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Formula Optimization of a Java Tea-Based Functional Drink Using Micro-Filtered Extracts

Brigitta Andien Alverina[1)], Christofora Hanny Wijaya[1)]*, Vallerina Armetha[1,2)], Helen Julian[3)]

[1)] Division of Food Science and Technology, Faculty of Engineering and Technology, IPB University, Bogor, Indonesia
[2)] Division of Chemical Engineering, Design, and Smart Manufacture, Faculty of Engineering and Technology, IPB University, Bogor, Indonesia
[3)] Chemical Engineering Department, Bandung Institute of Technology, Bandung, Indonesia

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ABSTRACT

Microfiltration (MF) is a non-thermal technique for microbial removal; however, it may also alter sensory and physicochemical properties, as compounds larger than the membrane surface or pore size are excluded. This study aimed to optimize the formulation of Java tea-based functional drink prepared with microfiltered extracts to achieve a balanced composition with optimum antioxidant and antihyperglycemic activities and sensory acceptability. The results showed that the 0.2 μm MF reduced microbial counts to below the detection limit (<1×10^0 CFU/mL). Filtration also altered the physicochemical properties, causing significant changes in color, pH, particle size, polydispersity index, and total dissolved solids. A D-optimal mixture design was employed using Design-Expert version 13, considering five factors: proportion of Java tea, sappan wood, ginger, lime, and kaffir lime extract. A total of 25 formulations were evaluated for antioxidant activity, antihyperglycemic potential, and sensory acceptability. The developed models exhibited strong predictive performance, except for aroma, which was excluded from optimization. The optimum formulation was chosen based on the highest desirability index (0.936). The verified optimized formulation resulted in an antioxidant activity of 588 ppm AEAC, an α-glucosidase inhibition of 59.9%, and a hedonic taste score of 6.7 on a 9-point scale. Compared with pasteurized or commercial counterparts, the optimized microfiltered formulation showed no detectable microbial colonies, higher bioactivity than the pasteurized sample, and superior color and overall sensory acceptability.

Keywords: antihyperglycemic, antioxidant, formula optimization, Java tea-based functional drink, microfiltration

INTRODUCTION

Traditional herbal beverages, such as "jamu", have been consumed for generations as part of Indonesian cultural practices for health maintenance (Harmayani *et al.,* 2019). In line with this tradition, advances in food science and increasing awareness of diet-related health issues have encouraged the reformulation into functional products to prevent degenerative diseases, including type-2 diabetes mellitus (T2DM). In Indonesia, a Java tea-based functional drink developed by Wijaya *et al*. (2007) incorporates extracts from locally sourced medicinal plants (e.g., Java tea leaves, sappan wood, ginger, curcuma, lime, and kaffir lime), exhibited significant efficacy in moderating postprandial blood glucose levels in T2DM patients (A'yuni *et al.,* 2022; Wijaya *et al.,* 2018). However, its large-scale production is constrained by high microbial loads in fresh extract, frequently exceeding 10^4 CFU/mL (Wijaya *et al.,* 2018). To ensure safety, current manufacturing practices rely on thermal pasteurization and chemical preservatives (Wijaya *et al.,* 2018). Heat treatments often compromise heat-sensitive bioactive compounds and alter sensory attributes, while chemical preservatives may conflict with consumer expectations for clean-label functional foods (Ramírez-Moreno *et al.,* 2025). Consequently, there is a growing need for innovative processing strategies that ensure food safety.

Microfiltration (MF) membrane technology has emerged as a promising alternative to conventional thermal preservation methods, as it enables effective microbial removal while better retaining heat-sensitive bioactives (Anis *et al.,* 2019). Findings by Meutia *et al.* (2023) demonstrated that applying MF to Java tea-based functional drink extracts achieved complete microbial elimination, as no detectable colonies were observed in the filtrates, while concurrently inducing physicochemical alteration and reducing functional capacity, for instance, a 63.95%

*Corresponding Author: Email: channywijaya@apps.ipb.ac.id

decline in ginger antioxidant activity (from 36.06 to 13.01 ppm AEAC). These reductions are primarily attributed to the size-exclusion effect. Therefore, beyond adopting membrane-based processing, optimizing the proportion of medicinal plant extract is essential for balancing functional efficacy with consumer-preferred sensory attributes in Java tea-based functional drinks. Excessive concentrations may enhance bioactivity but often impair flavor profiles, color stability, or reduce consumer acceptability, whereas insufficient levels may compromise the intended health benefits (Rutkowska and Pasqualone, 2025). From an industrial perspective, achieving this balance is critical to develop nutritionally adequate and commercially viable products regarding taste, appearance, and overall market acceptance. This study aimed to optimize the formulation of Java tea-based functional drink using microfiltered extracts, targeting maximum antioxidant and antihyperglycemic activity, improving microbial safety, and enhancing sensory appeal. In addition, the optimum formula will be compared with the pasteurized formula and commercial Java tea-based functional drink.

MATERIALS AND METHOD

Materials

Java tea (*Orthosiphon aristatus* Bl. Miq), sappan wood (*Caesalpinia sappan* Linn), curcuma (*Curcuma xanthorrhiza*), ginger (*Zingiber officinale* Roscoe), lime (*Citrus aurantifolia* Swingle), and kaffir lime (*Citrus hystrix* DC) were sourced from Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia. α-glucosidase and DPPH (*2,2-diphenyl-1-picrylhydrazyl*) were purchased from Sigma-Aldrich (St. Louis, MO, USA). p-NPG (*p-nitrophenyl-α-D-glucopharanoside*), ascorbic acid, and methanol were purchased from Merck (Darmstadt, Germany). Plate count agar (PCA) medium was purchased from Oxoid (Hampshire, UK). Food-grade ethanol 70%-wt was purchased from Onemed (Indonesia). A Q3/2 MF hollow-fiber polypropylene membrane (pore size 0.2 *μ*m, thickness 200 *μ*m, hydrophobic) was purchased from 3M™ (USA). The Ethics Committee of IPB University approved this study to involve humans as panelists of sensory analysis (Approval No. 1768/IT3.KEPMSM-IPB/SK/2025).

Pasteurization using microfiltration

Each herbal extract (Java tea, sappan wood, ginger, curcuma, lime, and kaffir-lime) was subjected to MF. The MF procedure and system setup followed the method described by Meutia *et al.* (2023). Prior to MF, the tubing and membrane pores were sterilized with 70% ethanol for 3 min, followed by rinsing with deionized water. The operating pressure was maintained at a maximum of 2 bar using a booster pump (model KJ-2648, KOJINE, manufactured by Deng Yuan, Taiwan) maintained the operating pressure at a maximum of 2 bar. The resulting microfiltered extracts were subsequently used as ingredients for formula optimization.

Extract characterization

Fresh and microfiltered extracts were characterized for particle size and polydispersity index (PdI), total dissolved solids (TDS), pH, microbial load by the total plate count method, and color in the CIELAB system. All measurements were performed in duplicate.

Formula optimization

The proportion of Java tea, sappan wood, ginger, lime, and kaffir lime extract were selected as factors due to their functional and sensory properties. A D-optimal mixture design in Design-Expert software (version 13, Stat-Ease Inc., Minneapolis, USA) was applied to identify the optimum proportion of each factor selected. Table 1 shows the responses obtained from 25 formulations. The response data were then analyzed using analysis of variance (ANOVA) to assess the model's validity, ensuring only valid models were advanced to the optimization stage, at a significance level of $p<0.05$. The optimum formulation was selected according to the highest desirability index. The optimum formula was then verified to assess the model's validity in predicting new responses, in which responses were considered valid if they fell within the 95% prediction interval (PI).

Comparative evaluation

The optimized formulation prepared with microfiltered extracts (MF-OF) was compared with the optimized formulation subjected to thermal pasteurization (PAST-OF), and with a commercial Java tea-based functional drink (COM). The comparison was based on rating hedonic, microbial load by the total plate count method, antioxidant activity, and antihyperglycemic activity. All measurements were performed in duplicate.

Microbiological analysis

The total plate count (TPC) method was used to quantify microorganisms, following the procedure described in SNI ISO 4833-1:2013 (BSN, 2013). For microbiological analysis, 1 mL of each serial dilution was aseptically transferred into sterile petri dishes in duplicate. Plate count agar (PCA) medium, maintained at 44–47 °C, was poured into each dish and thoroughly mixed with the sample to ensure uniform distribution. After solidification, the plates were inverted and incubated (LEEC, UK) at 30±1 °C for 48±2 h. Colony counts within the range of 10–300 were considered valid, and the results were reported as colony-forming units per milliliter (CFU/mL).

Table 1. Responses of 25 formulations of Java tea-based functional drink prepared using mixture design

Run	Response					
	Hedonic Rating*				Bioactivity	
	Color	Aroma	Taste	Overall	Antioxidant Activity (ppm AEAC)**	Antihyperglycemic Activity (% Inhibition)***
1	6.8	6.9	6.0	6.3	510.35	7.58
2	7.3	7.1	7.4	7.4	506.57	40.44
3	6.5	7.0	6.8	6.8	529.42	34.88
4	7.3	6.9	6.3	6.3	626.42	26.28
5	6.4	6.8	6.7	6.8	595.33	33.34
6	5.6	5.7	3.9	4.6	493.87	13.81
7	6.0	7.3	5.3	5.9	450.92	9.37
8	6.9	6.5	6.5	6.8	547.35	26.80
9	6.4	7.1	6.1	6.1	406.50	22.57
10	7.3	7.1	7.4	7.4	569.22	43.51
11	7.3	7.1	7.4	7.4	559.47	41.92
12	7.1	6.4	5.9	6.4	522.67	43.07
13	7.4	7.6	6.2	6.8	515.35	9.20
14	6.1	5.7	5.3	5.6	421.93	0.84
15	6.5	7.3	5.9	6.0	384.35	20.81
16	6.2	6.3	5.2	5.3	427.13	10.93
17	6.9	7.4	7.0	6.8	372.37	26.59
18	7.3	7.1	7.4	7.4	600.68	42.41
19	7.0	6.9	4.5	4.9	416.70	12.97
20	6.6	5.7	4.7	4.9	378.85	6.30
21	7.3	7.1	7.4	7.4	430.15	39.66
22	6.3	5.7	6.3	6.3	383.48	19.78
23	3.3	5.7	4.4	4.3	390.27	11.99
24	5.6	6.3	6.3	6.4	365.68	16.73
25	6.6	6.0	6.3	6.3	557.98	68.67

Note: Each run corresponds to formulations generated by mixture design. *Hedonic rating scores were obtained using a 9-point hedonic scale (1= dislike extremely, 9= like extremely). **mg ascorbic acid equivalent/L. ***Inhibition percent of α-glucosidase activity

Physicochemical analysis

Physicochemical analyses included measurements of color, particle size and polydispersity index (PdI), total dissolved solids (TDS), and pH. Color was measured using a chromameter (CR-300, Konica Minolta Sensing, Japan) following the CIE color system (CIE 2018). The sensor was placed directly on the sample surface to prevent external light interference, and measurements were taken at three different points. Results were expressed in the CIELAB system. Color difference (ΔE) was calculated based on the standard Equation 1.

$$\Delta E= \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \ldots\ldots\ldots\ldots (1)$$

For particle size and PdI analysis, sample of extract was diluted with HPLC-grade water to achieve 20–80% transmittance, placed in a disposable cuvette, and analyzed in duplicate using a Litesizer DLS 500 (Anton Paar, Austria). Next, TDS were determined according to APHA (2023) using a portable TDS meter (DiST 1, Hanna Instruments, USA), with each sample analyzed in duplicate. Then, pH was measured following ISO 10523:2023 using a calibrated pH meter (Apera Instruments, USA). Approximately 30–50 mL of each sample was placed in a container, and measurements were duplicated.

Antihyperglycemic activity

The α-glucosidase inhibition assay was used to analyze antihyperglycemic activity, following the method of Mayur *et al*. (2010) with minor adjustments. The α-glucosidase enzyme (0.2 U/mL) derived from *Saccharomyces cerevisiae* was used as the inhibitory target. In a 96-well microplate (Biologix Europe GmbH, Germany), 10 *µ*L of sample or acarbose standard was mixed with 50 *µ*L of 0.1 M phosphate buffer (pH 7.0) and 50 µL of enzyme solution, then pre-incubated at 37 °C for 10 min. The reaction was initiated by adding 25 *µ*L of 0.5 mM p-nitrophenyl-α-D-glucopharanoside substrate and continued incubation for 30 min at 37 °C. It was then terminated by adding 100 *µ*L of 0.2 M sodium carbonate, which also developed color. Absorbance was measured in duplicate at 410 nm using a Varioskan Lux Multimode microplate reader (Thermo Scientific, USA).

Antioxidant activity

The antioxidant activity was analyzed using DPPH method following the procedure described by Kubo *et al*. (2002) and Molyneux *et al*. (2004), with slight modifications to suit the experimental conditions. 2.5 μL of either sample or the ascorbic acid standard was mixed with 187.5 μL of methanol and 10 μL of DPPH solution. The mixture was incubated in the dark for 30 min. Absorbance was subsequently measured at 517 nm using a Varioskan Lux Multimode microplate reader (Thermo Scientific, USA). A standard calibration curve was prepared using ascorbic acid at 100, 200, 400, 600, and 800 ppm concentrations. All measurements were performed in duplicate.

Sensory evaluation

Sensory evaluation was conducted with 70 untrained panelists using hedonic rating tests to understand consumer-driven product differentiation. The hedonic rating test followed Meilgaard *et al.* (2016) with minor adjustments to the product context. In the hedonic rating test, panelists evaluated four attributes (such as aroma, color, taste, and overall acceptability) using a nine-point categorical hedonic scale, where higher scores indicated greater preference.

RESULTS AND DISCUSSION

Microbial and physicochemical quality of microfiltered extracts

Based on microbiological analysis of individual extracts, most microfiltered extracts detected no microbial colonies (Table 2). The persistence of microbial load in ginger and curcuma extracts may be attributed to their distinct initial microbial load and physicochemical characteristics (i.e., TDS, viscosity), which compromise the effectiveness of flow dynamics-dependent microbial removal during MF. The TDS of ginger and curcuma extracts was significantly higher, with a higher initial microbial load than the other extracts. Flow interruptions (i.e., by particle detachment) during MF may facilitate the release and redistribution of microbes into larger pores, thereby reducing overall retention efficiency. TDS represents the particle content, which in high amounts may result in particle detachment through two mechanisms: (i) migration toward larger pores driven by sufficiently strong Brownian motion; or (ii) loss of adhesion to pore walls when Brownian motion is relatively weak. Moreover, fluctuations in transmembrane pressure, even under continuous flow, may lower the log reduction value, underscoring the critical role of flow stability in maintaining effective microbial retention by the membrane. These results align with the findings of Helling *et al.* (2019), in which variations in flow dynamics were shown to influence membrane performance and, consequently, microbial retention. These findings underscore the need for stable and continuous operation to ensure consistent microbial safety.

All individual extracts following microfiltration experienced significant color changes (ΔE), with the greatest differences observed in curcuma extracts, followed by ginger and lime extracts (Table 2). The L* increased after MF of extracts, indicating enhanced brightness, except for ginger extracts (darkened from 56.81 to 47.65), likely due to phenolic oxidation (such as gingerol derivatives and diarylheptanoid into melanin-like pigments) (Liu *et al.,* 2019). For a* values, most extracts showed minimal variation, whereas a significant change after MF was observed for Java tea, ginger, and curcuma extracts. The change shifted from greenish to reddish for Java tea and ginger extracts, and a declining color from reddish to near neutral for curcuma extracts, indicating the removal of polysaccharide-bound chromophores and phenolic oxidation. Ginger phenolics are highly susceptible to oxidation mediated by polyphenol oxidase (PPO), which polymerizes into melanin-like pigments (Lim and Wong 2019). The b* values decreased significantly in curcuma, lime, and Java tea extracts (Table 2), indicating diminished yellow chromophores, particularly curcuminoids, which tend to aggregate into macromolecular complexes and be separated during MF (De Oliveira *et al.,* 2015).

The pH of the extracts significantly changed after MF (p<0.05). Decreases were observed in Java tea and sappan wood, whereas increases were observed in ginger, lime, and kaffir lime. The pH increase by MF was associated with the retention of high-molecular-weight organic acids, reducing the free H^+ concentration; while the decreases reflected removal of basic compounds such as alkaloids and phenolics (Zhou *et al.,* 2018).

After MF, the extracts showed markedly smaller and more uniform particles, as reflected by lower PdI values (<0.3) (Table 2), indicating higher homogeneity and physical stability (Danaei *et al.,* 2018). These findings are corroborated by the efficient retention of larger particles by the 0.2 μm membrane, resulting in filtrates with greater uniformity and enhanced stability-an advantage for industrial applications, which reduces sedimentation during storage and maintains visual clarity over time. Correspondingly, TDS values declined after MF (Table 2), evidencing efficient colloidal and dispersed matter removal. The combined reduction in particle size, PdI, and TDS contributes to greater product clarity and lower flocculation or sedimentation risks, which may improve physical stability during storage and potentially extend shelf life by maintaining dispersion stability. These attributes are also relevant to consumer acceptance, as visually clear and stable beverages are generally perceived as higher quality, underscoring the practical value of MF in improving extract quality. However, MF may also lead to partial loss of bioactive compounds associated with larger particles, indicating a trade-off between food safety, physical quality, and potential reduction in bioactivity. Therefore, formula optimization is necessary to achieve a balanced composition between food safety, bioactivity, and consumer acceptability.

Formula optimization of Java tea-based functional drink

ANOVA results for sensory and functional responses are summarized in Table 3, with factor-response interactions illustrated in contour plots (Figure 1A–F). Quadratic models were initially applied to color, taste, and overall sensory attributes response; however, several terms exhibited high multicollinearity (VIF>10). Only significant terms were retained to enhance robustness while preserving model hierarchy (Myers *et al.,* 2016). The refined quadratic models showed good predictive performance, whereas the aroma failed to yield a significant model and was excluded from optimization. For functional parameters, linear models adequately describe antioxidant and antihyperglycemic activities, with moderate predictive strength for antioxidant activity and data transformation required for antihyperglycemic activity. From an industrial perspective, such validated predictive models offer a reliable statistical basis for formula optimization and consistent large-scale product quality.

Sensory response

The reduced quadratic model for color, taste, and overall responses, as summarized in Table 3, showed a highly significant (p<0.0001), indicating strong statistical reliability. Furthermore, a non-significant lack-of-fit (p>0.05) confirmed that the models fit the data well and adequately explain the observed variability. The relatively small difference between adjusted R^2 and predicted R^2 further supports model robustness. In contrast, the aroma model did not produce a reliable model. The high p-value (0.2412>0.05), a low adjusted R^2 value (0.0763), and a negative predicted R^2 value (-0.1932) showed that the model was not significant, with weak explanatory power and poor predictive performance. Given these limitations, the aroma response variable was excluded from the optimization stage. From an industrial perspective, this exclusion is essential, as unreliable models could compromise process standardization and quality control strategies, leading to inconsistencies in product aroma.

The predicted response surface is visualized in Figure 1A–D, whereas the polynomial equation for the sensory response based on hedonic rating data is summarized in Table 3. Those equations provide a quantitative framework for identifying which factors should be carefully controlled (indicated by negative coefficients), and which can be optimized (indicated by positive coefficients). Across significant models, lime extract emerged as the strongest positive driver. Meanwhile, trace plots offer more precise interpretation in mixture design by accounting for the constrained total proportion of factor ranges (Piepel *et al.,* 2021). This explains apparent discrepancies between model coefficients and trace plot patterns. Trace plot analysis revealed that sappan wood extract was the primary contributor to color, taste, and overall score, followed by lime extract. In contrast, kaffir lime extract exerted a relatively neutral effect. Conversely, higher proportions of Java tea and ginger tended to reduce the score. This difference reflects design range constraints: sappan wood extract varied broadly (10–23%), producing a more substantial impact, while lime extract was limited (1–2%), restricting its impact (Figure 2A–C). Integrating these findings, the optimization framework highlights lime and sappan wood as the key positive drivers for maximizing sensory quality, whereas the proportions of Java tea, ginger, and kaffir lime should be carefully controlled to avoid negative impacts.

Bioactivity response

The linear model was identified as the most suitable predictive model for both antioxidant and antihyperglycemic activity response. For antioxidant activity response, the model was statistically significant (p= 0.0105) with a non-significant lack-of-fit (p= 0.3252), indicating adequate agreement with observed data. Although R^2 was moderate (0.4670), this does not necessarily compromise the model's validity. As highlighted by Myers *et al*. (2016), R^2 in response surface methodology (RSM) is probabilistic rather than absolute. Furthermore, Ali and Aasim (2024) emphasize that in biological data, which tends to be complex and noisy, reliable models frequently yield moderate or even low R^2 values.

Similarly, the antihyperglycemic response model exhibited good statistical reliability. The model was significant (p= 0.0002) with a non-significant lack-of-fit (p= 0.4597). An R^2 value of 0.6466 and a small difference between adjusted R^2 and predicted R^2 (0.1419<0.2) further support the model's satisfactory predictive performance for estimating new responses within the same experimental range. Meanwhile, the Box-Cox analysis suggested an optimum lambda value (λ= 0.5), corresponding to a square root transformation of the antihyperglycemic data (Figure 3). This indicates that the original dataset does not fully meet linear model assumptions, particularly regarding variance homogeneity and residual normality. Applying the transformation stabilized variance and improved residual distribution, enhancing alignment between predicted and observed values (Myers *et al.,* 2016).

Table 2. Microbiology and physicochemical characteristics of fresh and microfiltered extracts

Parameter	Extracts											
	Java Tea		Sappan Wood		Ginger		Curcuma		Lime		Kaffir Lime	
	Fresh	Microfiltered	Fresh	Microfiltered	Fresh	Microfiltered	Fresh	Microfiltered	Fresh	Microfiltered	Fresh	Microfiltered
TPC*	1.4x10^{3}	<1x10^{0}	<1x10^{0} (<30 colonies)	<1x10^{0}	2.4x10^{4}	4.8x10^{2}	3.0x10^{4}	7.6x10^{1}	2.4x10^{4}	<1x10^{0}	1.8x10^{2}	<1x10^{0}
Color												
L^*	57.99±0.65^{a}	58.29±0.04^{a}	37.78±0.26^{a}	40.30±0.12^{b}	56.81±3.21^{a}	47.65±1.95^{b}	51.80±0.34^{a}	58.80±0.48^{b}	58.34±3.97^{a}	70.68±1.13^{b}	52.18±0.28^{a}	74.07±4.53^{b}
a^*	-0.80±0.02^{a}	-0.87±0.01^{b}	33.83±0.79^{a}	32.18±2.42^{a}	-6.24±0.63^{a}	9.66±0.81^{b}	16.25±0.36^{a}	-0.78±0.14^{b}	-1.03±0.30^{a}	-1.30±0.02^{a}	-9.55±0.05^{a}	-8.38±0.57^{a}
b^*	13.59±0.01^{a}	6.08±0.00^{b}	61.56±4.30^{a}	57.29±8.24^{a}	32.29±2.19^{a}	36.68±1.71^{a}	69.54±4.37^{a}	21.49±1.00^{b}	11.11±1.20^{a}	1.34±0.00^{b}	20.11±0.02^{a}	17.47±0.53^{b}
∆E		7.52		5.23		18.87		51.46		15.74		22.08
pH	5.79±0.03^{a}	5.68±0.01^{b}	6.03±0.00^{a}	5.94±0.01^{b}	6.39±0.03^{a}	6.48±0.01^{b}	4.50±0.00^{a}	4.50±0.01^{a}	2.17±0.02^{a}	2.23±0.03^{b}	2.13±0.01^{a}	2.20±0.02^{b}
Particle size (nm)	804.53±1.59^{a}	295.40±1.11^{b}	1685.96±2.10^{a}	846.72±0.74^{b}	5976.85±2.82^{a}	371.22±0.95^{b}	776.23±0.02^{a}	175.48±0.06^{b}	17045.84±1.79^{a}	292.45±1.02^{b}	654.78±1.03^{a}	621.08±0.20^{b}
PdI	0.22±0.01^{a}	0.12±0.020^{b}	0.52±0.00^{a}	0.37±0.01^{b}	0.28±0.01^{a}	0.26±0.01^{a}	0.29±0.02^{a}	0.24±0.00^{a}	1.71±0.00^{a}	0.33±0.01^{b}	0.34±0.01^{a}	0.24±0.01^{a}
TDS (ppm)	622.00±0.00^{a}	345.00±1.41^{b}	239.00±0.00^{a}	192.00±0.00^{b}	1759.50±0.71^{a}	727.00±7.07^{b}	1977.00±4.24^{a}	642.00±2.83^{b}	1270.00±4.24^{a}	574.00±5.66^{b}	1827.00±2.83^{a}	624.50±2.12^{b}

Note: All values are expressed as means ± standard deviation. Different superscript letters within the same row indicate significant differences (p<0.05). TPC= total plate count, PdI= polydispersity index, TDS= total dissolved solids, $L^*a^*b^*$= color coordinates (lightness, red–green axis, yellow–blue axis), ∆E= total color difference, particle size determined by dynamic light scattering (nm). *max. 10^4 CFU/mL

Table 3. Fit-summary ANOVA model for each response

Responses	Model	Polynomial Equation	Significant (p<0.05)	Lack of Fit (p>0.05)	R^2	Adjusted R^2	Predicted R^2	Adequate Precision
Hedonic rating: color score	Reduced quadratic	6.01A+7.26B+6.73C+12.96D+6.42E–7.72AC	<0.0001	0.2071	0.7244	0.6518	0.3237	13.0212
Hedonic rating: aroma score	Linear	6.33A+7.21B+6.81C+6.16D+4.26E	0.2412	0.6766	0.2302	0.0763	-0.1932	3.8952
Hedonic rating: taste score	Reduced quadratic	6.41A+7.04B+6.58C+11.47D–5.53E–8.39AC–19.28AD+16.52BE	<0.0001	0.1331	0.8345	0.7664	0.6512	12.1494
Hedonic rating: overall score	Reduced quadratic	6.46 A+7.06B+6.64C+11.38D-1.61E-7.73AC-18.93AD+12.95BE	<0.0001	0.1023	0.8452	0.7814	0.6732	12.8502
Antioxidant activity	Linear	412.25A+578.75B+504.59C+433.41D+48.63E	0.0105	0.3252	0.4670	0.3604	0.1319	6.6223
Antihiperglycemic activity	Linear	4.20A+7.55B+4.26C+5.13D–8.84E	0.0002	0.4597	0.6466	0.5759	0.4340	9.5780

Note: A= Java tea extract, B= sappan wood extract, C= ginger extract, D= lime extract, E= kaffir lime extract

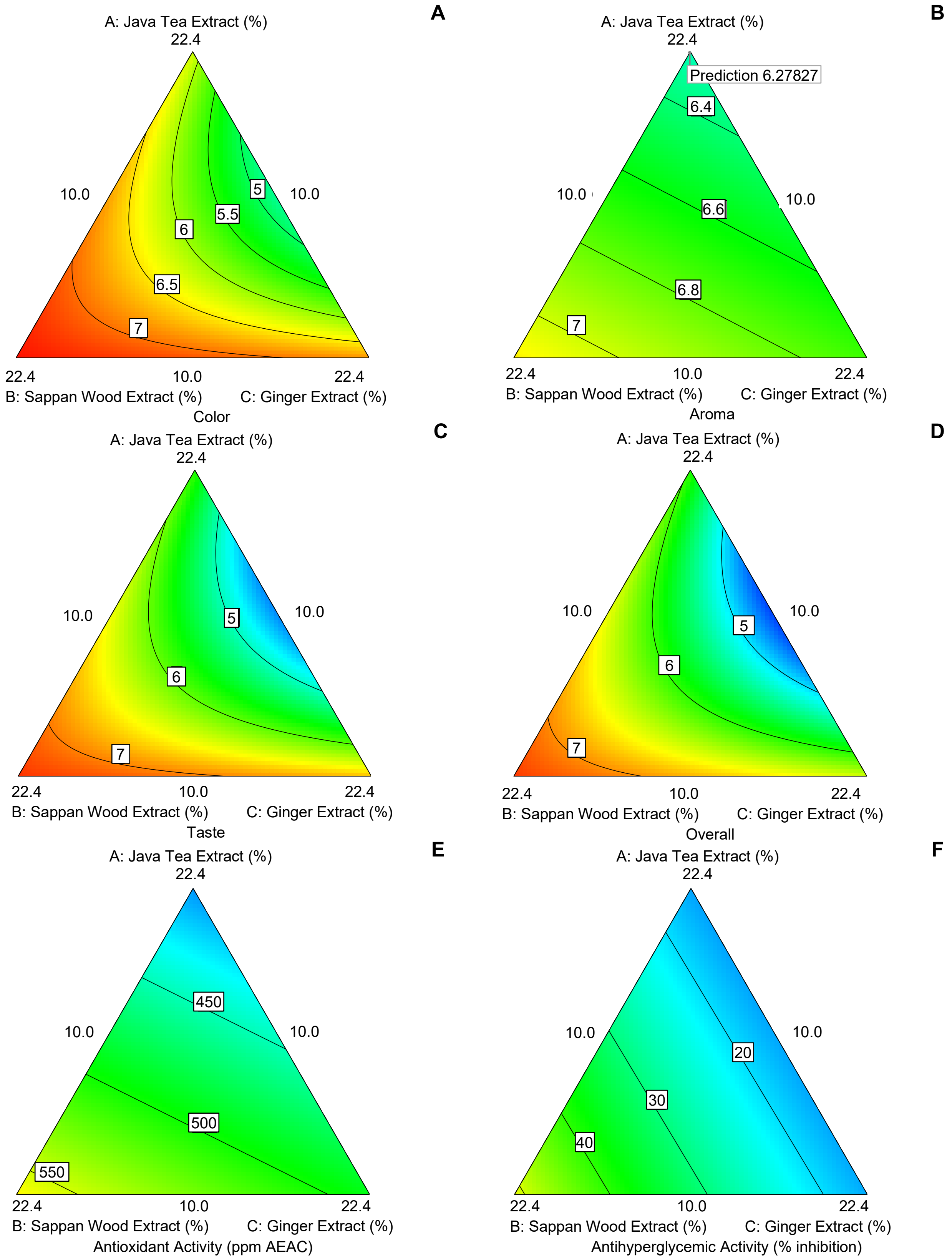

Figure 1. Contour plots showing the effect of three components on color (A), aroma (B), taste (C), overall (D), antioxidant activity (E), and antihyperglycemic activity (F)

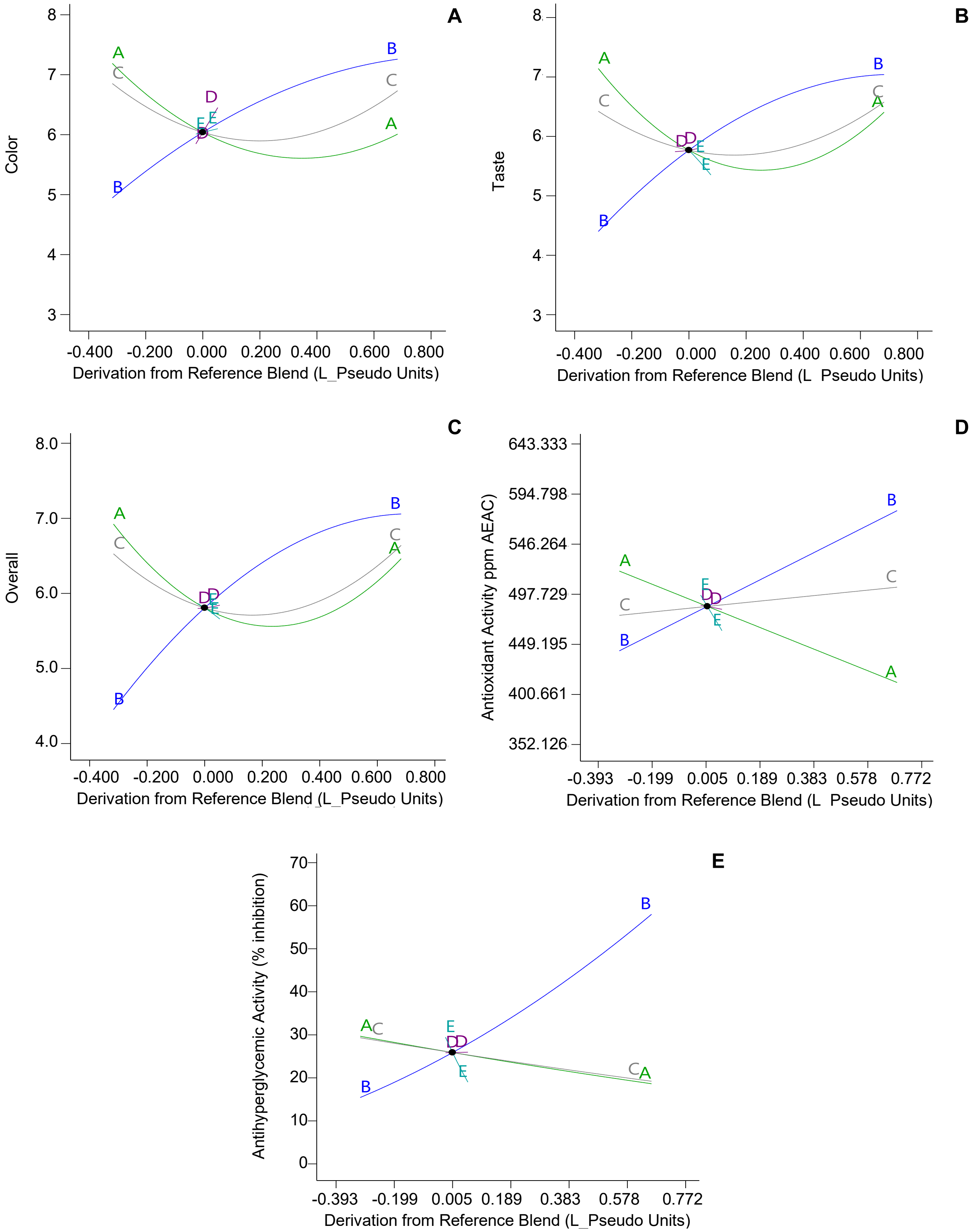

Figure 2. Trace (Piepel) plots showing the effect of blend components on color (A), taste (B), overall (C), antioxidant activity (D), and antihyperglycemic activity (E). Each plot illustrates the effect of deviation from the reference blend (x-axis) on the corresponding response value (y-axis). Letters A–E (described in Table 3 footnote)

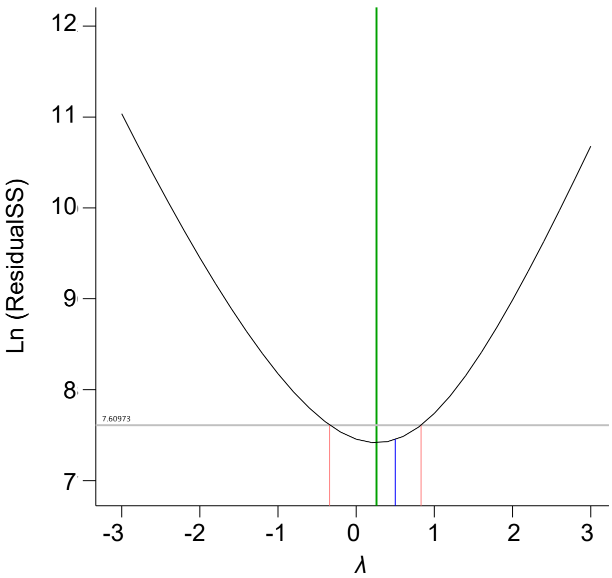

Figure 3. Box-Cox analysis results. The x-axis represents the transformation parameter (λ), and the y-axis represents the natural log of residual sum of squares. The vertical green line shows the optimal λ, and the red lines mark the 95% confidence interval. A square root transformation was applied as $\lambda \approx 0.5$ lies within this range

The predicted response surface is visualized in Figure 1E–F, whereas the polynomial equation for the functional response is summarized in Table 3. All extracts contributed positively to the responses when considered independently, except for kaffir lime in the antihyperglycemic response. Meanwhile, in mixture design, a more precise interpretation was achieved through trace plots, which account for the constrained total proportion of factor ranges. Across both functional responses, sappan wood extracts emerged as the dominant positive drivers. The trace plot also revealed a trade-off effect, in which increasing Java tea proportions reduced the response due to the displacement of sappan wood, whereas in antihyperglycemic activity, Java tea retained a supportive role but remained less influential than sappan wood. Ginger extract showed a neutral antioxidant response effect but a moderate positive effect in antihyperglycemic response. Lime and kaffir lime consistently trended downward in antioxidant activity, while kaffir lime also negatively affected antihyperglycemic activity (Figure 2D–E).

These findings align with the bioactive profile of sappan wood, which contains flavonoids, triterpenoids, phenolic acids, and glycosides (Asevedo *et al.,* 2024). Network pharmacology further supports the antidiabetic potential of sappan wood. Adnan *et al.* (2022) identified fisetin tetramethyl ether as a major constituent of sappan wood extract, demonstrating the highest affinity to the peroxisome proliferator-activated receptor (PPAR), a key regulator of glucose and lipid metabolism. PPAR activation contributes to glucose homeostasis and lipid regulation, thereby supporting glycemic control. Thus, the mechanistic evidence supports the mixture design outcome, confirming that the predicted positive effect of sappan wood aligns with its biochemical basis.

Verification of the optimum formula

The optimization yielded one optimum formulation with the highest desirability value of 0.936. The optimum formula was verified to assess the model's consistency in predicting new responses. The results of the formula verification are shown in Table 4. Model verification showed that all measured values fell within the 95% PI, demonstrating the model's reliability and accuracy in estimating new data within the defined variable range.

Comparison of Java tea-based functional drink

The microbiological, bioactivity, and sensory properties of the optimum formula prepared with microfiltered extracts (MF-OF) were compared with those of the optimum formula subjected to thermal pasteurization (PAST-OF), and commercial Java tea-based functional drink (COM) (Table 5). Total plate count characterization showed that no colonies were detected in MF-OF, verifying the effectiveness of 0.2 µm MF for removing microorganisms. In contrast, PAST-OF and COM still exhibited some microbial growth. However, all samples remain within the Indonesian national standard limit (BSN, 2022).

Table 4. Results of verification of the optimum formula

Response	95% PI Low	95% PI High	Prediction	Actual
Hedonic rating				
Color	6.0	8.4	7.2	7.0±1.5
Aroma	5.8	8.6	7.2	6.4±1.2
Taste	5.8	8.3	7.0	6.7±1.5
Overall	6.0	8.1	7.0	6.6±1.2
Bioactivity				
Antioxidant activity	420.7	731.1	575.9	588±2.97
Antihyperglycemic activity	25.7	97.5	55.8	59.94±0.92

Note: PI= prediction interval, Actual= experimental mean value (mean ± standard deviation), Prediction= model-predicted value

Table 5. Comparison of microbiological characteristics, bioactivity, and hedonic rating scores of three Java-tea based functional drink samples

Parameter	Sample		
	MF-OF	PAST-OF	COM
Total plate count (max. 10^4 CFU/mL)	$<1x10^0$	$1.7x10^0$	$1.5x10^0$
Bioactivity			
Antioxidant activity	$567.00±2.83^b$	$377.50±3.54^c$	$605.50±3.54^a$
Antihyperglicemic activity	$59.94±0.92^b$	$36.54±1.05^c$	$68.23±1.83^a$
Hedonic rating			
Color	$7.0±1.6^a$	$6.2±1.6^b$	$2.8±1.8^c$
Aroma	$6.4±1.2^a$	$6.5±1.6^a$	$5.1±2.6^b$
Taste	$6.7±1.5^a$	$5.3±1.8^b$	$6.8±2.2^a$
Overall	$6.6±1.2^a$	$5.5±1.7^b$	$5.9±2.3^b$

Note: MF-OF= optimum formula prepared with microfiltered extracts, PAST-OF= optimum formula subjected to thermal pasteurization, COM= commercial Java tea-based functional drink. Values are expressed as means ± standard deviation. Different superscript letters within the same row indicate significant differences (p<0.05)

The antioxidant and antihyperglycemic activities of MF-OF were comparable to those of COM and significantly higher than those of PAST-OF. These results indicate that MF better preserves heat-sensitive bioactive compounds than thermal pasteurization, although not as optimally as COM, which may have undergone standardization or enrichment. MF improved the formula's color and overall acceptability (MF-OF), with no significant effects on taste and aroma. These findings indicate that incorporating microfiltered extracts into formulations can improve overall sensory quality.

CONCLUSION

The application of microfiltration (MF) in Java tea-based functional drink extracts effectively reduced microbial loads in most extracts. Significant alterations in physicochemical characteristics were also observed, including shifts in color, pH, total dissolved solids, particle size, and polydispersity index, resulting in a more homogenous and stable extract solution. Optimization using a D-optimal mixture design successfully identified an optimal formulation with an antioxidant activity of 588 ppm AEAC, a α-glucosidase inhibition of 59.9%, a hedonic taste score of 6.7 on a 9-point scale, and a high overall desirability index of 0.936. Comparative analysis indicated that MF improved color and overall acceptability without significantly affecting taste and aroma, while maintaining microbial safety and causing a reduction in bioactivity. These findings highlight the effectiveness of integrating MF technology as a non-thermal pasteurization approach for producing high-quality functional beverages that deliver both enhanced bioactivity and sensory acceptability. The results may serve as a preliminary reference for future industrial applications for producing functional beverages that meet modern consumer demands for health-promoting properties, clean-label processing, and consistent product quality.

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