jotaf
Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Eylül/September 2022, 19(3)
Başvuru/Received: 27/12/21
Kabul/Accepted: 27/04/22
DOI: 10.33462/jotaf.1038037

http://dergipark.gov.tr/jotaf
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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of Green Tea Extract Concentrations on Sourdough Bread with *Lactiplantibacillus plantarum*

Farklı Konsantrasyonlarda Yeşil Çay Ekstraktının *Lactiplantibacillus plantarum* İlaveli Ekşi Hamur Ekmeği Üzerine Etkisi

Zühal ALKAY[1*], Elif ERŞAHİN[2], Muhammed Zeki DURAK[3], Osman SAĞDIÇ[4]

Abstract

In this study, sourdough breads were made using *Lactiplantibacillus plantarum* starter isolated from traditional sourdoughs. The main objective of this study was the effect of green tea powder extracts (0.25%, 0.50%, 0.75% and 1.0%) on *Lactiplantibacillus plantarum* added sourdough bread. The dynamic rheological properties of the doughs were determined. Bread production was carried out and then texture analysis, specific volume, color analysis, DPPH and total phenolic analysis were performed. Frequency scanning test was used to determine viscoelastic properties. $G' > G''$ was found in all doughs prepared using *Lb. plantarum* strain. The fact that $G' > G''$ indicated that all sourdoughs exhibited elastic properties. It was observed that the hardness values of sourdough breads containing green tea powder extracts were lower compared to the control bread. The hardness of the other breads varied between 161.9-267.6 g. Bread containing green tea powder extract at 0.75% concentration had the lowest hardness value. It was observed that the specific volumes of the breads increased as the green tea powder extract concentration increased. The specific volumes values for the concentrations (0.25,0.50,0.75,1.0 and control) were 3.39,3.80,3.95,5.21 and 4.45 (mL g^{-1}), respectively. In the color analysis of the breads, it was noted that as the ratio of green tea powder extract increased, the density increased. Another important parameter in our study is DPPH and total phenolic analysis. DPPH values of breads changed from 0.54 mmol g^{-1} to 1.46 mmol g^{-1}. The antioxidant value increased as the concentration of green tea powder extract increased in sourdough breads. Sourdough bread containing 1.0% green tea powder extract had the highest antioxidant value, while control bread (0.54) had the lowest value. The total phenolic content (TPC) of sourdough breads made with green tea powder extract varied from 10.67 to 104.65 ug g^{-1}. In the results, as the concentration of green tea powder extracts increased, the total phenolic content increased. A linear relationship between DPPH and total phenolic content was found. As a result, it can be said that green tea powder extracts improve the quality of sourdough breads.

Keywords: Green tea powder extract, *Lb. plantarum*, Sourdough bread, Antioxidant activity, Rheology

[1*]**Sorumlu Yazar/Corresponding Author:** Zuhal Alkay, Department of Food Engineering, Yıldız Technical University, İstanbul, Turkey. E-mail: zuhalalkay21@hotmaiil.com OrcID: 0000-0002-2420-3369

[2] Elif Erşahin, Department of Food Engineering, Tekirdağ Namık Kemal University, Tekirdağ, Turkey. E-mail: elifersahinn@gmail.com OrcID: 0000-0001-8849-2852

[3]Muhammed Zeki Durak, Department of Food Engineering, Yıldız Technical University, İstanbul, Turkey. E-mail: mzdurak@yildiz.edu.tr OrcID: 0000-0001-7245-1116

[4]Osman Sağdıç, Department of Food Engineering, Yıldız Technical University, İstanbul, Turkey. E-mail: osagdic@yildiz.edu.tr OrcID: 0000-0002-2063-1462

Atıf/Citation: Alkay, Z., Erşahin, E., Durak, M.Z., Sağdıç, O. Effect of Green Tea Extracts Concentrations on Sourdough Bread *with Lactobacillus plantarum*. *Tekirdağ Ziraat Fakültesi Dergisi,* 19 (3), 610-619.

Öz

Bu çalışmada geleneksel ekşi hamurlardan izole edilmiş *Lactiplantibacillus plantarum* starteri kullanılarak ekşi hamur ekmekleri yapılmıştır. Bu çalışmanın ana amacı, yeşil çay tozu ekstraktlarının (%0,25, %0,50, %0,75 ve %1,0) *Lactiplantibacillus plantarum* ekşi mayalı ekmek üzerindeki etkisi olmuştur. Hamurların dinamik reolojik özelliği belirlenmiştir. Ekmek üretimi gerçekleştirildi ve daha sonra tekstür analizi, spesifik hacim, renk analizi, DPPH ve toplam fenolik analizi yapılmıştır. Viskoelastik özelliklerin belirlenmesi amacıyla frekans tarama testi kullanılmıştır. *Lb. plantarum* suşu kullanılarak hazırlanmış tüm hamurlarda $G' > G''$ olmuştur. $G' > G''$ olması tüm ekşi hamurların elastik özellik sergilediği söylenebilir. Yeşil çay tozu ekstraktlarını içeren ekşi hamur ekmeklerinin sertlik değerlerinin kontrol ekmeği ile kıyaslandığında düşük olduğu görülmüştür. Ekmeklerin sertlik diğerleri 161.9-267.6 g arasında değişim göstermiştir. En düşük sertlik değerine % 0.75 konsantrasyonda yeşil çay tozu ekstraktı içeren ekmek olmuştur. Ekmeklerde yeşil çay tozu ekstraktı konsantrasyonu arttıkça spesifik hacimlerinin arttığı gözlemlenmiştir. Spesifik hacimler değerleri konsantrasyonların (0.25,0.50,0.75,1.0 ve kontrol) sırasına paralel olarak 3.39,3.80,3.95,5.21 ve 4.45 (mL g^{-1}) olmuştur. Ekmeklerin renk analizinde yeşil çay tozu ekstraktının oranı arttıkça koyuluğun arttığı kaydedilmiştir. Çalışmamızda bir diğer önemli parametre DPPH ve toplam fenolik analizi olmaktadır. Ekmeklerin DPPH değerleri 0.54 mmol g^{-1}'den 1.46 mmol g^{-1}'e değişmiştir. Ekşi hamur ekmeklerinde yeşil çay tozu ekstraktının konsantrasyonu arttıkça antioksidan değerinde artış olmuştur. %1.0 yeşil çay tozu ekstresi içeren ekşi mayalı ekmek en yüksek antioksidan değerine sahipken, kontrol ekmeği (0.54) en düşük değere sahip olmuştur. Toplam fenolik değerleri ise gallik asit cinsinden verilmiştir. Yeşil çay tozu ekstraktı ile yapılan ekşi hamur ekmeklerinin toplam fenolik içeriği (TPC) 10.67'den 104.65'e ug g^{-1} değişmiştir. Sonuçlarda yeşil çay tozu ekstraktlarının konsantrasyonu arttıkça toplam fenolik oranı artmıştır. DPPH ile Toplam fenolik arasında doğrusal bir ilişki bulunmuştur. Sonuç olarak, yeşil çay tozu ekstraktlarının ekşi mayalı ekmeklerin kalitesini artırdığı söylenebilir.

Anahtar Kelimeler: Yeşil çay tozu ekstraktı, *Lb. plantarum*, Ekşi hamur ekmeği, Antioksidan aktivite, Reoloji

1. Introduction

In recent years, it is known that one of the oldest functional foods developed by humans is bread (Bajerska et al., 2010). In particular, it was stated that sourdough bread came first among these breads. It is known that sourdough is mostly used to improve the properties and nutrition of wheat, rye, and oat bakery products. It has also been stated that sourdough fermentation improves the volume, texture, and flavor of fermented products. Additionally, it has been reported that it increases the shelf life of bakery products by slowing down the stalling (Torrieri et al., 2014; Yu et al., 2019; Karimi et al., 2020). Lactic acid bacteria and yeasts play a role as the main microorganisms in sourdough. Among these microorganisms, LABs affect the granular structure of starch and make the dough more compact by affecting the sourdough. It also makes it softer with less elastic tissue. Thanks to this, it increases the gas holding capacity (Yu et al., 2019; Karimi et al., 2020). Recently, the addition of sourdough to bread and the use of green tea extract in fermented products have attracted great interest. This interest is based on the desirable flavors of tea extracts and their positive effects on health. Also, the antioxidant activity and antimicrobial effects of green tea extracts (GTE) and epigallocatechin-3-gallate (EGCG) are important for delaying chemical rancidity and controlling microbial spoilage in foods (Nikoo et al., 2018). In many studies, it has been emphasized that green tea has strong antioxidant properties (Karori et al., 2007; Ning et al, 2017). It has been reported which green tea powder holds almost all catechins of fresh tea leaves (Ning et al, 2017). Green tea catechin polyphenols exhibit antioxidant properties as free radical scavengers (Wiseman et al., 1997; Bajerska et al., 2010). This study was conducted because of the positive effects on the health of both sourdough and the preparation of baked products with green tea powder extract, and the properties are rich in antioxidants. Green tea leaves and fresh tea leaves have been reported to retain almost all catechins. Green tea powder is a rich source of cellulose, protein and vitamins as well as bioactive components and has been noted to be included in biscuits, ice cream and beverages (Ning et al., 2017).

The main purpose of our study is to examine the effect of green tea extracts at different concentrations on sourdough bread. For this, the viscoelastic properties of the doughs were determined. Then, the bread making stage was carried out and the antioxidant properties, texture and specific volumes of the breads were evaluated.2. Materials and Methods

2.1. Material

Matcha green tea powder was purchased from Turkey Çaykur Ltd. Also, all chemicals were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich. The bacterial strain used in the study was isolated from sourdough previously brought from Manisa/Kırkağaç province in Turkey. It was then used in the study after it was identified by PCR (Alkay, 2021).

2.2. Methods

2.2.1. Extracting green tea powder

50 grams of green tea powder were allowed to shake in 1000 mL distilled water at room temperature for 24 h in a shaker incubator. After the incubation, the filtrate was filtered through Whatman filter paper and left to freeze at -80 ^{o}C for 24 h. It was left to dry in the lyophilizer for 72 h after freezing. The lyophilized extract was stored for use in further analysis.

2.2.2. Preparing sourdough samples

Lactic acid bacteria was incubated at 37^{o} C for 24 h in 5 mL of MRS broth. After incubation, it was incubated in 45 mL MRS broth under the same conditions. Thus, gradual development was achieved. After the incubation, the cells were centrifuged (at 5000 rpm 10 min, 4^{o} C) and the cell pellet was sedimented. The supernatant in the collapsed cell pellet was removed and diluted with sterile distilled water. The resulting suspension was inoculated into the dough at 10^7-10^8 log CFU g^{-1}. It was then fermented in an air conditioning cabinet at 24 h 30 ^{o} C in the presence of 80% humidity (Axel et al., 2016).

2.2.3. Rheology of dough prepared with different extracts

The viscoelastic behavior of dough containing sourdough with green tea powder extracts was performed according to the method determined by Yılmaz et al. (2016). A rheometer (Anton Paar, MCR 302, Austria) was used. Briefly, frequency scanning test was used to determine the viscoelastic properties of dough samples. For this, the deformation occurring in the range of 0.1-100 Pa and at a constant angular velocity of 10 rad/s at 25°C was investigated.

2.3. Making sourdough bread and analysis of bread

2.3.1. Bread making

Bread doughs were prepared using sourdough and green tea powder extracts in different concentrations obtained after 24 hours of fermentation. In the preparation of the control bread, 200 g wheat flour, 100 g sourdough, 120 mL water, 4 g salt, and 4 g commercial yeast were used. Then sourdough bread samples were prepared with green tea extracts at 0.25%, 0.5%, 0.75%, and 1% (per 100 g of wheat flour). The ingredients were mixed until the optimum consistency. It was cut into 160 g pieces. It was then rested for 15 minutes at room temperature and shaped and fermented at 30 ºC and 80% humidity for 45 minutes. The dough was baked in a 200 ºC oven for 45 minutes after the fermentation period.

2.3.2. Texture analysis in bread

Texture Profile Analysis (TPA) of bread samples was performed using a texture analyzer (TA.HD Plus Stable Micro Systems Ltd., Surrey, England). The bread was sliced to be 1.25 cm thick and placed in the test unit with 2 slices on top of each other. The pre-test speed was set as 5.0 mm s^{-1}, test speed 1 mm s^{-1}, 30% suppression, trigger type automatic force 5 g, waiting time between first and second printing 5 s. The results were given as the average of 3 parallel measurements (Yildirim and Arici, 2019).

2.3.2. Specific Volume

The volume of bread prepared in bread baking containers was measured with a Bread Volume Measuring Device (Şimşek Labortecnic, Ankara) with a volume of 400 mL. In this method, the volume of bread samples was determined by using the displacement of rapeseed grains. The results are given in mL g^{-1}.

2.3.3. Color analysis

Color changes of the color of the crumb and crust bread slices prepared by adding different concentrations of green tea extract were measured using the CR-400 colorimeter device (Konica Minolta Inc., Japan). Results were expressed as L^* denotes whiteness (value 100), a^* red (positive value), and b^* yellow (positive value) (Xing et al., 2021).

2.3.4. Determination of DPPH radical scavenging activities

Analysis of antioxidant (DPPH) in breads was performed according to the method used.by Zhou et al. (2019). The absorbance of each sample was read in a spectrophotometer (UV-1601 Shimadzu Corp. Japan) at 517 nm. Results are expressed in mmol TE g^{-1} as equivalent Trolox.

2.3.5. Determination of total phenolic (TPC) contents

For the total phenolic analysis, the breads were ground after drying at 45 ºC for 24 hours. The samples were weighed 1.25 g and vortexed in 25 mL of methanol and then left horizontally in a shaker for 24 h. Samples were centrifuged at 5000 rpm for 10 minutes at the end of 24 hours and the supernatant was taken with a syringe and passed through 0.22 µm PTFE syringe filters (Whatman). 100 µL extract, 100 µL methanol, 100 µL of Folin-Ciocalteu reagent and 700 µL of Na_2CO_3 were taken and vortexed rapidly. It was then incubated at room temperature for 30 minutes in the dark. At the end of the incubation, the samples were centrifuged again (8000 rpm, 3 minutes) and their supernatants were taken and read at 735 nm in the spectrophotometer. Results were given in gallic acid equivalent (µg GAE g^{-1}) (Venturi et al., 2019).

2.3.6. Statistical analysis

One-way analysis of variance (ANOVA) with Tukey's test was used to determine the difference between strains depending on the measured characteristics. Minitab version 17.3.1 (Minitab, Inc., State College PA, USA) and JMP version 9 were used.

3. Result and Discussion

3.1. Determination of the rheology of dough Prepared by Green Tea Extract

The viscoelastic properties of dough prepared by extraction of green tea powder were given in *Figure 1.*

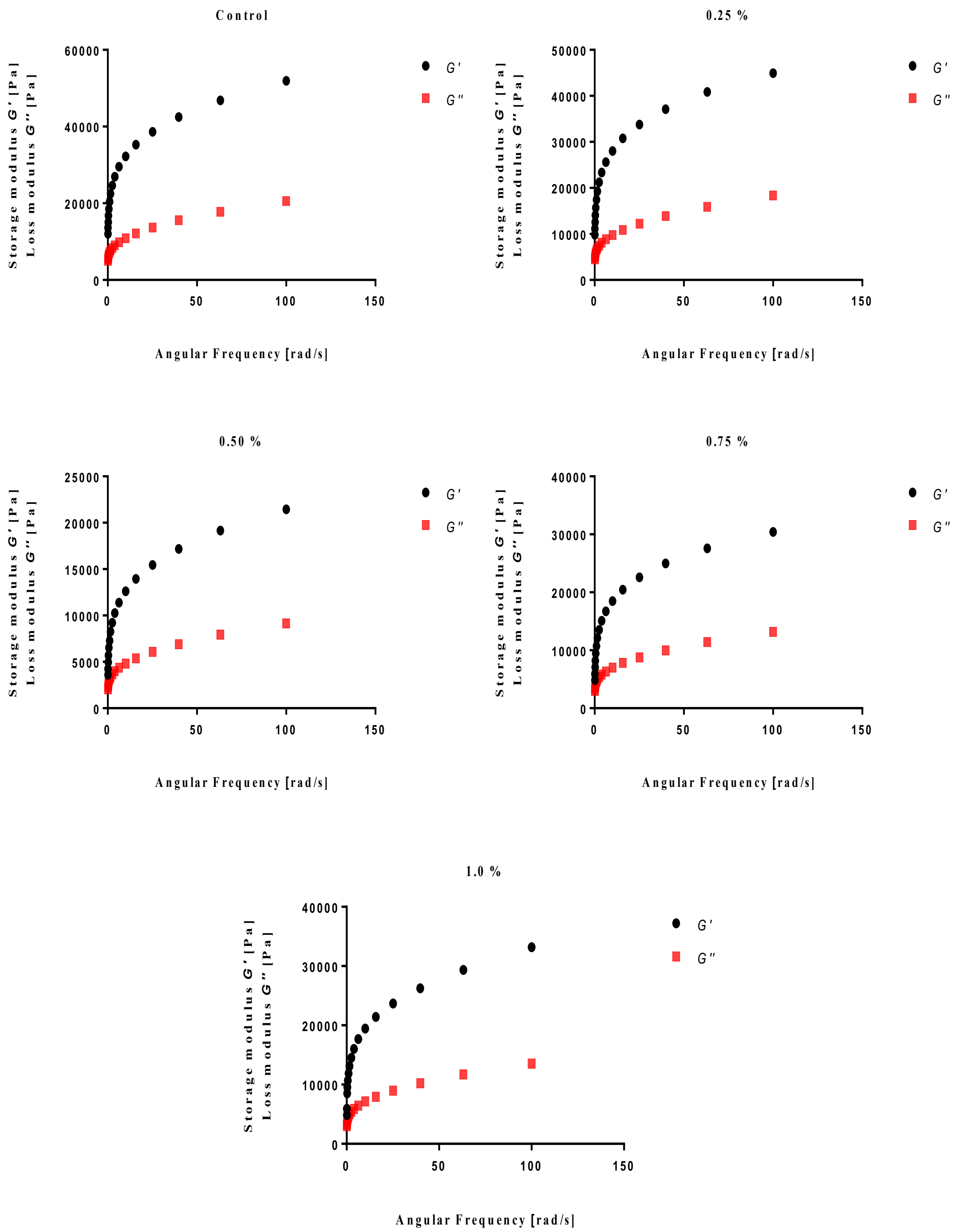

Figure 1. Rheograms of sourdough made with green tea extract in different concentrations

Extracts of different concentrations were used to be 0.25%, 0.50%, 0.75% and 1.0%. It was observed that the G' values of the prepared doughs were higher than the G'' values in the dynamic rheological properties. Since the elastic modulus (G') is higher than the viscous modulus (G''), it can be said that the elastic property of the dough is dominant (Yılmaz et al., 2017). It is known that dough rheology is a good indicator of dough mixing, sheeting, baking performance. Dough expansion and bread texture depend on the rheological properties of dough. It has been reported that these properties stem from gluten and starch components. It has been stated that by adding phenolic components to the dough, phenolic compounds interact with gluten and starch and this affects dough rheology. Also, phenolic antioxidants can establish the link by interacting with free radicals on gluten while mixing (Han and Koh, 2011a, b; Jackson and Hoseney, 1986; Schroeder, 1976; Xu et al., 2019).

In our results, green tea extraction caused softening of sourdough. Studies have shown that green tea extract has a high content of catechin, which acts as a reducing agent that causes SS bonds to turn into SH bonds. Therefore, the gluten web viscoelasticity also changes. Valková et al., (2020) stated that catechins can reduce the intermolecular SS bonds of gluten and the size of gluten polymers. As a result, it is assumed that the dough elasticity decreases, and the viscosity becomes soft.

3.2. Determination of textural properties and color measurements of sourdough breads

The bread crumb hardness is an important quality parameter and corresponds to the texture evaluated sensorially (Onacik-Gür et al., 2022). In addition, crumb hardness is an indicator of bread staling and is negatively associated with bread quality (Wang et al., 2007; Ning et al., 2017) and it has been noted that it plays an important role in the taste and consumer acceptance of bread, which is usually based on initial gelatinization (Zain et al., 2022). Studies have reported that the inclusion of green tea in bread increases the hardness, stickiness and bitterness of the bread. It was emphasized that when the amount of green tea extract added was increased, a decrease in brightness occurred (Ning et al., 2017; Zain et al., 2022). In our study, properties such as firmness, chewiness, flexibility, stickiness and specific volume of sourdough breads baked with different green tea extract concentrations were evaluated *(Table 1).* For the determination of texture profile analysis parameters, a tissue analyzer 36 (SMS TA.XT2 Plus, UK) with a 5 mm load cell and a 36 mm diameter cylindrical compression probe were used. In our data, sourdough bread containing 0.75% green tea extract had the lowest hardness value, while the control sourdough bread (without green tea extract) had the highest value. The springiness and cohesiveness values of sourdough bread containing 0.50% tea extract were higher compared to other breads. There was no significant difference between the cohesiveness values of sourdough breads.

Table 1 Textural properties of sourdoughs[1]

Bread Code	Hardness (g)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience	Specific volume (mL g^{-1})
0.25 %	253.71±6.48^{A}	1.32±0.47^{A}	0.89±0.02^{A}	224.66±0.03^{A}	297.46±106.4^{A}	0.59±0.03^{A}	3.39^{D}
0.50 %	235.95±7.32^{A}	1.48±0.08^{A}	0.89±0.01^{A}	208.61±2.12^{A}	264.20±77.48^{A}	0.61±0.05^{A}	3.80^{C}
0.75 %	161.97±16.68^{B}	1.05±0.09^{A}	0.87±0.01^{A}	141.45±12.41^{B}	148.29±0.02^{A}	0.57±0.00^{A}	3.95^{C}
1.0 %	264.38±2.26^{A}	1.17±0.26^{A}	0.87±0.01^{A}	231.69±3.09^{A}	314.5±3.45^{A}	0.58±0.00^{A}	5.21^{A}
Control	267.65±9.01^{A}	1.14±0.22^{A}	0.89±0.00^{A}	231.28±1.46^{A}	215.98±19.6^{A}	0.58±0.00^{A}	4.45^{B}

[1]Within the column, different superscript uppercase letters show differences between the strains.

It was observed that a green tea concentration of 1.0 % resulted in greater hardness in bread. Ning et al. (2017) reported that with the increase of GTE powder, the dough became stronger. Another study was conducted by Wang and Zhou (2004), and they stated that there were interactions between GTE catechins and wheat proteins during the bread-making process through hydrogen bonding. They stated that this causes a harder texture in bread. As seen in our study, the hardness of sourdough breads containing 0.25 %, 0.50 %, and 0.75 % green tea extracts was softer in terms of texture. It can be thought that as a result of the faster growth of bacteria and yeast with SPH, it increases gas production in bread and causes a more flexible bread texture (Karimi et al., 2020). In our results, it was seen that the specific volume increased with the increase of tea extraction and the values were higher than the control sourdough and there was significant difference in specific volume between the control and the sourdough breads with added green tea extract ($P < 0.05$). Ning et al., (2019) emphasized that there was no significant difference between the breads to which they added green tea extract at different concentrations and the control bread. However, it can be said that the specific volume of the control bread is higher than the other samples.

Figure 2. Image of bread types

After texture, another important parameter for bread is color. In our study, color analysis of bread was determined by determining *L*, a*, b** values *(Figure 3).* It was observed that the color became darker with the increase in green tea extracts. Ning et al., (2017) stated in their study that with the increase in the amount of green tea powder, the darkness of bread increased. They attributed the increase in color value to Maillard reaction, non-enzymatic browning, and conversion of heat-sensitive compounds. Onacik-Gür et al., (2022), studied the effect of green tea extract on rye bread. Bread crust and crumb color parameters were evaluated. They noted that the doughs with the addition of lactic acid were lighter in color when compared to the doughs with the addition of GTE. The addition of 0.5% GTE was found to darken the crust color. These parameters showed similar results with our study.

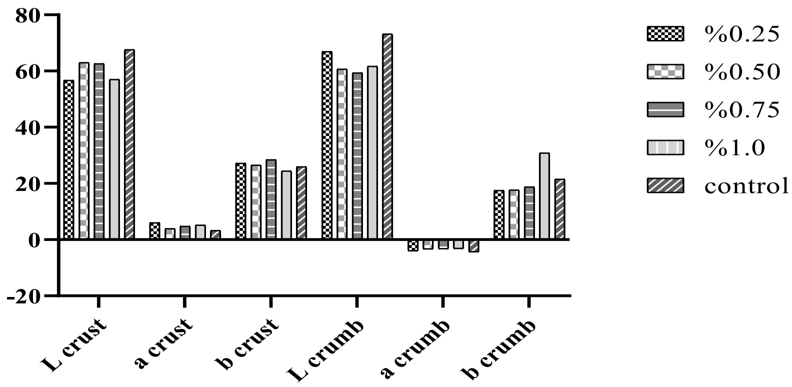

Figure 3. Color measurements of bread types containing different concentrations of green tea extract

3.3. DPPH and Total phenolic contents in the sourdough breads

It has been reported by many researchers that green tea contains antioxidant compounds (Karori et al., 2007; Ning et al., 2017). One of the important biological properties of polyphenolic compounds is antioxidant activity. Bread scavenges free radicals during baking. It is a mechanism for polyphenolic compounds to inhibit acrylamide formation in bread (Gębski et al., 2019; Ebbeling et al., 2018; Zain et al., 2022). Therefore, another important parameter in our study is to determine the antioxidant values of breads. Antioxidant properties of sourdough breads containing green tea extract in different concentrations are given in *Figure 4*. It has been observed that the addition of green tea extracts greatly increases the antioxidant properties of sourdough breads. DPPH values ranged from 0.54 to 1.46 mmol TE g^{-1}. Sourdough bread with 1.0% green tea powder extract had the highest antioxidant value, while control bread (0.54) had the lowest value. As the extraction amount of green tea powder increased, DPPH values increased. This may be due to green tea catechins. Recent studies have focused on the free radical scavenging ability of tea catechins (Bajerska et al., 2010). Besides, it is known that a strong relationship is established between the structure and activity of different compounds thanks to the DPPH method (Ning et al., 2017). Pan et al., (2022) examined the effect of catechins on wheat bread quality in their study. In the study, the antioxidant activity values in the bread crumbs in the control breads were 0.74 mg TE/g, while the antioxidant value in the catechin-containing bread crumbs was 14.36-19.82 mg TE/g. It has been stated that catechins increase the antioxidant activity of breads. In another study, Ning et al., (2017) reported that the antioxidant value of wheat flour pan bread with the addition of green tea powder was higher. Our results were similar.

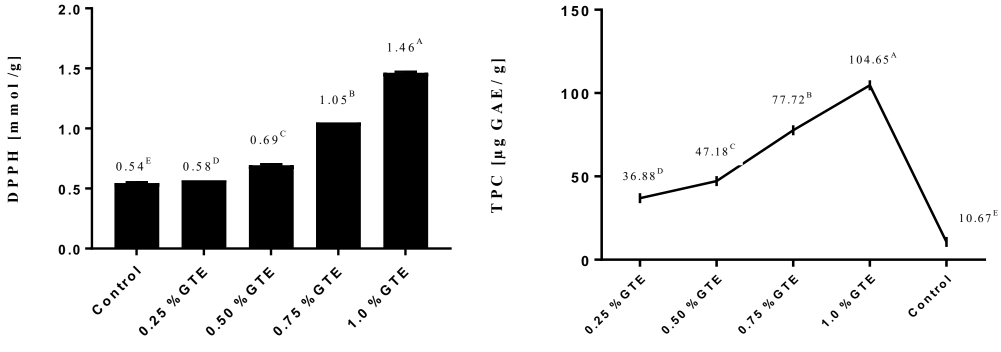

Figure 4. DPPH and total phenolic compounds of bread types

Another parameter important for green tea is total phenolic. In this study, the total phenolic content (TPC) of sourdough breads prepared with extracts of green tea powder changed from 10.67 to 104.65 (µg GAE g^{-1}). There was a linear correlation between DPPH values and TPC, and as the amount of extract of green tea powder increased, TPC increased. Ivanišová et al., (2018) enriched biscuit samples with green tea powder in their study. The total phenolic content of biscuit samples containing 1% green tea was 0.37 mg GAE/g, while the total phenolic content of the control sample was 0.09 mg GAE/g. The authors noted that tea leaves caused a significant increase in total phenolic concentrations.

4. Conclusion

This study examines the effect of different concentrations of green tea powder extract on *Lb plantarum* sourdough bread. Lactic acid bacteria and green tea powder extracts provided elastic properties to all doughs. Also, it was observed that the bread with 1.0% green tea powder extraction amount was harder, the inside color of the bread was greener, and the antioxidant activity was higher among all bread types. It can be said that as the concentration of green tea powder extract increases, the acceptability of breads decreases, and therefore these parameters should be optimized in future studies.

Acknowledgments

We thank Hakan Doğan and Danedolu team for their assistance in providing the sourdough samples

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