Selcuk Journal of Agriculture and Food Sciences

https://dergipark.org.tr/tr/pub/selcukjafsci

Research Article

(2026) 40 (1), 87-101 DOI:10.15316/selcukjafsci.1743642 e-ISSN: 2458-8377 Selcuk J Agr Food Sci

Utilizations Of Sourdough And Orange Peel Powder for The Production of Cookies with Increased Bioactive Value

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HIGHLIGHTS

- This study aims to investigate the potential use of both whole wheat flour sourdough and orange peel powder at concentrations of 1%, 3%, and 5% in cookie formulations.
- The study showed that both sourdough addition and orange peel powder caused significant changes in the moisture and fat content of cookie varieties compared to control samples, but there was no difference in ash content.
- As the orange peel concentration increased, the antioxidant content (DPPH and FRAP) of the cookie samples increased significantly.
- The addition of sourdough and orange peel powder also improved the color, physical, and textural properties of the cookie samples.

Abstract

The fortification of bakery products using orange peel powder—an underutilized fruit byproduct—and sourdough represents a novel approach to develop functional foods, as sourdough fermentation, driven by Lactic Acid Bacteria (LAB) and yeasts, enhances product quality, while orange peel powder contributes to improved flavor, texture, and bioactive properties. This study aimed to evaluate the impact of incorporating spontaneous sourdough (20% flour basis) and orange peel powder (1%, 3%, and 5% flour basis) as a dietary fiber source in cookie formulation on the nutritional composition, bioactive properties, sensory profile, and overall quality of the final product. Our results showed that both sourdough addition and orange peel powder caused significant changes ($p < 0.05$) in moisture (from 4.24 to 7.36) and fat content of cookie varieties compared to control samples, but there was no difference in ash content ($p > 0.05$). As the orange peel concentration increased, the antioxidant content (DPPH and FRAP) of the cookie samples significantly increased ($p < 0.05$). Sourdough and orange peel powder addition also improved the color, physical and textural properties of the cookie

Citation: Alkay, Z., Doğan, Ş. N., Tunçil, S., & Tunçil, Y. E. (2026). Utilizations Of Sourdough And Orange Peel Powder for The Production of Cookies with Increased Bioactive Value. *Selcuk Journal of Agriculture and Food Sciences*, 40 (1), 87-101. https://doi.org/10.15316/selcukjafsci.1743642

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Received date: 16/07/2025

Accepted date: 21/01/2026

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samples. Overall acceptability of sourdough and orange peel fortified cookies was not significantly different than control samples, indicating that range peel powder can be incorporated into cookie formulation to increase its bioactive content (mainly antioxidant capacity) up to 5% without significantly impacting the sensory attributes. Overall, these results show that cookies with increased bioactive value can be produced by incorporating sourdough and orange peel powder into formulation.

Keywords: Spontaneous sourdough; Whole wheat flour; Orange peel powder; Nutritional; Bioactive and technological properties

1. Introduction

Cookies are one of the most popular snacks consumed globally, with a significant presence in many cultures. The term "biscuits" or "cookies" typically refers to products made by baking soft wheat flour, sugar, and fat, along with minor ingredients like milk, salt, sweeteners, and aeration agents (Devi and Khatkar 2016). However, some variations consist of just butter, sugar, and flour (Krajewska and Dziki 2023). Several factors contribute to the universal appeal of cookies, including their wide consumption base, easy availability, reasonable price, good nutritional quality, diverse and pleasant flavors, and long shelf life. Compared to other bakery products, cookies occupy a dominant position in both production and consumption, leading to a significant global increase in their market share (Blanco Canalis et al. 2017). Cookie consumption is estimated to be approximately 13 kg per person annually worldwide (Blanco Canalis et al. 2017). According to the 2023 market research report, the Biscuit/Cookie market is projected to reach USD 104 billion globally in 2023, while this budget is expected to grow to USD 148.56 billion by 2030. Therefore, it is expected to exhibit a Compound Annual Growth Rate (CAGR) of 5.18% during the forecast period (Ali et al. 2024).

In the modern food industry, wheat is the dominant grain used for cookie production (Olojede et al. 2023). Although refined flour is widely used, there has been growing interest in using whole grain flours due to their higher nutritional contents (increased fiber, phenolics, and mineral levels). Despite this, lower processability and reduced sensory attributes of whole grain flour limit their utilization in cookie production (Alioglu and Özülkü 2024). To overcome these challenges, alternative strategies should be developed. One such alternative is the inclusion of sourdough in cookie formulations, which could add functionality to the final product.

Sourdough fermentation, recognized as one of the oldest food biotechnology approaches, is a traditional method used to increase the nutritional value, functionality, and processability of grains (Mohammadi-Kouchesfahani et al. 2019). Although sourdough fermentation has primarily been studied for its impact on the sensory, textural and shelf-life properties of bakery products (Gobbetti et al. 2014), it has recently gained great attention of both the scientific community and the food industry as a promising technology for developing innovative food products (including snack foods) with enhanced nutritional values (Maravić et al. 2024). In principle, sourdough fermentation represents the symbiotic interaction of various microorganisms, consisting of lactic acid bacteria (LAB) and yeasts (De Vuyst et al. 2023). During the fermentation process, protein and starch are hydrolysed, the pH is lowered and a more favorable environment for the growth of yeast is created. This leads to the release of amino acids during autolysis by yeasts, which further enhances LAB growth. Such interactions between microorganisms in sourdough for bakery products provide numerous benefits such as enhancing nutritional characteristics by making minerals and bioactive compounds bioavailable, improving the taste profile and lowering the glycemic index (Olojede et al. 2023). Additionally, metabolites produced during fermentation, such as organic acids and other bioactive compounds, can further improve the sensory and nutritional qualities of the product.

Moreover, research has shown that incorporating additional ingredients, such as orange peel, can further enhance the sensory properties and nutritional quality of snack foods. Orange peel, a byproduct of orange juice production, is typically discarded as waste, constituting approximately 50-60% of the processed fruit (Wilkins et al. 2007). Improper disposal of this waste can lead to environmental issues (Tahir et al. 2023). However, orange peel is rich in vitamins (especially vitamin C), minerals (e.g., iron, calcium, magnesium),

dietary fiber, and bioactive compounds like flavonoids, which are often found in higher concentrations in the peel than in other edible parts of the fruit (Rani et al. 2020). The high nutritional value and low cost of orange peel present an opportunity to incorporate it into various food products, including bakery goods, to improve both their nutritional and sensory profiles.

Although some studies have explored the use of orange peel flour in various food products, including bakery goods, research on the use of sourdough, particularly made from whole wheat flour, combined with orange peel flour in cookies is limited. Therefore, this study aims to investigate the potential of using both whole wheat flour sourdough and orange peel powder at 1%, 3%, and 5% concentrations in cookie formulations. The objective is to improve the taste, texture, nutritional value, and functional properties of cookies, thus contributing to healthier and more sustainable snack options.

2. Materials and Methods

Raw materials (whole wheat flour, powdered sugar, margarine, skimmed milk powder, salt, vanilla and baking powder, and orange) used in cookie production were purchased from local markets in Konya, Türkiye. For the orange peel powder, oranges were cut after being washed with tap water, followed by drying in a vacuum oven (Ecocell/EC 111, Germany) at 60 °C for 24 h. The dried samples were then ground with a coffee grinder and passed through 500 μm sieves, and stored at -20 °C until further use.

2.1. Preparation of sourdough and cookie formulation

Spontaneous fermentation of whole wheat sourdough was carried out by the following method: Whole wheat flour (187.5 g) was mixed with tap water (112.5 mL) to obtain a dough yield of 160 (dough yield = dough weight 100 flour weight^{-1}). The mixture was incubated at 30 °C for the first 24 h. On the second day, 30% of the fermented sourdough from the first batch was mixed with 168.5 g of whole wheat flour and 101.25 mL of water and used for backslopping, which was repeated every day for 4 consecutive days. Back-slopping was done until the pH became constant (approximately 3.5). Fermentation time was 24h for 4 days and incubated at 30 °C. Microbiological cultivation results, pH and TTA values are given in **Table 1**. For microbiological analysis, ten grams of sourdough were thoroughly homogenized with 90 mL of saline solution (FTS solution) containing 0.85% NaCl. Appropriate dilutions were then prepared and 20 μL aliquots were spread on Tryptic soy agar (Merck, Germany) plates using the spot method. These petri plates were then placed in an incubator at 30°C for 24–48 h and then colonies were counted. For yeast and mold enumeration, samples were plated on dichloran rose bengal chloramphenicol agar (DRBC, Merck, Germany) using the spread method and the petri dishes were incubated at temperatures between 25 for 5 days. For microbiological analysis, ten grams of sourdough were thoroughly homogenized with 90 mL of FTS solution containing 0.85% NaCl. Appropriate dilutions were then prepared and 20 μL aliquots were spread on Tryptic soy agar (Merck, Germany) plates using the spot method. These petri plates were then placed in an incubator at 30°C for 24–48 h and then colonies were counted. For yeast and mold enumeration, samples were plated on dichloran rose bengal chloramphenicol agar (DRBC, Merck, Germany) using the spread method and the petri dishes were incubated at temperatures between 25 and 30°C for a period of 4 to 5 days. Results are presented as logarithmic colony forming units per milliliter (log cfu g^{-1}). The pH analysis of the sourdough samples was conducted using an pH meter Inolab-pH meter (inoLab, Weilheim, Germany). The total titratable acidity (TTA) was determined through potentiometric titration employing 0.1 N NaOH until the pH reached 8.1, with the results expressed in lactic acid equivalents.

Table 1. Microbiological cultivation results (log cob g^{-1}), pH and TTA (%lactic acid) values of sourdough

Day	Total Mesophilic Aerobic Bacteria	Yeast	pH	TTA (% Lactic acid)
0	3.79±0.01	-	5.90±0.00	0.23±0.00
1	8.41±0.01	3.03±0.11	5.75±0.01	0.77±0.01
2	9.10±0.00	4.13±0.01	3.90±0.00	1.49±0.05
3	9.50±0.02	3.66±0.03	3.64±0.01	1.91±0.03

Results are expressed as mean± std.

In this study, 3 different test group cookies (**1SPK:** containing 1% orange peel powder and sourdough; **3SPK:** containing 3% orange peel powder and sourdough; **5SPK:** containing 5% orange peel powder and sourdough) and 5 different control cookies (**1C:** cookies made with 100% whole wheat flour and no sourdough; **2C:** cookies made with 100% whole wheat flour and sourdough; **1PKC:** control without sourdough and containing 1% orange peel powder; **3PKC:** control without sourdough and containing 3% orange peel powder; **5PKC:** control without sourdough and containing 5% orange peel powder) were produced. Sourdough (20%, based on flour weight) was used in the test samples and the flour type in sourdough and cookie ingredients were only whole wheat flour and cookie productions were carried out according to AACC Standard Method (No: 10-54.01). Modifications were made to this formulation and compared with the direct addition of whole wheat sourdough to the cookie formulation. The details of the formulation used in the study are given in **Table 2**. After all the raw materials were kneaded with a mixer for 5 min, the dough was rolled out to a thickness of 5.0 mm and shaped by cutting into 48.0 mm diameters. The cookie doughs were then baked in an oven (Vestel SF8401, Türkiye) at 180 ± 2 °C for 18 min. The samples were then kept at room temperature (approximately 2 h) to cool before performing the following analyses.

Table 2. Contents of cookie formulations

	Control groups *Kontrol Grupları*					Test groups *Test Grupları*		
	1C	2C	1PKC	3PKC	5PKC	1SPK	3SPK	5SPK
Whole wheat flour (g)	100	100	99	97	95	99	97	95
Sourdough (g)	-	20	-	-	-	20	20	20
Orange peel flour (g)	-	-	1	3	5	1	3	5
Powdered sugar (g)	40	40	40	40	40	40	40	40
Margarine (g)	40	40	40	40	40	40	40	40
Milk powder (g)	1	1	1	1	1	1	1	1
Salt (g)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Baking powder (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vanilla (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water (mL)	40	40	40	40	40	40	40	40

1C: Control (cookies made with 100% whole wheat flour, without sourdough), **2C:** (cookies made with 100% whole wheat flour, containing sourdough),
1PKC: (control without sourdough, containing 1% orange peel powder), **3PKC:** (control without sourdough, containing 3% orange peel powder),
5PKC: (control without sourdough, containing 5% orange peel powder), **1SPK:** (containing 1% orange peel powder and sourdough),
3SPK: (containing 3% orange peel powder and sourdough), **5SPK:** (5% orange peel powder and sourdough).

2.2. Proximate analysis

The approximate composition (moisture, fat, and ash content) of the cookie samples was determined and analyzed according to standard procedures (Olcay and Demir, 2020). Briefly, for moisture content, approximately 3 g of sample was weighed into containers brought to a constant weight. After being incubated at 102 °C (Nuve, KD200, Ankara, Turkey) for 3 hours, it was weighed again and the results were calculated. Crude fat content was determined using a Soxhlet system (SER 148; Velp Scientifica, Usmate, Italy) where hexane was utilized as a solvent (Olcay and Demir 2020). To determine the ash content, 3 g of samples were weighed into crucibles and burned in a muffle furnace (WiseTherm Digital Furnace, Wisd Laboratory Instruments) at 550°C until completely burned (AACC 1990).

2.3. Color measurements

Color analysis was measured using a Hunter colorimeter (Minolta Chroma Meter CR-400, Osaka, Japan). The color results of the cookie samples were measured at three different points. The color values of the cookies were recorded as L^* (lightness), a^* (redness) and b^* (yellowness) (Alkay et al. 2024).

2.3. Physical and textural parameters of cookies

For the diameter and thickness values, each cookie was measured using a digital micrometer (0.001 mm, Mitutoyo, Minoto-Ku, Tokyo, Japan) and the average values were calculated. The spreading rate of the cookie samples was calculated by dividing the cookie diameters by the thickness (Ertaş and Aslan 2020).

The hardness and fracturability of the cookie samples were measured using a texture analyzer (TA-TX2i, Stable Micro System, Surrey, England). After the calibration of the three point bend rig probe, a sample was placed on the table of the texture analyzer and the test was performed using the following conditions: load cell: 5 kg, pre-test speed: 0.1 mm s^{-1}, post-test speed: 10.0 mm s^{-1}, distance: 5 mm, trigger force: 50 g (Şirin et al. 2022; Alkay et al. 2024).

2.4. Total phenolic contents and antioxidant capacities

Extraction of phenolics

10 mL of acidified methanol solution (HCl/methanol/water, 1:80:10, v/v/v) was added on 1 gram of cookie samples. The mixture was then left in a shaking water bath (24 ± 1 °C) for 2 hours, followed by centrifugation (Awel centrifugation, France) at 3000 rpm for 10 minutes. The supernatant was then used in subsequent analyses (Total Phenolic contents, DPPH, FRAP) (Olcay and Demir 2020).

Total phenolic contents

To determine the phenolic content, briefly; 100 µL of supernatant was mixed and 1900 µL of pure water and 2.5 mL of Lowry C (prepared freshly as mixture (50 mL Lowry A + 1 mL Lowry B); Lowry A: 2% agueous Na_2CO_3 in 0. 1 M NaOH; Lowry B: 0.5% $CuSO_4$ in 1% $NaKC_4H_4O_6$ solution) were added. Then, it was kept at room temperature for 10 min. 250 µL of folin from Folin-ciocalteu reagent diluted with pure water at a ratio of 1:3 was then added. The mixture was kept in the dark for 30 min and reading was made at 760 nm in a spectrophotometer (Biochrom / Libra S22, Cambridge, England). The results were expressed as gallic acid equivalent (mg gallic acid per kg dry weight, GAE) (Vitali et al. 2009).

DPPH and FRAP analysis

Antioxidant capacities of the samples were determined using two different methods **1)** 2-2-Diphenyl-2-picrylhydrazyl (DPPH) method, and **2)** Ferric ion reducing antioxidant potential (FRAP) method, according to Olcay and Demir (2020) and Gao et al. (2000), respectively. Briefly; 100 µL of supernatant was taken and 900 µL of Tris HCl and 2 mL of DPPH solution were added. Then, it was kept at room temperature for 30 min. Reading was done at 517 nm in the spectrophotometer (Biochrom / Libra S22, Cambridge, England) device. The results were expressed as Trolox equivalent (µmol gallic acid per g dry weight, TE) (Olcay and Demir, 2020). FRAP antioxidant activity analysis solution was prepared by mixing 300 mmol/L acetate buffer, 10 mmol/L diammonium salt, 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 20 mmol L^{-1} $FeCl_3.6H_2O$ solution at a ratio of 10:1:1, respectively. 700 µL analysis solution was added to 50 µL of sample extract and kept in the dark for 5 min. Absorbance values were determined at 593 nm in a spectrophotometer (Biochrom / Libra S22, Cambridge, England). The results were expressed in trolox equivalent (Gao et al. 2000).

2.5. Sensory evaluation

Sensory analysis of cookie samples was performed by 9 untrained, healthy panelists aged between 21 and 32 years. Cookie samples were evaluated by panel members for color, taste, odor, appearance, fragility and overall acceptability. A hedonic scale ranging from one to five points was used for sensory evaluation of cookie samples (1: unacceptable, 5: acceptable).

2.6. Statistical analysis

Analyzes for cookie samples were performed in triplicate and the results obtained are given as mean±std. JMP statistical program (SAS Institute Inc., Cary, NC, USA) version 5.0.1 was used for statistical analysis. Means of main sources of variation were compared at $p < .05$. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA, USA) was used for data visualizations. The correlation analysis results of the total phenolic

and the antioxidant activity of cookies were expressed as Pearson's correlation coefficients by ORIGIN 2024 software (OriginLab Corporation).

3. Results and Discussion

3.1. Proximate composition of the cookie samples

The proximate composition (moisture, crude fat, ash) of the cookies are given in **Table 3.** The moisture contents of the cookie varieties varied between 4.24-7.36%. Although the moisture content in the samples containing sourdough (1SPK, 3SPK, 5SPK) was generally higher than the controls (1C, 2C, 1PKC, 3PKC, 5PKC), no statistical difference was observed ($p > 0.05$). The high moisture content of the cookie samples containing sourdough (1SPK, 3SPK, 5SPK) is consistent with the study conducted by Sahin et al. (2019) who reported 6.67-6.83%. These parameters may vary depending on the sourdough fermentation. The increase in moisture content may be due to mechanical changes in the flour, which may have a greater gas retention capacity that may reduce evaporation during baking (Olojede et al. 2023). This is also confirmed in previous studies that showed that heterofermentative lactic acid bacteria and yeasts present in sourdough may affect gas production (Abedfar and Sadeghi 2019). In addition, proteolytic and amylolytic activities, which cause increases in sourdough acidity, result in better gas retention capacity, expanded gluten network and increased porosity (Abedfar and Sadeghi 2019). Another important factor is the decrease in moisture content of the control samples (1PKC, 3PKC, 5PKC) as the orange peel powder content increases (**Table 3**). This decrease in our data is consistent with the study (biscuit enriched with pearl-millet and orange peels) conducted by Obafaye and Omoba (2018) who reported that the decrease in moisture content as the orange peel powder content increases. On the other hand, the moisture contents (4.24-5.80%) of the samples produced in this study were higher, while the fat contents (17.80-18.74%) were lower than those reported by Belose et al. (2021), who produced cookies with refined wheat flour and orange peel powder and reported the moisture content as 4.14-4.19% and the fat content as 23.06-25.86%. These differences can be attributed to both our use of sourdough and the type of flour used by Belose et al. (2021). Moisture loss or gain is one of the most important factors controlling the shelf life of foods, as it controls microbiological/enzymatic spoilage, drying/softening of food, condensation inside the packages and the development of mold as a result (Nwosu and Akubor 2018).

Another important parameter in terms of proximate composition was fat content. The fat contents of the cookie samples were between 17.80 – 20.03%, with the samples of 1SPK and 1PKC having the highest and lowest fat values, respectively (**Table 3**). Short-chain fatty acids produced by sourdough microorganisms (Longoria et al. 2020) could be a contributing factor to the highest fat value of 1SPK. Furthermore, sourdough addition could retain more fat in the structure, which is not released during the baking. Nutritionally important fats most likely form a complex with carbohydrates/proteins, which contribute to improving the storage time of baked goods. In addition, fat provides a lubrication effect that improves the mouthfeel, flavor and palatability of foods (Inyang et al. 2018; Olojede et al. 2023). When the data of our study were also evaluated in terms of orange peel powder, cookie varieties containing orange peel powder (1PKC) had lower fat content (17.80%, 18.74%, 18.51%, respectively) compared to the control sample (1C). This aligns with the findings of Nwosu et al. (2022) who reported that 16.78% exhibiting a lower fat content profile compared to the control biscuit sample made from wheat flour.

The ash values of the cookie samples varied between 1.88% and 2.23% and were found to be statistically insignificant ($p > 0.05$). The ash contents in the samples were not affected by both orange peel powder (fiber source) and sourdough ratio (enrichment type), but a partial increase in ash content was observed as the orange peel powder increased. This can be attributed to the fact that orange peel is rich in potassium, calcium, magnesium and phosphorus (Ramashia et al. 2024). The findings of the present study are consistent with those reported by Kopec et al. (2011), who indicated that the ash content of bread samples containing dietary fiber and sourdough ranged between 1.66% and 2.49%. However, when compared with the results of Maravić et al. (2022), who reported that the addition of 30% sourdough to cake formulations resulted in an ash content identical to that of the control samples (1.28%), the ash values obtained in this study were relatively higher (Olojede et al. 2023).

Table 3. Proximate composition of cookie samples

Samples *Örnekler*		Content		
		Moisture	Crude Fat	Ash
Control Groups *Kontrol Grupları*	1C	5.95±0.09^{AB}	18.86±0.05^{B}	2.10±0.09 ^{A}
	2C	6.67±0.88^{A}	18.17±0.14^{CDE}	1.88±0.24 ^{A}
	1PKC	5.80±0.22 ^{AB}	17.80±0.10^{E}	2.05±0.02 ^{A}
	3PKC	5.44±0.13 ^{AB}	18.74±0.10^{BC}	2.23±0.03 ^{A}
	5PKC	4.24±0.32^{B}	18.51±0.01^{BCD}	2.22±0.00 ^{A}
Test groups *Test Grupları*	1SPK	7.24±0.80^{A}	20.03±0.33^{A}	2.15±0.05 ^{A}
	3SPK	7.36±0.72 ^{A}	18.13±0.08^{DE}	2.08±0.05 ^{A}
	5SPK	7.13±0.35 ^{A}	18.11±0.09^{DE}	2.15±0.04 ^{A}

1C: Control (cookies made with 100% whole wheat flour, without sourdough), 2C: (cookies made with 100% whole wheat flour, containing sourdough),
1PKC: (control without sourdough, containing 1% orange peel powder), 3PKC: (control without sourdough, containing 3% orange peel powder),
5PKC: (control without sourdough, containing 5% orange peel powder), 1SPK: (containing 1% orange peel powder and sourdough), 3SPK: (containing 3% orange peel powder and sourdough), 5SPK: (5% orange peel powder and sourdough).
Results are expressed as mean± std. Different superscripted capital letters in the same column denote significant differences according to the Tukey HSD test ($p < .05$).

3.2. Total phenolic contents and antioxidant capacities of cookie samples

Total phenolic contents and antioxidant activities of cookie samples were given in **Figure 1.** Total phenolic contents of cookies varied between 4.7-5.2 mg GAE kg^{-1} (**Figure 1**). Although it has been previously reported that the use of sourdough in bakery products (such as bread) increased antioxidant activity and total phenolic content (Hayta and Hendek Ertop 2017), no statistical difference ($p > 0.05$) was observed among our samples. In a study on the production of cookies enriched with pomegranate peel, total phenolic contents were reported to be 90.7–161.9 mg GAE 100 g^{-1} (Ismail et al. 2014). The results of Al-janabi et al. (2024) showed an increase in phenolic concentration with increasing the percentage of dried tangerine peel powder addition. The results of Al-janabi et al. (2024) showed an increase in phenolic concentration with increasing the percentage of dried tangerine peel powder addition. These inconsistencies in the studies could be attributed to the different extraction methods employed for the analysis of phenolics. On the other hand, a low correlation was observed between phenolic compounds and antioxidant activity.

On the other hand, significant differences were observed in the antioxidant capacities of the samples in both DPPH and FRAP tests. Specifically, gradual increase in the orange peel addition resulted in gradual increase in the antioxidant capacities of cookie samples. This could be attributed to the high antioxidant capacities of phenolics of fruit peels. This could be further attributed to the fact that LAB activities in sourdough microflora during yeast fermentation cause both structural disintegration of grain cell walls and enzymatic disintegration of matrix compounds (Omoba and Isah 2018). Moreover, due to presence of reducing sugar in orange peel (Sharma and Gujral 2014), addition of orange peel powder could cause the formation of higher amount of Maillard reaction compounds such as melanoidins that have antioxidant capacity (Xu and Chang 2008; Sharma and Gujral 2014). This could be another contributing factor to the elevated antioxidant capacity of orange peel containing cookies. It is worth noting here that previous studies reported that fruit peel extracts have the ability to scavenge free radicals, chelate metals, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Obafaye and Omoba 2018). Similar results were also observed when antioxidant capacity of the samples were examined through FRAP analysis (**Figure 1**). This is further confirmed with Pearson's correlation analysis that there is a strong ($p < 0.05$) positive correlation between DPPH and FRAP analysis with a correlation coefficient (r) of 0.98 (**Figure 2**).

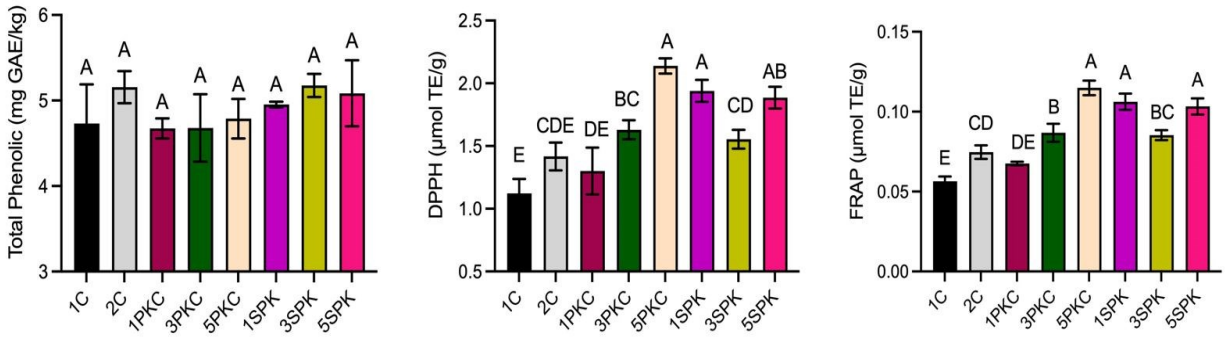

Figure 1. Bioactive components (total phenolic content, DPPH and FRAP) of cookie varieties. 1C: Control (cookies made with 100% whole wheat flour, without sourdough), 2C: (cookies made with 100% whole wheat flour, containing sourdough), 1PKC: (control without sourdough, containing 1% orange peel powder), 3PKC: (control without sourdough, containing 3% orange peel powder), 5PKC: (control without sourdough, containing 5% orange peel powder), 1SPK: (containing 1% orange peel powder and sourdough), 3SPK: (containing 3% orange peel powder and sourdough), 5SPK: (5% orange peel powder and sourdough).

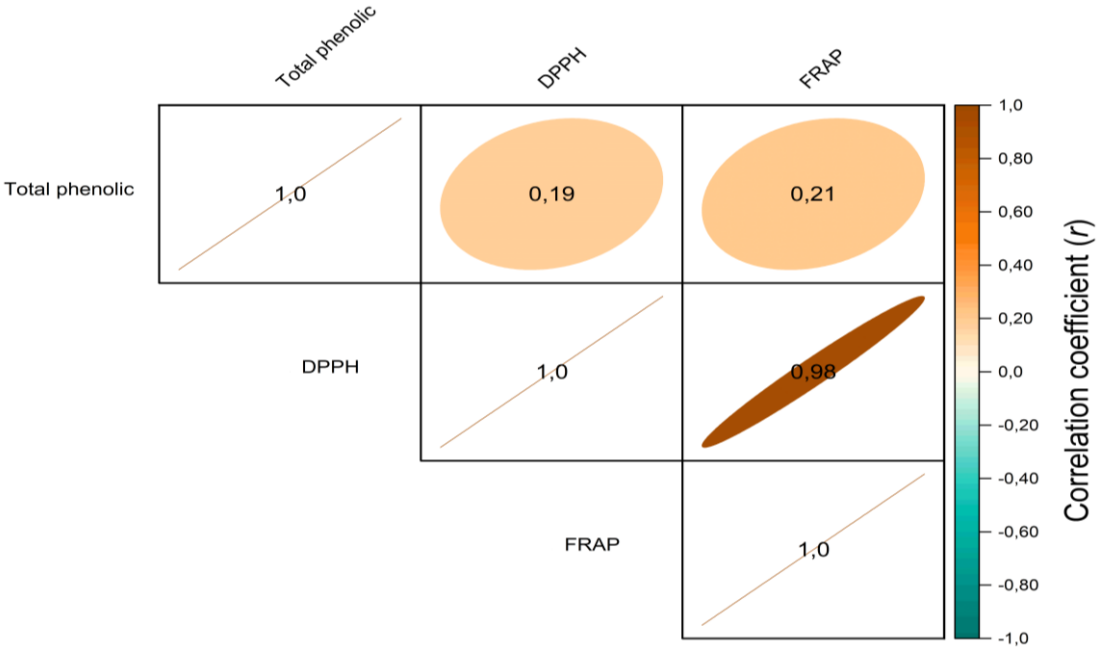

Figure 2. Pearson's correlation analysis of the total phenolic and the antioxidant activity (DPPH, FRAP) of cookies. (The Pearson correlation coefficient (*r*) indicates that there is a negligible correlation for *r*: 0.00–0.10, weak correlation for *r*: 0.10–0.39, moderate correlation for *r*: 0.40–0.69, strong correlation for *r*: 0.70–0.89, and very strong correlation *r*: 0.90–1.00)

3.3. Determination of physical (color, diameter, thickness, spreading rate) of cookie samples

The color analysis (L^*, a^*, b^*) of the produced cookie varieties was evaluated objectively using colorimeter (**Figure 3b**) in which L^* value indicates the darkness to lightness, while a^* value represents the spectrum from green to red; and b^* shows the range from blue to yellow. L^* represents lightness, defined as the perceived brightness of an object compared to a perfectly white object (0/+100). The a^* value indicates red for a positive value and green for a negative value (-100/+100), while the b^* value indicates yellow for a positive value and blue for a negative value (-100/+100). Color property is considered as the primary important parameters for the consumer to prefer a product (Arslan Unal and Ozkaya 2024). When the color values in our study are examined, the L^* value (lightness) was the highest in 1C (60.49) and 5SPK samples (59.55), while the lowest was in 5PKC sample (56.23) (**Figure 3b**). It was observed that the L^* value decreased compared to the control as the orange peel powder increased. Another important point for color is the a^* and b^* values. When compared

to the control sample (a^*: 5.10, b^*: 21.18), it was recorded that the a^* and b^* values significantly ($p < 0.05$) increased as the orange peel powder concentration increased. The decrease in L^* value and the increase in a^* and b^* values in our study are consistent with the study of El Makhzangy et al. (2024). This may be due to the fact that both the bran layer of whole wheat flour and the orange peel pigments are concentrated in the final product. Furthermore, the dark and red color of the cookie samples may be the result of the Maillard reaction, known as non-enzymatic browning, which occurs when reducing sugars and proteins are heated together (Al-Janabi and Yasen 2022; Arslan Unal and Ozkaya 2024).

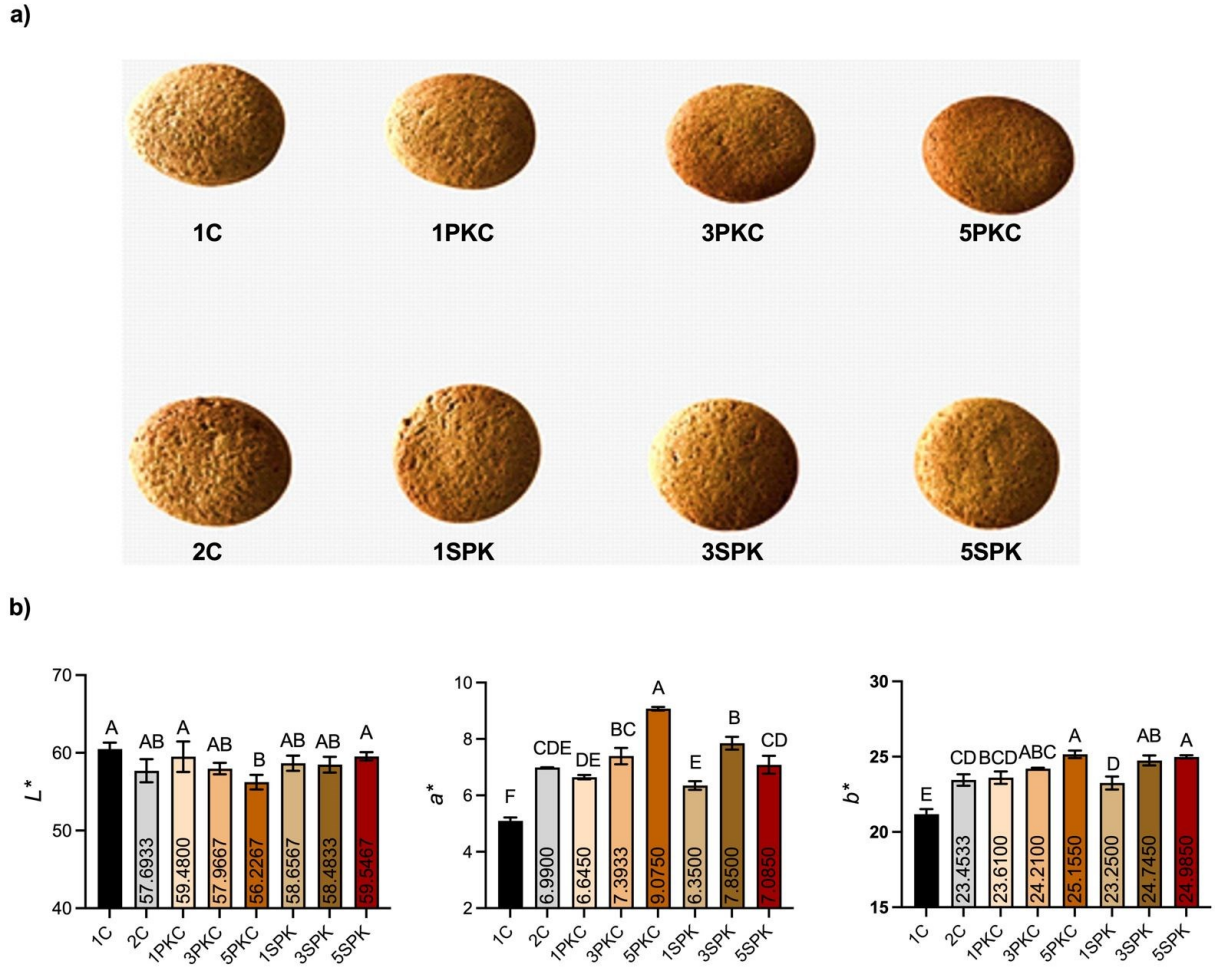

Figure 3. a) General appearances of cookies, **b)** L^* (brightness), a^* (redness), b^* (yellowness) values. Mean values with the same letter are not significantly different ($p > 0.05$, Tukey's multiple comparison test). 1C: Control (cookies made with 100% whole wheat flour, without sourdough), 2C: (cookies made with 100% whole wheat flour, containing sourdough), 1PKC: (control without sourdough, containing 1% orange peel powder), 3PKC: (control without sourdough, containing 3% orange peel powder), 5PKC: (control without sourdough, containing 5% orange peel powder), 1SPK: (containing 1% orange peel powder and sourdough), 3SPK: (containing 3% orange peel powder and sourdough), 5SPK: (5% orange peel powder and sourdough)

In addition to color properties, other physical properties (diameter, thickness, spreading rate) of the cookie samples were also evaluated (**Figure 4**). Our results revealed that the diameter of cookies showed a decreasing trend (49.0-47.8 mm) with the increase of orange peel powder substitution level. This situation was similar in the diameter values (48.9-46.9 mm) of sourdough and orange peel powder added samples. These decreases might be attributed to the polyphenols and fibers contained in orange peel because proteins can form complexes with polyphenols through hydrogen bonding between a carbonyl group of peptide residue and a hydroxyl group of phenols (Song and Yoo 2017), which can effectively strengthen dough networks and consequently improve the viscoelastic moduli in dough. In addition, the water slipperiness can be reduced because of the competition between fiber and gluten for water absorption with the addition of orange peel. Moreover, the fiber in orange peel can act as a filler in the viscoelastic matrix of dough, which can lead to an

increase in viscoelastic property (Han et al. 2021). On the other hand, it is observed that the thickness values of the sourdough and orange peel powder added samples increased (7.00 -7.85 mm) and these changes were reflected in the spreading ratio (**Figure 4**). These findings are consistent with the study of Alioğlu and Özülkü (2024), who observed a decrease in the spreading factor of the biscuits when sourdough was added to the formulation (spreading ratio of biscuit samples with whole wheat flour and certain concentrations of sourdough added was 6.9-5.8 mm). Physical properties such as the spreading ratio of cookies can be affected by various factors including protein, lipid and fiber content, water binding capacity and processes occurring during baking (Arepally et al. 2020). Therefore, considering that sourdough fermentation leads to changes in protein and starch (Siepmann et al. 2018), these differences can be attributed to the fermentation process (Maravić et al. 2024). Another difference may arise from differences in the matrix used in the cookie formulation (Alioğlu and Özülkü 2024).

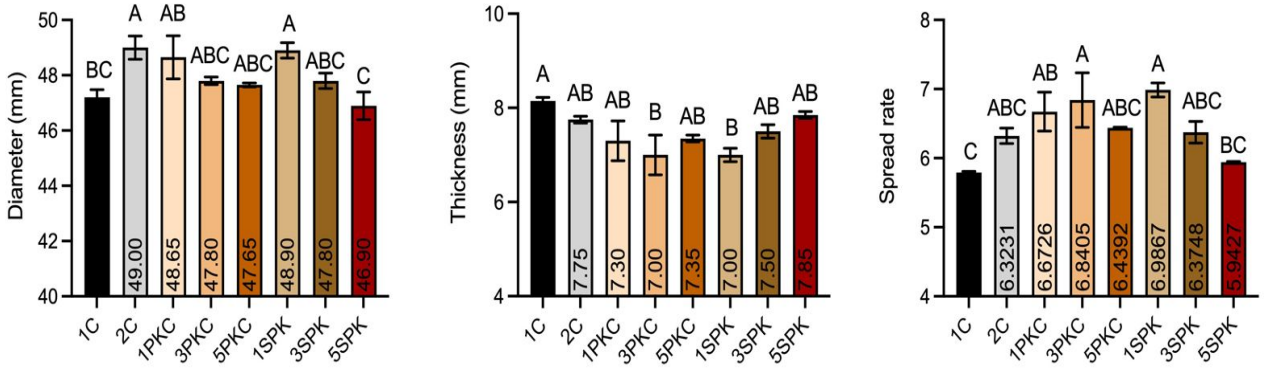

Figure 4. Diameter, thickness, spreading rates of cookies. Mean values with the same letter are not significantly different ($p > 0.05$, Tukey's multiple comparison test). 1C: Control (cookies made with 100% whole wheat flour, without sourdough), 2C: (cookies made with 100% whole wheat flour, containing sourdough), 1PKC: (control without sourdough, containing 1% orange peel powder), 3PKC: (control without sourdough, containing 3% orange peel powder), 5PKC: (control without sourdough, containing 5% orange peel powder), 1SPK: (containing 1% orange peel powder and sourdough), 3SPK: (containing 3% orange peel powder and sourdough), 5SPK: (5% orange peel powder and sourdough)

3.4. Textural (hardness, fracturability) parameters of cookie samples

Textural profile is important in determining the quality attributes of cookie samples. Hardness, which is important in terms of textural properties, represents a critical structural property of cookies that significantly affects sensory perception and overall product quality (Ramashia et al. 2024). Therefore, in our study, the textural properties (hardness and fracturability) of day 1 cookie samples were examined and compared with the control sample (1C). It was found that both the addition of orange peel powder and sourdough caused an increase in hardness values in cookie varieties, with the 5SPK sample revealing the highest hardness value (6361 *g*) (**Figure 5**). This could be attributed to the fact that the incorporation of dietary fiber into flour can significantly influence dough properties and cookie texture due to the high water absorption capacity of fiber. Dietary fibers compete with starch and gluten for water during heat treatment, which can interfere with proper gluten network formation and starch gelatinization. This competition reduces dough aeration and may lead to a lower effective protein functionality, ultimately resulting in a denser structure with reduced volume and increased hardness in the final baked product (Özçelik and Gör 2024; Kausar et al. 2024). Moreover, the presence of fiber increases water binding and reduces moisture availability in the dough matrix, contributing to drier, more crumbly, and crispier cookies. Given these effects, higher hardness values are expected in the cookies produced in the present study (Dhal et al. 2023).

Another important feature in textural properties is fracturability. Fracturability of cookies indicates the crispness of the product (**Figure 5**). While sample 2C (28.10 mm) had the highest breakability value, there were statistically significant differences between samples 3PKC (26.06 mm), 5PKC (25.51 mm), 1SPK (25.90 mm) ($p < 0.05$). One study indicated that the addition of Japanese quince to cookie samples caused a decrease in the breakability of cookies, probably due to the high fiber concentration, including pectin and cellulose-like

polysaccharides (Krajewska and Dziki 2023). Low breakability reflects the highest crispness of the products, sometimes the opposite is true. In our study, the addition of sourdough significantly affected the breakability of cookies, leading to higher values. This is consistent with the study of Maravic et al. (2024).

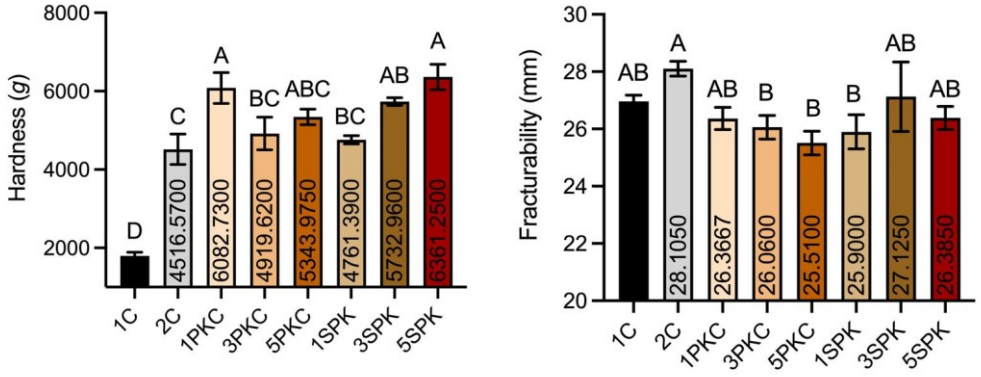

Figure 5. Textural (hardness, fracturability) profiles of cookie samples. 1C: Control (cookies made with 100% whole wheat flour, without sourdough), 2C: (cookies made with 100% whole wheat flour, containing sourdough), 1PKC: (control without sourdough, containing 1% orange peel powder), 3PKC: (control without sourdough, containing 3% orange peel powder), 5PKC: (control without sourdough, containing 5% orange peel powder), 1SPK: (containing 1% orange peel powder and sourdough), 3SPK: (containing 3% orange peel powder and sourdough), 5SPK: (5% orange peel powder and sourdough)

3.5. Sensory analysis

Consumer preferences based on the sensory profiles of sourdough-enriched cookies and control samples without sourdough are presented in **Figure 6**. Radar charts was employed to visually represent both the unique and overlapping sensory characteristics among the cookie samples.

Cookies containing sourdough and those formulated with orange peel powder were evaluated and compared with the control sample lacking sourdough. Regarding color, the highest mean scores were observed in samples 5SPK (4.33) and 2C (4.00).

Taste scores ranged from 2.75 for the 5PKC sample to 3.50 for samples 1C and 2C. A decreasing trend in taste preference was observed with increasing levels of orange peel powder, which may be attributed to the presence of limonene—a terpenoid compound found in citrus peels that imparts a characteristic bitter or pungent flavor. This finding aligns with the observations of Obafaye and Omoba (2018), who reported reduced palatability in biscuits enriched with orange peel powder.

In terms of appearance, the 5SPK sample achieved the highest rating (4.33), whereas the control sample (1C) received the lowest score (3.33). Notably, all sourdough-enriched samples were rated more favorably than the control in appearance, suggesting that sourdough fermentation positively influenced visual appeal. Furthermore, the addition of sourdough was associated with an increase in overall acceptability across the cookie samples.

The positive sensory impact of sourdough is well-documented and is primarily attributed to its influence on dough properties, which in turn enhances flavor, texture, and general acceptability (Ismail et al. 2014). During sourdough fermentation, proteolysis occurs, where proteins are hydrolyzed into polypeptides and subsequently into free amino acids. This biochemical transformation, modulated by starter cultures, contributes to flavor development through enzymatic breakdown and the formation of volatile compounds during baking (Olojede et al. 2024).

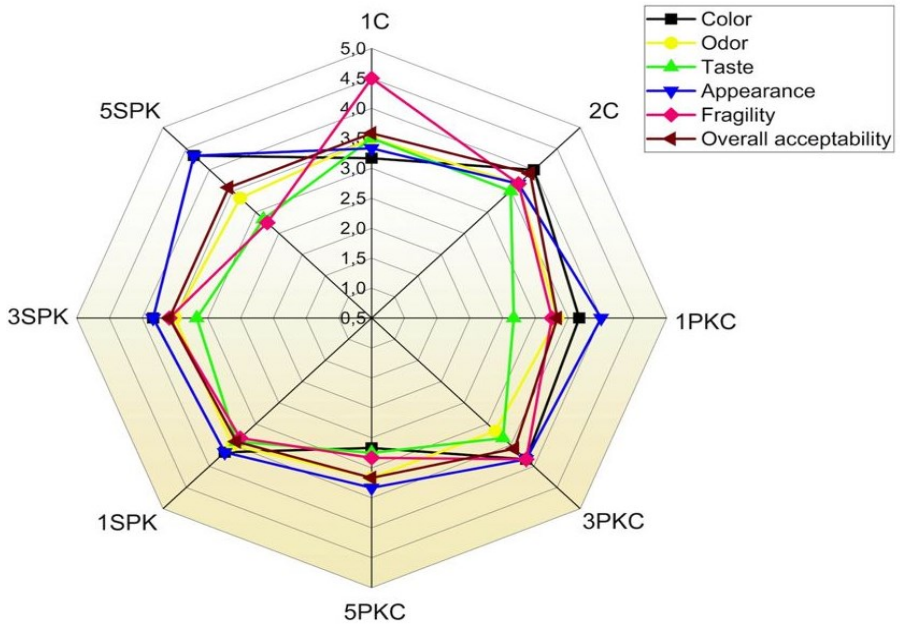

Figure 6. Spider web graph showing sensory attributes of cookie samples. 1C: Control (cookies made with 100% whole wheat flour, without sourdough), 2C: (cookies made with 100% whole wheat flour, containing sourdough), 1PKC: (control without sourdough, containing 1% orange peel powder), 3PKC: (control without sourdough, containing 3% orange peel powder), 5PKC: (control without sourdough, containing 5% orange peel powder), 1SPK: (containing 1% orange peel powder and sourdough), 3SPK: (containing 3% orange peel powder and sourdough), 5SPK: (5% orange peel powder and sourdough)

4. Conclusions

The study showed that the addition of both whole wheat spontaneous sourdough and orange peel powder to the cookie varieties had a significant effect on moisture and fat contents, but no effect on ash and total phenolic contents. It was found that the antioxidant values of cookies were significantly increased when enriched with sourdough and orange peel powder compared to the control samples. Sourdough and orange peel powder addition also improved the color, physical and textural textural properties of the cookie samples. Although addition of sourdough and orange peel impacted the some parameters in sensory attributes of cookie samples, overall acceptability of sourdough and orange peel fortified cookies were not significantly different than control samples, indicating that range peel powder can be incorporated into cookie formulation to increase its bioactive content (mainly antioxidant capacity) up to 5% without significantly impacting the ovarall sensory attributes. Overall, these results show that cookies with increased bioactive value can be produced by incorporating sourdough and orange peel powder into formulation.

Author Contributions: Conceptualization, Z.A and Y.E.T; methodology, Z.A., Ş.D. and Y.E.T; validation, Z.A. and Y.E.T.; formal analysis, Z.A., Ş.D., S.T.; investigation, Z.A., Ş.D. and Y.E.T.; writing—original draft preparation, Z.A. and Y.E.T.; writing—review and editing, Z.A., Ş.D., S.T. and Y.E.T.; supervision, Y.E.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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