

Research Article

Development of kombucha beverage by using fruit peels

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Abstract: Kombucha, a popular fermented tea beverage, undergoes biotransformation using a symbiotic culture of yeasts, fungi, and bacteria, yielding acetic acid. In response to the rising global demand for fruit-based food products, substantial amounts of fruit waste, including peels, seeds, and pulps, have contributed to environmental pollution. Peel waste, a significant byproduct generated by the fruit and vegetable industry and households, leads to nutrient and financial losses while posing environmental challenges. This study aimed to optimise kombucha formulations by incorporating orange peel (OP) and pomegranate peel (PP) to improve key parameters: antioxidant activity and sensory properties. Employing simplex lattice mixture design (Design Expert 13.0.1 software), an optimal formulation was achieved, comprising 75.00% OP and 25.00% PP, with a desirability value of 1.00. This innovative kombucha formulation, utilising OP and PP, offers multiple advantages, including biological activity, sustainability, and alignment with consumer preferences. The incorporation of OP and PP enhances the antioxidant activity and sensory properties thus further augmenting the renowned health benefits associated with fermented tea. By combining the zesty and citrusy notes of orange with the sweet and tart flavours of pomegranate, this novel product delivers a refreshing beverage that resonates with health-conscious consumers. The development of orange and pomegranate peel kombucha represents a compelling response to the growing demand for sustainable and nutritious beverages.

Keywords: kombucha, orange peel, pomegranate peel



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1. INTRODUCTION

Everywhere in the world, kombucha is consumed by a large population. The biotransformation of sugared tea created this healthy beverage by yeasts, fungi, and bacteria that produce acetic acid. The word "kombucha" has its roots in tea (cha) and Japanese seaweed (kombu). The kombucha's microbial composition is influenced by the types of yeasts and bacteria used, the environmental and climatic conditions in which they were grown, and its starting microbiome (Ivanišová et al., 2019). It has long been recognised that kombucha tea provides revitalising and cleansing effects. Additionally, kombucha tea was first ingested in East Asia for its curative properties, including treating digestive issues. Moreover, kombucha's popularity skyrocketed as it was promoted as a healthy fermented food with benefits comparable to yoghurt consumption (Kaewkod et al., 2022). Most kombucha formulas use tea as the primary infusion, including black tea, green tea, oolong tea, and others. To the author's

knowledge, no scientific study has been published on dried orange (*Citrus sinensis* L.) peel infusions formulated with dried pomegranate (*Punica granatum* L.) peel as the substrate for kombucha fermentation while using simplex-lattice mixture design optimisation technique to create a formulation for kombucha. However, some publications have been published on using other herbal teas as a medium for creating kombucha beverages. Therefore, in the present research, dried orange and pomegranate peels kombucha beverages were produced. The 4 formulations were optimised using two parameters: antioxidant activity and sensory properties. The results of these fundamental studies are to develop novel food products made from food waste that have biological properties and can benefit human health when consumed.

2. METHODOLOGY

2.1 Preparation of orange and pomegranate peels

The fruit peels were obtained from the fruit that was purchased at the local market for orange mandarin and pomegranate fruit. Both of the peels were washed and treated with 2% of sodium carbonate to prevent any mold growth during the processing and storage. The orange peel (OP) was dried for 90 hours, until it achieved a constant moisture content of 13.66% and water activity of 0.5197aw. Meanwhile, the pomegranate peel (PP) was dried for 48 hours until it achieved a constant moisture content of 7.50% and water activity of 0.2078aw.

2.2 Preparation of dried orange and pomegranate peels kombucha formulations

The kombucha starter culture, SCOBY, was purchased from a commercial source. The starter culture employed in this study is composed of cellulose layer and sour broth, and it was kept in a refrigerator (4°C) (SCOBY floating on the liquid surface) (Jakubczyk et al., 2020). The sour broth was prepared by steeping 1% of tea leaf (Lipton Brand) in 1000 mL of filtered hot water for 10 minutes, then 10% of sugar was added, the solution was allowed to cool down and the 50 g of SCOBY to the solution. The solution was allowed to ferment for 14 days. For steeping method, as the procedure suggested by Jakubczyk et al. (2020), 1 L distilled water (90°C) was combined with 100 g of sugar (100 g/L, 10%) and 10 g of tea leaf (10 g/L, 10%). However, since the study is aiming to produce the dried orange and pomegranate peels kombucha beverages, the mixture of the dried OP and PP as the medium of infusion were formulated according to percentages generated by the Design Expert software (Stat-Ease, Inc., Version 13.0, Minneapolis, USA) (Table 1). The three formulation mixture of OP and PP was weighted (10 g) according to percentages generated by the simplex-lattice mixture design and was placed in the tea bag for the preparation of the kombucha sample and one sample (control) was also prepared. A sterile conical flask was used to infuse the solution for 10 minutes. The dried OP and PP brew infusion was cooled down to 30°C and then put into clean glass bottles after being filtered with nylon filters. The SCOBY was added along with the sour broth solution (10%). The dried orange and pomegranate peels kombucha formulations were incubated at room temperature $28^{\circ}\text{C} \pm 1$ for 14 days of fermentation.

Table 1. Experimental Design for percentage of Dried Orange Peel and Dried Pomegranate Peel Powder obtained from Simplex-Lattice mixture design

Sample	Factors (%)	
	X ₁	X ₂
Formulation 1 (F1)	0.25	0.75
Formulation 2 (F2)	0.5	0.5
Formulation 3 (F3)	0.75	0.25
Formulation 4 (F4) (control)	0	0

Note: X₁: Dried Orange Peel, X₂: Dried Pomegranate Peel

2.3 Antioxidant activity and sensory properties of dried orange and pomegranate peels kombucha

2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of kombucha was measured according to (Zubaidah et al., 2018). Approximately, 7.8864 mg of DPPH was dissolved in 100 mL of 95% ethanol and was stirred using a magnetic stirrer and placed in an Amber Schott bottle. Then, 0.6 mL of the kombucha sample was mixed with 4.5 mL of a 0.1 mM DPPH solution. After 30 minutes of dark incubation, the mixture was tested for absorbance at 517 nm using the sample blank as the control. Antioxidant activity was assessed by measuring changes in absorbance, and was reported as a percentage of DPPH radical scavenging activity. Each assay was carried out two times. Ethanol and the DPPH solution was used as controls (Wan Sapawi et al., 2019). Results were displayed as a percentage of DPPH radical inhibition. The % of DPPH inhibition has been used to express the antioxidant of tested sample:

$$\text{Scavenging Activity (\%)} = [A_0 - A_s / A_0] \times 100$$

where:

A₀: absorbance of DPPH solution at 517 nm without tested sample (Blank); A_s: absorbance of DPPH solution at 517 nm with tested sample.

The hedonic test was conducted to determine the consumer preference for the product attributes (odour, colour, taste and overall acceptability). First, 30 panelists from UiTM Kuala Pilah campus staff and students were assessed the sensory properties of 4 formulations of dried orange peel and pomegranate peel kombucha beverages. The samples were coded in a clear plastic cup. The panelists were then assigned coded sample order using randomisation. The panelists were asked to assess the odour, colour, taste and overall acceptability of the sample. To minimise the impact of lingering flavour, the panelists were advised to rinse their mouths twice with water in between samples. Between sample testing, plain biscuits and water were provided to cleanse the palate. Finally, the panelists were assigned a 9-point hedonic rating to each sensory feature. Scale 1 is the lowest score, and scale 9 is the highest.

3. FINDINGS

The four kombucha formulations were analysed on day 14. These analyses included DPPH radical scavenging activity and hedonic test sensory evaluation. The results for analysis were presented in a table and graph.

3.1 DPPH radical scavenging activity

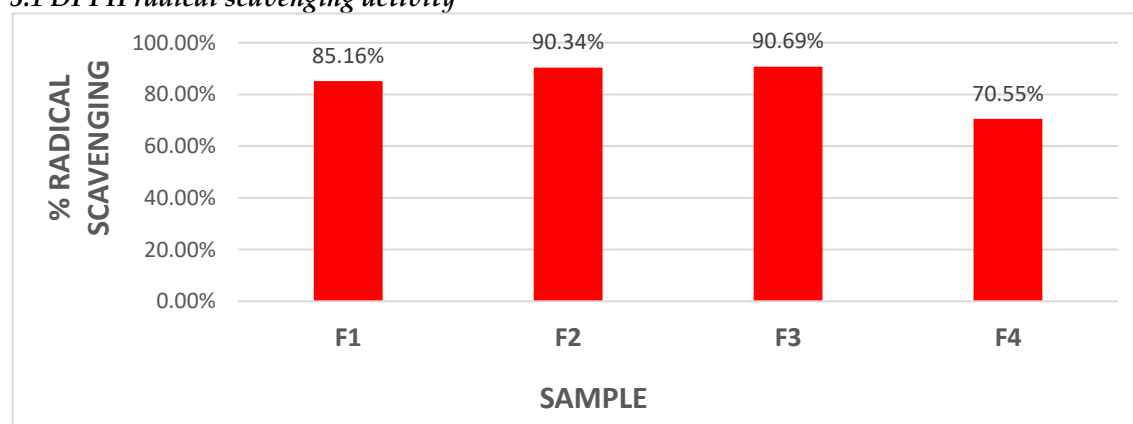


Figure 1. DPPH radical scavenging activity of the orange and pomegranate peels kombucha formulations on day 14

The assessment of the antioxidant activity of the four formulations under investigation indicated that the % DPPH radical scavenging activity ranged from 70.55% to 90.69% (as presented in Table 2). After 14 days of fermentation, F3 (0.75% OP:0.25% PP) exhibited the highest DPPH radical scavenging activity followed by F2 (0.50% OP:0.50% PP), F1 (0.25% OP:0.75% PP) and F4. The results indicated that the highest percentage of OP exhibited the highest antioxidant activity. However, F4 recorded the lowest DPPH radical scavenging reading due to no presence of the fruit peels in the sample. Therefore, the presence of OP and PP in the kombucha formulations contribute to their antioxidant activity compared to the control sample.

3.2 Sensory properties (Hedonic test)

Table 2. Summary total score Hedonic test of orange and pomegranate peels kombucha formulations on day 14

SAMPLE	ATTRIBUTES			
	<i>Odour</i>	<i>Colour</i>	<i>Taste</i>	<i>Overall Acceptability</i>
F1	103	145	116	125
F2	162	173	172	175
F3	153	169	167	173
F4	137	139	171	163

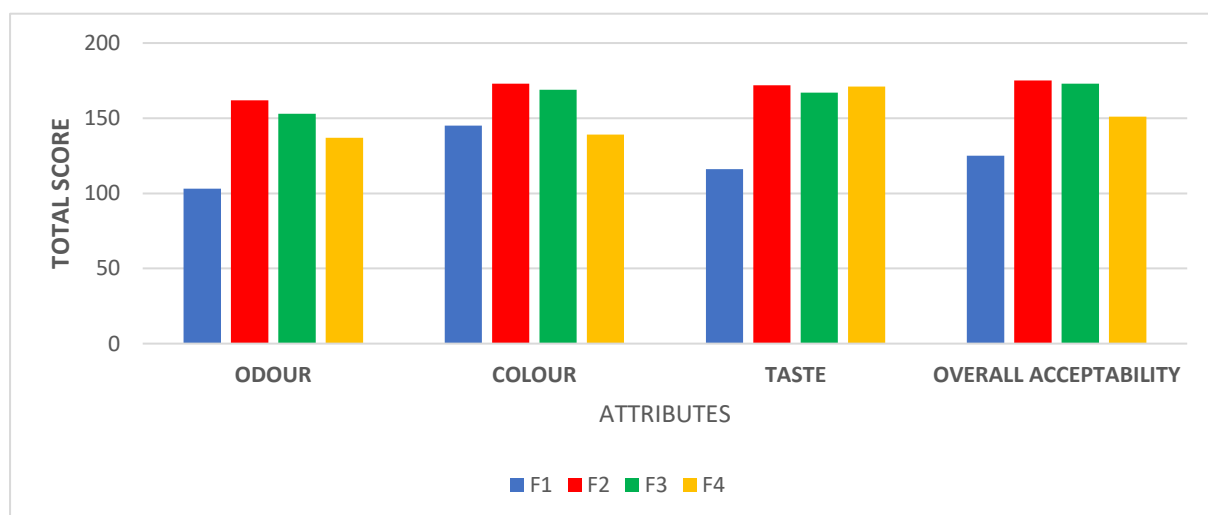


Figure 2. Sensory evaluations (Hedonic test) of the orange and pomegranate peels kombucha formulations at day 14

These scores reflect the perception and preference of the panelists who participated in the hedonic test. Higher scores indicate a more favourable evaluation of the respective attribute. In terms of odour, F2 received the highest score (162), followed by F3 (153), F4 (137), and F1 (103), suggesting that F2 was perceived as having the most appealing odour among the formulations. Regarding colour, F3 obtained the highest score (169), followed by F2 (173), F1 (145), and F4 (139), indicating that F3 was perceived as having the most visually appealing colour. For taste, F1 received the highest score (176), followed closely by F2 (172), F3 (167), and F4 (171). This indicates that F1 was perceived as having the most enjoyable taste among the formulations. When considering overall acceptability, F2 received the highest score (175), followed by F3 (173), F4 (163), and F1 (125). This suggests that F2 was perceived as the most acceptable formulation overall by the panelists.

3.3 Simplex-lattice mixture design

During the experimental design and analysis, the focus is on finding the mixture proportions that maximise the desirability value. This involves assessing the effects of different mixture components and their interactions on the response variable. Table 3 and Figure 3 present the optimised solutions for all formulations in the study.

Table 3. Optimised solution for all formulation generated by simplex-lattice mixture design

Number	OP	PP	R1	R2	R3	R4	R5	Desirability	
1	0.75	0.25	91.495	26.475	174.33	177.67	181.667	1.00	Selected

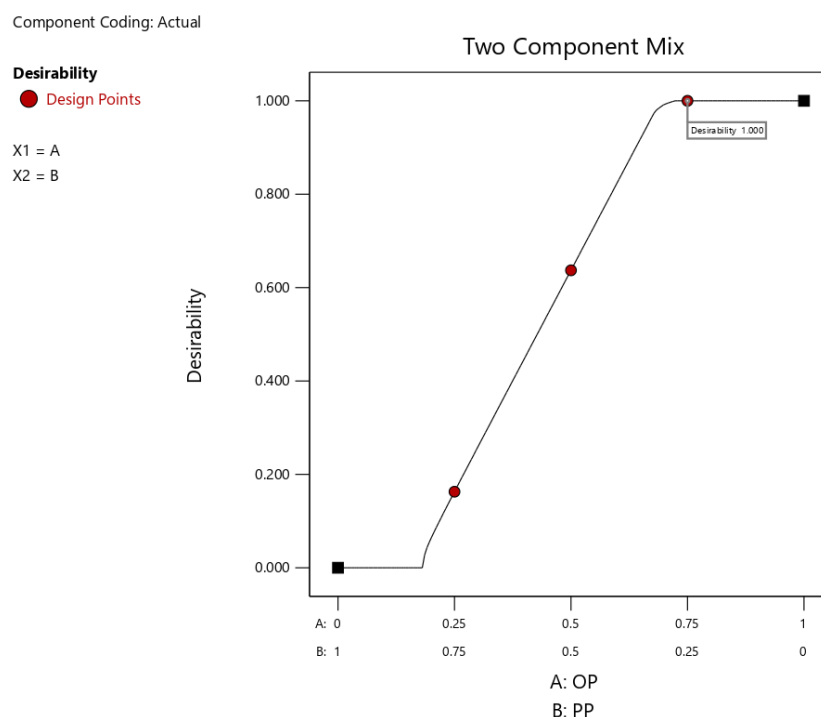


Figure 3. Optimised formulation plotted against desirability for all formulations

The response variables considered are % DPPH radical scavenging activity (R1), odour (R2), colour (R3), taste (R4) and overall acceptability (R5). The desirability value was 1.00 or 100.00% with formulation generated was 75.00% of orange peel and 25.00% of pomegranate peel.

4. DISCUSSION

In general, the addition of OP and PP exhibited the highest DPPH radical scavenging activity compared to the non-treated samples (control). These findings suggest that the fermentation duration and the composition of substrates made from a combination of OP and PP have a substantial impact on the qualities and beneficial compounds of kombucha. The evaluation of antioxidant potential revealed that the concentration of antioxidant compounds in the final stage of fermentation ranged from 70.55% to 90.69% DPPH radical scavenging activity. OP is known to be rich in specific phenolic chemicals, including naringin, hesperidin, narirutin, and neo hesperidin, which are characteristic flavanone glycosides found in citrus (Saini et al., 2022). PP on the other hand, contains ellagic acid, gallic acid, caffeic acid, chlorogenic acid, syringic acid, ferulic acid, vanillic acid, p-coumaric acid, and cinnamic acid (Chen et al., 2020). These bioactive compounds contribute to the increased antioxidant properties of kombucha.

The hedonic test results provide insights into the evaluation of different formulations (F1, F2, F3, and F4) based on their sensory attributes, including odour, colour, taste, and overall acceptability. Looking at the scores obtained for each attribute, we can observe some patterns. In terms of odour, F2 received the highest score (162), indicating that it was perceived as having a more pleasant smell compared to the other formulations. F1 received the highest score for taste (176), suggesting that it was perceived as the most enjoyable in terms of flavour. F2 also obtained the highest score for overall acceptability (175), indicating that it was the most preferred formulation overall by the evaluators. When it comes to colour, F3 received the highest score (169), suggesting that it was perceived as visually appealing. These results highlight the differences in sensory characteristics among the formulations and provide valuable information for further development and improvement based on consumer preferences.

The experimental design and analysis in this study aimed to determine the optimal mixture proportions that would maximise the desirability value. The effects of different components and their interactions on various response variables were assessed. The response variables considered included DPPH radical scavenging activity and sensory properties. The desirability value obtained was 1.00 or 100%. The formulation generated consisted of 75.00% orange peel and 25.00% pomegranate peel.

5. CONCLUSION

In conclusion, the research focused on the development of kombucha utilising orange peel and pomegranate peel as key components. The experimental design and analysis aimed to identify the optimal mixture proportions that would maximise the desirability value, considering various response variables such as DPPH radical scavenging activity and sensory properties. The desired desirability value of 1.00 was F3, consisting of 75% orange peel and 25% pomegranate peel. These findings suggest that this formulation holds potential as the optimised formulation for the development of kombucha using orange peel and pomegranate peel. It exhibited favourable characteristics based on the evaluated response variables. Further studies and validation are recommended to fully assess the sensory attributes, shelf stability, and potential health benefits of the developed kombucha formulation. Additionally, investigating the bioactive compounds and their mechanisms of action in orange peel and pomegranate peel could provide valuable insights for future research and product development in the field of functional beverages.

Acknowledgements: The authors are also grateful to the Faculty of Applied Science, Universiti Teknologi MARA Cawangan Negeri Sembilan, Kampus Kuala Pilah for the use of laboratory facilities.

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