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Research Article

Probiotic Shelf Life, Antioxidant, Sensory, Physical and Chemical Properties of Yogurts Produced with *Lactobacillus acidophilus* and Green Tea Powder [1]

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Abstract

The aim of the present study was to determine the effects of the addition of green tea powder (GTP) in the production of yogurt on survival of *Lactobacillus acidophilus* (P) and the yogurt properties. Four yogurt groups (yogurt without P and GTP, Control, C; yogurt with P and without GTP, PC; P + 1% GTP; and P + 2% GTP) were produced. The yogurt samples were stored at 4°C. The addition of GTP into milk did not affect the viability of yogurt bacteria during fermentation. The highest count of *L. acidophilus* was detected in P + 2% GTP. The *L. acidophilus* count was high up to the 7th day (7.54 log cfu/g). Yeast and mold were not counted (<2 log cfu/g) in any yogurt sample during storage. GTP has antioxidant properties that could be attributed to the presence of phenolic and flavonoids compounds. The panelists preferred the PC and P + 1% GTP samples during the storage period. As a result of this research, we can suggest the consumption and production of probiotic yogurt with 1% GTP supplement.

Keywords: *Yogurt, Green tea powder, Lactobacillus acidophilus, Shelf life, Antioxidant activity, Phenolic compounds*

Lactobacillus acidophilus ve Yeşil Çay Pudrası İle Üretilen Yoğurtların Probiyotik Raf Ömrü, Antioksidan, Duyusal, Fiziksel ve Kimyasal Özellikleri

Öz

Bu araştırmanın amacı, yoğurt üretiminde yeşil çay pudrası (GTP) ilavesinin *Lactobacillus acidophilus* (P) canlılığı ve yoğurt özellikleri üzerine etkilerini belirlemekti. Bu amaçla, dört farklı yoğurt çeşidi üretildi [P ve GTP ilavesiz yoğurt (Kontrol, C); sadece P içeren yogurt (PC); P + %1 GTP ilaveli yoğurt ve P + %2 GTP ilaveli yoğurt]. Yoğurt örnekleri 4°C'de muhafaza edildi. Süte GTP ilavesi, fermantasyon sırasında yoğurt bakterilerinin canlılığını etkilemedi. En yüksek *L. acidophilus* sayısı P + %2 GTP ilaveli yoğurtta tespit edildi. *L. acidophilus* sayısı 7. güne kadar yüksekti (7.54 log kob/g). Depolama süresince yoğurt örneklerinin hiçbirinde maya ve küf bulunmadı (<2 log kob/g). GTP, fenolik ve flavonoid bileşiklerinin varlığına bağlanabilecek antioksidan özelliklere sahipti. Panelistler depolama süresince PC ve P + %1 GTP örneklerini daha çok beğendiler. Bu araştırma sonucunda, %1 GTP katkılı probiyotik yoğurt üretimi ve tüketimini önerebiliriz.

Anahtar sözcükler: *Yoğurt, Yeşil çay pudrası, Lactobacillus acidophilus, Raf ömrü, Antioksidan aktivite, Fenolik bileşikler*

INTRODUCTION

Demand for functional foods is growing rapidly due to increased awareness of consumers about the impact of food on health. Yogurt, one of the best known of the foods that contain probiotics, is a popular food [1,2]. Probiotics such as *Lactobacillus acidophilus* are most commonly incorporated into yogurts worldwide. Considering that

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probiotic properties are strain-dependent, possible beneficial effects of consuming yogurt containing *L. acidophilus* are controlling various types of diarrhea and urogenital infections, alleviating lactose intolerance, preventing gastro-intestinal diseases, lowering serum cholesterol levels, anticarcinogenic activity, reducing allergic symptoms, antioxidative properties, stimulation of the immune system, improving resistance to various diseases [2,3]. The number of probiotic bacteria that are required to produce the health benefits is not entirely clear, but to exert the beneficial health effects, the count of probiotic bacteria in the food product should be adequately high 10^6-10^8 cfu/mL or g during shelf life [4,5]. In recent years, there has been increasing interest in the use of natural food additives. Probiotic foods can be supplemented with other active components with the goal of providing additional functional properties.

Green tea and green tea components are known to provide a wide range of benefits to human health [2,6]. Green tea is the least processed form and thus, retains all the healthy ingredients in their natural forms. Many studies have evaluated tea, tea polyphenols and tea extracts as factors for decreasing the risk of cardiovascular diseases and various types of cancer [7,8]. The beneficial effects of green tea have been attributed to the strong antioxidative and other health benefits of the rich green tea phenolic compounds, known as tea catechins [6-9]. Tea polyphenolics such as catechins are known to possess an antimicrobial effect against many microorganisms including pathogens, but these compounds do not inhibit lactic acid bacteria (LAB) [7,10]. Preliminary studies were conducted to see the effects of the addition of tea extract [8] and tea infusions [7,11] to milk on properties of yogurt during its production by fermentation.

Green tea powder (GTP) was selected in this study on the basis of the benefits of green tea to human health and the fact that it is a widely consumed beverage worldwide. The aims of the present study were to study the possibility of manufacturing a new functional probiotic yogurt. We investigated whether the addition of GTP would increase the nutritive value and functionality of yogurt and evaluated the effect of GTP addition on the survival of *L. acidophilus* and other LAB as well as the physical, chemical, sensory characteristics and antioxidant capacities of yogurts. It is possible that the use of GTP in yogurt technology can contribute to improvements in the quality, safety and functionality of probiotic yogurt. Previously, the effects of green tea supplementation on the yogurt were studied [12], but to our knowledge, this is the first study of the use of GTP for the supplementation of probiotic yogurt.

MATERIAL and METHODS

Materials

Fresh tea leaves were harvested and processed at the Atatürk Tea and Horticultural Research Institute (Rize, Turkey). The harvested green tea leaves were steamed, rolled, dried, ground and sieved. The particle size of GTP used as supplement was less than 355 µm. Raw cow's milk was purchased from Atatürk University Pilot Dairy Plant (Erzurum, Turkey). *Lactobacillus acidophilus* DSMZ 20079 was imported from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The direct-to-vat system yogurt culture (YC-350) *(Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus)* (Peyma-Hansen, Istanbul, Turkey) was used as the yogurt starter.

Manufacture of the Yogurt Samples

Yogurt samples were manufactured from cow's milk. The production of yogurt samples is shown in *Fig. 1* for clarity. The YC-350 culture *(Streptococcus thermophilus* and *Lactobacillus bulgaricus)* and *L. acidophilus* (3.2×10^8 cfu/g) was added (except for the Control sample). The yogurt samples were cooled and stored at 4°C for 28 days (for microbiological analysis) and 21 days (for physicochemical analysis). The yogurt samples produced by the inoculation of milk were divided into four groups (C: Control group yogurt; PC: yogurt with *L. acidophilus* and without GTP; P+1% GTP: yogurt with *L. acidophilus* and 1% GTP (w/v); P+2% GTP: yogurt with *L. acidophilus* and 2% GTP (w/v) *(Fig. 1)*.

Microbiological Analysis

The effect of the addition of GTP at various levels (0%, 1% and 2%) on the survival of the *L. acidophilus* strain during the production of yogurt was investigated. In addition, the effects of the use of *L. acidophilus* and GTP at various concentrations on the viability of yogurt cultures in the production of yogurt were examined. For each sample, 10 g of yogurt was diluted in 90 mL of 0.85% (w/v) NaCl solution and homogenized in a sterile polyethylene bag by using a Stomacher (Mayo HG400 Stomacher, Milan, Italy) for 2 min. Serial dilutions were made in 0.1% peptone in 0.85% NaCl, and all determinations were made in duplicate [13]. The numbers of yeast and mold (Dichloran Rose-Bengal Chloramphenicol Agar [DRBC Agar]; Merck) were determined according to Harrigan [13]. MRS, M17 and MRS bile agars (Merck) were used for the enumeration of LAB and determined according to Vinderola and Reinheimer [14]. The agar plates were incubated for 5-7 days at room temperature (yeast and mold), 3 days at 35-37°C in an anaerobic jar *(L. bulgaricus, S. thermophilus* and *L. acidophilus)*. The microorganisms were counted on the 1st, 7th, 14th, 21st and 28th days of the storage.

Physical and Chemical Analysis

The yogurt and milk samples were analyzed for total solids, protein and fat analysis using the method of AOAC [15]. The pH of the samples was measured using a pH meter (Mettler-Toledo AG 8603 Schwerzenbach, Switzerland). Titratable acidity (lactic acid, %) and syneresis were determined according to Atamer and Sezgin [16]. The syneresis, pH,

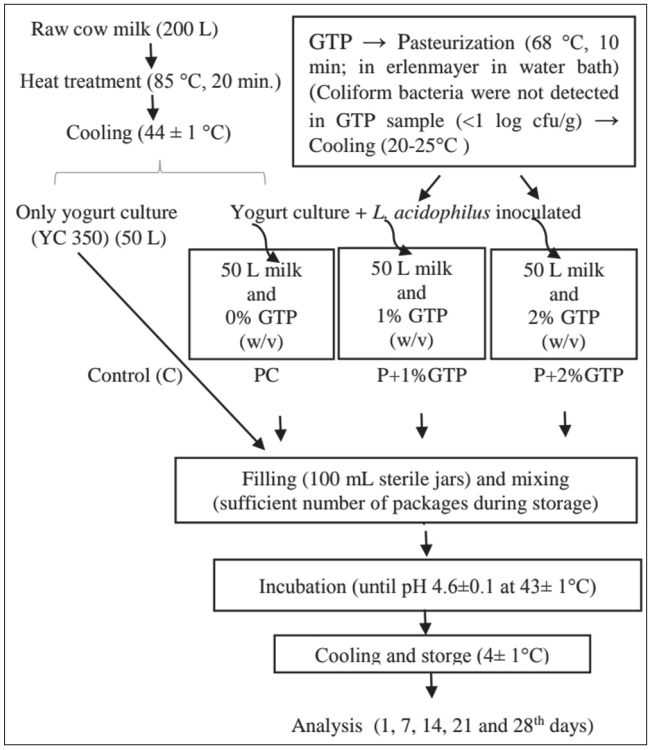

Fig 1. Production of Control and GTP yogurt groups

C: Control: (probiotic and GTP free); **PC:** Probiotic control: (with probiotic and GTP-free), **P+1% GTP:** (probiotic + 1% GTP); **P+2% GTP:** (probiotic + 2% GTP)

titratable acidity and sensory properties were assessed once a week. Dry matter, fat and protein contents of samples were determined at the beginning of the storage.

Antioxidant Analysis

Reducing Power Abilities: The Fe^{3+}-reducing ability of the yogurt samples and GTP was assayed using the $Fe^{3+}(CN)_6$-$Fe^{2+}(CN^-)_6$ reduction method [17]. The cupric ion (Cu^{2+})-reducing power was used as a second method for the analysis of the samples. The Cu^{2+}-reducing capability was analyzed according to the spectroscopic method of Apak et al.[18] with a modification. Another reducing power assay is the ferric-reducing antioxidant power, which is based upon reduction of the Fe^{3+}-TPTZ complex under acidic conditions [19].

Radical-Scavenging Abilities: The DPPH radical-scavenging activity of samples was determined according to the method of Blois [20]. The ABTS radical-scavenging activity of the samples was analyzed using the method described by Re et al.[21]. The DMPD radical-scavenging ability of the samples was analyzed according to the method described by Fogliano et al.[22]. The superoxide radical ($O_2^{\cdot-}$)-scavenging activity of the samples was analyzed in accordance with slight adjustments [23].

Metal-Chelating Ability: The metal-chelating ability of samples was determined according to the method of Gülçin and Daştan [24]. For this purpose, various concentrations of sample were mixed 0.25 mL $FeSO_4$ solution (2 mM), and 2,2′-bipyridine solution (0.2% in 0.2 M HCl) were used. The absorbances of the samples were recorded spectrophotometrically at 562 nm. EDTA is used as a standard ferrous ion (Fe^{2+}) chelator.

Total Phenolic and Flavonoid Contents: The total phenolic contents of samples were assessed using the Folin-Ciocalteu method [25] according to the equation Absorbance (λ_{760}) = 0.0021 × total phenols (gallic acid equivalent [GAE; µg]). The total phenolics in foods were estimated using the Folin-Ciocalteu method, which relied on the transfer of electrons from phenolic compounds to the FCR in alkaline medium. The content of total phenolics was calculated on the basis of a graph (r^2: 0.9706) that was prepared using gallic acid and expressed as micrograms of GAE. Total flavonoid contents of samples were estimated using a colorimetric assay as described in previous studies [23].

Sensory Analysis

The consumer acceptability properties were evaluated using a sensory evaluation of the yogurt by a jury of fifty panelists (age 20-50-years old) who were experienced and familiar with yogurt and green tea taste. Coded yogurt samples were stored at 4±1ºC and were scored on the 1st, 7th, 14th and 21st days of storage. Five parameters, colour-appearance, odour, flavour, sourness and general acceptability were evaluated using a sensory rating scale of 1-9 (1 for extreme dislike, to 9 for extreme like). The tests were conducted at city center (Erzurum, Turkey), in their houses by consumers or by students and teaching staff of the Atatürk University Food Engineering Department. Sensory analysis was made at room temperature and under fluorescent lamps. Sensory analysis forms were given. After that, the yogurt samples taken from the refrigerator were given to the panelists one by one. All yogurt samples were presented to the panelists in glass jars (100 mL). The samples were presented in random order. Water was provided to the panelists for palate cleansing between the samples [26].

Statistical Analysis

The experiments were conducted in a completely randomized design in a factorial arrangement: four treatments of yogurt (C, PC, P+1% GTP and P+2% GTP), four storage periods (1st, 7th, 14th and 21st days) and two replicates. All data were analyzed statistically using SPSS 17.0 program (SPSS Inc., Chicago, IL, USA). ANOVA and Duncan's Multiple Range Test were used to determine significant differences among the results.

RESULTS

The gross chemical and physical properties of the raw milk and GTP are shown in *Table 1*. The yogurt samples were

analyzed for total solids, protein and fat at the beginning of the storage period. The addition of GTP significantly affected the gross chemical composition of the yogurt samples *(Table 2)*.

The results of microbiological analysis of the yogurt samples were shown in *Table 3*. The count of *L. acidophilus* showed significant (P<0.05) differences among the probiotic yogurt groups. In the case of 1% GTP addition to the 21st day, up to 28 days with the addition of 2% GTP, the probiotic property was maintained *(Table 2)*. On the other hand, the storage period had also a significant effect on the count of *L. acidophilus* (P<0.05) *(Table 3)*. The results showed a significant decrease in *L. acidoph*ilus in probiotic treatment during storage time. The addition of GTP to the milk before the yogurt fermentation did not have a negative effect on yogurt formation. But, the results showed that both the treatment and storage period had significant effects (P<0.05) on the counts of *L. bulgaricus* and *S. thermophilus (Table 3)*. In the present study, it was found that the treatment and storage period did not have a significant effect (P>0.05) on the counts of yeast and mold *(Table 3)*.

The yogurt samples showed potent Fe^{3+} reducing capability, and these differences were statistically important (P<0.01). The cupric ion (Cu^{2+}) reducing power of same concentration (30 µg/mL) of the yogurt samples and the standard reducing compounds is shown in *Table 4*. It was determined that Cu^{2+} reducing capacity of the yogurt samples was increased dependent on the concentration (10-30 µg/mL). In this study, the final method used to assess the reducing ability was the Fe^{3+}-TPTZ reducing power, which offers a well-known index of the antioxidant, or reducing, potential of plant samples or pure compounds. The DPPH radical scavenging of the yogurt samples and GTP are

Table 1. *The gross chemical properties of raw milk, GTP and fresh yogurt samples*

Materials and Yogurt Samples		Total Solids (%)	Fat (%)	Protein (%)	Ash (%)	Acidity (%)	pH
Material	Milk	12.37	3.45	3.40	0.64	0.17	6.65
	GTP	92.70	-	19.44	4.13	-	-
Yogurt samples	C	12.61±0.17^{ab}	3.90±0.14^{c}	3.36±0.03^{a}	-	-	-
	PC	12.37±0.04^{a}	3.70±0.14^{bc}	3.56±0.03^{a}	-	-	-
	P +1% GTP	13.13±0.23^{b}	3.50±0.14^{ab}	3.61±0.01^{a}	-	-	-
	P + 2% GTP	14.11±0.31^{c}	3.20±0.00^{a}	4.03±0.29^{b}	-	-	-

C: *Control (probiotic free and GTP free);* ***PC:*** *with probiotic and GTP free;* ***P+1% GTP:*** *probiotic + 1% GTP);* ***P+2% GTP:*** *probiotic + 2% GTP*
Mean values followed by different letters in the same column are significantly different (P<0.05)

Table 2. *The syneresis, acidity and pH of the yogurt samples during the storage*

Yogurt Samples	Storage Time (days)	Syneresis (mL/25g)	Titratable Acidity (lactic acid, %)	pH
C	1	10.95±0.7^{b,B}	0.71±0.01^{a,A}	4.71±0.01^{c,AB}
	7	11.30±0.1^{b,B}	0.74±0.01^{a,B}	4.50±0.00^{b,B}
	14	9.9±0.1^{c,A}	0.79±0.01^{a,C}	4.28±0.04^{a,A}
	21	11.3±0.4^{d,B}	0.82±0.01^{b,C}	4.31±0.01^{a,B}
PC	1	10.75±0.1^{b,B}	0.74±0.01^{a,A}	4.69±0.09^{c,A}
	7	11.45±0.2^{b,C}	0.78±0.01^{b,B}	4.42±0.01^{b,A}
	14	10.2±0.0^{d,A}	0.85±0.01^{b,C}	4.23±0.04^{a,A}
	21	10.7±0.1^{cd,B}	0.83±0.01^{c,C}	4.20±0.00^{a,A}
P+1% GTP	1	10.75±0.4^{b,BC}	0.81±0.0^{b,B}	4.80±0.01^{c,B}
	7	11.15±0.1^{b,C}	0.80±0.01^{b,B}	4.60±0.03^{b,C}
	14	9.65±0.1^{b,AB}	0.87±0.0^{c,C}	4.45±0.03^{a,AB}
	21	9.2±0.8^{a,A}	0.73±0.0^{a,A}	4.45±0.00^{a,C}
P+2% GTP	1	8.8±0.0^{a,A}	0.71±0.01^{a,A}	4.96±0.00^{c,C}
	7	9.65±0.2^{a,B}	0.80±0.01^{b,B}	4.88±0.02^{bc,D}
	14	8.8±0.0^{a,A}	0.84±0.01^{b,C}	4.58±0.17^{a,B}
	21	9.4±0.3^{bc,B}	0.83±0.01^{bc,BC}	4.72±0.00^{ab,D}

C: *Control (probiotic free and GTP free);* ***PC:*** *with probiotic and GTP free;* ***P+1% GTP:*** *probiotic + 1% GTP);* ***P+2% GTP:*** *probiotic + 2% GTP*
Horizontal column, lower cases (a-d) express differences between yogurt samples (P<0.05); Vertical column, capital letter (A-C) express differences between storage periods (P<0.05)

Table 3. *The changes in microbiological characteristics of yogurt samples during the storage period (log cfu/g)*

Yogurt Samples	Storage Time (days)	*L. bulgaricus*	*S. thermophilus*	Yeast and Molds	*L. acidophilus*	
C	1	7.55±0.10	8.96±0.01	<2	<2	
	7	7.37±010	8.91±0.01	<2	<2	
	14	8.29±0.12	9.04±0.03	<2	<2	
	21	7.69±0.10	8.66±0.05	<2	<2	
	28	7.78±0.03	8.81±0.04	<2	<2	
PC	1	6.65±0.10	8.69±0.06	<2	7.55±0.07	
	7	7.45±0.01	8.77±0.02	<2	7.89±0.06	
	14	8.04±0.01	8.78±0.08	<2	6.52±0.04	
	21	7.41±0.02	8.40±0.02	<2	5.08±0.05	
	28	6.91±0.02	8.68±0.04	<2	<4	
P+1% GTP	1	7.32±0.05	8.28±0.04	<2	6.63±0.06	
	7	8.18±0.02	8.86±0.04	<2	7.00±0.02	
	14	7.59±0.10	8.72±0.08	<2	6.95±0.02	
	21	7.75±0.04	8.72±0.02	<2	6.92±0.05	
	28	6.97±0.05	8.18±0.02	<2	5.71±0.06	
P+2% GTP	1	6.22±0.06	8.21±0.39	<2	6.59±0.10	
	7	7.95±0.05	8.25±0.44	<2	7.72±0.02	
	14	7.84±0.05	8.72±0.05	<2	6.38±0.05	
	21	7.30±0.03	8.18±0.03	<2	7.11±0.03	
	28	7.90±0.01	8.40±0.03	<2	6.95±0.06	
Yogurt samples	**n**					
C	10	7.74±0.33a	8.88±0.14a	<2±0.0a	-	
PC	10	7.29±0.51d	8.66±0.15b	<2±0.0a	6.21±1.56c	
P+1% GTP	10	7.56±0.43b	8.55±0.29b	<2±0.0a	6.64±0.51b	
P+2% GTP	10	7.44±0.69c	8.35±0.29c	<2±0.0a	6.95±0.49a	
Storage period						
1	8	6.93±0.57e	8.53±0.35b	<2±0.0a	6.92±0.49b	
7	8	7.74±0.36b	8.70±0.33a	<2±0.0a	7.54±0.43a	
14	8	7.94±0.28a	8.81±0.15a	<2±0.0a	6.62±0.27c	
21	8	7.54±0.20c	8.49±0.24b	<2±0.0a	6.37±1.01d	
28	8	7.39±0.48d	8.52±0.27b	<2±0.0a	5.56±1.33e	
Source	**D.F.**	**ANOVA**			**D.F.**	**ANOVA**
Sample (S)	3	**	**	**	2	**
Storage Period (SP)	4	**	**	**	4	**
S × SP	12	**	**	**	8	**
Error	20				15	

C: *Control (probiotic free and GTP free);* ***PC:*** *with probiotic and GTP free;* ***P+1% GTP:*** *probiotic + 1% GTP);* ***P+2% GTP:*** *probiotic + 2% GTP*
Averages of the same column values (each section separately) by the same letter did not differ significantly from Duncan's multiple range tests at 5% significance; ***(a-e)***
Mean ± SD, values followed by the same letters within a column are significantly different at P<0.05

summarized in *Table 4*, which shows the half-maximal radical scavenging concentrations (IC_{50}) of the samples and GTP and the standards, trolox and α-tocopherol. A lower IC_{50} value demonstrates a higher DPPH· scavenging activity. As shown in *Table 4,* the yogurt samples and GTP were efficient $ABTS^{\cdot+}$ scavengers in a concentration-dependent manner (10-30 µg/mL, r^2: 0.957). The content of phenolic compounds in the yogurt samples was expressed as milligrams of GAE *(Table 5)*.

The scores for the sensory properties of the yogurts are presented in *Table 6*. The supplementation of the yogurts with the *L. acidophilus* and GTP significantly affected (P<0.05) the sensory scores. Generally, the GTP yogurts received lower scores. In terms of sourness, no statistically significant differences (P>0.05) were detected among the samples. Even at the end of the 21st day, all samples were evaluated as favorable, and statistically there were no differences (P>0.05) among the yogurt samples.

Table 4. *Determination of absorbance values of reducing ability of 30 µg/mL concentration of yogurt samples and GTP by ferric ions (Fe^{3+})reducing, FRAP methods, and cupric ions (Cu^{2+}) reducing capacity, half maximal concentrations (IC_{50}, µg/mL) of yogurt samples and GTP and standards for radical scavenging abilities including DPPH•, $ABTS^{•+}$, $DMPD^{•+}$, $O_2^{•-}$ and metal (Fe^{2+}) chelating effects*

Antioxidants, Yogurt Samples and GTP	Fe^{3+}-Fe^{2+} Reducing		Cu^{2+}-Cu^{+} Reducing		Fe^{3+}-TPTZ Reducing		DPPH• Scavenging		$ABTS^{•+}$ Scavenging		$DMPD^{•+}$ Scavenging		O_2 Scavenging		Fe^{2+} Chelating	
	λ_{700}	r^2	λ_{450}	r^2	λ_{593}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
α-tocopherol	1.078±0.004	0.9424	1.375±0.003	0.9898	1.769±0.008	0.9383	13.58	0.9901	6.18	0.9688	57.75	0.9648	31.50	0.9748	19.80	0.9373
Trolox	1.088±0.007	0.9844	1.982±0.006	0.9711	2.505±0.008	0.9441	11.01	0.9811	7.14	0.9788	22.35	0.9830	18.73	0.9277	7.96	0.9011
EDTA							-	-	-		-		-		1.86	0.9972
C	0.167±0.003	0.9388	0.303±0.004	0.9915	0.615±0.007	0.9717	99.01	0.9560	53.30	0.9923	28.07	0.9438	33.01	0.9205	53.44	0.9462
PC	0.186±0.005	0.9109	0.248±0.007	0.9689	0.697±0.006	0.9889	77.11	0.9919	49.50	0.9511	36.47	0.9385	63.01	0.9366	20.38	0.9628
P+1% GTP	0.510±0.007	0.9399	0.293±0.007	0.9616	0.528±0.006	0.9636	98.85	0.9872	43.31	0.9318	25.66	0.9952	36.47	0.9942	77.01	0.9878
P+2% GTP	0.564±0.006	0.9755	0.470±0.008	0.9528	0.469±0.010	0.9480	63.02	0.9660	31.50	0.9006	27.72	0.9910	23.10	0.9848	53.37	0.9511
GTP	1.426±0.006	0.9292	0.994±0.006	0.9770	1.601±0.011	0.9101	43.31	0.9922	23.10	0.9889	11.55	0.9458	19.25	0.9782	23.14	0.9318

Table 5. *Total phenolics and flavonoids contents of yogurt samples and GTP as gallic acid equivalent (GAE) and quercetin equivalent (QE)*

Antioxidants	Total Phenolics (µg GAE)	Total Flavonoids (µg QE)
C	2.13	2.23
PC	7.86	3.30
P+1% GTP	26.68	6.01
P+2% GTP	40.18	7.76
GTP	1275	9.30

C: *Control (probiotic free and GTP free);* ***PC:*** *with probiotic and GTP free;* ***P+1% GTP:*** *probiotic + 1% GTP);* ***P+2% GTP:*** *probiotic + 2% GTP;* ***GTP:*** *Green tea powder*

DISCUSSION

To obtain GTP, harvested green tea leaves were dried, ground and sifted. For this reason, the dryness and protein ratio of GTP is very high. Increasing the GTP level increased total solids (between 12.37 and 14.11%) and protein (between 3.36 and 4.03%) contents of all samples. The initial and final pH values showed that the pH values of the yogurt samples were higher in the samples that had been supplemented with higher levels of GTP *(Table 2)*. The lactic acid produced by Lactobacilli inhibits the growth of other organisms and lowers the pH of the product in these products. *L. acidophilus* grows in low pH (<3.5), anaerobic conditions and undergoes fermentation only [27]. Acidity was found to be higher in yogurt containing *L. acidophius* than in control. The increase in pH may have resulted from the presence of various basic compounds in the GTP. A similar result was found by Najgebauer-Lejko [2]. Conversely, lower pH values were found for tea infusion-supplemented yogurts, but the level of fortification had little effect on that parameter Najgebauer-Lejko [7]. The pH values of the yogurt samples supplemented with GTP were higher than the average value determined for the other yogurts. The result obtained here differed from the study of green tea infusions by Najgebauer-Lejko et al.[7]. The differences between the initial and final pH values decreased with increasing concentrations of GTP *(Table 2)*. During the storage period, the mean acidity values were 0.74%, 0.78%, 0.84%, and 0.80% lactic acid on the 1st, 7th, 14th and 21st days, respectively *(Table 2)*. The mean syneresis values were 10.8, 10.7, 10.2, and 9.2 mL/25 g for the C, PC, P+1% GTP, and P+2% GTP samples, respectively. Syneresis decreased with the increase of dry matter in the yogurt samples. A similar result was also reported by Cakmakci et al.[28]. *L. acidophilus* had no significant effect on the composition of yogurt samples *(Table 1, Table 2)*. The lowest syneresis value was observed on the 14th of storage *(Table 2)*.

The increasing level of GTP used in the production of probiotic samples increased the number of *L. acidophilus.* As it can be understood from these results, the increase of GTP supplementation has further stimulated the development of *L. acidophilus.* This could be due to the fact that the GTP promoted the growth of *L. acidophilus*. Indeed, Lee et al.[29] reported that tea phenolics had significant effects on the intestinal environment probably by acting as metabolic prebiotics. Similarly, Ankolekar et al.[10] and Zhao and Shah [9] reported that green tea extracts containing *L. acidophilus* did not inhibit the growth of beneficial LAB. In another study, green tea extracts were found to permitted the survival of the selected probiotic strains better than the salt solution López de Lacey [30]. It can make an important contribution to know the effect of green tea on *L. acidophilus*, in terms of its survival to gastric and intestinal conditions, its adhesion properties, and antagonisms with pathogens. Also, as the GTP rate added in yogurt production increases, these effects may have increased.

Use of *L. acidophilus* alone in yogurt production (no GTP) strain lost viability and decay to <4 log cfu/g in the 28th day. The *L. acidophilus* count was high up to the 7th day, as previously also found by Turgut and Cakmakci [31]. However, the *L. acidophilus* count tended to decrease after the 7th day of storage. Nevertheless, it maintained its probiotic properties (>10^6 cfu/g) [4,5] until the 14th day of storage. It is thought that the initial inoculum level can be increased

Table 6. *Sensory characteristics of yogurt samples during storage (score 1: poor; 9: excellent)*

Sensory Properties	Storage Period (days)	Types of Yogurt			
		C	PC	P+1% GTP	P+2% GTP
Colour & appearance	1	8.43 ± 0.53^{b}	8.71 ± 0.49^{b}	6.33 ± 1.63^{a}	6.83 ± 1.60^{a}
	7	8.56 ± 0.53^{b}	8.78 ± 0.44^{b}	7.50 ± 1.06^{a}	8.00 ± 1.00^{ab}
	14	8.50 ± 0.58^{b}	8.75 ± 0.50^{b}	7.50 ± 0.58^{ab}	6.75 ± 1.50^{a}
	21	8.33 ± 0.58^{bc}	9.00 ± 0.00^{c}	7.50 ± 0.50^{ab}	6.50 ± 1.32^{a}
Odour	1	$8.42\pm0.79^{b,A}$	$8.57\pm0.79^{b,A}$	$5.50\pm2.07^{a,A}$	$5.83\pm2.14^{a,A}$
	7	$8.22\pm0.67^{a,A}$	$8.22\pm0.67^{a,A}$	$7.89\pm0.78^{a,B}$	$8.00\pm0.87^{a,B}$
	14	$8.00\pm0.00^{a,A}$	$8.25\pm0.50^{a,A}$	$7.75\pm1.41^{a,B}$	$7.00\pm1.41^{a,AB}$
	21	$7.67\pm0.58^{a,A}$	$8.00\pm0.00^{a,A}$	$8.5\pm0.5^{a,B}$	$7.27\pm1.1^{a,AB}$
Flavour	1	$8.57\pm0.53^{b,B}$	$9.00\pm0.00^{b,C}$	$4.33\pm2.07^{a,A}$	$4.33\pm2.07^{a,A}$
	7	$7.78\pm0.67^{a,AB}$	$8.06\pm0.88^{a,AB}$	$7.63\pm0.48^{a,B}$	$8.30\pm0.80^{a,B}$
	14	$8.25\pm0.50^{ab,B}$	$8.62\pm0.48^{b,BC}$	$7.50\pm0.58^{a,B}$	$7.75\pm0.87^{ab,B}$
	21	$7.33\pm0.29^{a,A}$	$7.57\pm0.51^{a,A}$	$8.23\pm0.68^{a,B}$	$7.00\pm1.00^{a,B}$
Sourness	1	8.43 ± 0.53^{B}	8.57 ± 0.53^{A}	7.33 ± 1.97^{A}	6.50 ± 2.59^{A}
	7	7.28 ± 0.44^{A}	7.44 ± 0.68^{A}	7.44 ± 1.72^{A}	6.92 ± 2.28^{A}
	14	7.25 ± 0.96^{A}	7.50 ± 1.73^{A}	6.50 ± 1.73^{A}	6.42 ± 1.65^{A}
	21	7.33 ± 0.58^{A}	8.17 ± 0.76^{A}	8.17 ± 0.76^{A}	7.00 ± 0.00^{A}
General acceptability	1	$8.00\pm0.82^{b,A}$	$8.14\pm0.90^{b,A}$	$5.83\pm1.94^{a,A}$	$4.83\pm2.14^{a,A}$
	7	$7.67\pm0.50^{a,A}$	$8.06\pm0.88^{a,A}$	$7.67\pm0.75^{a,B}$	$8.31\pm0.58^{a,B}$
	14	$8.00\pm0.00^{ab,A}$	$8.55\pm0.53^{b,A}$	$7.75\pm0.50^{ab,B}$	$7.30\pm0.89^{a,B}$
	21	$7.50\pm0.50^{ab,A}$	$8.00\pm0.00^{b,A}$	$8.40\pm0.66^{b,B}$	$6.80\pm0.72^{a,B}$

C: *Control (probiotic free and GTP free);* ***PC:*** *with probiotic and GTP free;* ***P+1% GTP:*** *probiotic + 1% GTP);* ***P+2% GTP:*** *probiotic + 2% GTP*
Horizontal column (a-c): *differences between yogurt types, P<0.05;* ***Vertical column (A-C):*** *differences between storage period, P<0.05*

to maintain the number of *L. acidophilus* required for probiotic properties for a longer time.

Najgebauer-Lejko et al.[7] and Ankolekar et al.[10] also stated that tea polypenols have antimicrobial effects against many microorganisms including pathogens, but these compounds do not affect the development of lactic acid bacteria. The highest average *L. bulgaricus* and *S. thermophilus* counts were determined in C samples. However, the average counts of *L. bulgaricus* and *S. thermophilus* were higher than 7.0 and 8.0 log cfu/g in other yogurt groups, respectively. On the other hand, all yogurt samples maintained high levels of the starter bacteria during the 4-week cold storage. However, there was a slight decrease in the count of starter bacteria during last 2 week of storage. This decline could be due to the increase in organic acid production. Similarly, it was reported in several studies that the accumulation of organic acids as a result of growth and fermentation is an important factor in the loss of cell viability [4,32]. Yeast and mold were not counted (<2 log cfu/g) in any yogurt sample during storage periods. This result is effective in extending the shelf life of yogurt. Because the shelf life of yogurt depends on the hygienic conditions during processing and packaging [33].

Various plants are sources of functional food components. One of the most important functions of these compounds is their antioxidant effect, which increases the importance of plants. Our study supplies valuable results on the antioxidant capacity of C, PC, P+1% GTP, P+2% GTP and GTP as indicated by several bioanalytical methods including measurements including ferric ions (Fe^{3+}), Cupric ions (Cu^{2+}) and Fe^{3+}-TPTZ reducing abilities, the DPPH, ABTS, DMPD and $O_2^{\bullet-}$ radical scavenging activities, the ferrous ions (Fe^{2+})-chelating activity, and the total phenolic and flavonoid contents.

Reducing power of bioactive compounds or food components reflects the electron-donating capacity and is associated with antioxidant activity [34]. The reducing ability of food or plant materials can be determined by means of the direct reduction of Fe^{3+} to Fe^{2+}. The Fe^{3+} reducing capacity of the yogurt samples, and standard antioxidants increased consistently with increasing concentrations of the samples. The Fe^{3+}-reducing capacity of the yogurt samples and both standards (as absorbance values) demonstrated the following order: GTP > α-tocopherol ≈ trolox > P+2% GTP > P+1% GTP > PC > C) at the same concentration (30 µg/mL). The results proved that yogurt samples had marked Fe^{3+}-reducing ability. In this method, the reducing capacity of food constituents measure direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. Addition of free Fe^{3+} to the reduced product leads to the formation of the intense Perl's Prussian blue complex, $Fe_4[Fe(CN\text{-})_6]_3$, which has a strong absorbance at 700 nm [34].

The Cu^{2+} reducing capacity of the yogurt samples and standard reducing agents at the same concentration (30 µg/mL) demonstrated the following order: trolox > α-tocopherol > GTP > P+2% GTP > C ≈ P+1% GTP > PC. The results clearly showed that cupric ion (Cu^{2+})-reducing ability was similar to the ferric ion (Fe^{3+})-reducing ability. Cu^{2+} reducing assays are based on the reduction of Cu^{2+} to Cu^{+} by the combined action of all antioxidants or reducing in aqueous-ethanolic medium (pH 7.0) in the presence of neocuproine food constituents yielding a Cu^{+}-complexes with maximum absorption peak at 450 nm.

According to the results obtained from FRAP (Fe^{3+}-TPTZ) assay *(Table 4)*, the reducing power of yogurt samples, and standard antioxidants decreased in the following order: trolox > α-tocopherol > GTP > PC ≈ C > P+1% GTP) ≈ P+2% GTP. In this method, higher absorbance values indicate a greater capacity to reduce the Fe^{3+}-TPTZ complex. FRAP values are calculated by measuring the absorbance increase at 593 nm and relating it to a ferrous ions standard solution or to an antioxidant standard solution. The change in absorbance is proportional to the combined FRAP value of the antioxidants in the food constitutes [35].

Free radical chain reactions are widely accepted as a common mechanism of lipid peroxidation. Radical-scavenging compounds may directly react with and quench peroxide radicals to terminate the peroxidation chain reactions and protect the quality and stability of foods [36]. The total radical-scavenging capacities of the yogurt samples and GTP were analyzed and compared to those of α-tocopherol and trolox using the DPPH·, $ABTS^{\cdot+}$, $DMPD^{\cdot+}$ and $O_2^{\cdot-}$ radical scavenging methods. The IC_{50} values for samples in this analysis were α-tocopherol < trolox < GTP < P+2% GTP < P+1% GTP < PC < C. The results show that the concentration of $ABTS^{\cdot+}$ (P>0.01) decreases substantially due to the scavenging capacity of all samples.

Another assay that is very similar to the use of the $ABTS^{\cdot+}$ is the $DMPD^{\cdot+}$ assay. The UV-visible spectrum of $DMPD^{\cdot+}$ had a maximum absorbance at 505 nm. This assay is particularly suitable for hydrophilic antioxidants, but is less sensitive to hydrophobic bioactive compounds, while the opposite case applies for the other two tests. As in the previous both DPPH· and $ABTS^{\cdot+}$ radical scavenging methods, the samples efficiently scavenged the $DMPD^{\cdot+}$ radical in concentration-dependent manners (10-30 µg/mL). The IC_{50} values for the yogurt samples, GTP, and standards were calculated as GTP < trolox < P+1% GTP < P+2% GTP < C < PC < α-tocopherol *(Table 4)*.

Superoxide anion radicals ($O_2^{\cdot-}$) are biologically toxic and are deployed by the immune system to kill invading micro-organisms. Also, this radical species exhibits limited chemical reactivity, but can generate more dangerous species, including singled oxygen and hydroxyl radicals, which cause the peroxidation of lipids [27]. As shown in *Table 4*, the IC_{50} value for the $O_2^{\cdot-}$ radical-scavenging of the yogurt samples and GTP were found to be 33.01 µg/mL (r^2: 0.9205), 63.01 µg/mL (r^2: 0.9366), 36.47 µg/mL (r^2: 0.9385), 23.10 µg/mL (r^2: 0.9848), 19.25 µg/mL (r^2: 0.9782), respectively. On the other hand, the IC_{50} values for trolox and α-tocopherol were found to be 31.50 µg/mL (r^2: 0.9748) and 18.73 µg/mL (r^2: 0.9277), respectively. The $O_2^{\cdot-}$ scavenging effects of the samples and standards on the $O_2^{\cdot-}$ increased in the following order: Trolox ≈ GTP > P+2% GTP > α-tocopherol > C > P+1% GTP > PC. A lower value of the IC_{50} indicates a greater $O_2^{\cdot-}$ scavenging activity.

The yogurt samples and GTP were also effective in chelating metal ions. The IC_{50} values for the samples and standard metal chelator compounds including EDTA demonstrated the following order: EDTA > trolox > α-tocopherol > PC > GTP > P+2% GTP ≈ C > P+1% GTP. These results clearly show that the Fe^{2+} ion-chelating effect of the yogurt samples was similar to that of α-tocopherol but lower than that of trolox. Lower IC_{50} values indicate higher metal chelation capacity.

Polyphenols are a broad group of phytochemicals that have antioxidant properties. The total phenolic compounds in the yogurt samples demonstrated the following order: C < PC < P+1% GTP < P+2% GTP < GTP *(Table 5)*. Phenolic compounds are likely to contribute to the radical-scavenging activity of the GTP extracts [37]. Furthermore, the total flavonoid compounds in yogurt samples were determined as milligrams of quercetin equivalents (QE). As shown in *Table 5*, the total flavonoids in the samples demonstrated the following order: C < PC < P+1% GTP < P+2% GTP < GTP.

Sensory evaluation is the most important criterion for acceptance or rejection of a food [38-40]. The incorporation of GTP into the yogurt with *L. acidophilus* had a significant influence on the notes received in the sensory evaluation. Statistical analysis showed that significant differences were found between the C and PC samples and the GTP yogurts. According to the C and PC yogurt samples, the color scores of the yogurt samples with GTP added were found to be lower. Similar results were found in yogurt with kiwi marmalade, which has green color similar to GTP [41]. The GTP addition caused a significant change from the usual color of plain yogurt. In 21 days of storage, probiotic yogurt samples with 1% GTP and 2% GTP received similar scores. However, all yogurt samples received well-acceptable color and appearance scores *(Table 6)*. The panelists noted that the yogurt with *L. acidophilus* and 2% GTP had a markedly bitter and astringent taste during the storage. As a reason, it can be said that the bitter and astringent taste of GTP penetrates into the yogurt. Catechins are water-soluble, thus giving bitterness and astringency to green tea infusion. Modification of catechins is effective on the color, taste and aroma of teas [42]. For example, it has been reported that the conversion from ester catechins to non-ester catechins can reduce the bitterness and

firmness of green tea [42,43]. In particular, flavour scores in fresh yogurts with GTP were found to be very low *(Table 6)*. Thus, the increase in flavour scores in other periods can be attributed to some biochemical transformations of catechins and other phenolic compounds. In this research, compared to C, generally PC and P+1% GTP samples were more preferred by the sensory evaluation panelists during the storage period.

We concluded that GTP has antioxidant properties that could be attributed to the presence of phenolic and flavonoid compounds. A positive effect of GTP on *L. acidophilus* was observed that depended of the amount of GTP. GTP stimulated the probiotic activity. In conclusion, GTP could be successfully used as a functional additive for selected probiotic yogurts to enhance the health benefits of the yogurt. Addition of GTP in the manufacture of yogurt is recommended because GTP is a natural herbal product with a wide range of beneficial health and nutritional properties; this makes this yogurt a new functional food. The addition of GTP produced a new kind of probiotic yogurt that retained an acceptable quality during storage for 3 weeks. To our knowledge, this is the first study of the use of GTP for the supplementation of yogurt with *L. acidophilus*. Sensory properties are one of the most important factors in the acceptance of a new food product. When all the research results are taken into consideration, it can be said that probiotic yogurt with 1% GTP supplement can be consumed for 21 days.

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Conflict of Interest Statement

There are no conflicts of interest to declare.

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