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Development of Java Tea based Functional Drink: Scale-up Formula Optimization based on the Sensory and Antioxidant Properties

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ABSTRACT

A Java tea-based functional drink was modified by using simplicia extracts rather than fresh ingredients to facilitate mass production. However, the modification can impact a product's nutraceutical efficacy as well as its sensory properties. This study aimed to optimize the formula for the modified Java tea-based functional drink in terms of both maximum antioxidant properties (efficacy) and sensory properties (color, taste, and aroma). Formula optimization was performed by response surface methodology (RSM) using *Design Expert®* 7 software. Java tea extract (JE), sappan wood extract (SE), ginger extract (GE), and lime extract (LE) contents were identified as important factors for optimization because of the antioxidant and desirable sensory properties of these ingredients. Sensory evaluation was conducted using the *balanced incomplete block design* (BIBD) method. Ultimately, a Java tea-based functional drink was successfully formulated from simplicia extracts with optimum sensory and antioxidant properties. The antioxidant activity of the optimum formula was 335.69 ± 48.30 ppm ascorbic acid equivalent *antioxidant* capacity and the sensory acceptance scores (on a scale of 1–7) were 5.6 for color, 5.8 for taste, 5.2 for aroma, and 5.2 for overall attributes (corresponding to 'like slightly' to 'like moderately'). Simplicia extracts in appropriate combinations can be used instead of fresh ingredients for the large-scale production of a Java tea-based functional drink while maintaining efficacy and palatability.

INTRODUCTION

Functional foods believed to promote general health and reduce the risk of diseases are becoming increasingly popular, including various herbal drinks. Many of these herbal drinks are produced from traditional medicinal ingredients such as Java tea. Research on Java tea-based functional drinks has shown promising health benefits such as antihyperglycemic activity (Indariani *et al.*, 2014). Furthermore, these drinks are rich in antioxidants (Wijaya *et al.*, 2010). A Java tea-based functional drink prepared using fresh ingredients was reported to possess 532.307–726.818 ppm ascorbic acid equivalent *antioxidant* capacity (AEAC) (Wijaya *et al.*, 2007; Wijaya *et al.*, 2011).

Pilot production of a Java tea-based functional drink using fresh ingredients has been conducted at both 10-L (Wazirroh *et al.*, 2013) and 100-L scales (Wijaya *et al.*, 2014), and both trials yielded promising results for eventual commercialization. However, utilization of fresh ingredients is often limited by availability and short shelf-life. To produce this tea on an industrial scale, extracts of Java tea, sappan wood, ginger, and curcuma were modified using simplicia instead of fresh ingredients.

Modification of extraction processes and ingredients may impact the functional efficacy of the product as well as its sensory properties. Therefore, this study aimed to optimize the formula of a Java tea-based functional drink made from simplicia extracts according to sensory and antioxidant properties.

MATERIAL AND METHODS

Plant Materials

Java tea (*Orthosiphon aristatus* (Blume) Miq., specimen No. BMK0076042016), sappan wood (*Caesalpinia sappan* L, specimen No. BMK0120092016), ginger (*Zingiber*

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officinale Roscoe, specimen No. BMK0043052015), lime (*Citrus aurantifolia* (Christm.) Swingle, specimen No. BMK0202092016), lemon (*Citrus limon* (L.) Osbeck, specimen No. BMK0201092016), kaffir lime (*Citrus histrix* DC, specimen No. BMK0189092016), and curcuma (*Curcuma xanthorrhiza* Roxb, specimen No. BMK0042072014) were identified and deposited at the Tropical Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia.

Chemical materials

Ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, ethanol, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium carbonate, sodium acetate trihydrate, and acetic acid (glacial) 100% were purchased from Sigma-Aldrich. Acarbose (Glucobay) was purchased from a local pharmacy. α-Glucosidase sourced from *Bacillus stearothermophilus* (G3651) and 4-nitrophenyl α-D-glucopyranoside (PNPG) N1377 were purchased from Sigma-Aldrich.

Preparation of extracts

Extract of java tea, sappan wood, ginger, lime, lemon, kaffir lime, and curcuma were produced by selected toll manufacturing. Java tea, sappan wood, ginger, and curcuma were then extracted in boiling water and filtered through membranes under pressure. Lime, lemon, and kaffir lime were extracted from fresh ingredients using a screw pressing machine. All the collected extracts were pasteurized to meet microbial requirements and stored at 10°C or colder.

Extract characterization

All the extracts were analyzed for physical, chemical, and microbiological characteristics. Physical characteristics examined included appearance, pH, specific gravity, and solubility. Chemical characteristics examined were heavy metal content and antioxidant activity. The total plate counts of fungi, *E. coli*, *S. aureus*, *Salmonella sp*., and *P. aeruginosa* were also examined as microbial characteristics.

Formula optimization

The optimal formula was determined by response surface methodology (RSM) using mixture design and analyzed by *Design Expert*® 7. The concentrations of Java tea extract (JE), sappan wood extract (SE), ginger extract (GE), and lime extract (LE) were selected as variables because of their antioxidant and sensory properties. The constraints of each factor were previously established during preliminary trials. The Java tea-based functional drink was then formulated by adding fixed concentrations of curcuma extract, lemon extract, kaffir lime extract, sucrose, aceculfame-K, sucralose, flavor enhancer, and xathan gum in water. Twenty-one experimental formulations were prepared to determine the formula with optimal antioxidant activity and sensory properties. Analysis of variance (ANOVA) was used to validate the models from *Design Expert*® 7, with significance set at $p < 0.05$. After obtaining the optimum formula, we conducted verification analyses and measured antihyperglycemic activity by the α-glucosidase inhibition method.

Analysis of antioxidant activity

Antioxidant activity was measured as described by Kubo *et al*. (2002) and Molyneux (2004) with minor modifications. The analysis was performed using an Elisa reader (Biotek) using ascorbic acid as the standard. The results are expressed as ppm AEAC. Each measurement was performed in triplicate.

Sensory evaluation

Sensory evaluation was conducted according to the *balanced incomplete block design* (BIBD) method described by Cochran and Cox (1957). In this study, the BIBD was arranged for 21 numbers of block (b), 5 numbers of units per block (k), 5 numbers of replication (r), and 21 numbers of treatments (t). By following this design, the efficiency factor was 0.84 (high numbers indicate greater precision).

Hedonic ratings were performed by 21 screened panelists who evaluated taste, color, aroma, and overall attributes of the Java tea-based drink on a 7-point scale: (1) dislike extremely, (2) dislike moderately, (3) dislike slightly, (4) neither like nor dislike (neutral), (5) like slightly, (6) like moderately, (7) like extremely. Responses were then analyzed by *Design Expert*® 7 to determine the optimum beverage formula.

Analysis of antihyperglycemic activity

Antihyperglycemic activity was measured using the α-glucosidase inhibition method as described by Mayur *et al*. (2010) with minor modifications. The analysis was performed using an Elisa reader (Biotek). Briefly, a beverage sample (10 µL) was mixed with 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of 0.5 mM 4-nitrophenyl *α*-D-glucopyranoside, and 25 µL of *α*-glucosidase solution (0.04 Unit/mL) in microplate wells. The mixture was incubated at 37°C for 30 min and the reaction terminated by adding 100 µL of 0.2 M sodium carbonate solution. Blanks with enzymes replaced by buffer were prepared to correct background absorbance. Negative controls with sample replaced by methanol were also prepared. Positive controls contained Acarbose (10 ppm). Each measurement was performed in triplicate.

RESULT AND DISCUSSION

Extract characterization

Characteristics of extracts are presented in Table 1. Characterization of simplicia extracts was useful for determining extract ingredients. Extract characteristics met the standard requirements of PerKa BPOM No. 12 (2014) from The Indonesia National Agency of Drug and Food Control.

Mixture design analysis of Java tea-based functional drink

Five responses (antioxidant activity, color, taste, aroma and overall attributes) for formula optimization of the Java tea-based functional drink were analyzed by *Design Expert*® 7. The Responses for each formula are presented in Table 2.

Effect of formula optimization on antioxidant activity

The antioxidant activity of the 21 experimental Java tea-based drink formulas varied between 116 and 313 ppm AEAC (Table 3). The polynomial equation of antioxidant activity revealed interaction effects among ingredients. Based on D-optimal mixture design analysis, a quadratic model of antioxidant activity was derived that fit the data with statistical significance ($p < 0.05$, $R^2 = 0.8202$). The response surface 3D graph of antioxidant activity is shown in Figure 1. The mathematical models of each individual response are presented in Table 3.

Table 1: Characteristics of extracts from selected toll manufacturing.

Extract Name	Java tea	Sappan Wood	Ginger	Curcuma	Kaffir lime	Lime	Lemon
Physical appearance	Brown to dark brown liquid	Reddish brown liquid	Brown to dark brown liquid	Yellowish brown liquid	Yellowish white to yellow liquid	Light yellow liquid	Light yellow liquid
pH	6.66	6.24	6.86	6.43	3.00	2.87	3.01
Specific gravity (g/mL)	1.0011	1.0017	1.0168	1.0019	1.0372	1.0359	1.0287
Heavy metal							
Pb (≤10 mg/kg)	conform	conform	conform	conform	conform	conform	conform
Cd (≤0.3 mg/kg)	conform	conform	conform	conform	conform	conform	conform
As (≤5 mg/kg)	conform	conform	conform	conform	conform	conform	conform
Hg (≤0.5 mg/kg)	conform	conform	conform	conform	conform	conform	conform
Microbiology							
TPC (max. 10^4 CFU/g)	10 cfu/g	70 cfu/g	90 cfu/g	50 cfu/g	320 cfu/g	10 cfu/g	20 cfu/g
Fungi (max. 10^3 CFU/g)	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
E. coli (negative)	negative	negative	negative	negative	negative	negative	negative
S. aureus (negative)	negative	negative	negative	negative	negative	negative	negative
Salmonella sp. (negative)	negative	negative	negative	negative	negative	negative	negative
P. Aeruginosa (negative)	negative	negative	negative	negative	negative	negative	negative
Antioxidant (ppm AEAC)	380.44	952.47	480.83	297.30	953.24	226.42	335.23
Solubility in water	conform	conform	conform	conform	conform	conform	conform

Table 2: Experimental design and responses for Java tea-based functional drink optimization.

Treatments	Factors (%)				Responses				
	JE	GE	SE	LE	Antioxidant Activity (ppm AEAC)	Sensory Properties			
						Color	Taste	Aroma	Overall
run 1	*	*	*	*	239.13	4.8	4.4	5.4	4.6
run 2	*	*	*	*	131.40	4.8	4.4	4.8	4.6
run 3	*	*	*	*	278.26	5.6	4.6	5	5
run 4	*	*	*	*	157.49	4.8	5.4	4.6	4.8
run 5	*	*	*	*	116.91	5.2	5.2	5.4	5.4
run 6	*	*	*	*	243.96	6	4.2	5	4.8
run 7	*	*	*	*	177.78	5.8	5.2	5.6	5.6
run 8	*	*	*	*	265.22	5	4.6	4.8	4.6
run 9	*	*	*	*	213.53	5	5.2	4.8	5.2
run 10	*	*	*	*	199.03	5.8	4.8	5	5
run 11	*	*	*	*	242.03	5.2	3.8	5	4.2
run 12	*	*	*	*	157.00	4	5.6	4.6	5
run 13	*	*	*	*	220.77	3.8	5.2	5.4	4.8
run 14	*	*	*	*	251.69	4.6	5.2	4.6	5
run 15	*	*	*	*	313.04	5.4	5.4	5.6	5.6
run 16	*	*	*	*	227.05	4	5.2	5.2	5.4
run 17	*	*	*	*	255.56	4.6	5	5.2	5.2
run 18	*	*	*	*	216.43	4.6	5	5.2	5.2
run 19	*	*	*	*	206.28	5.2	5	4.6	5
run 20	*	*	*	*	265.70	4.6	5.4	5.2	4.6
run 21	*	*	*	*	131.88	3.2	3.4	4.6	3.4

JE = Java tea extract, GE = Ginger extract, SE = Sappan wood extract, LE = Lemon extract, AO = Antioxidant activity.

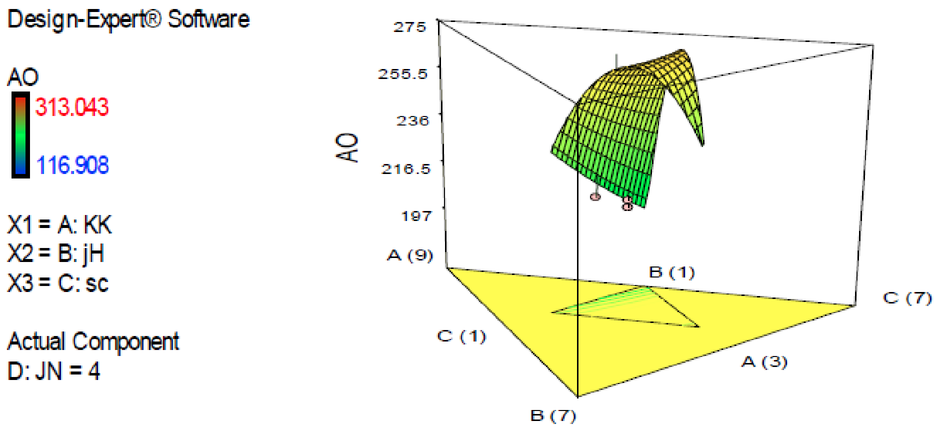

Fig. 1: Three-dimensional graph of antioxidant activity response.

Table 3: Mathematical models for each individual response.

Responses	Models	Polynomial equation	R-squared	Predicted R-squared	Adjusted R-squared	Adequate precision	Lack of fit
Antioxidant activity	Quadratic	-116.23JE-190.31GE-134.63SE-167.23LE+47.46JE*GE+30.14JE*SE+ 18.3JE*LE1.50GE*SE+36.92GE*LE+ 44.61SE*LE	0.8202	0.0804	0.6743	8.058	Sig
Taste	Mean	-	-	-	-	-	-
Color	Mean	-	-	-	-	-	-
Aroma	Mean	-	-	-	-	-	-
Overall	Mean	-	-	-	-	-	-

Effect of formula optimization on sensory evaluations

In contrast to drugs and supplements, sensory characteristics are critical factors determining public acceptance of functional foods and drinks. In this study, sensory evaluation of the Java tea-based functional drink was performed by screened panelists. Evaluation followed a balanced incomplete block design. Color, taste, aroma, and overall attributes were used as responses for formula optimization (Table 2). All sensory attributes were fitted with a mean model (Table 3) indicating that color, taste, aroma, and overall attributes were not significantly influenced by the component factors.

Formula optimization

To achieve the optimum formula, each individual factor and response was ranked according to importance (Table 4). Antioxidant activity, taste, and color were considered to be of maximum importance (5, +++++) because of the major influences of these factors on both functionality and sensorial acceptance, while the other factors and responses were considered to be of intermediate importance, ranging from 3 (+++) to 4 (++++).

Based on RSM analysis, the optimal formula for a Java tea-based functional drink produced from simplicia extracts was determined according to antioxidant and sensory properties. The optimal formula has a desirability value of 0.85 (Table 5), a predicted antioxidant content of 277.23 ppm AEAC, and predicted sensory acceptance scores of 4.87 for taste, 4.86 for color, 5.03 for aroma, and 4.90 for overall attributes. These ratings range from “neither like nor dislike” to “like slightly” (Table 6).

Table 4: Importance criteria for Java tea-based functional drink optimization.

Component/Response	*Goal*	Minimum	Maximum	*Importance*
JE	*in range*	20	40	3 (+++)
GE	*in range*	6.8	20	3 (+++)
SE	*in range*	6.8	26.7	3 (+++)
LE	*in range*	15.3	33.3	3 (+++)
Antioxidant activity (ppm AEAC)	*maximize*	116.908	313.043	5 (+++++)
Color	*in range*	3.2	6	5 (+++++)
Aroma	*in range*	4.6	5.6	4 (++++)
Taste	*maximize*	3.4	5.6	5 (+++++)
Overall	in range	3.4	5.6	4 (++++)

Verification

Results from the prediction formula were compared to actual experimental results for verification (Table 7). The actual antioxidant activity (335.69 ± 48.30 ppm AEAC) was reasonably close to the predicted value (277.23 ppm AEAC). Thus, as predicted, the utilization of simplicia extracts influenced product efficacy (Table 6). Moreover, the measured antioxidant activity was lower than that of previous pilot-scale production runs using fresh ingredients, where levels of 709 ppm AEAC (Waziiroh *et al.*, 2013) and 910.97 ppm AEAC (Wijaya *et al.*, 2014) were obtained. This decrease in antioxidant activity is likely the result of utilizing simplicia extracts. These results are in accordance with the findings of Herreros *et al*. (2010) that the effects of heating on the antioxidant activities of spices and vegetable products are influenced by the type of raw materials and processing conditions. Reductions in antioxidant activity and phenolic compounds by sterilization were also reported for a herbal drink by Techaratanakrai *et al*. (2009). The heat exposure involved in simplicia extract production can indeed reduce antioxidant activity, but this drawback is compensated by the ease of industrial production.

Table 5: Suggested optimized formulae.

No	JE	GE	SE	LE	AO	taste	color	aroma	overall	Desirability
1	*	*	*	*	277.2303	4.866667	4.857143	5.028571	4.904762	0.852155
2	*	*	*	*	273.9374	4.866667	4.857143	5.028571	4.904762	0.848625
3	*	*	*	*	262.7117	4.866667	4.857143	5.028571	4.904762	0.836129
4	*	*	*	*	256.5069	4.866667	4.857143	5.028571	4.904762	0.828888

Table 6: Predicted responses for the optimized formula.

Responses	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
AO	277.23	17.99	237.64	316.82	35.14	199.90	354.56
Taste	4.87	0.12	4.61	5.12	0.58	3.66	6.08
Color	4.86	0.15	4.54	5.18	0.72	3.35	6.36
Aroma	5.03	0.07	4.88	5.18	0.34	4.31	5.75
Overall	4.90	0.11	4.68	5.13	0.51	3.84	5.96

Table 7: Comparison of predicted to actual experimental results.

Responses	Prediction	Verification
Antioxidant activity (ppm AEAC)	277.23	335.69 ± 48.30
Taste	4.87	5.8
Color	4.86	5.6
Aroma	5.03	5.2
Overall	4.90	5.2

Although antioxidant activity decreased, the optimized formula for this Java tea-based functional drink prepared using simplicia extracts retains antioxidant activity equivalent to 60–70 mg of ascorbic acid (vitamin C). According to US Food and Drug Administration (FDA) regulations, products labeled as high in antioxidants must contain 20% or more of the recommended daily intake of vitamin C (minimum 30 mg) (FDA, 2017).

Sensory ratings were 5.6 for color, 5.8 for taste, 5.2 for aroma, and 5.2 for overall attributes, corresponding to "like slightly" to "like moderately" on the 7-point scale. These results are within the 95% prediction interval of the models (Table 6). All parameters (antioxidant activity and sensory attributes) were close to corresponding model predictions, thereby validating the RMS models.

Antihyperglycemic activity of the optimized formula

The antihyperglycemic activity of the optimum formula as measured by α-glucosidase inhibition assay was 31.98% ± 0.21%, similar to the 29.91% measured from a Java tea-based functional drink made from fresh ingredients (Wijaya *et al*., 2014). Indariani *et al*. (2014) reported that Java tea-based functional drinks are enriched in phenols and that total phenols are strongly correlated with both antihyperglycemic and antioxidant activities. However, the current study did not reveal such a correlation, as antihyperglycemic activity did not differ between a Java tea-based functional drink made from simplicia extracts and that made from fresh ingredients. However, the fruit extracts used in both formulations (lime, kaffir lime, and lemon) were extracted from fresh ingredients and all are reported to have antihyperglycemic activities (Wijaya *et al*., 2010; Tadera *et al*., 2006; Ladaniya, 2008; Kim *et al*., 2000). The utilization of fresh fruit extracts is therefore expected to maintain the antihyperglycemic activity of Java tea-based functional drinks whether made from simplicia extracts or fresh ingredients.

CONCLUSION

A Java tea-based functional drink was successfully formulated from simplicia extracts with optimal sensory and antioxidant properties. The antioxidant activity of the optimum formula was 335.69 ± 48.30 ppm AEAC and the sensory acceptance ratings for color, taste, aroma, and overall attributes (on a 7-point scale) ranged from 5.2 to 5.8. The antihyperglycemic activity of the optimum formula was 31.98% ± 0.21% as measured by the α-glucosidase inhibition method. Thus, simplicia extracts are suitable for the mass production of Java tea-based functional drinks.

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