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Effective against obesity, production of probiotic yogurt containing green tea and *Lactobacillus gasseri*

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Abstract

The aim of this study was to produce a probiotic yoghurt (from cow's milk) effective against obesity, in which 2%, 3% and 4% green tea (*Camellia sinensis*) was added and *Streptococcus thermophilus* and *Lactobacillus gasseri* cultures were used in a 1/1 ratio. On all storage days (1, 7, 14, 21), yoghurt samples showed statistically significant differences in terms of pH (4.34-4.76), titratable acidity (1%-1.24%), dry matter (17.84%-20.70%), serum separation (20.80%-28%) and water holding capacity (49.8%-56.9%). The difference between storage days is also significant. Phenolic substance content increased as the amount of green tea increased. During storage, phenolic content varied between 33.18-45.29 gallic acid (GLA) mg/kg in control yogurt, and 203.14-565.43 GLA mg/kg in green tea-containing yogurts. The decrease in the L* value and the increase in the a* and b* values were caused by the addition of green tea and the increase in the amount of green tea added. Increasing the amount of added green tea resulted in an increase in the flow behavior index. The sample containing 2% green tea was the most liked among the green tea samples. In that sample, Lb. *gasseri* number was above the lower limit (1.53×10^6 - 9.47×10^6 cfu/g) for a food to be considered probiotic during storage. It was concluded that in order for a product to maintain its probiotic properties during storage, the rate of inoculated starter culture should be high. The amount of *Lb. gasseri* added to the milk from which yoghurt will be made should be increased.

Keywords: probiotic yoğurt, green tea, *Lactobacillus gasseri*, obesity

Introduction

The fact that the fat tissue in the person's body is more than necessary indicates that the person is obese. Obesity affects the quality of life of individuals negatively and can shorten the life span. The prevalence of obesity is increasing day by day in many places in the world [1]. Obesity negatively affects all aspects of human life and is associated with many diseases [2]. Excessive calories consumed than calories expended is the main cause of obesity and overweight [3]. Intestinal microbiota affects nutrient absorption and energy distribution. Therefore, it plays a role in the pathogenesis of obesity and metabolic syndromes [4]. Consuming foods high in fat and energy changes the number and composition of intestinal bacteria [5]. As a result, an unhealthy flora called dysbiosis occurs [6]. Caloric intake, intestinal permeability, increase in the levels of pro-inflammatory cytokines and endotoxemia occur in the intestine whose microbial balance is disturbed [7]. In some studies, it has been observed that the use of prebiotics and probiotics changes the intestinal flora and provides weight loss [5].

Consumption of *Lb. gasseri*, a probiotic bacterium, is effective against obesity as it reduces body weight, BMI, waist-hip circumferences and body mass. The anti-obesity effect of *Lb. gasseri* is associated with its ability to settle in the human gut and improve the intestinal environment. Such properties of *Lb. gasseri*, together with its ability to reduce lipid absorption, result in a reduction in abdominal fat and other body dimensions [8]. Because of these beneficial effects on health, *Lb. gasseri* is widely used in various fermented food products and dietary supplements [9].

In a study, the weights of rats fed with *Lb. gasseri* BNR (10^9-10^{10} cfu) + sucrose, sucrose and normal diet for 10 weeks were examined. As a result, it was determined that the white adipose tissue and body weights of rats fed *Lb. gasseri* BNR were significantly reduced. In conclusion, we show that the anti-obesity effects of *Lb. gasseri* BNR17 can be attributed to high expression of fatty acid oxidation-related genes and low leptin levels [10].

As a chemical structure, green tea (*Camellia sinensis*) contains alkaloids, lignin, flavanol glycosides, carbohydrates, amino acids, minerals and vitamins, and it also consists of high amounts of polyphenols (catechins) [11]. Epidemiological evidence and several randomized controlled intervention studies show an inverse relationship between tea consumption (predominantly green tea) and body fat levels and waist circumference [12]. Caffeine and catechins and (-)-epigallocatechin-3-gallate, which are found in green tea, are effective against obesity [13]. It has been determined that green tea reduces food intake. It interrupts lipid emulsification and absorption. It also suppresses adipogenesis and lipid synthesis. It increases thermogenesis, fat oxidation and fecal lipid excretion. Thus, energy consumption increases [14]. In a study, it was determined that feeding obese rats with yogurt to which green tea was added was effective in losing weight compared to the control group [15].

The aim of this study was to produce probiotic yoghurt that is thought to be effective against obesity by using *S. thermophilus* and *Lb. gasseri* cultures and adding green tea at different rates. In this study, the effects of *Lb. gasseri* and green tea on some properties of produced probiotic yoghurts were investigated.

Material and Methods

Daily pasteurized cow's milk used in the production of probiotic yogurts with green tea was procured from Ak Gıda San. ve Tic A. Ş. (Pamukova/Sakarya). Pasteurized milk was stored at +4 °C until the moment of application, taking care not to break the cold chain. The energy value of milk used in yogurt production was 64 kcal/100 mL. The carbohydrate (g), sugar (g), protein (g), salt (g), fat (g), saturated fat (g), content of milk were 4.5, 4.5, 3.1, 0.1, 3.7, 2.4/100 mL, respectively. *S. thermophilus* and *Lb. gasseri* were obtained from SFA Arge ve Özel Sağlık Hizmetleri Ltd. Şti. (İstanbul). Skimmed milk powder was obtained from Gemici Gıda Tic Ltd Şti (Esenler/Istanbul). The energy value of skimmed milk powder was 352 kcal/100 g. The protein, fat and carbohydrate (lactose) contents of the milk powder were 37, 1 and 52 g/100 g, respectively. Green tea was obtained from the General Directorate of Tea Enterprises (Rize).

Production of yoğurt

For yogurt production, 200 mL of 4000 mL pasteurized milk was heated to 42 °C and then 10 g *Lb. gasseri* and 10 g *S. thermophilus* were added in a 1/1 ratio. The culture was activated for 30 min at 42 °C. Skimmed milk powder at the rate of 5% of total milk (4000 mL) was added to 3800 mL of milk, mixed until clumping disappeared and heated until it reached 90 °C and kept at the same temperature for 10 minutes. Since the pasteurization temperature applied to the purchased pasteurized milk was not known exactly, it was deemed appropriate to apply heat treatment again. Since the ideal thing is to pasteurize the milk to be used to make yoghurt at 90 ºC for 10 minutes [16], this process was carried out. The milk was divided equally into four containers, green tea was not added to the milk in the first container, 2%, 3%, 4% of dried leafy green tea was added to the milk in the other containers, and after waiting for 10 minutes, the milk was filtered. When the milk reached the yogurt fermentation temperature (42 °C), the activated culture was equally distributed to the milk. Yogurt milk mixed for homogenization was divided into plastic containers in quantities that can be used for analysis, and then left to fermentation in an incubator at 42 °C. During the fermentation, the pH was measured regularly. The fermented yogurts were stored under refrigerator conditions (at 4 °C) for 21 days. Yogurts were analyzed on the 1st, 7th, 14th and 21st days of storage. Analyzes were performed in 3 replications.

Physicochemical analyzes

Probiotic yoghurt samples were tested for pH using a benchtop meter (Isolab 616.11.001, Germany) calibrated with pH buffer solutions. Titratable acidity was determined in % lactic acid [17]. The amount of dry matter was determined according to AOAC (2012) [18].

For determination of serum separation (Syneresis %), 25 g of yogurt sample was placed through filter paper and kept at 4 °C for 2 hours. Next, the separated serum was weighed. Serum separation was calculated according to the following formula [19].

Serum Separation=(V1) / (V2) x 100

V1: Amount of serum separated (mL)
V2: Amount of yogurt (g)

For analysis, 10 g of yoghurt samples were taken and transferred to falcon tubes and centrifuged at 5000 rpm at 4 °C for 20 minutes, then the supernatant was removed and the pellet was weighed. The formula to determine water retention capacity (%) is shown below [20].

Water Holding Capacity (%) = ((Tare+pellet)- tare) / (Sample amount) x 100

Green tea leaves are rich in phenolic compounds [21]. In this study where green tea leaves are used, it is necessary to analyze the phenolic content of the samples. The total phenolic content was determined by measuring the color of phenolic compounds in alkaline medium with Folin-Ciocalteu solution in a spectrophotometer. 100 µl of yogurt sample and 500 µL of Folin-Ciocalteu reagent were added to the test tubes, which contained 7.5 ml of distilled water, then agitation was performed and waited for 3 minutes. Then, 1 mL of 0.25 mL of saturated Na_2CO_3 solution was added and made up to 10 mL with distilled water. Yogurt samples were kept in the dark for 1-1.5 hours. Then, readings of yogurt samples relative to the blank were performed at 720 nm in a spectrophotometer (Shimadzu Corporation UV-1208, Japan). This method was used by modifying the methods of Singleton *et al*. (1999) [22]. The results were calculated as mg/kg in gallic acid.

Color analyses of yogurt samples was performed on the 1st day of storage. Hunter Lab (Konica Minolta CR-5, Japan) model color measuring device was used for color analysis. As a result of the analysis, yoghurt samples were examined for L*, a* and b* values.

Rheological analyses

Yoghurt samples were examined for rheological properties on the 1st, 7th, 14th and 21st days of storage. Analysis was carried out at 20ºC using TA Discover rheometer (TA DHR-2, USA) equipped with a cone and plate sensor (40 mm diameter and 0.500 mm gap adjustment) was determined. For each sample, independently repeated measurements were taken and data processing was done using a TA software package. Rheological analyzes were tested with TA rheometer Data Analysis software (V3.0) with suitable models to measure the properties of yogurts. Dynamic oscillation tests were performed to characterize the viscoelastic properties of yoghurt. Frequency sweeps between 0.01 and 100 Hz were then performed at a constant shear stress of 1% Pa in the linear viscoelastic region (LVR) to obtain the dynamic complex viscosity (η*), the elastic modulus (G'), the viscous modulus (G") [23].

Sensory analyses

Sensory evaluation of yoghurts was carried out by a group of panelists on the first day of storage. This group consisted of 6 experienced academicians from the Department of Food Engineering. The panelists were asked to rate 1-9 for the "Appearance", "Color", "Flavour", "Odour", "Consistency on Spoon", "Consistency in Mouth" and "Overall Acceptability" characteristics of the yogurt samples.

Microbiological analyses

Sterile water with salt (NaCl, 0.85% weight/volume) was used for dilution preparation, and spread plate technique was used to grow microorganisms. The number of *S. thermophilus* was determined after aerobic incubation (48 hours at 37 ºC) on M-17 Agar (Oxoid, UK). *Lb. gasseri* number was determined after anaerobic incubation (48 hours at 37 °C) in a 5% CO_2 incubator (Panasonic MCO-170AICUV, Japan) on MRS Agar (Oxoid, UK) adjusted to pH 5.2 [24]. The results are expressed as "log cfu/g".

Statistical analysis

In evaluating the physicochemical and microbiological analysis results, the difference between the groups was determined using the univariate general linear model procedure. The difference between the groups in the evaluation of the sensory and color analysis results was determined by applying one-way ANOVA analysis. In evaluating the rheological analysis results, the difference between the groups was determined using the univariate general linear model procedure. DUNCAN multiple comparison test was used to determine the degree of difference. SPSS statistical software program (version 18; SPSS, Inc., Chicago, IL, USA) was used in the evaluation [25].

Results and Discussion

Physicochemical and microbiological properties

The acidification performance of probiotic bacteria is low compared to normal yoghurt culture. In this case, pH values will be higher than in normal yoghurt [26]. Additionally, according to a study, some components in tea leaves have an inhibitory effect on the growth and metabolism of yoghurt bacteria [27]. For this reason, it was thought that the fermentation of the yogurt samples would be completed between the pH values of 4.65-4.95. Fermentation took 13.5 hours. It was observed that fermentation in green tea-added yoghurt samples was completed at higher pH values than in control yoghurt (Table 1).

Table 1: pH values of yogurt samples during fermentation

Samples	Beginning	6th hour	8th hour	10th hour	12th hour	13.5th hour
C	6.48±0.04 Aa	6.34±0.04 Ab	6.23±0.03 Ac	5.51±0.03 Cd	4.94±0.04 De	4.66±0.03 Cf
2%GT	6.46±0.03 ABa	6.37±0.03 Ab	6.15±0.04 Bc	5.86±0.04 Bd	5.81±0.04 Ae	4.82±0.03 Bf
3%GT	6.44±0.03 ABa	6.38±0.02 Ab	6.25±0.03 Ac	6.01±0.04 Ad	5.33±0.03 Ce	4.89±0.04 Af
4%GT	6.42±0.03 Ba	6.32±0.04 Ab	6.19±0.03 ABc	5.96±0.04 Ad	5.53±0.04 Be	4.94±0.04 Af

C: Control, 2%GT:Sample with 2% green tea added, 3%GT:Sample with 3% green tea added, 4%GT:Sample with 4% green tea added

*a,b,c,d,e,f: Small letters in the same row show the statistical difference between the fermentation times of each yogurt sample. (p<0.05).

*A,B,C,D: Capital letters in the same column indicate differences between yogurt samples at the same fermentation time (p<0.05).

The pH value of the probiotic yogurt produced using *Lb. gasseri* in the study of Zhou *et al.* (2021) [28] at the end of the fermentation lasting for 8 hours, and the pH value of the set-type yogurt produced by adding green tea extract in the study of Shokery *et al.* (2017) [29] at the end of the fermentation, as in our study, it was higher than it should be.

It has been stated that catechins obtained from phenolic compounds inhibit the growth of lactic acid bacteria [30]. The sensitivity of LAB to phenolic compounds depends on the bacterial type and strain. In addition, the chemical structure and concentration of polyphenols are also important [30,31].

During storage, the pH values of yoghurt samples containing green tea were higher than the control sample. The pH values of yoghurt samples during storage are shown in Table 2. The pH values of green tea yogurts increased until the 14th day of storage and then decreased (p<0.05). The addition of a plant extract rich in phenolic compounds causes a slow decrease in the pH of yoghurt. This indicates an increase in the buffering capacity of yoghurt, which resists pH changes despite the accumulation of organic acids [32]. A similar situation was observed in many studies in which yogurts with green tea were produced [30,33-35]. In the study of Baltova & Dimitrov (2014) [36], yogurt samples were produced by adding human-derived *Lb. gasseri* 4/13 strain to traditional starter cultures as an auxiliary culture. At the end of the first 24 hours of storage, it was determined that the yogurt samples had pH values of 4.70-4.69. The pH values decreased during storage, but after 21 days, they reported that the pH was in the range of 4.38-4.32. The reason why the pH in their study was lower than in ours may be the use of *Lb. bulgaricus* and no green tea added.

The titration acidity value of the control yogurt was found to be slightly higher than the green tea yogurt samples in general. Titration acidity values of yogurt samples during storage are shown in Table 2. The difference between storage days in all yoghurt samples in terms of titratable acidity is statistically significant. Additionally, the difference between yoghurt samples on all storage days was found to be statistically significant (p<0.05). Titratable acidity values of yogurt should be between minimum 0.6% and maximum

1.5% (wt% as lactic acid) specified in Turkish Food Codex (2009) [37]. According to the analysis results, the titratable acidity values of the yoghurt samples were in accordance with the communiqué. In a study, it was determined that the acidity value decreased as the amount of green tea added in bioyogurts increased [30]. In another study, the storage period of the control yogurt ended on the 21st day, while the storage period of the yogurt produced by adding *Lb. gasseri* and having lower titration acidity ended on the 35th day [38].

Dry matter change (%) of yogurt samples during storage is shown in Table 2. In general, an increase in dry matter content % was observed as green tea content and storage time increased ($p<0.05$). The reason why the dry matter rate increases with the increase in green tea content may be that green leaf tea leaves particles in the yoghurt milk during the infusion. In the study of Çakmakçı *et al*. (2019) [34], as in our study, in probiotic yoghurt production, as the amount of green tea added increased, the dry matter rate also increased. With the increase in the dry matter content, the nutritional value of the yogurt will increase as the nutritive components in the yogurt increase [39]. Defects such as lack of aroma, weak clot formation and loose consistency occur in yogurt with low dry matter content. Therefore, yogurts with high dry matter are of higher quality [40].

It was determined that the serum separation % values decreased with the prolongation of the storage period (Table 2). According to the 21st day data, it was determined that the serum separation value was the highest in the 4% green tea sample (24%) and the least in the control sample (20.8%). Serum separation is one of the rheological properties of yogurt [41]. Serum separation, also called syneresis, is expressed as the separation of the liquid phase held in the protein network of yogurt and the water or serum seen in yogurt [41,42]. Serum separation, which is common in fermented milk products such as yogurt, is an undesirable defect and is an important quality criterion [41]. Serum separation analysis in yogurt is performed to determine the stability of the clot [43]. In the studies, as in our study, it was determined that the serum separation increased as the amount of green tea added increased [44-46]. Jeong *et al*. (2018) [45] stated that the reason for the increased serum separation was probably due to compounds such as polyphenols in the content of green tea. The excess presence of polyphenols in green tea may cause serum separation by reducing the gel matrix that limits the yoghurt serum. Even a small drop in pH leads to a reduced load that weakens colloidal stability [47]. It can increase serum release from the gel matrix. In addition, Lucey (2002) [48] explained that postacification is one of the factors that can increase whey production in yogurts.

It was determined that as the amount of added green tea increased, the WHC (%) during storage decreased. The water retention capacity change of yoghurt samples during storage is shown in Table 2. WHC, which affects the viscosity of yoghurt, plays an important role in the storage of yoghurt and its preference by consumers. WHC is caused by water retention and aggregation of protein particles due to the effect of gravity [28]. The WHC of normal yogurt is at the highest value between pH 4.2-4.6 [49]. In our study, since the pH value was higher than 4.6 with the addition of green tea, it was thought that the WHC of yogurt samples might decrease as the green tea ratio increased. The highest WHC value during storage was determined in the control sample. In addition, the starter culture used was thought to affect the water holding capacity of the yogurt samples. In a study, it was determined that the water holding capacity of yogurt samples containing *Lb. gasseri* was low [28]. It was determined that the results obtained in these studies were similar to our study.

Table 2: Physicochemical and microbiological values of yogurt samples during storage

Samples	Storage time (day)	pH	Titreatable Acidity	Dry Matter (%)	Syneresis (%)	Water Holding Capacity (%)	Phenolic Substance (gallic acid) mg/kg	*Lactobacillus gasseri* (log cfu/g)	*Streptococcus thermophilus* (log cfu/g)
C	1	4,44±0,04 Bb	1,13±0,01 Ab	17,84±0,16 Cb	27,20±0,20 Bb	54,30±0,44 Ac	45,29±7,14 Da	4,79±0,04 Ba	8,72±0,03 ABb
	7	4,34±0,04 Bc	1,20±0,01 Aa	17,88±0,12 Cb	28,00±0,20 Aa	55,60±0,36 Ab	34,48±2,18 Db	3,63±0,30 Cc	8,84±0,16 Aab
	14	4,55±0,04 Ba	1,22±0,02 Aa	17,88±0,12 Cb	24,00±0,00 Ac	56,90±0,26 Aa	33,26±2,15 Db	4,80±0,04 Ca	8,99±0,08 Aab
	21	4,45±0,04 Cb	1,24±0,02 Aa	18,34±0,02 Da	20,80±0,20 Dd	55,00±0,17 Ab	33,18±2,27 Db	4,14±0,14 Cb	9,16±0,29 Aa
2% GT	1	4,46±0,05 ABc	1,15±0,01 Aa	17,99±0,10 Cc	28,00±0,00 Aa	53,50±0,30 Bb	251,15±16,23 Cc	6,97±0,04 Aa	8,59±0,11 Ba
	7	4,61±0,04 Ab	1,10±0,02 Bb	18.58±0,02 Bb	28,00±0,00 Aa	52,60±0,26 Bc	348,29±9,37 Cb	6,31±0,02 Abc	8,63±0,08 Aa
	14	4,71±0,04 Aa	1,13±0,02 Bab	18,56±0,04 Bb	24,00±0,00 Ab	54,60±0,26 Ba	409,55±22,19 Ca	6,18±0,02 Ac	8,51±0,50 Aa
	21	4,70±0,04 ABa	1,14±0,02 Ba	19,98±0,02 Ba	21,20±0,20 Cc	53,90±0,36 Bb	203,14±6,59 Cd	6,46±0,22 Ab	8,83±0,11 Ba
3% GT	1	4,49±0,04 ABc	1,17±0,04 Aa	18,52±0,06 Bc	28,00±0,20 Aa	52,70±0,36 Ca	352,10±21,49 Bc	6,34±0,33 Aa	8,78±0,07 Aa
	7	4,62±0,03 Ab	1,04±0,02 Cc	18,56±0,19 Bc	27,20±0,00 Cb	51,70±0,36 Cb	383,80±4,19 Bb	5,28±0,17 Bb	7,99±0,41 Bb
	14	4,73±0,04 Aa	1,11±0,03 Bb	18,96±0,19 Ab	23,20±0,20 Bc	52,30±0,30 Cab	499,27±17,87 Ba	5,41±0,05 Bb	8,41±0,12 Aa
	21	4,73±0,03 Aa	1,10±0,02 Cbc	19,77±0,18 Ca	22,00±0,00 Bd	50,30±0,26 Cc	317,80±17,29 Bd	6,45±0,08 Aa	8,71±0,05 Ba
4% GT	1	4.53±0,03 Ac	1,08±0,02 Ba	18,92±0,14 Ab	27,20±0,20 Bb	51,30±0,17 Da	446,52±11,73 Ab	6,10±0,85 Aa	8,67±0,07 ABa
	7	4,58±0,03 Ac	1,00±0,02 Db	18,93±0,17 Ab	27,60±0,20 Ba	50,70±0,30 Db	458,25±7,90 Ab	6,17±0,15 Aa	8,43±0,11 ABa
	14	4,76±0,04 Aa	1,03±0,02 Cb	18,97±0,03 Ab	24,00±0,20 Ac	51,20±0,26Da	565,43±29,00 Aa	4,47±0,03 Db	8,28±0,53 Aa
	21	4,66±0,04 Bb	1,11±0,02BCa	20,70±0,09 Aa	24,00±0,00 Ac	49,80±0,30 Cc	425,08±12,23 Ab	5,50±0,02 Ba	8,70±0,08 Ba

C: Control, 2%GT:Sample with 2% green tea added, 3%GT:Sample with 3% green tea added, 4%GT:Sample with 4% green tea added

*a,b,c,d: Small letters in the same row indicate the statistical difference between the storage days of each yogurt sample ($p<0.05$).

*A,B,C,D: Capital letters in the same column indicate differences between yogurt samples on the same storage days ($p<0.05$).

The phenolic content of the control yogurt was lower than that of the other yogurts and decreased regularly during storage. It was observed that the total phenolic substance value increased with the increase in the green tea ratio in the yogurt samples (Table 2). In addition, the degradation of milk proteins by yogurt bacteria causes an increase in the total phenolic content of yogurt samples. This is because some amino acids, such as thyroside amino acid, have a phenolic side chain [50]. Studies have shown that the total phenolic content of yogurts produced with the addition of green tea increases as the ratio of green tea increases [29,30,33,34,51,52]. The reason for the decrease in total phenolic substance values on the 21st day of storage is because lactic acid bacteria can reduce the total phenolic content in green tea, and some polyphenols in yogurt samples can be hydrolyzed by microflora [53].As a result of acidity developing during or after incubation in yogurt production, microorganisms use phenolic acids such as ferulic and coumaric acids, resulting in the formation of different phenolic compounds such as vanillic and p-hydroxybenzoic acid [50]. However, phenolic compounds can interact with caseins or whey proteins, resulting in the formation of soluble and insoluble complexes. This, in turn, causes a decrease in the phenolic value [54]. In one study, as in this study, the phenolic content, which increased until the 14th day of storage, then decreased [33].

It was determined that the highest number of *Lb. gasseri* during storage was in the sample containing 2% green tea. The control sample had the lowest number of *Lb. gasseri*. The difference between yogurt samples in terms of *Lb. gasseri* numbers on storage days and *Lb. gasseri* numbers on all storage days was statistically significant in all yogurt samples ($p<0.05$). Generally, the highest *Lb. gasseri* numbers were determined on the 1st day of storage (Table 2). During storage, the number of *S. thermophilus* of the control yogurt showed a regular increase. Statistically insignificant and non-regular changes were observed in the numbers of *S. thermophilus* of green tea samples ($p>0.05$) (Table 2). While sample with 2% green tea added remained probiotic during all storage days, the other green tea added samples preserved their probiotic properties in the first days of storage. The control sample had the lowest *Lb. gasseri* count, indicating that green tea favorably promoted the growth of *Lb. gasseri*. In one study, *Lb. gasseri* K7 (1%, v/v) was inoculated into milk medium with and added 1% yeast extract. The number of *Lb. gasseri* was 8.7 log cfu/mL in the first 10 hours of fermentation [55]. That number than the number of *Lb. gasseri* in our study. The reason may be yeast extract or the number of *Lb. gasseri* inoculated. In one study, yogurt was produced by adding *Lb. gasseri* 4/13 to standard cultures. The *Lb. gasseri* number was higher than in our study during storage. This may be due to standard cultures or the number of *Lb. gasseri* inoculated. According to that study, *Lb. gasseri* 4/13 strain had high adhesion to human epithelium and was capable of lowering cholesterol concentration. Additionally, that study found that *Lb. gasseri* 4/13 strain had beneficial potential in stimulating the immune system. The strain has been successfully applied as a co-culture in yogurts. Yoghurt products enriched with *Lb. gasseri* 4/13 had high concentrations of live *Lb. gasseri* 4/13 cells. Those yoghurts were also accepted in terms of taste [36]. During storage, *S. thermophilus* was the dominant species with 7.99-9.16 log cfu/g. Addition of green tea did not affect the number of streptococci. The numbers of *S. thermophilus* in the studies performed on yogurts with green tea were similar to those in our study [30,34].

The difference between yogurt samples in terms of L*, a* and b* values was found to be statistically significant ($p<0.05$) (Table3). It was determined that the L* value decreased in all yogurt samples as the green tea addition increased. An increase in a* and b* values was observed with the increase in the amount of green tea added.

Table 3: Color values of yogurt samples

Samples	L*	a*	b*
C	92,51 A	-0,85 D	12,79 D
2%GT	90,34 B	-0,58 C	14,28 C
3%GT	89,86 C	-0,38 A	14,50 B
4%GT	89,21 D	-0,43 B	15,09 A

C: Control, 2%GT:Sample with 2% green tea added, 3%GT:Sample with 3% green tea added, 4%GT:Sample with 4% green tea added *A,B,C, D: Capital letters in the same column indicate differences between yogurt samples ($p<0.05$).

The reason for the decrease in the L* value as the amount of green tea added is increased, is because enzymatic oxidation of polyphenols, especially tea catechins, occurs during tea fermentation. This leads to the formation of a number of colored chemical compounds such as theaflavins and thearubigins, which can affect the color of the product obtained with tea [56]. In previous studies, as in this study, the L* value of yogurts decreased as the amount of green tea added to yogurt milk increased. L* values differ between studies depending on the amount of green tea added and the characteristics of the milk used [29,30,35,56]. It has been determined that the a* and b* values of yogurts increase with the increase in the amount of green tea added in the studies [29,30,35,56].

Rheological and sensory properties

The rheological properties of green tea added probiotic yogurt samples are shown in Table 4. The flow behavior index increased with the addition of green tea and the increase in its ratio. The highest storage modulus (G') and loss modulus (G") values were determined in the sample containing 3% green tea on the 1st and 7th days of storage, and the lowest G' and G" values were determined in the control sample on the 7th and 14th days of storage. The rheological properties of yogurt are very important in product development, quality control, process design, transportation and storage [57]. The G' value, which represents the elasticity of yogurt, is a measure of the deformation energy stored in the sample during shear [58]. In this study, the viscosity modulus of the yogurt samples, G", was lower than (G') during storage, although it followed a similar profile to (G'). This indicated that all yogurt types had an elastic or solid-like character. Flow index values (n) indicate how far from the Newtonian behavior. If the n value is between 0 and 1, it is stated as pseudoplastic flow (shear thinning), 1 as fluid Newtonian, and above 1 as dilatant flow (shear thickening) [59].

Table 4: The consistency coefficient (K), flow behavior index (n) and R2 values of probiotic yogurt samples with green tea added at different rates

Days	Samples	G’			G’’		
		n’	K’	R^2	n’	K’	R^2
1	Control	0.24±0.0065 Cb	228.2±4.89 Bb	0.96	0.16±0.002 Da	75.33±0.40 Ca	0.99
	2%GT	0.26±0.008 Ba	226.71±6.36 Ba	0.95	0.18±0.002 Ca	79.04±0.43 Ba	0.99
	3%GT	0.25±0.005 BCb	288.87±4.60 Aa	0.98	0.20±0.001 Bb	108.24±0.37 Aa	0.99
	4%GT	0.30±0.009 Aa	171.94±5.51 Cd	0.96	0.20±0.001 Ac	68.83±0.23 Dd	0.99
7	Control	0.23±0.008 Cb	201.69±5.17 Bc	0.94	0.16±0.003 Cab	62.38±0.55 Db	0.98
	2%GT	0.25±0.008 Bab	210.98±5.39 Bb	0.95	0.17±0.002 Bb	72.8±0.45 Cc	0.99
	3%GT	0.25±0.005 Bb	287.46±4.99 Aa	0.98	0.20±0.001 Aa	102.63±0.38 Ab	0.99
	4%GT	0.28±0.007 Ab	209.58±5.12 Bc	0.97	0.20±0.001 Ab	79.6±0.29 Bc	0.99
14	Control	0.26±0.010 Ba	171.14±5.81 Cd	0.92	0.16±0.003 Dbc	55.98±0.49 Dc	0.98
	2%GT	0.26±0.008 Bab	210.61±5.51 Bb	0.95	0.18±0.0015 Ca	74±0.35 Cb	0.99
	3%GT	0.28±0.008 Aa	212.52±5.84 Bb	0.98	0.19±0.001 Bb	76.22±0.35 Bd	0.99
	4%GT	0.26±0.005 Bc	257.4±4.55 Ab	0.98	0.21±0.001 Aa	95.54±0.25 Ab	0.99
21	Control	0.23±0.008 Cb	243.20±6,42 Ba	0.93	0.16±0.003 Dc	75.34±0.70 Ca	0.97
	2%GT	0.24±0.008 Bb	209.90±5,59 Db	0.94	0.16±0.002 Cc	71.88±0.52 Dd	0.98
	3%GT	0.27±0.007 Aa	221.58±5,43 Cb	0.96	0.20±0.001 Ab	79.08±0.36 Bc	0.99
	4%GT	0.23±0.006 Cd	285.12±5,22 Aa	0.98	0.18±0.001 Bd	106.48±0.33 Aa	0.99

C: Control, 2%GT:Sample with 2% green tea added, 3%GT:Sample with 3% green tea added, 4%GT:Sample with 4% green tea added *a,b,c,d: Small letters in the same row indicate the statistical difference between the storage days of each yogurt sample (p<0.05). *A,B,C,D: Capital letters in the same column indicate differences between yogurt samples on the same storage days (p<0.05).

In our study, it was determined that all yogurt samples exhibited pseudoplastic flow (thinning by shear) since the n value was between 0 and 1. A good yogurt should have non-Newtonian flow [60]. In this study, increasing the amount of added green tea increased the flow behavior index. After the 14th day of storage, the flow behavior indexes decreased. The consistency coefficient values (K) correspond to the viscosity. Seasonal changes in temperature, protein and ion values, processing, food composition, incubation and storage conditions affect viscosity changes [59]. The K value of the yoghurt sample with 3% green tea added was highest on the 1st and 7th days of storage. On the 14th and 21st days, the K value of the 4% green tea sample was the highest. This showed the highest thickening ability, indicating increased mesh flexibility and rearrangement of gel structures. The n value was highest for the sample with 4% green tea on Days 1 and 7 of storage, and for the sample with 3% green tea on days 14 and 21 of storage. In one study, the flow behavior index (n) of 1% and 2% green tea powder added yogurts was higher than control, 0.01% and 0.02% green tea powder added yogurts. On the contrary, the consistency coefficient (K) was less [44] In other study, the addition of *Lactobacillus gasseri* LGZ 1029 to *Streptococcus thermophilus* CGMCC 1.2741 caused a decrease in the n value and an increase in the K value [28].

In the sensory evaluation results, it was determined that the scores of all characteristics of the yogurt samples generally decreased as the amount of green tea added was increased (Figure 1). The difference between the yogurt samples in terms of appearance, color, odour, consistency on spoon and consistency in mouth scores was found to be statistically insignificant (p>0.05). The difference between yogurt samples in terms of flavour and general acceptability was found to be statistically significant (p<0.05). Flavour scores and general acceptability scores of the control and 2% green tea-containing yogurt samples, and the general acceptability scores of 3% and 4% green tea-containing yogurt samples were statistically similar. The increase in the ratio of green tea caused a decrease in the likability of the yogurt samples, especially the addition of green tea as a taste caused the yogurt sample to have a more bitter taste.

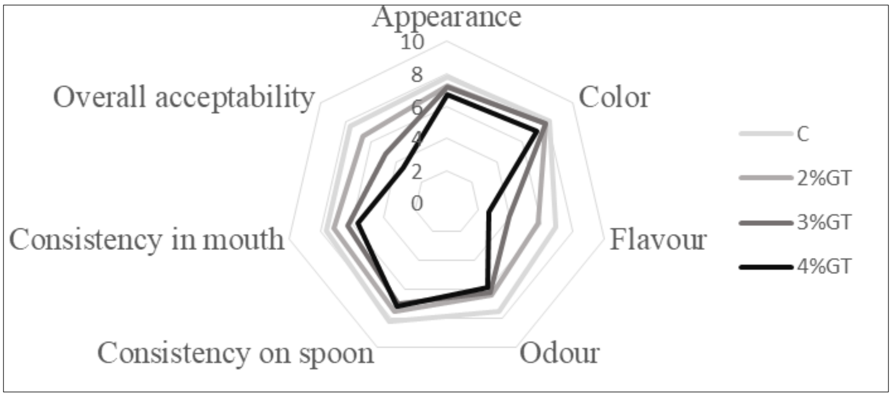

Fig 1: Sensory analysis results of yogurt samples

In the studies, it was observed that the appearance, taste and general acceptability scores decreased with the addition of green tea and the increase in the added ratio [46,52]. It was also observed that the consistency score decreased as well as the taste score [35]. In one study, it was determined that 1% green tea added yogurt had lower values than the control sample in terms of taste, smell, appearance, color and general acceptability [29]. In their study, Mikky *et al.* (2021) [38] determined that the taste and appearance scores of drinking yoghurts produced using standard yoghurt culture + *Lb. gasseri* were lower ($p < 0.05$) than those of drinking yoghurts produced using standard yoghurt culture. Baltova & Dimitrov (2014) [36] reported that yogurt enriched with *Lb. gasseri* 4/13 has a well-accepted taste. Zhou *et al.* (2021) [28], it was determined that the flavor and texture properties of yogurts containing mixed strains were better than yogurts containing single strain. Previous study results support the results of this study.

Conclusion
In this study, it was determined that the number of *Lb. gasseri* in yoghurt containing 2% green tea was higher than the number of *Lb. gasseri* in other yoghurts than the number of microorganisms evaluated as probiotic (at least 10^6 cfu/g) during storage. On the other hand, yogurt containing 4% green tea showed probiotic properties on the 1st and 7th days of storage, while it lost its probiotic property on the other days. The number of *Lb. gasseri* in the control yogurt was below the limit value on all days of storage. It was concluded that the green tea used in the ideal ratio supports the development of *Lb. gasseri*. In order for yogurts to show probiotic properties for a longer time, *Lb. gasseri/S. thermophilus* ratio may not be 1/1 but at least 1.5/1. Yogurts with high phenolic content were produced with the addition of green tea. For yogurt consumption with high phenolic content, yogurts should be consumed before the 14th day of storage. The proportion of green tea may be less in order to increase the sensory general acceptability. In yogurts to be produced by adding 3% or more of green tea, other methods can be investigated to mask the bitterness of green tea.

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