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Total Phenolic Content (TPC) and Antioxidant Activities (AOA) of Orange (*Citrus Sinensis*) and Lemon (*Citrus Limon*) Peels Extract on Shelf Life of Handmade Donut

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Abstract

Citrus fruits such as oranges (Citrus sinensis) and lemons (Citrus limon) produce considerable peel waste. Peels have high concentrations of bioactive compounds such as phenolic acids and flavonoids, with antioxidant and antibacterial activity. In this work, orange peel extracts have been evaluated for use as a natural source of antioxidants in lieu of synthetic antioxidants such as butylated hydroxyanisole (BHA). Citrus peels have been subjected to Ultrasonic Assisted Extraction (UAE) with ethanol for extraction of phenolic compounds. Total phenolic content (TPC) and antioxidant activity have been determined with Folin-Ciocalteu and DPPH tests, respectively. Analysis revealed that peels of lemons contained a high TPC (7.564 mg GAE/g) and antioxidant activity (75.79% inhibition) compared to peels of oranges (4.764 mg GAE/g, 67.72% inhibition). Hand-made doughnuts have been evaluated for preservability. Lemon peel extracts extended shelf life for eight days, and peel extracts of oranges extended for seven days, in contrast to six days for the control. BHA extended for nine days. There was no significant antimicrobial activity, but statistics confirm citrus peel extracts as antioxidants and preservatives naturally. Extraction efficiency and yield of bioactive compounds can be optimized in future studies. Further studies in antibacterial activity, types of bacteria, and testing must be conducted. Investment value will be determined through food preference tests. Triple testing, particularly DPPH and Folin-Ciocalteu, must be conducted for accuracy. Confirmatory tests for citrus peel extracts for processing for food will make them practically useful. This work will transform citrus peels into functional food additives in an attempt to mitigate environmental concerns and maximize food use in a sustainable manner.

1. Introduction

Among all fruits consumed globally, Citrus species are generally consumed, especially oranges (*Citrus sinensis*) and lemons (*Citrus limon*) and most of their use involves peels that are considered wastes from the industry, mainly because of juice extraction [1]. This large amount of peels involves an environmental problem and a resource that is scarcely used. Citrus peels are rich in bioactive compounds like phenolic acids, flavonoids, and

essential oils that have been proven to exhibit high antioxidants and antimicrobial activities. Valorization of peels may provide a sustainable solution for reducing waste and simultaneously offer natural food preservatives [2].

Antioxidants are considered important in preventing the oxidation process, which is one of the major reasons for food spoilage [3]. While synthetic antioxidants, such as BHA, are widely used in the industry, because of the possible health and environmental risks they create, much interest has grown toward using more natural sources [4]. It was pointed out that peels of citrus fruits, especially lemons, contain a higher concentration of bioactive compounds compared with other fruits [5]. These compounds are not only effective in neutralizing free radicals but also confer other health benefits, including anti-inflammatory and cardioprotective effects [6]. Despite the known benefits of citrus peels, their practical application as natural preservatives in food products have not been fully explored.

Most of the recent studies is focused on the chemical composition and antioxidant capacity of citrus peels, with little emphasis on practical applications in food preservation. This represents a gap in research that calls for further investigation on how these natural compounds can be effectively used to prolong the shelf life of food products. Moreover, only limited studies have compared the antioxidant activities of peels from different citrus species, especially regarding their application as natural preservatives.

The main purpose of the research was to analyse the TPC and AOA of orange and lemon peels and their efficiency as natural preservatives for extending the shelf life of handmade donuts. This study was conducted to find answers to several questions such as which among the citrus peels exhibited higher antioxidant activity, can these extracts be used effectively to extend the shelf life of food products, is it can be proven that the lemon peel extract, which has a higher phenolic content, will prove better in antioxidant property than the orange peel extract, which making it more effective as a natural preservative.

By answering these questions, this study would be contributing to the development of sustainable methods of food preservation and less reliance on synthetic preservatives by utilizing the wastes of citrus peels. This will not only reduce the environmental hazard of waste citrus peel but also conform to the growing consumer demand for natural and eco-friendly food products. Such results may be further exploited by wider uses of citrus peel extracts in food industry, offering the twin advantages of reduction of wastes and value addition to foods.

2. Methodology

2.1 Sample Preparation and Extraction of Peels by using Ultrasonic Assisted Extraction (UAE)

Orange and lemon were obtained from the nearby local markets. The fruits were washed, and the peels were separated from the fruits and cut into smaller pieces using a kitchen knife. The fruit peels were then dried in the drying oven at 40°C for 24 hours. Afterward, the dried peels were grounded to get in the fine powder form. Approximately 10 g of orange and lemon peel powder were extracted according to [7] method with some modifications by using UAE and 150 ml of 90% ethanol as the solvent. The power used for extraction was constant at 60% amplitude, while the time of extraction were 30 minutes. The extracted sample were then separate from the solid residue using Whatman No. 1 filter paper. The filtered extracts were centrifuged at 5,000 rpm and 4°C for 15 minutes. Then, the sample were subjected into a rotary evaporator at 40°C to remove the solvent in the sample. The equation below was used to determine the extraction yield percentage.

$$Extraction\ yields\ (\%) = \frac{mass\ of\ extracted\ product}{mass\ of\ raw\ material} \times 100 \quad (1)$$

2.2 DPPH Radical Scavenging Assay

The DPPH radical scavenging assay method were applied according to [8] with modifications in this study to determine the antioxidant activity of the extracted sample. 5 mL of 0.1 mM DPPH were prepared in ethanol and combined with 1 mL of extracted sample. To protect the DPPH solution from light, the test tubes were covered with aluminium foil. Next, the test tubes were incubated at 37°C in the dark for 30 minutes. The absorbance of the reaction mixture was measured at 517 nm with a UV-VIS spectrophotometer. The scavenged DPPH will be calculated as:

$$DPPH\ scavenging\ activity\ (\%\ in\text{□}ibition) = \frac{Ab - As}{Ab} \times 100 \quad (2)$$

Progress in Engineering Application and Technology Vol. 6 No. 1 (2025) p. 625-634

Where,
A_b is absorbance of blank
A_s is absorbance of sample

2.3 Folin-Ciocalteu Reagent Method

The Folin-Ciocalteu reagent method were applied in order to determine the total phenolic content of the sample. Firstly, 3.16 ml of distilled water was added into test tubes. Then, 0.2 mL of Folin-Ciocalteu phenol reagent were added along with 40 µL of peels extract in each test tubes. 0.6 mL of 7.5% sodium carbonate was added to the mixture and gently vortexed. All the test tubes were incubated for 2 hours at room temperature. After incubation period, the absorbance of the developed blue complex will be measured spectrophotometrically at 765 nm against a blank using a UV-VIS spectrophotometer. The standard calibration curve will plot using gallic acid and the total polyphenol content were expressed as gallic acid equivalents.

2.4 Antimicrobial Test

For the antimicrobial activity, disk diffusion method was use. First, bacterial lawn was prepare by streaking *E.coli* culture on the Mueller-Hinton agar. 5 μL of extract were spotted alternately on both sides of the paper discs and allowed to dry before the next 5 μL was spotted to ensure precise impregnation. After that, the paper disc was placed on the bacterial lawn. The positive control of Ampicillin disc was also placed. All discs were fully dried before the application on the bacterial lawn. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition (ZOI) around the discs. Antibacterial activity will be expressed as the mean zone of inhibition diameters (mm) produced by the peel extract.

2.5 Application of Peel Extract on Handmade Donut

Citrus peel extracts were applied to the handmade donut using common donut making recipes with some modifications. To make the handmade donuts, 1 cup of warm milk were added with 2 and 1/4 teaspoons (1 packet) of active dry yeast and 1/4 cup of granulated sugar, 1/4 cup of melted butter into the large mixing bowl. The mixture was sat for about 5 minutes, or until it becomes frothy. Next, 4 cups of all-purpose flour and 1 teaspoon of salt were gradually added. Then, the peels extract was added separately and thoroughly stirred. The mixture was mixed until dough formed. The dough kneaded on a lightly floured surface for about 5 minutes, or until it becomes smooth and elastic. The dough was placed in a bowl, covered with a clean kitchen towel, and let it rise in a warm place for about 1 to 1 and 1/2 hours, or until it has doubled in size. Once risen, the dough was rolled out on a floured surface to about 1/2-inch thickness. A donut cutter was used to cut out the donuts. The cut donuts were placed on a parchment-lined baking sheet, covered with the towel, and let them rise for another 30 minutes. Then, the donuts were deep fried in hot oil until golden brown.

3. Results and Discussions

3.1 Extraction Yield

The extraction yield of orange and lemon peels using ethanol as the solvents are shown in Table 1. The yield of extraction was calculated by using the equation below.

$$Extraction\ yields\ (\%) = \frac{mass\ of\ extracted\ product}{mass\ of\ raw\ material} \times 100 \quad (3)$$

Table 1: Extraction yield of orange and lemon peels

Solvent	Citrus Peels	Yield (%)
Ethanol	Orange	43.7
	Lemon	38.9

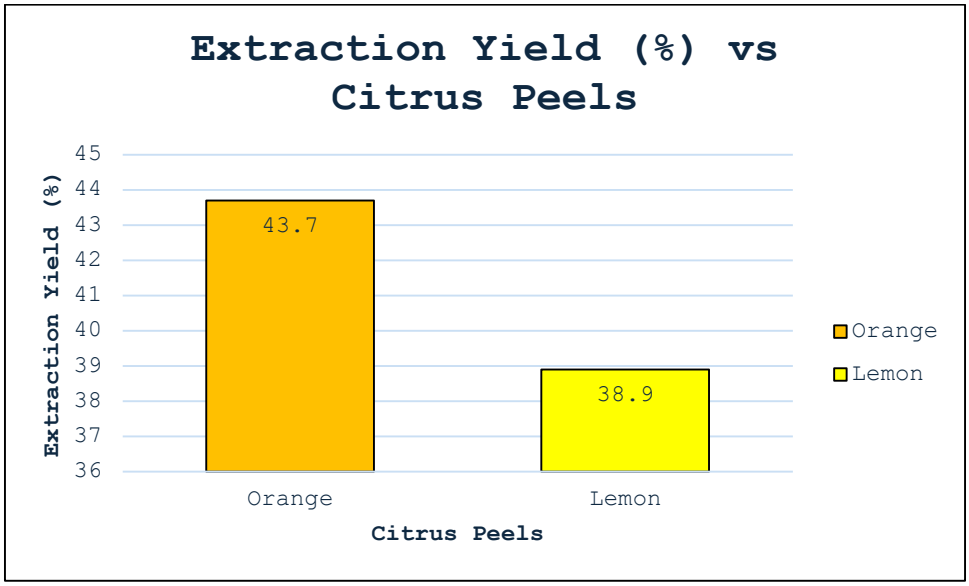


***Fig. 1*:** Graph of extraction yield against citrus peels

Based on the table and figure above, results showed the extraction yield of orange peels is the highest with the value of 43.7%. The mass of extracted product obtained from orange peels is 4.37 gram while lemon peels is 3.89 gram. Thus, lemon peels extraction yield is 38.9%, lower than orange peels extraction yield. These difference in extraction yield can be due to many factors for example extraction efficacy, number of soluble components, solvent used for extraction, and citrus varieties [8].

Extraction efficiency plays a key role in yielding. Orange peels had a higher yield (43.7%) than lemon peels (38.9%), likely due to differences in their physical and chemical properties, such as porosity and fiber content, which affect solvent penetration and compound release [9]. The proportion of soluble components also impacts yield. Orange peels may contain more extractable compounds like essential oils, flavonoids, and phenolics, making them more soluble in the solvent used [10]. Solvent choice is another factor. Ethanol, used in this study, effectively extracts bioactive compounds based on its polarity [11]. However, its compatibility varies by compound. Orange peel compounds may be more ethanol-soluble, enhancing extraction efficiency, whereas lemon peel compounds may be less soluble, leading to a lower yield.

3.2 DPPH Radical Scavenging Assay

After extraction of antioxidants from orange and lemon peels, the antioxidants were tested to determine the antioxidant activity by using DPPH radical scavenging assay method. The results obtained from the test are shown in Table 2 below.

***Table 2*:** Antioxidant activity of orange and lemon peels

Sample	DPPH scavenging activity (% inhibition)
Orange peels	67.72
Lemon peels	75.79

Progress in Engineering Application and Technology Vol. 6 No. 1 (2025) p. 625-634

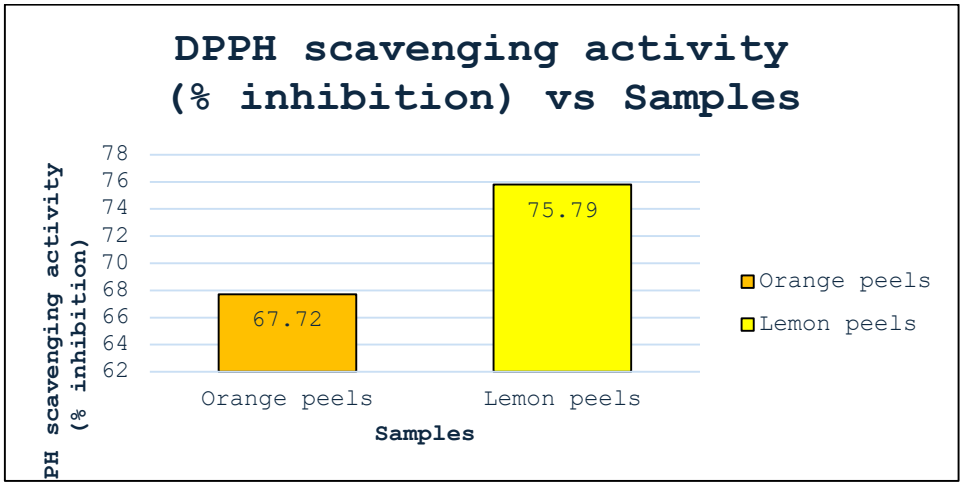


Fig. 2: Graph of DPPH scavenging activity against samples

It was showed in the graph that lemon peel extract showed higher DPPH scavenging activity than orange peel extract, indicating its higher antioxidant activity. This prove that lemon peel extract is more active against free radicals. In other words, when combined with DPPH solution, antioxidants in peel extracts donated hydrogen atoms or electrons to free radicals until neutralized and transformed into DPPH-H. This action was characterized by the violet colour turning into yellow, which, at a decrease in absorbance at 517 nm, provided the degree of free radical neutralization and therefore quantified the antioxidant activity. The results agree with other works such as [5], which reported the same activity in orange peel extracts but with a higher activity from lemon peel extracts. [12] also agreed that lemon peels have higher antioxidant properties, thus supporting the results of this study, with 75.79% DPPH scavenging activity for lemon peels. One of the limitations of this work is the sole utilization of the DPPH assay, which, though effective, probably may not describe the full spectrum of antioxidant activities of the extracts. Additional assays could round out this understanding. These findings show that lemon peel extract may prove to be an effective natural antioxidant for food preservation and can serve as an alternative to synthetic preservatives. This contributes to a significant reduction in food losses and ensures environmental sustainability.

3.3 Folin-Ciocalteu Reagent Method

Folin-Ciocalteu reagent method was applied to determine the total phenolic content (TPC) of orange and lemon peels extract. The amount of total phenolic content of the peels extract was calculated by using the standard curve of gallic acid which is shown below. The amount of total phenolic content is tabulated in Table 4.3.

$$y = 0.131x - 0.1814 \quad (4)$$

Where,
y = Absorbance of sample
x = equivalent concentration of phenolic compounds in the sample

Table 3: Total phenolic content (TPC) of orange and lemon peels extract

Sample	Total Phenolic Content (mg GAE/g)
Orange peels	4.764
Lemon peels	7.564

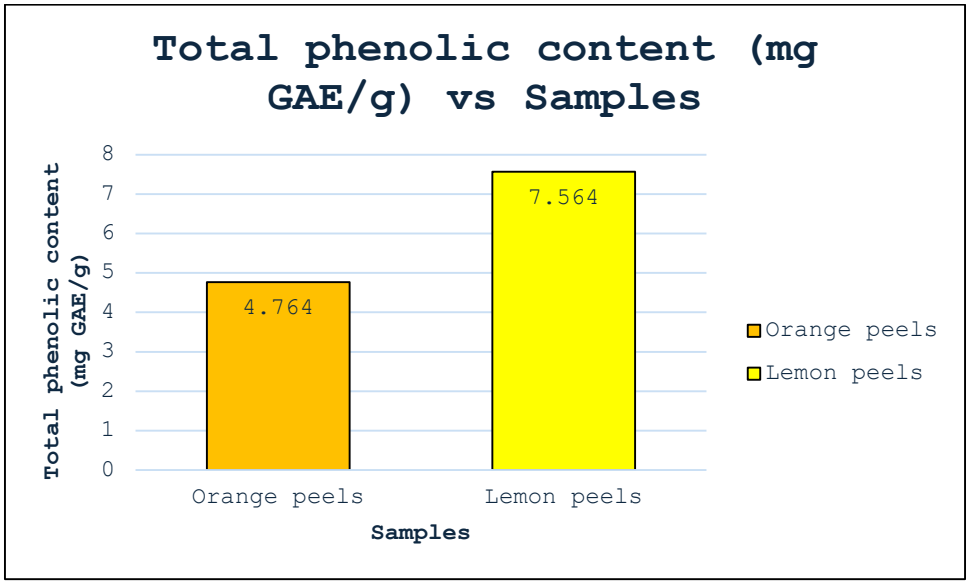


Fig. 3: Graph of total phenolic content against samples

Figure 3 indicates that the total phenolic content value in lemon peel extract is higher, with an average of 7.564 mg GAE/g, while orange peel extract has only 4.764 mg GAE/g. Similar studies, such as one conducted by [5], have also documented that lemon peels generally possess a high amount of phenolic compounds owing to their bioactive contents comprising flavonoids and phenolic acids. The variation in total phenolic content may be due to the different phytochemical profiles inherent in each citrus species, extraction methods, and conditions of fruit growth, among other environmental factors [13]. The Folin-Ciocalteu method adopted here relies on the reduction of phosphotungstic or phosphomolybdic acid complexes by phenolics into a blue complex, whose intensity at 765 nm is proportional to the phenolic content [14]. The lemon peel extract was more intense in blue colour, therefore indicating a higher phenolic content than that of orange peel extract, as shown in Figure 4.

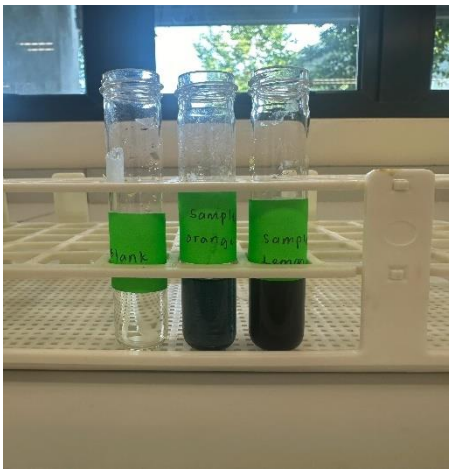


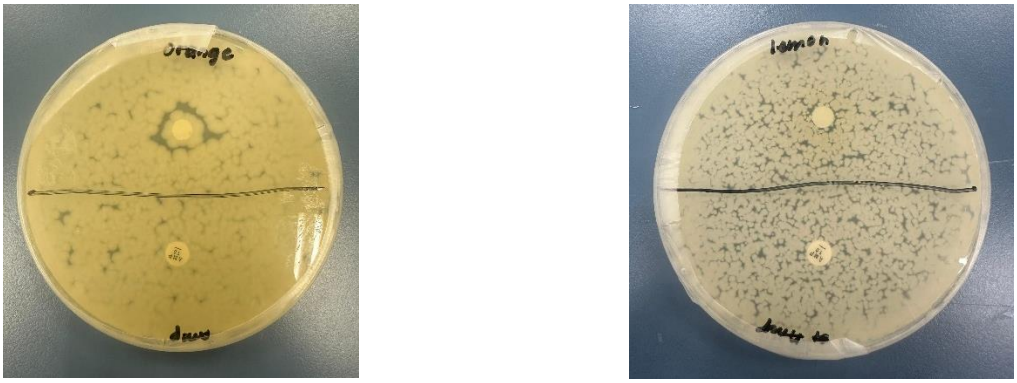
Fig. 4: Blue colour intensity of samples

This could mean that lemon peel extract may be more effective for applications requiring antioxidants, such as food preservation and health supplements. However, orange peel extract also demonstrated significant phenolic content, making it a viable option. A very reasonable positive relation between the total phenolic contents and the antioxidant activities can be reasoned from structural features such as phenolic compounds hydroxyl group donors of hydrogen atoms or electrons for free radicals' neutralization; hence, showing enhanced antioxidant action [15]. Compounds such as flavonoids and phenolic acids are highly involved in this process through their high efficiency in the neutralization of free radicals, thus enhancing the antioxidant properties of the extracts. This relationship indicates that phenolic compounds are important in enhancing the antioxidant capacity of plant-based samples [16].

Progress in Engineering Application and Technology Vol. 6 No. 1 (2025) p. 625-634

3.4 Antimicrobial Test

In this test, the antimicrobial activity of the extracted antioxidant from both orange and lemon peels were tested for further assessment in their capabilities to inhibit bacterial growth by implementing disc diffusion method. The results from this test will show the clear area around the antimicrobial agent which is the antioxidant where bacteria are unable to grow. The clear area is also known as the zone of inhibition (ZOI). The obtained results are presented below in Figure 5.



(a) **(b)**

***Fig. 5*:** Result of disc diffusion method of the peels extract (a) Orange extract; (b) Lemon extract

The obtained results showed that the peel extracts neither of orange nor lemon exhibited any clear zone of inhibition against bacterial growth, which meant that no marked antibacterial activity was expressed under the given conditions. The fact can be related to a few aspects that could affect such an outcome for example efficiency in the extraction assisted by ultrasound that would interfere with the actual bioavailability of antimicrobial substances, the proper extraction of phenolic or essential oil components can provide insufficient quantity in action, and remaining residual solvents such as ethanol interfere with the potentiality of active ingredients. The disc diffusion method used also has its limitations, especially when dealing with hydrophobic substances like essential oils. The diffusion of such non-polar molecules through agar may be restricted, leading to lower detectable activity despite the presence of active compounds. According to [16], contamination during handling or preparation of samples could also have impacted the test results and, hence, the effectiveness of the extracts. However, the orange peel extract showed some promises as a natural antioxidant with a slight clear area around it. Although the orange peel extract had a lower DPPH scavenging activity and total phenolic content than lemon peel extract, it is still promising for antioxidant usage.

3.5 Application of Peels Extract on Handmade Donut

The application of peels extract on handmade donuts was focused on the observation of rate of fungi formation on the donut. This is to distinguish the potential of the peels extract to act as a natural preservative for food. The results of observations throughout 14 days are shown below in Table 4. From all the samples, the blank sample was the first to have fungi formation on the donut. The fungi formation was circled on the figure. This means the donut shelf life that does not contain any preservatives is only 6 days long. On day 7 of donut storage, a little of fungi formation was spotted on the orange donut sample which was circled in the figure. This finding shows that orange peels extract can serve as a natural preservative because it can longer the shelf life of the donut by one day. On the eighth day of storage, lemon donut sample started to have fungi formation on the surface. This finding can support with the results of previous test which indicated that lemon peels extract is a better natural preservative compared to orange peels. This is due to the amount of total phenolic content of lemon peels extract is higher than orange peels extract. Thus, lemon peels extract can exhibit better antioxidant activity to longer the donut shelf life. And lastly, the formation of fungi was started to be seen on the BHA (synthetic preservative) donut sample on the day 9 of storage. This observation implies that synthetic preservatives have better performance as a food preservative even though it can longer the shelf life of donut by one day longer than lemon peels.

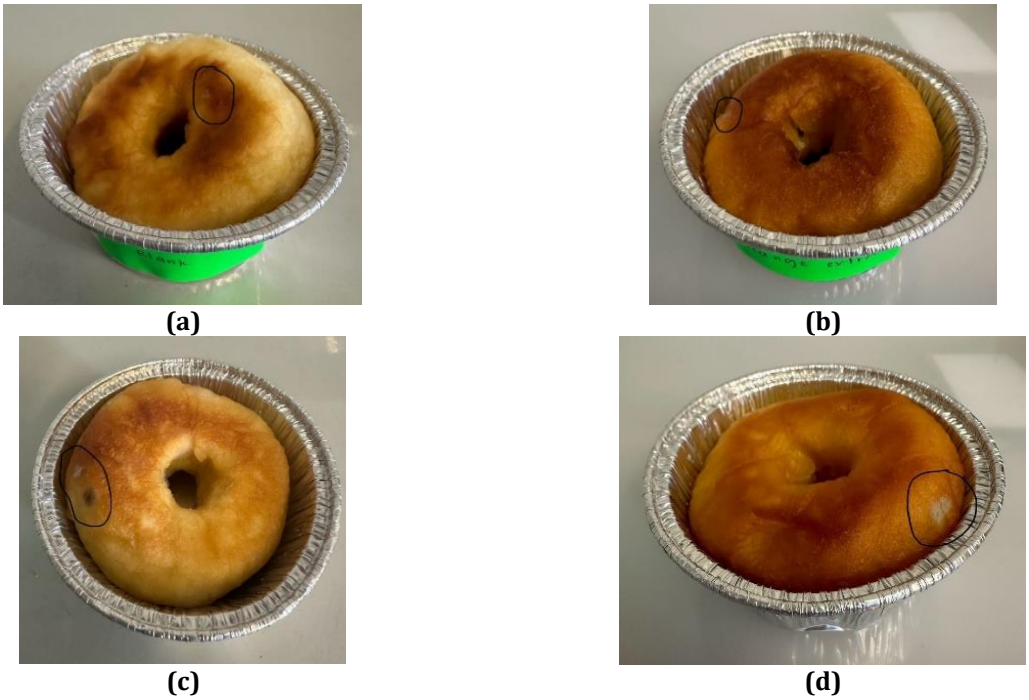


***Fig. 6*:** Observation of donuts throughout 14 days (a) Blank donut sample on day 6; (b) Orange donut sample on day 7; (c) Lemon donut sample on day 8; (d) BHA donut sample on day 9

4. Conclusion and Recommendations

4.1 Conclusion

This study proved that peels from citrus fruits, specifically orange (*Citrus sinensis*) and lemon (*Citrus limon*), have the potential as natural antioxidants and food preservatives. The peels of lemons were found to exhibit higher total phenolic content and antioxidant activities compared to orange peels, due to their higher concentration of bioactive compounds such as flavonoids and phenolic acids. In the DPPH assay, the lemon peel extract showed the highest capacity for free radical scavenging. Its higher amount of phenolics, as determined by the Folin-Ciocalteu method, enhanced its effectiveness as a natural preservative. These extracts practically demonstrated a potential in shelf life extension for homemade donuts. The lemon peel extract application showed that growth of fungi was significantly delayed by up to eight days compared to seven attained using the orange peel extract and the control. These findings may encourage more use of natural preservatives as they are less harmful and more eco-friendly options to their artificial alternative such as BHA. Although there are several limitations concerning their antibacterial properties, the study highlights the role of citrus peels in contributing toward sustainable food preservation. The approach adds value to waste and addresses environmental problems while at the same time improving the quality of food, contributing to studies of natural antioxidants and preservatives that can also find wider uses in the food industry.

4.2 Recommendations

Future studies should look on optimizing the extraction method to increase the yield and efficacy of bioactive compounds extracted from citrus peels. To improve the bioavailability of active compounds, several extraction solvents, circumstances, and procedures could be used. Furthermore, more research into the antibacterial capabilities of citrus peel extracts is suggested, with a focus on diverse bacterial strains and improved testing procedures to overcome potential limitations of the disc diffusion method. Investigating the utilization of citrus peel extracts in various food products and conducting sensory evaluations will also provide useful information about their economic viability. Other than that, to get more accurate and precise data, it is recommended to perform the test in triplicate especially DPPH radical scavenging activity and Folin-Ciocalteu reagent method test. Finally, research on the economic feasibility and scalability of employing citrus peel extracts in industrial food processing would help promote their practical use.

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Conflict of Interest

Authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

This journal requires that all authors take public responsibility for the content of the work submitted for review. The contributions of all authors must be described in the following manner:

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Shahmi Danial, Nor Faizah;* ***data collection:*** *Shahmi Danial;* ***analysis and interpretation of results:*** *Shahmi Danial, Nor Faizah;* ***draft manuscript preparation:*** *Shahmi Danial. All authors reviewed the results and approved the final version of the manuscript.*

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