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Optimizing the utilization of pomelo (*Citrus maxima* (Brum.) Merr.) seeds as a quality dietary fiber

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Abstract:
Orange seeds, often overlooked as waste, have hidden potential since fiber derived from them contains numerous biochemical substances that can enhance the nutritional value of food. We aimed to investigate the impact of pomelo seed fiber on the biscuit dough's properties (starch and gluten), physicochemical characteristics, and biochemistry, as well as the product's shelf life.
We studied three types of samples: control (no dietary fiber), biscuits with dietary fiber from pomelo (*Citrus maxima* (Brum.) Merr.) seeds, and biscuits with wheat germ fiber. Scanning electron microscopy was employed to analyze rubbery starch and gluten in the dough, while response surface methods were used to optimize the biscuits' strength via a central composite design. The product's shelf life was determined based on microbial contamination levels. ANOVA test and Tukey's Honestly Significant Difference post hoc test were performed to assess the differences in physicochemical and biochemical properties.
Citrus seed fiber influenced rubbery starch and gluten properties, causing significant differences ($p < 0.05$) in fracturability, total dietary fiber, and Trolox equivalent antioxidant capacity among the three samples. The biscuits enriched with citrus seed fiber contained flavonoid compounds and acylserotonin, with acyl-*N*ω-methylserotonin dominating in the C22 and C24 homologs. Despite varied evaluations in texture and aroma, the biscuits with citrus seed fiber were well-received for their taste and boasted an extended shelf life (> 12 months).
Dietary fiber obtained from *C. maxima* seeds not only enhanced the nutritional value of the biscuits but also paved the way for innovative healthy food opportunities.

Keywords: Acyl-*N*ω-methylserotonins, citrus seeds, dietary fiber, N-serotonin, rubbery gluten, rubbery starch, nutritional value

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INTRODUCTION

Orange seeds, often overlooked and dismissed as waste, possess untapped potential waiting to be fully explored. These seeds are a rich source of various biochemical substances, including fatty acids, limonoids, tocopherols, phytosterols, dietary fiber, and flavonoids. Extensive research has revealed their pharmaceutical potential, including anticancer, anti-hematopoiesis, antifertility, and hepatoprotective properties [1–8]. Furthermore, these valuable biochemical compounds can enhance the nutritional value and quality standards of food products [9]. The diverse range of biochemical compounds found in orange seeds makes them a compelling subject for further investigation as potential food additives.

While it is widely acknowledged that biochemical compounds found in orange seeds have potential to enhance the nutritional value and dietary fiber content, there is an ongoing debate among researchers regarding the precise impact of incorporating these ingredients into food products. Notably, the addition of dietary fiber derived from orange seed flour has been shown to influence various properties, including emulsion properties, water absorption in dough, biscuit friability, and the microfibril structure of dough [10–12]. However, there has been a notable absence of research investigating the effects of dietary fiber on the characteristics of rubbery starch and gluten in dough. This represents a significant gap in the existing body of research on the subject.

The production of dietary fiber from orange seeds has traditionally relied on older methods, such as those employed by Akpata & Akubor and Yilmaz & Karaman, involving the removal of seed shells and oil [10, 11]. Orange seed shells and their oil contain a unique compound, N-serotonin, which is relatively rare in the seeds of other plants [13]. In this research, we undertook a novel approach to producing orange seed fiber, which retains the seed shells and oil.

The wealth of antioxidant compounds and pectin fiber in orange seeds becomes particularly evident when they are processed into dietary fiber and subjected to thorough testing. In this research, we assessed their influence on the properties of rubbery starch and gluten in the dough, a concept that has already been extensively studied and well-established [14]. Furthermore, we scrutinized the physicochemical attributes of the final product, namely biscuits. We also conducted optimization trials utilizing the central composite design methodology.

Orange seed oil exhibits potential as an antibacterial agent, although its antimicrobial activity is relatively less potent compared to that of orange fruit and peel oil [15]. Research on *Citrus aurantifolia* seeds using chloroform, methanol, and ethanol has demonstrated robust antibacterial efficacy [16]. Additionally, the ethanol extract of *Citrus paradisi* Macf. seeds from the *Rutaceae* family has exhibited remarkable antifungal activity [17]. However, some studies have suggested that orange seed extract possesses even stronger antibacterial properties [18, 19]. In this study, we assessed the antimicrobial attributes of pomelo (*Citrus maxima* (Brum.) Merr.) seeds. This assessment was designed to determine the shelf life of products by evaluating microorganism contamination levels.

The core focus of our research revolved around the utilization of pomelo seeds to elevate the quality of biscuits. To achieve this, we set two primary objectives: (i) scrutinizing the influence of citrus seed fiber on the properties of rubbery starch and gluten in the dough, and (ii) evaluating the impact of citrus seed fiber on the physicochemical, biochemical, and shelf life characteristics of the biscuits. For this, we employed fiber derived from pomelo seeds along with dietary fiber sourced from wheat seeds as integral components of our study materials.

STUDY OBJECTS AND METHODS

Materials. The following materials were procured from a local grocery store: pomelo seeds (*Citrus maxima* (Brum.) Merr.), wheat flour, and dietary fiber extracted from wheat seeds. Additionally, we obtained various chemical reagents and solutions essential for our experiments, including HOBT solution (N-hydroxybenzotriazole), hexane, 95% alcohol, tetrahydrofuran, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide), triethylamine, ethyl acetate, NaOH, HCl, dimethylformamide, H_2SO_4, and Nucleophile. We also acquired specific chemical solutions to conduct tests for flavonoids, phenolics, protein, fat, and ash contents, as well as antioxidant substances.

Producing pomelo (*C. maxima*) seed powder. First, we thoroughly cleaned and dried pomelo seeds in an oven at 50°C for 4 h. Then, we ground the seeds to break down all of their components, including the outer shell. The resulting product underwent further drying in the oven until its moisture content reached below 10%. To ensure a uniform fineness of the flour, we sifted it through a 100-mesh sieve. Any remaining coarse particles were subjected to additional grinding and sifting until a consistent flour fineness was achieved.

Extracting dietary fiber from pomelo seed powder. The pomelo seed flour was combined with distilled water (1:20) and homogenized at 12 500 rpm for 10 min. To optimize extraction efficiency and product quality, ultrasonic treatment was applied using a UP400St ultrasonic device (Hielscher Ultrasonics) with an amplitude of 90% for 10 min. It was followed by a 5-min break to allow the mixture's temperature to reach 45°C. The resulting mixture was subsequently filtered through a filter cloth with a mesh size of 0.150 mm. Any remaining solid residue was carefully rinsed five times with water before being dried under vacuum conditions at 50°C for 4 days.

Biscuit production. All the ingredients for making biscuits, including wheat flour (40%), shortening (14%), sugar (8%), dietary fiber (3%), and salt (2%), were thoroughly mixed to ensure even distribution. Water (33%) was added to the mixture, and it was blended using a mixer at medium speed for 15 min. The dough was then molded using a biscuit machine with minimal capacity to form wet weights of 7 g per dough portion. These portions were baked in a rotary oven at 160°C (20 rpm) for 30 min. After baking, the biscuits were promptly transferred to a room with a temperature of 14°C and a relative humidity of about 55%. To preserve their freshness, the biscuits were individually wrapped in plastic packaging with resealable zippers as needed.

The samples with dietary fiber were divided into two distinct groups: those containing wheat fiber and those enriched with citrus seed fiber. In addition, we conducted analyses on the control samples that did not contain any dietary fiber.

Physical analysis of the biscuits. The surface color of three randomly selected biscuit samples was evaluated using a WR-10 QC colorimeter (China), while their hardness and brittleness were assessed using PRE MAD-TPA texture analysis (Brookfield Engineering Laboratories, Inc., USA). The physical appearance of the dietary fiber dough and its impact on the adhesive properties of starch and gluten were examined using an Axia Chemi scanning electron microscope (Fisher Scientific, USA). Images displaying the biscuit texture and pores were captured using a cellphone.

Furthermore, we conducted an optimization test using two factors, temperature and heating time, to determine the friability of biscuits with citrus seed

fiber. For this test, we employed response surface methods and a central composite design.

Chemical analysis. ***The extraction of N-acylserotonin.*** The established protocol for synthesizing N-acylserotonin compounds has been previously executed and subsequently validated by Kruk *et al.* [13, 20]. In this procedure, the fatty acids contained in the dietary fiber were dissolved in 18 mg of HOBT (N-hydroxybenzotriazole) solution and 1 mL of tetrahydrofuran. Then, 22 µL of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) was added to the mixture and stirred. Concurrently, serotonin hydrochloride was dissolved in 500 µL of dimethylformamide, and 12 µL of triethylamine was introduced into the mixture. The resulting mixture was stirred and then subjected to extraction using 5 mL of ethyl acetate and 5 mL of water. The reaction product was subsequently collected, evaporated, and reconstituted in 2 mL of peroxide-free anhydrous tetrahydrofuran.

The hydrolysis of N-acylserotonin. The method of hydrolyzing N-acylserotonin has previously been employed by Kruk *et al.* and Trela-Makowej *et al.* [13, 20]. The compound fractions $C_{33}H_{56}N_2O_2Na^+$ and $C_{35}H_{60}N_2O_2Na^+$ were subjected to hydrolysis in a concentrated solution of HCl and tetrahydrofuran (1:9, v/v) at 105°C for 1 h and subsequently evaporated to dryness. The resultant fatty acids contained in 5-mL Wheaton glass vials were then combined with serotonin. In parallel, 100 mg of lemon seed oil was dissolved in 1 mL of ethanol, followed by the addition of 100 µL of saturated NaOH in water. The mixture was heated for 1 h at 90°C using a thermoblock. Subsequently, the solution was neutralized with HCl, and the fatty acids were extracted with ethyl acetate after dilution with water. Upon evaporation under a nitrogen atmosphere, the obtained fatty acids were also combined with serotonin.

N-acylserotonin was identified in dietary fiber extracts by assessing peak areas on HPLC chromatograms using a GSYS0002 instrument (Gilson Inc., USA) in conjunction with the synthetic standards of known concentrations. The HPLC setup involved a reverse-phase Nucleophil 100 C18 column and a mobile phase consisting of CAN, MeOH, and H_2O (70:7.5:1, v/v). The chromatographic separation was carried out at a flow rate of 1.4 mL/min.

The nutritional components were analyzed as follows. The moisture content was assessed using an HC103 moisture analyzer (Metler Toledo), while water activity was measured with an MS2100 water activity moisture meter. The crude protein content was determined following method 46–12 of the Association of Official Analytical Chemists (AOAC). The fat and ash contents were quantified using the AOAC's method 30–10 [21]. All the results obtained from these analyses were reported as percentages based on dry weight. Additionally, the Kjedahl method served as a correction factor for any remaining protein levels.

The capacity of both free and bound phenolic compounds was evaluated by using phenolic extracts. The extract was obtained as outlined in [22] to be subsequently concentrated and reconstituted in 3 mL of deionized water. A membrane filter was utilized to purify the deionized water. Free and bound phenolics were determined by measuring the Trolox equivalent antioxidant capacity (TEAC) as described in [23].

Microbiological test. In our study, we conducted periodic assessments of microbial growth in the three biscuit samples, with observations made once every month over a span of 12 months. These evaluations involved quantitative testing for bacteria using the total plate count method, as well as for yeast and mold [24].

Respondent selection. Randomly selected employees of a biscuit company located in the Bandung region of West Java, Indonesia, were invited to provide their feedback and opinions regarding our research biscuits. The level of respondent acceptance was assessed using a 1–5 Likert scale, with the following interpretations: 1 – "strongly disagree", 2 – "disagree", 3 – "neutral", 4 – "agree", and 5 – "strongly agree".

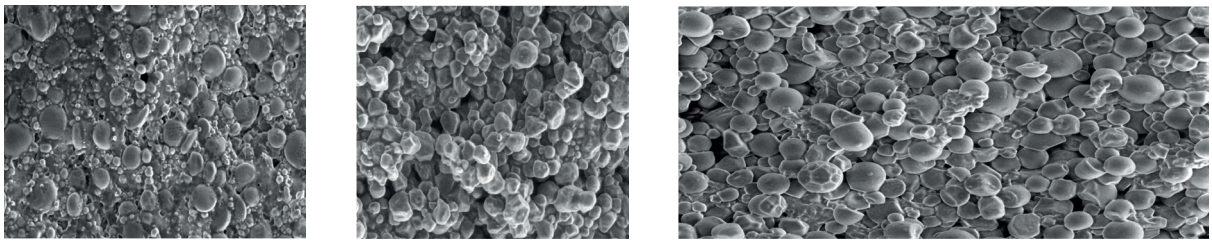
Statistical analysis. The data analyses were conducted using IBM SPSS Statistics, version 26 (SPSS Inc., Chicago, IL, USA). An analysis of variance (ANOVA) was employed, followed by the Tukey Honestly Significant Difference post hoc test to discern variations in the biscuit biochemical levels. The results were presented in the format of mean ± standard deviation, and statistical significance was determined at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Dietary fiber and dough characteristics (starch and gluten granules). Incorporating 3% of dietary fiber and 33% of water into the biscuit dough significantly altered its properties, particularly starch and gluten granules. As illustrated in Fig. 1, the control sample exhibited a strong binding between starch and gluten granules at multiple points. Additionally, liquefied gluten was observed, which effectively bound starch granules from the flour. The control dough sample showed minimal voids, or empty spaces.

The presence of dietary fiber extracted from wheat grain significantly impacted the rubbery starch and gluten phases. The standard practice involves adding water to the mixture to achieve these phases. According to Fig. 1, the rubbery starch and gluten phases were clearly distinguishable in the control dough (without dietary fiber). In contrast, the wheat fiber dough displayed reduced stickiness and elasticity compared to the control sample. A similar behavior was also evident in the dough containing citrus (pomelo) seed fiber.

The incorporation of dietary fiber has a notable impact on the structure of starch and gluten granules within the dough. Blanshard previously indicated that



a b c

Figure 1 Physical appearance of starch and gluten granules in biscuit dough on Scan Electron Microscopy within 50 µm: a – Control; b – Wheat dietary fiber; c – Citrus seed dietary fiber

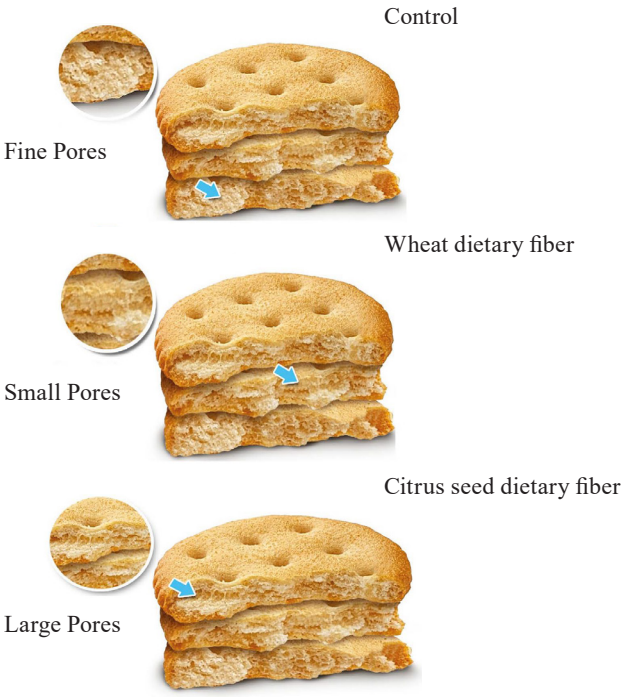


Figure 2 Physical appearance of the biscuits baked at 160°C for 40 min. Biscuit thickness ranged from 5.01 ± 0.15 mm on 50× magnification on a cellphone camera

in good bread dough, a water content of over 20%, with no heating, resulted in a rubbery consistency involving both starch and gluten [14]. This state leads to the amalgamation of starch and gluten. In our study, the control samples displayed conditions that align with Blanshard's ideal criteria for bread production [14]. Conversely, in the wheat fiber and pomelo seed fiber samples, the liquefaction of starch and gluten was not as evident, resulting in discernible differences in tensile strength and brittleness when the biscuits were subjected to breaking.

Physicochemical chemical analysis and antioxidant composition. The doughs (Fig. 1) were then baked in the oven at 160°C for 40 min. We deliberately enlarged (50×) the images of the resulting biscuits (Fig. 2) to see the differences in their pores. As can be seen, the pores of the control sample were small in diameter (0.1 mm) but evenly distributed on all the sides. The wheat fiber sample had large pores (0.2 mm), as well as small ones evenly distributed on all the sides. In the pomelo seed fiber samples, almost all the surfaces were dominated by large pores (± 0.3 mm), with some smaller pores observed as well.

According to Table 1, we observed no notable differences ($p > 0.05$) among the three samples in terms of weight loss, thickness, or the contents of protein, fat, ash, and soluble dietary fiber. Specifically, no significant disparities were detected between the control and the wheat fiber samples, while a significant difference ($p < 0.05$) was identified in the citrus seed fiber sample. This discrepancy was also evident when examining color (b^*), TEAC (bound), water activity, and moisture.

Significant distinctions ($p < 0.05$) were observed among the three samples in terms of color (a^*), fracturability, total dietary fiber, insoluble dietary fiber, and TEAC (free). We focused our attention on achieving similar levels of fragility in the citrus seed fiber sample as those found in the control and the wheat fiber samples. Maintaining a consistent level of brittleness is crucial to prevent difficulties in the subsequent process, specifically during the packing phase, where powdery characteristics can be problematic.

All the three samples contained antioxidants equivalent to Trolox, with the citrus seed fiber sample exhibiting the highest content of both free (9.11 ± 0.24 µmole Trolox/g) and bound (8.21 ± 0.19 µmole Trolox/g) TEAC. Additionally, we found that the biscuits enriched with citrus seed fiber also contained flavonoid compounds that were absent in the other two samples. These flavonoid compounds included eriocitrin (free/bound), rutin (free/bound), naringin (free/bound), hesperidin (bound), neohesperidin (free), and naringenin (free). Our findings align closely with the results reported by Yilmaz & Karaman [11].

Baking time and temperature optimization for fragile biscuits. We aimed to optimize the baking of biscuits containing citrus seed fiber with a fracturability value of 4326 ± 56 g force. This value was exceptionally high and it was crucial for the packaging process. Therefore, we sought to attain a fracturability value that was equal or close to the values of the control sample (7011 ± 34) and the wheat fiber sample (5990 ± 56). To achieve this, we employed two variables, temperature and time, aiming to establish the standard fracturability values equivalent to those

Table 1 Physicochemical characteristics and antioxidant composition of the biscuits

Property	Control (no fiber)	Wheat fiber	Citrus seed fiber
Weight loss, %	14.58 ± 0.34[a]	15.21 ± 0.41[a]	15.01 ± 0.21[a]
Thickness, mm	4.80 ± 0.10[a]	4.89 ± 0.12[a]	5.01 ± 0.15[a]
Color *L*	64.32 ± 0.23[a]	68.21 ± 0.13[b]	66.64 ± 0.34[ab]
*a**	8.90 ± 0.18[c]	6.70 ± 0.21[b]	5.54 ± 0.26[a]
*b**	28.86 ± 0.21[b]	28.27 ± 0.42[b]	25.32 ± 0.27[a]
Fracturability, g force	7011 ± 34[c]	5990 ± 56[b]	4326 ± 56[a]
Hardness, g force	4221 ± 35[a]	6218 ±65[b]	5738 ± 47[ab]
Moisture, %	2.65 ± 0.21[a]	3.01 ± 0.26[ab]	4.03 ± 0.31[b]
Water activity, A_w	0.15 ± 0.02[b]	0.13 ± 0.01[ab]	0.10 ± 0.01[a]
Protein, % dry weight	11.37 ± 0.28[a]	11.07 ± 0.31[a]	11.27 ± 0.26[a]
Fat, % dry weight	14.34 ± 0.29[a]	14.61 ± 0.45[a]	14.06 ± 0.36[a]
Ash, % dry weight	1.54 ± 0.10[a]	1.62 ± 0.26[a]	1.59 ± 0.36[a]
Total dietary fiber, % dry weight	5.12 ± 0.35[a]	10.28 ± 0.13[c]	7.42 ± 0.36[b]
Insoluble dietary fiber, % dry weight	3.23 ± 0.19[a]	8.04 ± 0.23[c]	5.23 ± 0.16[b]
Soluble dietary fiber, % dry weight	3.03 ± 0.15[a]	2.98 ± 0.23[a]	3.08 ± 0.14[a]
TEAC-free, µmole Trolox/g	5.41 ± 0.11[b]	2.49 ± 0.18[a]	9.11 ± 0.24[c]
TEAC-bound, µmole Trolox/g	2.43 ± 0.17[a]	2.67 ± 0.15[a]	8.21 ± 0.19[b]

TEAC – Trolox equivalent antioxidant capacity

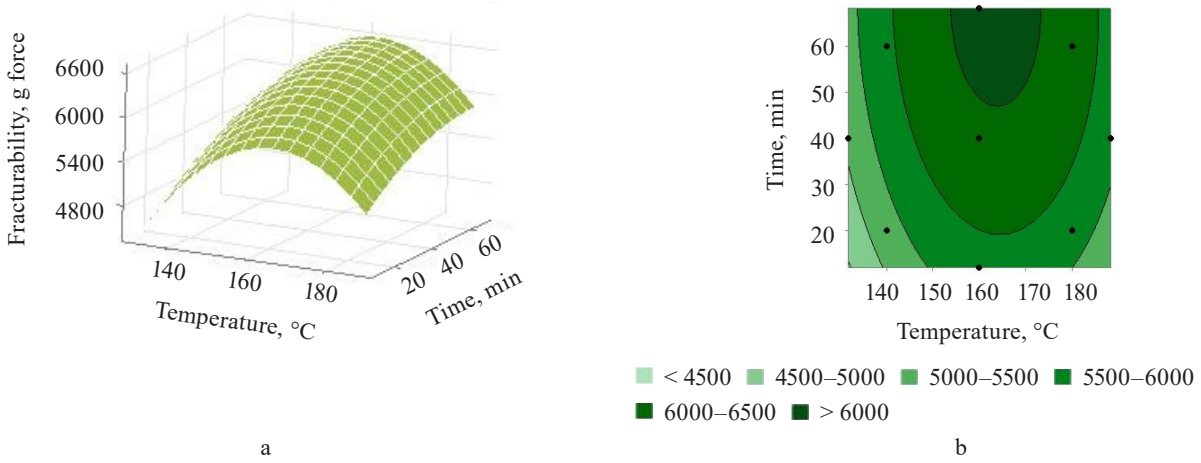


Figure 3 The influence of baking temperature and time on the fracturability of biscuits. The surface visual plot (a) and contour plot (b) of the citrus seed fiber samples

of the control sample. The outcomes of our analysis are presented in Fig. 3.

We conducted a series of experiments utilizing response surface methodology and a central composite design, which were repeated 13 times. In these experiments, we systematically explored a temperature range from 140 to 180°C and a time range from 20 to 60 min to analyze their combined impact on fracturability values. Our findings indicated a significant correlation between time and temperature, leading us to identify optimal fracturability values within the time window of 50–60 min and the temperature range of 155–170°C.

As a result of the optimization test conducted with response surface methodology, the fracture strength of the citrus seed fiber samples increased from 4326 to 6000 g force, approaching the values of the control and the wheat fiber samples.

The composition of phenolic acids, flavonoids, and acylserotonin. The phenolic test conducted on the three biscuit samples revealed no statistically significant differences ($p > 0.05$) for various phenolic compounds, including gallic, caffeic, syringic and syringic (bound), *p*-coumaric and *p*-coumaric (bound), *trans*-ferulic acid, and *trans*-2-hydroxycinnamic acids (Table 2).

When comparing the control with the citrus seed fiber sample, significant differences ($p < 0.05$) were observed in the levels of gallic acid (bound), 3,4-hydroxybenzoic acid, and 3,4-hydroxybenzoic acid (bound). Additionally, vanilic acid was only present in the citrus seed fiber sample. Conversely, vanilic acid (bound) exhibited the opposite trend, with significant differences in its levels between the citrus seed fiber sample and the control.

Table 2 The composition of phenolic acids, acylserotonin, and flavonoids

Biochemical	Control	Wheat fiber	Citrus seed fiber
Phenolic acids, mg/g sample			
Gallic acid	2.70 ± 0.34[a]	2.42 ± 0.41[a]	2.20 ± 0.49[a]
Bound	0.13 ± 0.02[a]	0.16 ± 0.02[a]	0.23 ± 0.01[b]
3,4-Hydroxybenzoic acid	0.410 ± 0.015[a]	0.470 ± 0.018[a]	0.74 ± 0.02[b]
Bound	0.001 ± 0.000[a]	0.0070 ± 0.0001[a]	0.037 ± 0.002[b]
Vanilic acid	n.d.	n.d.	0.028 ± 0.002
Bound	0.019 ± 0.001[a]	0.020 ± 0.002[a]	n.d.
Caffeic acid	0.314 ± 0.020[a]	0.29 ± 0.01[a]	0.32 ± 0.01[a]
Bound	0.23 ± 0.01[a]	0.21 ± 0.01[a]	n.d.
Syringic acid	0.103 ± 0.001[a]	0.1060 ± 0.012[a]	0.115 ± 0.001[a]
Bound	0.109 ± 0.002[a]	0.1170 ± 0.013[a]	0.105 ± 0.002[a]
p-Coumaric acid	0.118 ± 0.010[a]	0.108 ± 0.020[a]	0.115 ± 0.030[a]
Bound	0.114 ± 0.020[a]	0.112 ± 0.020[a]	0.105 ± 0.010[a]
Sinapic acid	n.d.	n.d.	n.d.
Bound	0.049 ± 0.001[a]	0.051 ± 0.002[a]	n.d.
trans-Ferulic acid	4.12 ± 0.40[b]	2.10 ± 0.21[a]	n.d.
Bound	2.06 ± 0.12[a]	2.16 ± 0.21[a]	1.98 ± 0.01[a]
trans-2-Hydroxycinnamic acid	0.53 ± 0.01[a]	0.61 ± 0.02[a]	0.58 ± 0.01[a]
Bound	0.12 ± 0.01[a]	0.14 ± 0.02[ab]	0.16 ± 0.01[b]
Acylserotonin, mg/g sample			
ai-C21 (18-Methyleicosanoic)	n.d.	n.d.	0.70 ± 0.02
Me-C20	n.d.	n.d.	0.40 ± 0.02
n-C21	n.d.	n.d.	0.30 ± 0.01
Me-C21	n.d.	n.d.	1.90 ± 0.02
n-C22	n.d.	n.d.	1.40 ± 0.40
ai-C23 (20-Methyldocosanoic acid)	n.d.	n.d.	0.70 ± 0.21
Me-C22	n.d.	n.d.	3.30 ± 0.24
n-C23	n.d.	n.d.	1.10 ± 0.13
Me-ai-C23	n.d.	n.d.	0.40 ± 0.02
Me-C23	n.d.	n.d.	3.70 ± 0.21
n-C24	n.d.	n.d.	0.60 ± 0.21
ai-C25 (22-Methyltetracosanoic acid	n.d.	n.d.	0.50 ± 0.01
Me-C24	n.d.	n.d.	2.20 ± 0.62
n-C25	n.d.	n.d.	1.10 ± 0.02
iso-C26 (24- Methyleicosanoic acid)	n.d.	n.d.	0.90 ± 0.03
Me-C25	n.d.	n.d.	1.90 ± 0.02
n-C26	n.d.	n.d.	1.20 ± 0.02
ai-C27 (25-Methylhexacosanoic acid)	n.d.	n.d.	0.20 ± 0.01
Me-C26	n.d.	n.d.	2.50 ± 0.17
n-C27	n.d.	n.d.	0.10 ± 0.01
iso-C28 (26-Methyleicosanoic acid)	n.d.	n.d.	0.20 ± 0.02
Me-C27	n.d.	n.d.	0.20 ± 0.01
n-C28	n.d.	n.d.	0.40 ± 0.01
Flavonoids, mg/g sample			
Eriocitrin	n.d.	n.d.	0.074 ± 0.004
Bound	n.d.	n.d.	0.1140±0.0005
Rutin	n.d.	n.d.	1.0440 ± 0.0001
Bound	n.d.	n.d.	2.622 ± 0.006
Naringin	n.d.	n.d.	1.455 ± 0.095
Bound	n.d.	n.d.	0.232 ± 0.004
Hesperidin	n.d.	n.d.	n.d.
Bound	n.d.	n.d.	0.250 ± 0.001
Neohesperidin	n.d.	n.d.	0.315 ± 0.006
Bound	n.d.	n.d.	n.d.
Naringenin	n.d.	n.d.	0.129 ± 0.001
Bound	n.d.	n.d.	n.d.

n.d. – not detected

The citrus seed fiber sample contained several flavonoid biochemicals that were absent in the control and wheat fiber samples. These included eriocitrin, rutin, naringin, hesperidin, neohesperidin, and naringenin, both in their free and bound forms. Notably, naringin exhibited the highest concentration among these compounds. Additionally, the citrus seed fiber sample lacked free hesperidin, bound neohesperidin, and bound naringenin. Phenolic acid compounds, including gallic acid (bound), 3,4-hydroxybenzoic acid (free and bound), and *trans*-2-hydroxycinnamic acid (bound), were prominently present in the citrus seed fiber sample. These results were similar to those reported by Yilmaz & Karaman [11].

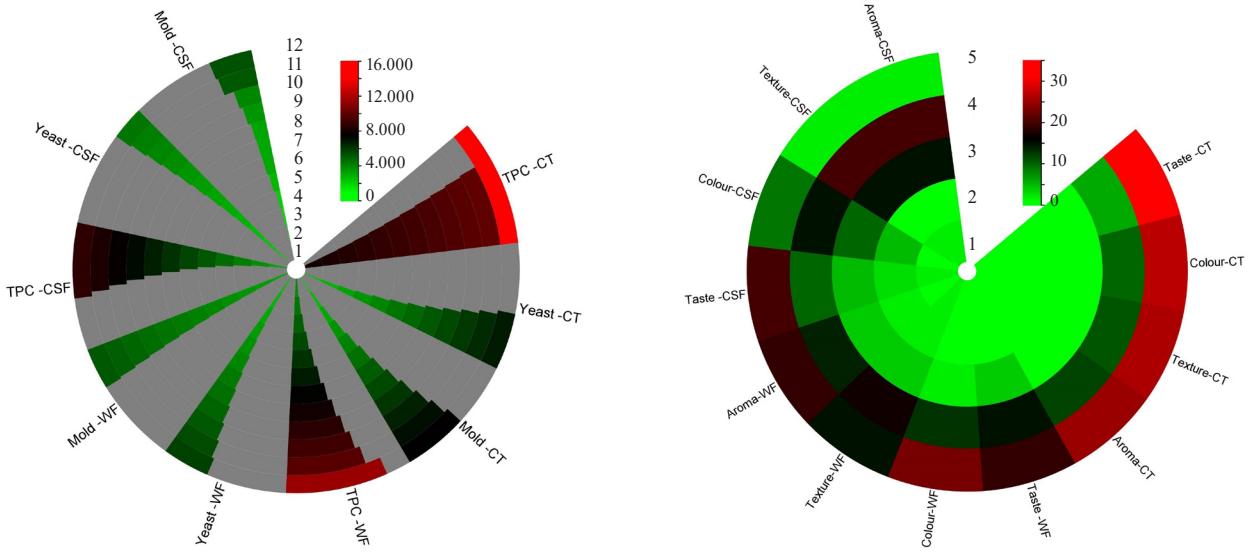
The acylserotonin compound was exclusively identified in the citrus seed fiber sample and was absent in the control and wheat fiber samples. These findings reinforced the results of Kruk *et al.*, who reported that citrus seeds contain serotonin (5-hydroxytryptamine, 5-HT) compounds, which are relatively rare in other plant seeds [13]. Having analyzed acylserotonin levels within the citrus seed fiber samples, we found a prevalence of long-chain acyl-methylserotonin. This dominance was evident through the elevated values of compounds such as Methyl-C22-serotonin, Methyl-C23-serotonin, Methyl-C24-serotonin, Methyl-C25-serotonin, and Methyl-C26-serotonin, all of which exceeded 1.5 mg/g in the sample content.

The distribution of acylserotonin compounds in the citrus seed fiber samples indicated that normal-chain acylserotonin came second in terms of prevalence. This was evident through the presence of compounds such as n-C22-serotonin (1.4 mg/g), n-C23-serotonin (1.1 mg/g), n-C25-serotonin (1.1 mg/g), n-C26-serotonin (1.2 mg/g), and other normal-chain acylserotonin compounds with concentrations below 1 mg/g. In contrast, branched-chain acylserotonin compounds were detected in lower quantities, with ai-C21-serotonin present at 0.7 mg/g and ai-C27-serotonin at the lowest concentration of 0.2 mg/g. Additionally, long- and branched-chain acylserotonins were identified, albeit in very small amounts, exemplified by the compound methyl-anteiso-C23-serotonin (Me-ai-C23) at 0.4 mg/g.

Microorganism contamination analysis. Over the course of 12 months, we conducted regular observations of bacterial contamination, including the total plate count, as well as yeast and mold levels (Fig. 4). These assessments were conducted on a monthly basis. The maximum allowable limits for microbial contamination in biscuits are established by the Indonesian Food and Drug Supervisory Agency [25]. According to their guidelines, the permissible limits are 1×10^4 CFU/g for the total plate count, as well as for the total yeast and mold contamination.

In the control samples, bacterial contamination had peaked at the 11th month, reaching its maximum allowable limit of 1×10^4 CFU/g. By the 12th month, bacterial contamination had further increased to 1.4×10^4 CFU/g. The overall yeast and mold contamination in these samples had already reached 1.2×10^4 CFU/g by the 10th month. Consequently, when considering microbial contamination, the product's effective age in the control samples could be regarded as only 10 months.

The shelf life of the wheat fiber biscuits extended to 12 months, even with a microbial contamination level of 1.15×10^4 CFU/g and total yeast and mold contamination of 1.1×10^4 CFU/g. In contrast, the citrus seed fiber biscuits exhibited lower bacterial contamination at 8.7×10^3 CFU/g by the 12th month, reaching total yeast and mold levels of 9.8×10^3 CFU/g. Notably, the biscuits containing citrus seed fiber boasted a shelf life exceeding 12 months. Thus, citrus seed fiber effectively



TPC: total plate count; CT: control sample (without fiber); WF: fiber from wheat seeds; CSF: citrus (pomelo) seed fiber

a b

Figure 4 Heatmap diagrams of microorganism contamination (a) and sensory evaluation (b) on a five-point Likert scale

extended the product's longevity with regards to microbial contamination, as illustrated in Fig. 4a.

While our study did not explicitly focus on microbial aspects, these results bolster the findings from previous research, indicating the positive impact of citrus seed fiber on the product's longevity and quality [16, 17, 19].

Sensory evaluation of the biscuits. A total of 40 respondents were randomly selected to evaluate the biscuits' quality in terms of taste, color, aroma, and texture. Figure 4b indicates a strong preference for the control biscuits among the respondents, as evidenced by the dominant red color in the chart. In the case of wheat fiber biscuits, the respondents particularly appreciated their color (57.5%) and aroma (47.5%). As for the biscuits with citrus seed fiber, the respondents showed a preference for their taste (50%), with some positive feedback regarding their color (22.5%) and a smaller proportion appreciating their texture (5%) and aroma (5%). While these biscuits may not be as popular as the control or wheat fiber samples, there is still potential for enhancing their quality, especially in terms of texture, aroma, and color.

Despite variations in texture, aroma, and taste among the biscuits enriched with dietary fiber from pomelo seeds, these biscuits continue to receive favorable ratings, particularly in terms of taste. The addition of dietary fiber significantly enhances their nutritional value by incorporating vitamins, minerals, and antioxidants [21]. This not only enriches the product's nutritional profile but also offers consumers the chance to enjoy delicious biscuits while reaping the benefits of plant dietary fiber.

CONCLUSION

Adding citrus dietary fiber to the biscuit dough significantly influenced the structure of starch and gluten, resulting in distinct characteristics. While the typical rubbery texture associated with starch and gluten was not readily observed in the citrus seed and wheat seed samples, these structural differences had a profound impact on the strength and texture of the biscuits. The samples with citrus seed fiber predominantly had large pores and were very fragile, requiring only 4326 ± 56 g force to break. Their fracture strength was substantially improved to 6000 g force by subjecting them to heating at temperatures between 155–170°C for 50–60 min, thus enhancing their overall texture and strength.

Our research underscores the beneficial impact of incorporating citrus seed fiber into biscuits, particularly in terms of boosting their antioxidant content and TEAC (both free and bound forms). The samples with citrus seed fiber contained flavonoid compounds that were absent in the wheat fiber samples and the control biscuits without dietary fiber. These compounds are believed to possess the potential to inhibit bacterial, mold, and yeast contamination, consequently extending the shelf life of biscuits beyond 12 months.

Pomelo (*Citrus maxima*) seed dietary fiber is a unique source of serotonin, a compound rarely encountered in the seeds of other plants. The predominant forms of serotonin in this fiber were acyl-*N*ω-methylserotonins, particularly in the C22 and C24 homologues, where long-chain bonds (C20-C28) were prevalent.

CONTRIBUTION

All authors involved in the research analysis and manuscript writing process.

CONFLICT OF INTEREST

There is no conflict of interest between the author.

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