



Optimization of lemon grass-scent leaf-sorghum stem sheath infusion using mixture experiment

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ARTICLE INFO

Keywords:

Lemon grass, Scent leaf, Sorghum stem sheath
Optimization, Surface response
Physicochemical properties

ABSTRACT

Optimization of lemon grass-scent leaf-sorghum stem sheath infusion using a mixture experiment was determined in this study. Lemon grass and scent leaf were washed and steamed for five minutes while the sorghum stem sheath was washed and the three components were dried in the dehydrator at 50 °C for 18 hrs. The components were milled separately and the blends were mixed according to Design Expert software. The mixture was packaged in an infusion bag and immersed in boiled water for 10 min. The infusion was subjected to physicochemical, antioxidant, and antinutrients analyses. It was discovered that an increase in lemon grass-scent leaf-sorghum stem sheath caused an increase in the carotenoid, total dissolved solids, viscosity, specific gravity, turbidity, phenolic compound, infusion rate, tannin, oxalate, alkaloid contents while the increase in the components decreased the flavonoid and DPPH contents of the infusion. However, the Design Expert Software optimized the process based on the constraints given, and the optimum conditions for the independent variables were generated with values of 8.03%, 76.98% and 14.99% for sorghum stem sheath, lemon grass and scent leaf respectively.

Introduction

Lemon grass (*Cymbopogon citratus*, family: Poaceae) is a tall aromatic perennial plant which contains bioactive compounds such as phytochemicals, antioxidants, macronutrients, minerals, vitamins, and essential oils [12,14,32]. Ranade and Thiagarajan [32] and Nambiar and Matela [27] further classified the use of lemon grass bioactive compounds in folk medicine, cosmetics, and food ingredients. However, Nambiar and Matela [27] reported its use in treating anti-inflammatory, vasorelaxant, diuretic, treating ringworm infestation, gastrointestinal disturbances, fevers and hypertension. Lemon grass contains monoterpene aldehyde called citral which are used in flavoring soft drinks, scenting detergents and soaps, as a fragrance in perfumes and cosmetics, as insect repellent and carminative [35]. Lemon grass is widely used in herbal infusions and other non-alcoholic beverages in baked food, and also in confections [35]. Lemon grass contains several important bioactive compounds which are useful in several health issues. According to Olorunnisola et al. [29], lemon grass infusion was regarded as

a nontoxic and non-mutagenic infusion

Likewise, *Ocimum gratissimum* L. (Lamiaceae) which is known as scent leaf, African basil or clove basil is common in Africa, South America and Asia, and are aromatic herb used in ethnomedicinal management of a range of inflammatory disorders [25,28]. It has been used extensively in the traditional system of medicine in many countries for headaches, fever, diarrhea, pneumonia etc. [31]. The plant was reported to have essential oils which are useful in cosmetics, food industry, perfumery and pharmaceutical industries [30].

Sorghum bicolor (L.) Moench belongs to the family Poaceae and is ranked among the cereal crops. Apart from the use of Sorghum grains in food and biofuel production, the sheath, leaves, stalk and the roots are also used as food additives and for pharmaceutical purposes [4]. Sorghum bicolor stem extract according to Adedeji et al. [2] consists of natural antioxidants which prevent degenerative diseases, cell damage and lipid oxidation. *Sorghum caudatum*'s leaf sheath was reported to be rich in polyphenols which are used as antianemic and in the food industry [3].

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<https://doi.org/10.1016/j.meafoo.2023.100104>

Received 1 May 2023; Received in revised form 10 July 2023; Accepted 9 August 2023

Available online 12 August 2023

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Table 1
Descriptive statistics on the physicochemical, antioxidant and anti-nutrient properties of the lemongrass-scent leaf-sorghum stem sheath infusion.

Run	Sorghum Stem Sheath%	Lemon Grass%	Scent Leaf%	Carotenoid mg/g	Total Dissolved Solid mg/L	Viscosity mm ² /s	Specific Gravity	Turbidity NTU	Oxalate %	Tannin %	Alkaloid %	Flavonoid %	Infusion Rate mm/min	Total Phenolic mg GAE/g	DPPH %
1	9.50	75.50	15.00	0.2048	666	1.25	1.002	83	0.019	0.046	0.040	0.81	19.63	79.30	27.54
2	8.00	82.00	10.00	0.3023	566	1.24	1.002	81	0.011	0.024	0.039	0.83	18.88	80.57	25.28
3	8.00	62.00	30.00	0.2333	623	1.34	1.001	83	0.028	0.085	0.054	0.80	20.76	92.00	25.27
4	10.00	80.00	10.00	0.2059	569	1.27	1.002	85	0.014	0.048	0.043	0.83	19.96	79.76	27.69
5	10.00	80.00	10.00	0.2059	569	1.27	1.002	85	0.014	0.048	0.043	0.83	19.96	79.76	27.69
6	9.00	61.00	30.00	0.2333	622	1.32	1.001	83	0.028	0.085	0.054	0.80	20.76	92.00	25.28
7	8.50	66.50	25.00	0.2338	624	1.33	1.001	84	0.028	0.086	0.055	0.81	20.70	93.00	25.20
8	9.00	71.00	20.00	0.2148	606	1.32	1.001	84	0.022	0.072	0.052	0.81	20.21	83.91	26.76
9	8.00	82.00	10.00	0.3023	566	1.24	1.002	81	0.011	0.024	0.039	0.83	18.88	80.57	25.38
10	9.00	61.00	30.00	0.2333	620	1.33	1.001	84	0.029	0.085	0.055	0.81	20.77	93.00	25.27
11	9.50	65.50	25.00	0.2339	625	1.35	1.001	85	0.029	0.087	0.056	0.82	20.79	93.00	25.30
12	8.50	76.50	15.00	0.2048	567	1.25	1.002	83	0.019	0.048	0.040	0.82	19.69	79.52	27.78
13	10.00	60.00	30.00	0.2333	623	1.34	1.001	83	0.028	0.085	0.054	0.80	20.76	92.00	25.28
14	8.00	62.00	30.00	0.2333	624	1.34	1.001	84	0.028	0.086	0.055	0.80	20.78	93.00	25.29

The use of natural and readily available plants with high nutritional and medicinal values as tea infusion had been suggested as a way to enrich the diet of consumers. Lots of plants such as lemon grass, scent leaf, Parquetina, herbal tea, ginger, spices, food waste etc. had been used as infusion and reports showed that they have valuable nutrients which promote or improve the health of consumers [1,13,15,19,20,29]. They are cheap source of plant materials with high phytochemical and antioxidant properties. The use of these three plants blends (Lemon grass, scent leaf and sorghum stem sheath) for infusion had not been researched. However, the plants have been reported to have different bioactive compounds which could promote the health of the consumers. Moreover, mixture of the plants for infusion production may have positive effects on the compositions of the infusion and the right mixture to give the optimum requirements would be determined. Therefore, this paper presents the optimization of the lemon grass-scent leaf-sorghum stem sheath infusion using a mixture experiment.

Material and methods

Lemon grass and Scent leaves were obtained at National Horticultural Research Institute (NIHORT) Ibadan, Oyo State, Nigeria, while the sorghum stem sheath was purchased in a local market at Ilorin, Nigeria. Lemon grass and scent leaves were washed, steamed for 5 min and dried at 40 °C in the dehydrator for 18 hrs. Sorghum stem sheath was washed and dried at 40 °C in the dehydrator and milled. The raw materials were milled separately and packaged in airtight plastic container. The three raw materials were blended in different proportions according to Design Expert software. Fourteen samples were generated as shown in Table 1. One gram of the blended sample each was packaged in infusion bag and kept in Ziplock polythene bag. The modified method described by Olorunnisola et al. [29] was used for lemon grass-scent leaf-sorghum stem sheath infusion. Each infusion bag was immersed in 150 ml of boiled water for 10 min.

Analyses

Carotenoid was determined with the method of Kumari et al. [23], total dissolved was determined using Hannah meter (Hi 9813-5 model) while specific gravity was done using picnometer method. A turbidity meter (Kapur Scientific digital turbidity meter) was used to measure turbidity. Tannin, alkaloid, oxalate and flavonoid were analyzed using the methods of Ifemeje et al. [18], Al-Wahsh et al. [5] and Ejikeme et al. [11] respectively, while the Infusion rate was calculated. Total phenolic and DPPH were determined using Amin et al. [6] and Ayala-Soto et al. [7] methods.

Statistical analysis

The optimal experimental design of the mixture experiment was adopted. The design consist of 3 mixture components (sorghum stem sheath having a range of values between 8 and 10%; Lemon grass value ranged between 60 and 82%; scent leaf between 10 and 30%. The final mixture of the components is expected to be 100%. Dependent variables such as carotenoid, total dissolved solids, viscosity, specific gravity, turbidity, oxalate, tannin, alkaloid, flavonoid, infusion rate, total phenol content as well as DPPH scavenging properties were evaluated as responses. The statistical analyses were performed using Design Expert (State-Ease Inc, Minneapolis, MN, USA) software. The experimental data were fitted to a quadratic model to express the response variables as a function of independent variables using the equation below;

Table 2
Analysis of Variance (ANOVA) for the fitted polynomial model of the independent variables.

Response	Source	Df	Sum of Squares	Mean Square	F-value	P-value
Carotenoid	Model	5	0.011	0.002202	8.91	0.0040
	Residual	8	0.001977	0.0002472		
	Lack of fit	4	0.001977	0.0004943		
	Pure error	0.000	0.000	0.000		
	Total	13	0.013			
TDS	Model	2	6503.45	3251.72	5.44	0.0228
	Residual	11	6580.55	598.23		
	Lack of fit	7	6578.05	939.72		
	Pure error	4	2.50	0.63		
	Total	13	13,084.00			
Viscosity	Model	2	0.020	0.010	30.05	0.0001
	Residual	11	0.003696	0.0003360		
	Lack of fit	7	0.003646	0.0005209		
	Pure error	4	0.000050	0.00001250		
	Total	13	0.024			
SG	Model	2	0.000002969	0.0000021484	35.53	0.0001
	Residual	11	0.0000004596	0.0000004178		
	Lack of fit	7	0.0000004596	0.0000006566		
	Pure error	4	0.000	0.000		
	Total	13	0.000003429			
Turbidity	Model	5	17.58	3.52	7.32	0.0074
	Residual	8	3.84	0.48		
	Lack of fit	4	2.84	0.71		
	Pure error	4	1.00	0.25		
	Total	13	21.43			
Oxalate	Model	5	0.0006563	0.0001313	45.53	0.0001
	Residual	8	0.000009681	0.000001210		
	Lack of fit	4	0.000009181	0.000002295		
	Pure error	4	0.000000500	0.0000001250		
	Total	13	0.000660			
Tannin	Model	5	0.007347	0.001469	45.53	0.0001
	Residual	8	0.0002582	0.00003227		
	Lack of fit	4	0.0002577	0.00006441		
	Pure error	4	0.00000050	0.0000001250		
	Total	13	0.007605			
Alkaloid	Model	2	0.0005769	0.0002885	33.54	0.0001
	Residual	11	0.00009459	0.000008599		
	Lack of fit	4	0.00009359	0.00001337		
	Pure error	4	0.0000010	0.0000002500		
	Total	13	0.0006715			
Flavonoid	Model	2	0.001557	0.0007784	22.17	0.0001
	Residual	11	0.0003861	0.00003510		
	Lack of fit	7	0.0003361	0.00004802		
	Pure error	4	0.0000500	0.00001250		
	Total	13	0.0001943			
Response	Source	Df	Sum of Squares	Mean Square	F-value	P-value
Infusion Rate	Model	5	6.13	1.23	39.20	0.0001
	Residual	8	0.25	0.031		
	Lack of fit	4	0.25	0.062		
	Pure error	4	0.00025	0.0000625		
	Total	13	6.38			
Total Phenolic Compound	Model	2	472.23	236.11	45.35	0.0001
	Residual	11	57.27	5.21		
	Lack of fit	7	56.27	8.04		
	Pure error	4	1.00	0.25		
	Total	13	529.50			
DPPH Radical Scavenging	Model	2	9.41	4.70	7.25	0.0098
	Residual	11	7.14	0.65		
	Lack of fit	7	7.14	1.02		
	Pure error	4	0.000250	0.00006250		
	Total	13	16.54			

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

$$+ \beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 + \beta_{13} X_1 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

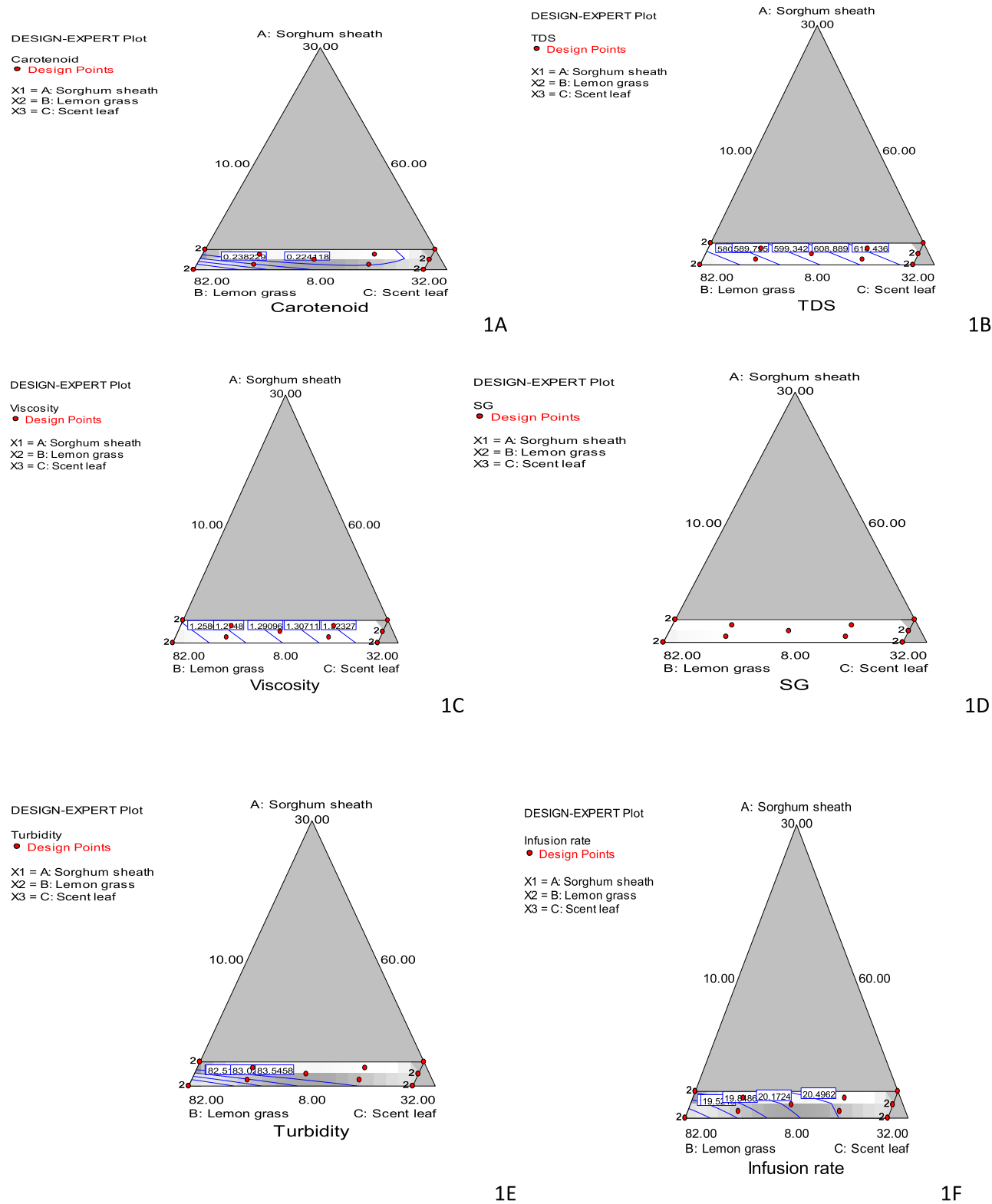


Fig. 1. Surface response of the physicochemical properties of the infusion.

Where Y = dependent variables, X_1 X_2 X_3 are independent variables
 β_{11} = Coefficient of independent variables

Results and discussion

Physicochemical, antioxidant and antinutrient of lemon grass-scent leaf-sorghum stem sheath infusion

Physicochemical, antioxidant and anti-nutrient properties of the lemon grass-scent leaf-sorghum stem sheath infusion are shown in Table 1. The sample with 82% lemon grass had the highest carotenoids while the sample with 75% lemon exhibited the highest total dissolved solid. Viscosity increased with an increase in scent leaf substitution. There were insignificant changes in the specific gravity of the infusion but infusion with 10:80:10% sorghum stem sheath, lemon grass and scent leaf was more turbid. Oxalate, alkaloid, infusion rate and total phenolic compounds increased with increase in scent leaf addition. DPPH scavenging activities were more pronounced in infusion with more lemon grass than with other components. The infusions from the plant mixtures contain antioxidant properties which chelate free radicals in the body thereby preventing certain diseases in the body [34]. The antinutrient contents of the infusion were low and could not cause problems to the health of the consumer. Joshua et al. [20] studied the properties of *Parquetina Nigrescens* (Parquetina) and *Cymbopogon Citratus* (Lemon grass) infusion and reported low values for tannin, flavonoids and alkaloid contents of the infusion. Consumption of infusion from the lemon grass-scent leaf-sorghum stem sheath could boost the health of the consumers.

Model fitting and effects on mixture experiment

The analysis of variance showed that the quadratic model was the highest-order model with significant terms when compared with other models, and was effective in describing the carotenoid content, turbidity, oxalate, tannin, and infusion rate of the infusion mixture. However, the response surface linear model was significant and effective in describing the total dissolved solids, viscosity, specific gravity, alkaloid content, flavonoid content, total phenolic compound and DDPPH radical scavenging.

Effect of mixture experiment on the carotenoid content of the infusion

ANOVA (Table 2) shows that the quadratic model was effective in describing the carotenoid content of the infusion samples ($F = 8.97$; $P = 0.0040$). There was only 0.40% chance that an F-value this large could occur due to noise. The effectiveness of the model was also ascertained using the coefficient of determination (R^2). An R^2 value of 0.8477 implies that about 84.77% of variations in the observed values of carotenoid content of the infusion product were explained by the model [21]. A low coefficient of variation (CV) value of 6.72% indicates that the experiment was precise and reliable [10,16]. An adequate precision value of 8.727 indicates an adequate signal, thus, this model can be used to navigate the design space [10].

The effect of sorghum stem sheath, lemon grass and scent leaf shows that the linear, mixture components are significant terms ($F = 10.65$; $P = 0.0056$) influencing the carotenoid content of the infusion. An increase in sorghum sheath, lemon grass, and scent leaf resulted in an increase in carotenoid content of the infusion mixture (Fig. 1A). This effect could be expressed in the equation as shown;

$$\text{Carotenoid content} \left(\frac{\text{mg}}{\text{g}} \right) = +2.40X_1 + 0.29X_2 + 0.24X_3 \quad (1)$$

From the study, it could be deduced that the plants are sources of carotenoid which are beneficial to the consumer. Carotene is vitamin A precursors which help to prevent blindness in consumers and also

function as antioxidant thereby scavenging free radicals in the body

Effect of mixture experiment on the total dissolved solids of the infusion

The analysis of variance (Table 2) shows that the linear model ($F = 5.44$; $P = 0.0228$) was effective in describing the effect of sorghum sheath, lemon grass, and scent leaf of the infusion product. The model was predictable ($R^2 = 0.4971$), reliable (CV = 4.04%) and considered an adequate to make predictions (Adequate precision = 5.835). However, the total dissolved solids of the infusion sample were influenced by the linear mixture of sorghum sheath, lemon grass, and scent leaf ($F = 5.44$; $P = 0.0228$). Fig. 1B shows that total dissolved solids are dependent on sorghum sheath, lemon grass and scent leaf. An increase in the amount of sorghum sheath, lemon grass and scent leaf resulted in an increased in total dissolved solids. Eq. (2) indicated that sorghum sheath, lemon grass and scent leaf have a positive effect on total dissolved solids of infusion samples.

$$\text{Total Dissolved Solid} \left(\frac{\text{mg}}{\text{L}} \right) = 738.79X_1 + 570.70X_2 + 626.56X_3 \quad (2)$$

Total dissolved solid showed the amount of solute that released into the infusion from the plants used. The more the total dissolved solid in the infusion, the more turbid and viscous the infusion becomes.

Effect of mixture experiment on the viscosity of the infusion

The viscosity of the infusion samples was modeled using response mixture linear model which was significant ($F = 30.05$; $P = 0.00010$) and was effective in describing the viscosity of the infusion samples. The model was predictable ($R^2 = 0.8453$) and capable of describing 84.53% of variability in the observed values of viscosity of the infusion samples. The model was reliable (CV = 1.41), adequate (Adequate precision = 12.779) and can be used to make predictions and navigate within the design space. The effect of sorghum sheath, lemon grass and scent leaf infusion production shows that the linear components of the independent variables are the terms influencing the viscosity of the infusion product. The response surface effect shows a positive effect of the sorghum sheath, lemon grass and scent leaf on the viscosity of the infusion product (Eqn 3). An increase in these components increased the viscosity of the infusion (Fig. 1C).

$$\text{Viscosity} \left(\frac{\text{mm}^2}{\text{s}} \right) = +1.44X_1 + 1.24X_2 + 1.34X_3 \quad (3)$$

An increase in viscosity of the infusion could be due to an increase in the total dissolved solid of the infusion.

Effect of mixture experiment on the specific gravity (SG) of the infusion

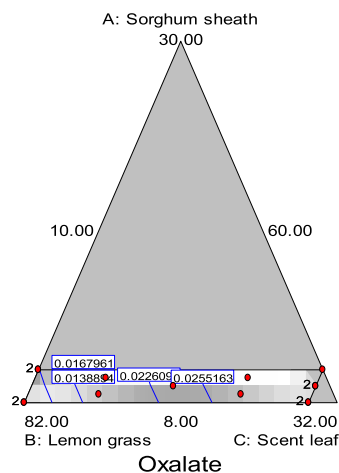
The specific gravity of the infusion mixture was modeled using a linear model because a linear model was significant ($F = 35.53$; $P = 0.0001$) and effective in describing the specific gravity of the infusion samples. There was only a 0.01% chance that a model F-value this large could occur due to noise. The mixture linear model was reliable (CV = 0.02) and predictable ($R^2 = 0.8659$) explaining 86.54% variability in the observed value of specific gravity of the infusion samples. The adequate precision values of 11.790 indicates an adequate signal, hence, this model could be used to navigate the design space. The effect of sorghum sheath, lemon grass and scent leaf on the specific gravity of infusion shows that only the linear mixture of the independent variables had a significant effect ($F = 35.53$; $P = 0.001$) on the specific gravity of the infusion samples. Eqn (4) shows that sorghum sheath, lemon grass and scent leaf had a positive effect on the specific gravity of infusion samples. An increase in concentration/quantity of independent variables increased the specific gravity of the infusion samples (Fig. 1D).

$$\text{Specific Gravity} = +1.0X_1 + 1.0X_2 + 1.0X_3 \quad (4)$$

DESIGN-EXPERT Plot

Oxalate
● Design Points

X1 = A: Sorghum sheath
X2 = B: Lemon grass
X3 = C: Scent leaf

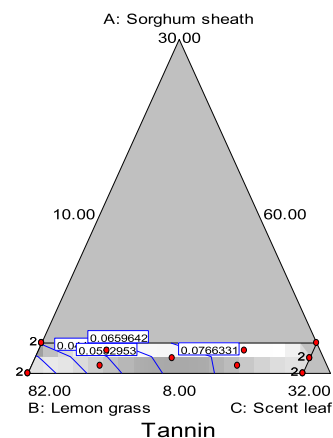


2A

DESIGN-EXPERT Plot

Tannin
● Design Points

X1 = A: Sorghum sheath
X2 = B: Lemon grass
X3 = C: Scent leaf

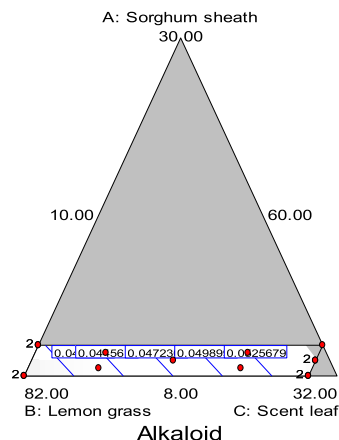


2B

DESIGN-EXPERT Plot

Alkaloid
● Design Points

X1 = A: Sorghum sheath
X2 = B: Lemon grass
X3 = C: Scent leaf

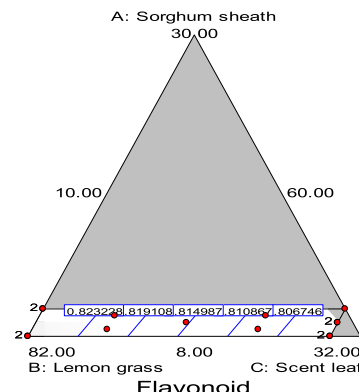


2C

DESIGN-EXPERT Plot

Flavonoid
● Design Points

X1 = A: Sorghum sheath
X2 = B: Lemon grass
X3 = C: Scent leaf

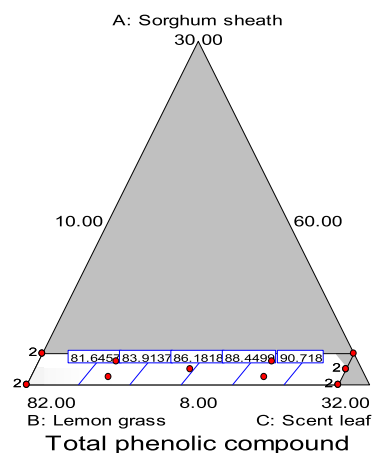


2D

DESIGN-EXPERT Plot

Total phenolic compound
● Design Points

X1 = A: Sorghum sheath
X2 = B: Lemon grass
X3 = C: Scent leaf

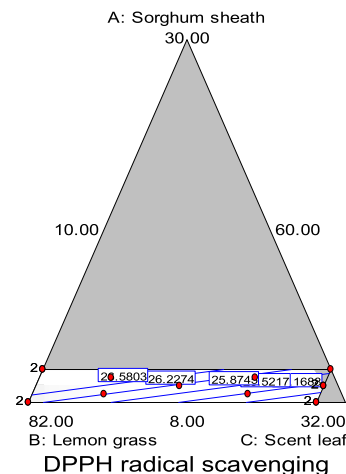


2E

DESIGN-EXPERT Plot

DPPH radical scavenging
● Design Points

X1 = A: Sorghum sheath
X2 = B: Lemon grass
X3 = C: Scent leaf



2F

Fig. 2. Surface response of the antioxidant and anti-nutrient properties of the infusion.

Effect of mixture experiment on the turbidity of the infusion

The turbidity of the infusion samples was modeled using mixture quadratic model. This is because the quadratic model was significant (F

$= 7.32$; $P = 0.0074$) and effective in describing the turbidity of the infusion sample. There was only a 0.74% chance of error. The model was reliable ($CV = 0.83\%$), predictable ($R^2 = 0.8206$) and explaining 82.06% of variation in the turbidity of the infusion samples. The adequate precision value of 8.332 indicates adequate signal, thus, the mixture

quadratic model can be used to navigate the design space. The effect of independent variables on the turbidity of the infusion indicates that only the linear mixture component was significant ($F = 17.62$; $P = 0.0012$) influencing the turbidity of the infusion samples.

The equation expressing this effect is shown in Eq. (5)

$$\text{Turbidity (NTU)} = +113.07X_1 + 80.96X_2 + 83.56X_3 \quad (5)$$

The positive coefficients of the independent variables indicate positive effect. This implies that increasing the quantity of sorghum sheath, lemon grass or scent leaf would result in increased turbidity of the infusion samples (Fig. 1E).

Effect of mixture experiment on the infusion rate of the infusion

The infusion rate was modelled using mixture quadratic model. This was because the quadratic model was significant ($F = 39.20$; $P = 0.0001$) and effective in describing the infusion rate of the infusion samples. The model was fitted, predictable ($R^2 = 0.9608$), and effective in describing 96.085 (CV = 0.88%) and adequate to navigate the design space (adequate precision = 16.886).

The effect of the independent variables showed that only the linear mixture components are significant ($F = 97.20$; $P = 0.0001$) terms influencing the infusion rate of the infusion samples. Eq. (6) shows a positive effect of the independent variables.

$$\text{Infusion rate } \left(\frac{\text{mm}}{\text{min}}\right) = +56.47X_1 + 18.88X_2 + 20.94X_3 \quad (6)$$

This effect could be shown in Fig. 1F, increasing the amount of sorghum sheath, lemon grass, and scent leaf resulted in increased in the infusion rate of the infusion samples. Rate of infusion is temperature-time dependent. An increase in temperature and exposure time may decrease the bioactive components in the infusion [1]. This was also demonstrated by Klepacka [22] for tea infusion and Etheridge and Derbyshire [13] for herbal infusion.

Effect of mixture experiment on the oxalate content of the infusion

The oxalate content of the infusion samples was modeled using a quadratic model which was significant ($F = 108.47$; $P = 0.0001$) and effective in describing the oxalate content of the infusion samples. There was only 0.01% chance that an F value this large could occur due to noise. The quadratic model was reliable (CV = 5.00%) and predictable ($R^2 = 0.9855$) describing 98.55% of variations in the observed values of oxalate content of the infusion. The model was considered adequate in making precision (accurate precision = 24.40) and could be used to navigate the design space. The effect of sorghum sheath, lemon grass, and scent leaf on oxalate content of the infusion shows the linear mixture component ($F = 26.80$; $P = 0.0001$) and interaction of lemon grass and scent leaf ($F = 10.74$; $P = 0.0112$) influenced the oxalate content of the infusion samples. Eq. (7) shows the positive effect of linear component and interaction component.

$$\text{Oxalate content (\%)} = +0.003094X_1 + 0.0011X_2 + 0.028X_3 + 0.017X_2X_3 \quad (7)$$

This positive effect implies that an increase in the number of independent variables resulted in increased oxalate content of the infusion samples (Fig. 2A). Consumption of food with high oxalate contents had been reported to cause kidney stone in consumers [22] but the oxalate contents of the infusion from the mixture experiments were low and could not pose any hazard to human health.

Effect of mixture experiment on the tannin content of the infusion

The tannin content was modeled using a mixture quadratic model. The model was significant ($F = 45.53$; $P = 0.0001$). There was only a 0.01% chance that an F-value this large could occur due to noise. The

model was effective in describing the oxalate content of the infusion samples. The model was reliable (CV = 8.75), predictable ($R^2 = 0.9661$), and adequate in making decisions (adequate precision = 17.266). The effect of the independent variables showed that the linear mixture components are significant ($F = 111.50$; $P = 0.0001$) model terms influencing the tannin content of the infusion samples. These components exhibited a positive effect on the tannin content of the infusion samples (Eqn 8).

$$\text{Tannin (\%)} = +1.28X_1 + 0.023X_2 + 0.089X_3 \quad (8)$$

An increase in the amount of sorghum sheath, lemon grass, and scent leaf resulted in increase in tannin content of the infusion samples (Fig. 2B). Tannin and phenol are phenolic compounds which have beneficial effects on consumers. It has been reported that the phenolic compounds has antioxidant properties which chelate the free radicals thereby preventing diseases such as cancer, cardiovascular diseases and other health related diseases [17,26,33].

Effect of mixture experiment on the alkaloid content of the infusion

The mixture linear model was significant ($F = 33.54$; $P = 0.0001$) and effective in describing the alkaloid content of infusion mixture. There was a 0.01% chance that an F-value this large could occur due to noise. The linear model was predictable ($R^2 = 0.8591$) describing 85.91% of variations in the observed values of alkaloid content of the infusion mixtures, reliable (CV = 6.05) and can be used to navigate the design space (Adequate precision = 12.982). The effect of the independent variables on the alkaloid content shows that the linear mixture components was significant ($F = 33.54$; $P = 0.0001$) terms influencing the alkaloid contents of the infusion mixture. Eqn 9 shows a positive effect of sorghum sheath, lemon grass and scent leaf on the alkaloid content of the infusion mixture.

$$\text{Alkaloid content (\%)} = +0.06X_1 + 0.039X_2 + 0.056X_3 \quad (9)$$

This effect is shown in Fig. 2C. An increase in the amount of sorghum sheath, lemon grass and scent leaf increased the alkaloid content of the infusion mixture. Alkaloid is important compounds in human diet due to their stimulating effects on human organism and central nervous system [24]. Alkaloids also have anticancer, antimicrobial, anti-inflammatory, antifungi properties according to Kurek [24].

Effect of mixture experiment on the flavonoid content of the infusion

Again, mixture linear model was used to model the flavonoid content of the infusion mixture. The linear model was significant ($F = 22.17$; $P = 0.0001$) and effective in describing the data. There was only a 0.01% chance that an F-value this large could occur due to noise. The model was predictable ($R^2 = 0.8012$) and fitted; reliable (CV = 0.73) and adequate to navigate within the design space (Adequate precision = 9.353). The effect of the independent variables on the flavonoid content of infusion shows that only the linear mixture components are significant ($F = 22.17$; $P = 0.0001$) model terms influencing the flavonoid contents of the infusion mixture. The effect of independent variables on the flavonoid content of the infusion mixture is presented in Eqn (10). The independent variables showed a negative effect.

$$\text{Flavonoid (\%)} = +0.83X_1 - 0.83X_2 + 0.80X_3 \quad (10)$$

Thus increase in sorghum sheath, lemon grass and scent leaf decreased the flavonoid content of the infusion mixture (Fig. 2D). Flavonoid are class of natural plant with anti-ulcer, antibacterial and anti-inflammatory properties [8,9,13].

Effect of mixture experiment on the total phenol content of the infusion

Total phenol content was modeled using a mixture linear model. This

Table 3
Optimum conditions for the infusion formulation.

Parameter	Value
Sorghum stem sheath (%)	8.03
Lemon grass (%)	76.98
Scent leaf (%)	14.99
Carotenoid (mg/g)	0.25912
TDS (mg/L)	583.594
Viscosity (mm ² /s)	1.26531
Specific gravity (SG)	1.00175
Oxalate (%)	0.0179452
Tannin (%)	0.0478483
Alkaloid (%)	0.043081
Flavonoid (%)	0.821126
Infusion rate (mm/min)	19.5133
Total Polyphenol Compound (mg GAE/g)	82.8018
DPPH Radical Scavenging (%)	25.9458

is because the model was significant ($F = 45.35$; $P = 0.0001$) and effective in designing the data. There was only a 0.01% chance that a F-value this large could occur due to noise. The effectiveness of a model was also ascertained R^2 . R^2 value of 0.8918 implies that the mixture linear model was effective and was able to describe 89.18% variations in the observed values of total phenol content. The CV value of 2.64 indicates the reliability of the model, while adequate precision of 13.435 indicates that the model can be used to navigate the design space. The effect of the independent variables on the response (total phenol content) indicated that only linear mixture components are significant ($F = 45.35$; $P = 0.0001$). Model terms influencing total phenol content of the infusion samples.

Eq. (11) revealed that the independent variables had a positive effect on the response.

$$\text{Total Phenol content} \left(\text{mg} \frac{\text{GAE}}{\text{g}} \right) = +74.49X_1 + 79.38X_2 + 94.50X_3 \quad (11)$$

Moreso, an increase in the amount of sorghum sheath, lemon grass, and scent leaf, resulted in increased total phenol content of the infusion mixture (Fig 2E). Total phenol content of the infusion was higher than that reported for herbal infusion [15]. Total phenol content in infusion exhibited antioxidant properties which help to prevent degenerative diseases in man [15].

Effect of mixture experiment on the DPPH radical scavenging of the infusion

This was modelled using a mixture linear model. This was because the model was significant ($F = 7.25$; $P = 0.0098$) and effective in designing the DPPH Radical Scavenging. There was only 0.98% chance that an F-value this large could occur due to noise. The model was reliable (CV= 3.09%), predictable ($R^2 = 0.5685$), and can be used to navigate the design space (Adequate precision= 7.302). The effect of the independent variables on DPPH radical scavenging indicated that the linear mixture components was significant ($F = 7.25$; $P = 0.0098$) model terms influencing the DPPH radical scavenging of the infusion mixtures. Eq. (12) showed that only sorghum sheath had a positive effect on the DPPH radical scavenging. However, lemon grass and scent leaf had a negative effect on DPPH radical scavenging.

$$\text{DPPH radical Scavenging} (\%) = +1.30X_1 + 5.89X_2 - 7.60X_3 \quad (12)$$

An increase in sorghum sheath resulted in increased DPPH radical scavenging; while increased amount of lemon grass and scent leaf resulted in decreased amount of DPPH radical scavenging (Fig. 2F). DPPH is used to estimate the antioxidant properties of the infusion. It measures the scavenging activities of the antioxidants present in the infusion. Therefore, all the infusion showed appreciable amount of DPPH scavenging ability against the free radicals.

Optimization of the independent variables

The constraints for optimization of independent variables in range; the dependent variables (responses) such as carotenoid content, TDS, total phenol content and DPPH radical scavenging were set at maximum. The viscosity, specific gravity, turbidity and infusion rate were set in range. Finally, oxalate content, tannin, alkaloid, flavonoid were set at minimum. The Design Expert software takes into consideration and optimize the process according to the constraints given. It generates the optimum condition and expected responses based on desirability index as shown in Table 3.

Conclusion

The physicochemical, antioxidant and antinutrient composition of sorghum stem sheath-lemon grass-scent leaf infusion showed considerable amount of antioxidants as revealed by the DPPH scavenging activities and the phenolic compound present in the infusion. However, the antinutrient factors present in the infusion were low and could not cause any damage to the consumers. Total dissolved solids and viscosity increased with an increase in the dependent variables. To obtain the optimum conditions for the independent variables based on the constraints given, values of 8.03%, 76.98% and 14.99% for sorghum stem sheath, lemon grass and scent leaf were generated respectively.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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