined as the force required to disrupt the yogurt texture and it is the most important factor for determination of yogurt firmness [44]. The hardness of the samples ranged from 16 to 23. It initially increased and then decreased, indicating the proper synergistic property of propolis [45]. Sandoval et al. (2004) stated that carbohydrate molecules are able to firmly bond with water molecules and trap them due to increasing water absorption power, thus increasing the viscosity of the aqueous phase, resulting in increasing of resistance to the applied force. Also, the results revealed that an increase in the rate of homogenization and fat increased the hardness of the samples [46]. is important Yogurt viscosity an characteristic that affects its quality. Stirred yogurt is a homogenous and viscous substance, and this viscosity can be affected by influencing factors such as incubation temperature, fat and casein content, heat treatment of milk, acidity of milk, type of starter and additives [38]. In relation to the adhesiveness of yogurt samples, it can be said that the adhesion force is the force necessary to overcome the surface attraction force between the particles, the more the structure of the yogurt is preserved from hardness, the adhesiveness will be greater, which is an accordance with the result of the hardness [47]. Propolis has high adhesive strength, creating a good adhesiveness when mixed with yogurt because of its strong reaction with fats and proteins. In this study, adhesiveness at first increased and then decreased.

# **3.3.** Syneresis or separation of serum measurement

The results of the investigation of the effect of aqueous propolis extract on the syneresis of synbiotic yogurt inoculated with *L. casei* are shown in Table 4. The results indicated Table 4. Pagulta of magnetic of syneresis of diff that the effect of different percentages of propolis and also the day of storage on the yogurt syneresis was significant (p<0.05). The highest syneresis rate was found for sample A (0%) on days 1, 7, 14 and 21 (p<0.05). The lowest syneresis rate was found for sample E (4%) on days 1, 7, 14 and 21 (p<0.05).

Table 4- Results of measurement of syneresis of different synbiotic yogurt treatments inoculated with L. casei
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Turaturanta	Day 1	Day 7	Day 14	Day 21
Treatments		Syneresis		
А	$19.99 \pm 0.11^{a}$	$21.55\pm0.19^{a}$	$22.96\pm0.06^a$	$24.03\pm0.06^{\rm a}$
В	$19.99 \pm 0.11^{a}$	$21.22\pm0.19^{b}$	$22.22\pm0.38^{b}$	$23.44\pm0.38^{\text{b}}$
С	$19.55\pm0.38^{\mathrm{b}}$	$20.22\pm0.19^{\rm c}$	$22.92\pm0.06^{\text{b}}$	$22.59 \pm 0.42^{\circ}$
D	$18.99 \pm 0.11^{\circ}$	$19.25 \pm 0.12^{d}$	$20.11\pm0.19^{\rm d}$	$20.92\pm0.06^{e}$
E	$18.77 \pm 0.19^{\circ}$	$19.22\pm0.19^{d}$	$20.73 \pm 0.12^{\circ}$	$21.62\pm0.27^{\text{d}}$

Different letters in each column represent significant differences (p<0.05).

Treatments (percentage of propolis) are A: Control, B: 1%, C: 2%, D: 3% and E: 4%.

Syneresis in yogurt is an undesirable property and, along with water holding capacity, is considered as one of the quality indicators of yogurt during storage. The results showed that until 14th day of the storage, the syneresis of the samples significantly decreased because of increased solid matter and water absorption and holding property. As the concentration of propolis increases, the syneresis rate significantly decreases due to the involvement of water molecules in the structure of the propolis and the increase in the viscosity of the product [48]. The findings of the present study are consistent with the results obtained by other researchers who used gum in the formulation of different dairy products such as yogurt, yogurt drink and whipped cream and reported that the addition of beewax led to a decrease in syneresis and its higher concentration caused a significant decrease in the serum separation [49-51]. Our results are in agreement with the results obtained by Korkmaz et al. (2021) who found a decrease in syneresis in homemade yogurt prepared with propolis extract during storag [36].

Temiz et al. (2014) stated that most hydrocolloids cause an increase in viscosity due to their water absorption properties [52]. Hydrocolloids have high water absorption properties and this water absorption is positively correlated with hydrocolloid concentration [53]. The results of the present study are consistent with the results of Won Young et al., (2020). These researchers stated that the addition of olive leaf extract to yogurt reduces the amount of syneresis and increases the viscosity of the yogurt. Also, the amount of syneresis increases and viscosity decreases by increasing the storage time [38]. Syneresis in yogurt is influenced by the physical conditions of yogurt during the storage period. In this regard, adding extract leads to an increase in dry matter and as a result, the texture becomes firmer and the syneresis decreases [54].

#### 3.4. Sensory evaluation

#### 3.4.1. Aroma, taste, color and texture

As shown in Table 5, the effect of treatment and time and the interactive effects of treatment and time on total acceptance of the yogurt samples were significant (p<0.05). According to Table 5, it was observed that the panelists found significant differences in the aroma, taste, color and texture of the vogurt treatments among the control and propolis-containing yogurt treatments (p < 0.05). Based on the results of the sensory evaluation, the level of aroma, taste, color and texture of the vogurt treatments were determined and the lowest level of the desirability of yogurt belonged to sample E (4%) (p<0.05). During the storage time, the sensory desirability of aroma, taste, color and texture of the yogurt treatments significantly decreased, and at the end of the 21st day of storage, the lowest desirability was observed compared

to the first day of production (p<0.05). Also, according to the report of the evaluation team, the lowest amount of desirability of the texture of yogurt belonged to the control treatment (p < 0.05). During the storage time, the sensory desirability of texture of yogurt treatments decreased significantly, and at the end of the 21st day of storage, the lowest amount of sensory desirability of texture and firmness was observed compared to the first day of production (p < 0.05).

Table 5- Results of measurement of Aroma, Taste

and Color of different synbiotic yogurt treatments inoculated with L. casei					
Traatmonta	Day 1	Day 7	Day 14	Day 21	
meatments		Aroma			
А	$4.55\pm0.53^{\rm a}$	$4.01\pm0.77^{\rm a}$	$3.33\pm0.55^{\rm a}$	$3.11\pm0.33^{\rm a}$	
В	$4\pm0.70^{ab}$	$3.66\pm0.50^{ab}$	$3.01\pm0.01^{ab}$	$2.77\pm0.44^{ab}$	
С	$4\pm0.72^{ab}$	$3.66\pm0.51^{ab}$	$3.04\pm0.02^{ab}$	$2.55\pm0.52^{ab}$	
D	$4.22\pm0.66^{ab}$	$3.44\pm0.52^{ab}$	$2.66\pm0.50^{\rm b}$	$2.55\pm0.52^{\text{b}}$	
Е	$3.77\pm0.66^{\rm b}$	$3.33\pm0.50^{b}$	$2.55\pm0.52^{\rm c}$	$2.33\pm0.50^{\text{b}}$	
		Taste			
А	$4.44\pm0.52^{\rm a}$	$4\pm0.71^{\mathrm{a}}$	$3.33\pm0.5^{\rm a}$	$3.11\pm0.3^{\mathrm{a}}$	
В	$4.22\pm0.44^{ab}$	$3.44\pm0.52^{\rm b}$	$2.88\pm0.33^{ab}$	$2.44\pm0.5^{\text{b}}$	
С	$4.11\pm0.33^{ab}$	$3.33\pm0.50^{b}$	$2.66\pm0.5^{\rm b}$	$2.22\pm0.41^{\text{b}}$	
D	$3.77\pm0.44^{\text{b}}$	$3.33\pm0.52^{\rm b}$	$2.55\pm0.52^{\rm b}$	$2.22\pm0.40^{\text{b}}$	
Е	$3.77\pm0.44^{\text{b}}$	$3.33\pm0.50^{\rm b}$	$2.55\pm0.52^{\rm b}$	$2.22\pm0.40^{\text{b}}$	
Color					
А	$4.66\pm0.50^{\rm a}$	$4.22\pm0.66^{\rm a}$	$4\pm.001^{a}$	$3.77\pm0.44^{\rm a}$	
В	$4.66\pm0.50^{\rm a}$	$4.11\pm0.60^{\mathrm{a}}$	$3.77\pm0.44^{\rm a}$	$3.66\pm0.50^{\rm a}$	
С	$4.55\pm0.52^{\rm a}$	$4.11\pm0.60^{\rm a}$	$3.77\pm0.44^{\rm a}$	$3.33\pm0.50^{\rm a}$	
D	$4.55\pm0.52^{\rm a}$	$4.11\pm0.33^{\rm a}$	$3.66\pm0.70^{\rm a}$	$3.33\pm0.50^{\rm a}$	
Е	$4.55\pm0.52^{\rm a}$	$4\pm.50^{\mathrm{a}}$	$3.66\pm0.50^{\rm a}$	$3.33\pm0.50^{\rm a}$	
Texture					
А	$4.22\pm0.44^{\rm a}$	$4\pm0.001^{\text{b}}$	$3.77\pm0.66^{b}$	$3\pm0.50^{b}$	
В	$4.22\pm0.44^{\rm a}$	$4\pm0.000^{\rm b}$	$4\pm0.000^{ab}$	$3.11\pm0.33^{ab}$	
С	$4.33\pm0.50^{\rm a}$	$4.22\pm0.44^{ab}$	$4.22\pm0.44^{ab}$	$3.11\pm0.60^{ab}$	
D	$4.33\pm0.50~^{\rm a}$	$4.22\pm0.44^{ab}$	$4.22\pm0.44^{ab}$	$3.55\pm0.52^{\rm a}$	
E	$4.44\pm0.52^{\rm a}$	$4.33\pm0.50^{\rm a}$	$4.44\pm0.52^{\rm a}$	$3.55\pm0.52^{\rm a}$	

Different letters in each column represent significant differences (p<0.05).

Treatments (percentage of propolis) are A: Control, B: 1%, C: 2%, D: 3% and E: 4%.

#### **3.4.5.** Total acceptance

According to Figure 1, it was observed that the effect of treatment and time and the interactive effects of treatment and time on total acceptance of the yogurt treatments were significant (p<0.05). The panelists found no significant differences in the texture desirability of the yogurt containing propolis and control sample on days 1 and 7 (p>0.05). However, based on the results of sensory evaluation of evaluators in other treatments, the total acceptance of other treatments decreased significantly, and the least desirability was observed for sample E (4%) (p<0.05). During the storage, the total acceptance of the propoils-containing vogurt treatments decreased significantly, and at the end of day 21, the lowest total acceptance was observed compared to the first day of production (p<0.05).



Fig 1 -Results of measurement of total acceptance of different synbiotic yogurt treatments inoculated with L. casei Different letters in each column represent significant differences (p<0.05).

Treatments (percentage of propolis) are A: Control, B: 1%, C: 2%, D: 3% and E: 4%.

#### **3.5.** Viability of probiotics

The results of the viability of *L. casei* in different days with different concentrations of propolis are presented in Figure 2. Based on the results, the effect of time and propolis on the viability of probiotic

bacteria was significant (p<0.05). The count of *L. casei* was higher in shorter times of storage, and it decreased as the time days increased. Also, by increasing the concentration of propolis, the survival of *L. casei* increased and treatment E (4%) showed the highest survival of *L. casei* after 21 days of investigation.





Different letters in each column represent significant differences (p<0.05).

Treatments (percentage of propolis) are A: Control, B: 1%, C: 2%, D: 3% and E: 4%.

resulted in an increase in the count of

probiotic bacteria which decreased over

time. Our results are agreement with the

results obtained by other researchers [35,

36, 71-74], in which they studied viability

of probiotic bacteria in homemade yogurt

containing propolis extract and observed

the highest survival rate of Lactobacillus

species within 7 days in samples containing

propolis. Also, Prudêncio et al. (2014) in

their survey, stated that the limitation of

One of the most important characteristics of probiotic microorganisms is their viability and survival in various products in order to be present in the product at the time of consumption. According to the report of most researchers, at least 10<sup>6</sup> CFU/g of product is necessary to exert the health effects of probiotics. The probiotic species, temperature, stimulators growth and inhibitors, pH value, incubation time, inoculation level. metabolite concentrations and nutrient availability are the most important factors affecting the probiotic viability and survival in various products [55, 56]. The results of this study showed that increasing storage time was conversely related to bacterial survival. In the other words, the survival of probiotics decreased over time due to the effect of produced acid on the bacteria over time, which decreases the bacterial resistance and causes their death [57]. Another reason could be high secretion of alkaline substances to adjust the environment, which eventually leads to an increase in internal pH, thereby causing cell death [58-60]. Also, the researchers stated that with increasing storage time at low temperatures and decreasing the pH in probiotic vogurts, the viability of probiotics decreases [61-65]. It has been reported that glycosides improve the growth of probiotic bacteria. Also, the increase in the viability could be attributed to the high polyphenol content of propolis, as a natural compound of plant extracts, which directly affects the count of probiotic bacteria [66-69]. Faraji et al. (2012) investigated the optimization of the low-fat probiotic yogurt production process using a composite design. To optimize the formulation of low-fat probiotic yogurt, the effect of different concentrations of inulin, chitosan and xanthan at three levels (1, 2 and 3%) on the viability of L. acidophilus during 15 days of storage was studied. The results revealed that higher concentration of inulin and chitosan increased the growth and survival of *L. acidophilus* [70]. Similarly, the results showed that higher concentrations of honey and propolis

access to nutrients in the environment is one of the important factors in decreasing the count of probiotic bacteria [75]. Shahdadi et al. (2014) in their study reported that the population of probiotic bacteria decreases over time [76]. Faraji et al (2020) found that the count of L. acidophilus in all refrigerated samples decreased from day 1 to day 21. The most important factors affecting the reduction of the number of probiotic bacteria during refrigerated storage include the changes in acidity, the production of metabolites such as organic acids and hydrogen peroxide by traditional yogurt bacteria, as well as the access to nutrients in limited environment. Given the fact that in all treatments, the count of probiotic per gram of the product  $(10^6 \text{ CFU/g})$  exceeded the minimum recommended number in a probiotic product, all the samples could be claimed to have the healthful properties of a probiotic product. **4-** Conclusion In this study, the effect of propolis extract on physicochemical, sensory and microbial characteristics of synbiotic inoculated with L. casei was evaluated. The results showed that the addition of propolis extract has a positive effect on the physical

the

vogurt

and chemical properties of the product. In the samples containing propolis extract, pH and syneresis were lower, and on the other hand, the acidity level and also the viability of probiotic were higher than the control treatment. Although according to the results of the sensory evaluation team, the highest level of favorability was related to the control treatment, but due to the effects of propolis extract on textural (sensory) properties, as well as the viability of probiotic, it can be concluded that yogurt production with the addition of 4% propolis can be a functional food that consumer can benefit from its nutritional attributes.

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بررسی تاثیر عصاره بره موم بر ویژگی های فیزیکوشیمیایی، حسی و میکروبی ماست سین بیوتیک تلقیح شده با لا*کتوباسیلوس کازئی* 

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چکیدہ	اطلاعات مقاله
فرآوردههای لبنی پروبیوتیک همچون ماست یکی از مواد غذایی پرطرفدار در دنیا محسوب	
میشوند. همچنین ترکیبات فراسودمند از جمله پری بیوتیکها در کنار پروبیوتیکها در	تاریخ های مفاله :
محصولات مختلف مورد استفاده قرار می گیرند. یکی از ترکیبات فراسودمند بره موم بوده که	تاریخ دریافت: ۱٤۰۲/۱۰/۲۷
دارای خواص آنتی اکسیدانی، ضد التهابی، ضد قارچی، ضد ویروسی و ضد توموری بوده و	تاریخ پذیرش: ۱٤۰۲/۱۲/۲۰
علاوه بر این خواص، خود به عنوان یک پری بیوتیک مطرح میباشد. این مطالعه با هدف	
بررسی اثر عصاره بره موم بر ویژگیهای فیزیکوشیمیایی، حسی و میکروبی ماست سین	كلمات كليدى:
بيوتيک تلقيح شده با <i>لاکتوباسيلوس کازئی</i> انجام شد. اين مطالعه در پنج تيمار (شاهد بدون	بره موم،
بره موم (A)، ۱٪ بره موم (B) ، ۲٪ بره موم (C) ، ۳٪ بره موم (D)، ٤ ٪ بره موم (E)) و سه	
تکرار برای هر تیمار انجام شد. خواص فیزیکی و شیمیایی مانند فعالیت انتی اکسیدانی و	ماست،
پلی فنول کل، اسیدیته و pH، اب اندازی، بافت (سختی، چسبندگی، فنری و قابلیت جویدن)،	سين بيوتيک،
زنده مانی پروبیوتیکها و همچنین خواص حسی (مزه، بو، بافت، رنگ و پدیرش کلی)	پرى بيوتيك،
مونههای ماست در روزهای ۱، ۷، ۱۶ و ۱۱ اندازه خیری شدند. بالا نرین میزان مطلوبیت	5.1 <i>5</i>   1 <b>"</b> (V C "
حسی مربوط به بیمار شاهد بود. کمترین میزان PH در نمونه شاهد و ۱۰/ بود و با بیمارهای ۲/۰ ۳/۰ ۵/۱۰ ایداد: بابا ۱۰ این باد نام ۱۰۰ از ۱۰ میزان ۲۰	پروبيونيک، لا کنوباسيلوس کارنې
۱۰٫۱۱٫۱٫۱٫۱٫۱٫۱٫۱٫۱٫۱٫۱٫۲٫۲٫۲٫۲٫۲٫۲٫۲٫۲٫	DOI:10.22034/FSCT.21.152.77.
داد که از رمان و درصد بره موم بر بقای با دس پروبیو یک معنی دار بود. بیسترین تعداد <i>ن.</i> کانتر در ترمان کان در اداد شار به طرب کار در تران نتر ده گرفت که از بر در به مندان	* مسئول مكاتبات:
کارنی در نیمار ۲۰ مسامیه سد. به طور کنی می توان در امید خواج علی می دوان	<u>rzakarimi@gmail.com</u> ,
یک پری بیولیک کمی کورن در ماسک است مستور که می کورن در بهبود مورش مستوری. مرافته مایدت مفید و موثر باشد.	<u>rezakarimi@guilan.ac.ir</u>
و باخلی مانتگ ملینا و الولو باشد.	