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Optimization of total polyphenols and tannins content during extraction of Khaya tea (*Khaya senegalensis*): Effect of water volume, temperature and infusion time

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ABSTRACT: A study was carried out to determine the optimum effect of infusion temperature, time, and water volume on chemical composition of Khaya tea. Four grammes of Khaya tea was infused in incubators at different temperatures, for different times, and water volume. And treatments for infusion water volume consisted of control 100, 150 and 200 ml at different incubator temperature (45, 70, 95°C) at different time (5, 10, 15 min). A Box Behnken experimental design was used with three replicates for all evaluations. ANOVA was performed to obtain the regression equation that could predict the responses within given range. The chemical analysis of total polyphenols and tannins were done using spectrophotometric methods. The results of this study showed that the optimum conditions for the tea infusion were found to be150 ml of infusion water, temperature 85-95 °C, and infusion time 10-15 min. But the optimum condition that we used to infused khaya tea for the follow studied are 4 g of bark's powder of Khaya senegalensis in 150 ml of hot water at 95°C for 10 min The corresponding responses under these conditions were 1400 ± 50 mg/100 g Dry Matter and 69 ± 3.2 mg/100g Dry Matter respectively for the total polyphenol and tannins content of the Khaya Tea.

KEYWORDS: *Khaya senegalensis*, Khaya Tea, optimization, polyphenols, tannins, Box-Behnken design.

1 INTRODUCTION

Tea polyphenols have been reported to have various biological and pharmacological functions, such as an anti-HIV effect, antioxidant, antimutagenic, anticarcinogenic, antitopoisomerase, and obesity, and hypocholesterolemic activities [1]. Khaya is a tree of the family Meliaceae consisting of seven species which originated from tropical Africa. *Khaya senegalensis* is present in Mauritania, Senegal and right up to Northern Uganda and in Cameroon. Its bark is often used to treat certain illnesses such as malaria, head-aches, fever, smallpox, diarrhea, lumbago (back pain), rheumatism, wounds, etc. In addition to polyphenols such as alkaloids, saponins, flavonoids, tannins and DPPH anti-radical activity, Khaya bark is also rich in phenols, and some phragmalin limonoids such as khayanolides, khayanosides, 2,6-dihydrofissinolide and two mexicanolides named khayanone and 2-hydroxyseneganolide [2]. Khaya tea, a beverage produced from extracts of the bark of Khaya tree is even said to be effective in weight loss therapies of Cameroonian population. Many studies *in vitro* and *in vivo* showed that polyphenols have some bio-activity that influenced obésity [3], [4]. Therefore the objective of this work is to Optimize the parameters of Khaya Tea effect of water volume, temperature and infusion time on total polyphenols Extraction

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS AND KHAYA TEA PREPARATION

Fresh barks were harvested in September 2010 early in the morning (6 A.M.) from three mature trees located at Meskine, a locality close to Maroua, in the far north-region of Cameroon. The barks were coarsely crushed in a mortar with a pestle, dried in a ventilated-convention oven at 45°C for 24h, then ground in a hammer mill (Culatti Polymix, Germany) endowed with a sieve of pore size 250 µm. The powder obtained was used to prepare the beverage or Khaya tea obtained by infusion of 4 g of bark's powder of *Khaya senegalensis* in different water volume(100,150,and 200 ml), at different temperature (45,70, and 95°C) for different infusion time (5,10, and 15min), followed by filtration through a 250 µm pore size diameter sieve. The Box Behnken experimental design was composed of 15 experiments including 2^3 full factorial design points, and 7-centre points. The resultant filtrate served as Khaya tea extract and was used for further experiments throughout our study.

2.2 Measurement of Total Polyphenol Contents

The contents of total polyphenol in the khaya tea extracts were measured by the modified Folin-Ciocalteu assay carried out according to the method described by [5]. The extracts (100 µL) were mixed with 250µL of diluted Folin-Ciocalteu reagent (50% v/v). The mixture was allowed to stand at room temperature (25°C) for 5 min incubation after which 250µL of sodium carbonate (20% p/v) was added and completeted to 2000 µL with distilled water. The final mixture was incubated at room temperature (25°C) for 60 min and absorbance of the resulting solution measured at 725 nm. The contents of total polyphenol were calculated by comparing with an external standard calibration curve of (0,2g/L)gallic acid (R^2= 0.9954) and were expressed as gallic acid equivalents (GAE, g of gallic acid) per 100 g of sample.

2.3 Measurement of tannins Contents

A quantity of polyvinylpolyrrolidone (PVPP) (100mg) and 1.0 mL of khaya extract was added in 1.0 mL of water distilled tube. The mixture was incubated at 4°C for 15minutes and mixed, before centrifuged at 3000 trs for 10 minutes. The floating collected contain simples others phenols that tannins (the tannins was bonded to PVPP). The quantity of tannins of floating was measured like mentioned precedently. The composition of phenols no tannic reported at the dry matter was determined. The tannins content of sample was determined like this:

$$\text{Tannins content (\%)= Total phenols (\%) – No-tannics phenols (\%)} \quad \text{(a)}$$

2.4 Development of suitable design matrix

Statgraphics 5.0 was used to obtain the regression equation that could predict the responses within given range and Sigma plot 11.0 was used to optimized. The Box-Behnken design is a Response Surface Methodology (RSM) design that was employed to find the optimum experimental condition with definite values of key experimental determinants for the maximum yield of polyphenols and tannins in khaya tea extract. All the experiments were designed using Design of Experiments (DOE) in order to study the combined and individual effects of three influential experimental parameters on the extraction of polyphenols. These variables were A: infusion temperature (45, 70, 95°C), B: infusion time (5, 10, 15 min), C: water volume (100, 150, 200 ml). Each parameter had three levels which were -1, 0 and +1, shown in Tab. 1. A total of 15 sets of experiments were performed to determine significant factors for the extraction of polyphenols and tannins.

Table 1: Independent variables and their coded levels used in RSM studies

		Level	
Factors	Units	Low (-1)	High (1)
Infusion temperature	(°C)	45	95
Infusion time	(min)	5	15
Water volume	(ml)	100	200

Table 2: Design for three variables showing observed values of Polyphenols and tannins.

Run	Temperature:A (^{O}C)	TimeB: (min)	VolumeC: (ml)	Polyphenols: (mg/100g DM)	Tannins: (mg/100g DM)
1	0(70)	0(10)	0(150)	1107.45	58.83
2	1(95)	1(15)	0(150)	1395.65	52.97
3	-1 (45)	1(15)	0(150)	916.76	25.68
4	-1 (45)	0(10)	1(200)	979.04	50.13
5	0(70)	-1 (5)	-1(100)	709.07	36.83
6	1(95)	-1 (5)	0(150)	1309.58	38.93
7	0(70)	0(10)	0(150)	1101.89	57.03
8	0(70)	1(15)	-1(100)	898.67	32.08
9	0(70)	1(15)	1(200)	1555.06	51.17
10	1(95)	0(10)	-1(100)	606.47	54.35
11	0(70)	0(10)	0(150)	1105.48	58.57
12	1(95)	0(10)	1(200)	1593.12	98.17
13	-1 (45)	-1 (5)	0(150)	1006.16	43.15
14	-1(45)	0(10)	-1(100)	635.76	72.89
15	0(70)	-1 (5)	1(200)	1667.25	53.62

2.5 MATHEMATICAL MODELING

Analysis of variance (ANOVA) was performed for the independent and dependent values to obtain regression equations that could predict the responses within a given range. The generalized second order regression equation used in the response surface study was as follows:

$$\mathbf{Y_i = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i,j=1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2} \qquad \text{(b)}$$

Where $\mathbf{Y_i}$ is the predicted response, β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i, and X_j are the independent variables. For coded independent variables (A, B, and C), the selected polynomial equation could be expressed as:

$$\mathbf{Y_i = \beta 0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2} \qquad \text{(c)}$$

The design expert software was used to generate response surfaces and three dimensional (3D) plots. The adequacy and significance of the regression model was tested using ANOVA method. Test for significance on individual model coefficients and test for lack-of-fit was also estimated.

2.6 DETERMINATION OF OPTIMUM EXTRACTION AND VALIDATION OF THE FINAL MODEL

Optimum condition for the possible maximum extraction of polyphenols and tannins from barks of *K. senegalensis* depends on all the three parameters were obtained using the predictive equation of RSM. The software design expert was applied to search the optimum desirability of the response which is maximum of polyphenols and tannins. The verification of the validity and adequacy of the predictive extraction model with respect to all the three variables within the design space was done by performing a random set of 3 experimental combinations to study extraction. Three verification run experiments were previously and remaining three experiments were those which have not been used but are within the range of the levels defined previously. The experimental and predictive values of polyphenols and tannins were compared to validate the model.

3 RESULTS

3.1 CHEMICAL COMPOSITION OF *K. SENEGALENSIS* BARK POWDER

The chemical composition of *K. senegalensis* bark powder is presented in table 3.The bark powder was relatively low in moisture, and mainly composed of fibers. Ash was also highly represented but the protein level was average. This was the first time at the best of our knowledge the proximate composition of the bark of *Khaya senegalensis* was reported. The composition generally reflected the composition of bark of other plants reported in literature. In fact, our previous report on *Scorodophleus zenkeri* and *Hua gabonii* barks revealed range compositions of 9.7-96 g/ 100mg DM for ash, 10.2-14.2 g/ 100g

DM for proteins, 2.5-3 g/ 100g for lipids, and 3.2-20.5g/ 100g DM for available carbohydrate. Basically the structure of plants bark is mainly composed of fibers and may contain resin, calcium oxalate cristal, tannins, and secretory elements [6].

Table 3: Chemical Composition of K. senegalensis bark powder

Parameters	Tenor(mg/100gMS)
Moisture content(g/100g)	10. 20±0.03
Lipids	1.05±0.02
Avalable sugars	4.78±0.32
Proteins	4690±320
Ash	11000±200
Fibers	13000±126
Alcaloïds	164±5
Saponins	470±20

Repetition number =3; Average ± écart-type

Table 4: Identification of presents components on methanolic extract of K.senegalensis by HPLC method

Number	Components
1-	Tannic acids
2-	Rutines
3-	Cathéchines
4-	Isoquecétines

3.2 ANOVA ANALYSIS FOR POLYPHENOLS AND TANNINS

3.2.1 ANOVA ANALYSIS FOR POLYPHENOLS

As summarized in Table 3, the ANOVA analysis of response 1: Polyphenols, there is only a 0.01% chance that a “Model F-Value” could be large which may occur due to noise. Values of Probability > F less than 0.0500 indicate model terms are significant. In this case five effects: A, B, C, AA, BB, are significant model terms. The insignificant model terms can be eliminated to improve the model. In this study, backward elimination procedure was used to reduce the insignificant terms. The predicted R^2 of 0.9711 is in reasonable agreement with the adjusted R^2 of 0.9730. The adjusted R^2 value corrects the R^2 value for the sample size and for number of terms used in the model. The high adjusted R^2 value (0. 9730) obtained from ANOVA analysis indicating that the developed model is highly significant [7], [8]. This model shows standard deviation (SD), mean, absolute standard’s mean, and standard’s estimation value of 530.3900, 1105.8327, 0.5329 and 1.0522 respectively. Here, the calculated value of residual autocorrelation (R.A) of 1 order is 0.0548 and this lower value of R.A designates a better reliability of the model [9]. A correlation coefficient (R^2) of 0.9903 was obtained indicating high degree of correlation between the experimental parameters and response: total polyphenol [9].

Table 5: ANOVA table for response surface quadratic model (Response: polyphenols of Khaya Tea)

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value Prob > F	Decision
Model	557.2584	9	61.9176			
A:	9.6046	1	9.6046	8.6800	0.0321	**S**
B:	133.6800	1	133.6800	120.7500	0.0001	**S**
C:	145.3200	1	145.3200	131.2600	0.0001	**S**
AA	10.5283	1	10.5283	9.5100	0.0274	**S**
AB	0.5485	1	0.5485	0.5000	0.5129	N **S**
AC	0.0029	1	0.0029	0.0000	0.9613	N **S**
BB	257.5230	1	257.5230	232.6200	0.0000	**S**
BC	0.0376	1	0.0376	0.0300	0.8610	N **S**
CC	0.0135	1	0.0135	0.0100	0.9164	N **S**
Total error	5.5354	5	1.1071	-	-	
Total (corr.)	573.5080	14				
Mean =1105.8273			R- Squared = 0.9903			
Standard Deviation = 530.39			Adj R- Squared = 0.9729			
Mean Absolute standard = 0.5294			Pred. R-Squared = 0.9711			
Estimation of standard = 1.0522			Residual Autocorr. of 1 order = 0.0483			

***S**: significant ; **NS**: Non Significant*

The normal probability described in Fig. 1 which shows some scatters along the line which indicates that the residuals follow a normal distribution. This designates that the model satisfies the assumptions of the ANOVA which depicting the accuracy and applicability of RSM in optimizing all three parameters to maximize the extraction of total polyphenols.

The experimental results of the CCD design were fitted with a second order polynomial equation. The Eq.(d) depicts the empirical relationship between polyphenols of tea and the three independent variables in coded units obtained by applying RSM.

The source AA that the regression coefficient is (-1.68861), influenced significantly and negatively the general model equation and can be eliminated for optimized extraction of polyphenols.

Polyphenols contents = $11,2033 + 1,09571*A + 4,08779*B + 4,26204*C - 1,68861*A^2 + 0,370307*A*B - 0,0268296*A*C + 8,35139*B^2 + 0,0969852*B*C - 0,0604379*C^2$ (d).

In this case five effects A, B, C, AA, BB, are significant model terms. The insignificant model terms AB, AC, BC, CC can be eliminated to improve the model. And the relationship between significant model terms and polyphenol obtained by the application of RSM is given by equation (e):

Polyphenols contents $=11,2033+1,09571*A+4,08779*B+4,26204*C-1,68861*A^2 + 8,35139*B^2$ (e)

The quadratic equations used were delivered when others factors were kept at the center of design (coded value equal to zero).

Polyphenols contents $=11,67+4,08779*B+4,26204*C+ 8,35139*B^2$ (f)

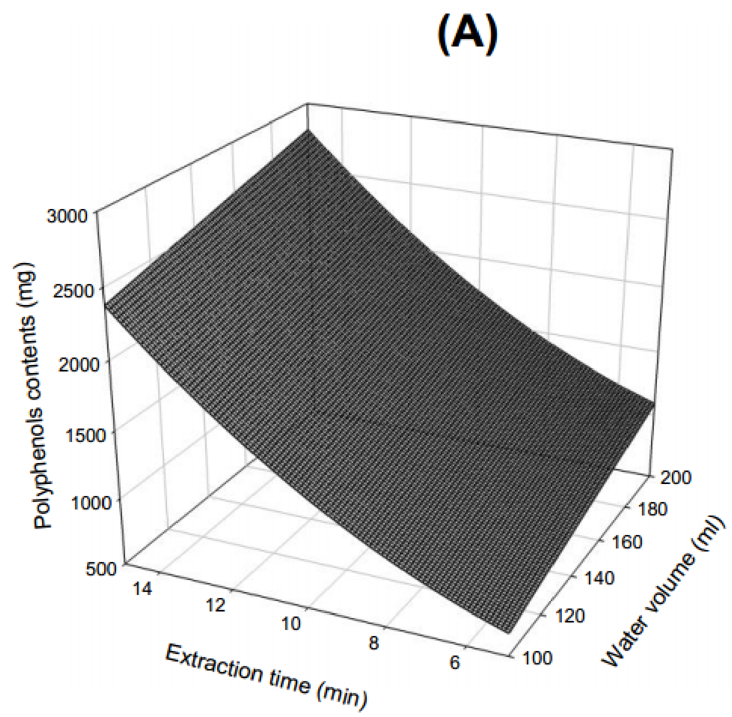


Figure 1(A): Response surface plots for the effects of extraction time and water volume on polyphenols of Khaya tea extracts.

For fig.1(A), the optimum zone of polyphenols contents (1000-3000 mg) are obtained for the parameters zone between 150-200 mL (water volume) and 8-15 minutes (time). It shows the effect of the interaction of extraction time and water volume on the polyphenols contents at a fixed extraction temperature of 0 level. Minimum polyphenols value was obtained at the lowest extraction time and reached the maximum value at 15 min of extraction time in the fixed water volume of 200 ml.

Polyphenols contents = 23,64248+1,09571*A+4,26204*C (g)

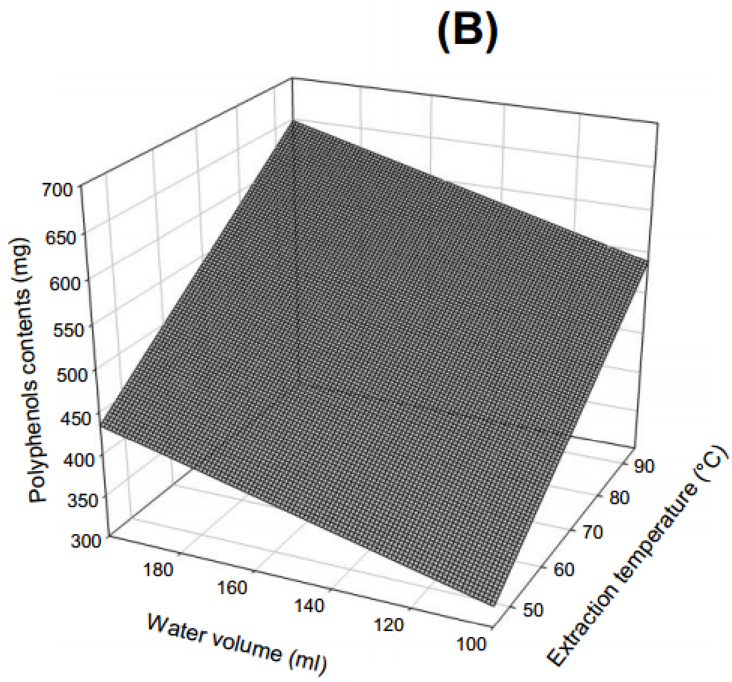


Figure 1(B): Response surface plots for the effects of water volume and extraction temperature on polyphenols of Khaya tea extracts.

For fig.1(B), the optimum zone of polyphenols contents (650-700 mg) are obtained for the parameters zone between 150-200 mL (water volume) and 80-95°C (temperature). And it shows the effect of the interaction of water volume and extraction temperature on the polyphenols contents at a fixed extraction time of 0 level. Minimum polyphenols value was also obtained at the lowest of water volume and reached the maximum value at 200 ml of water volume in the fixed extraction temperature of 95° C.

Polyphenols contents = $15{,}46534+1{,}09571*A+4{,}08779*B+4{,}26204 + 8{,}35139*B^2$ (h)

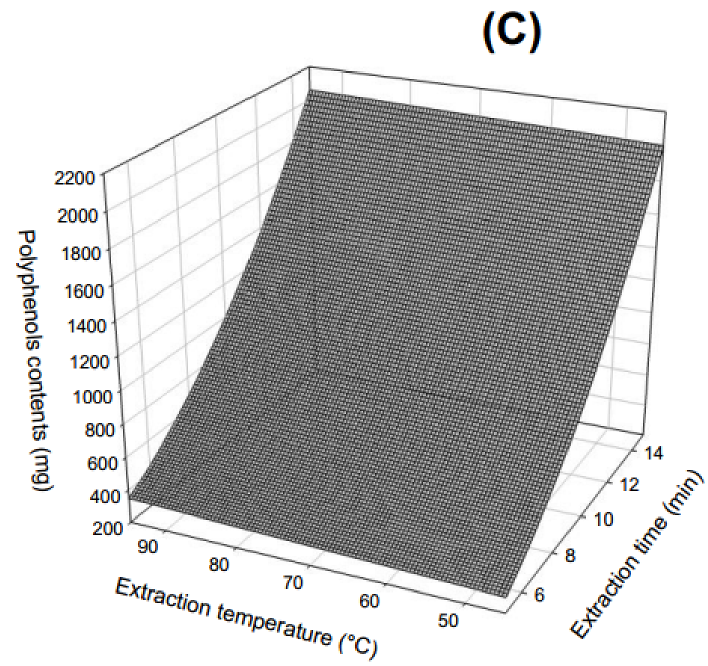


Figure 1(C): Response surface plots for the effects of extraction time and extraction temperature on polyphenols of Khaya tea extracts.

For fig.1(C), the optimum zone of polyphenols contents (800-2200 mg) are obtained for the parameters zone between 10-14 minutes (time) and 45-95°C (temperature). It shows the effect of the interaction of extraction time and extraction temperature on the polyphenols contents at a fixed water volume of 0 level. Minimum polyphenols value was obtained at the lowest extraction time and reached the maximum value at 15 min of extraction time in the fixed extraction temperature of 95 ° C. Moreover, we have found that water volume (C) was the most significant factor affecting the responses at the level of $p < 0.05$. Significant increase in polyphenols content is observed in first with the increase of both extraction time and volume water (Fig. 1(A)), in second with the increase of both water volume and extraction temperature (Fig. 1(B)), and in third with the increase of both extraction time and temperature (Fig. 1(C)).

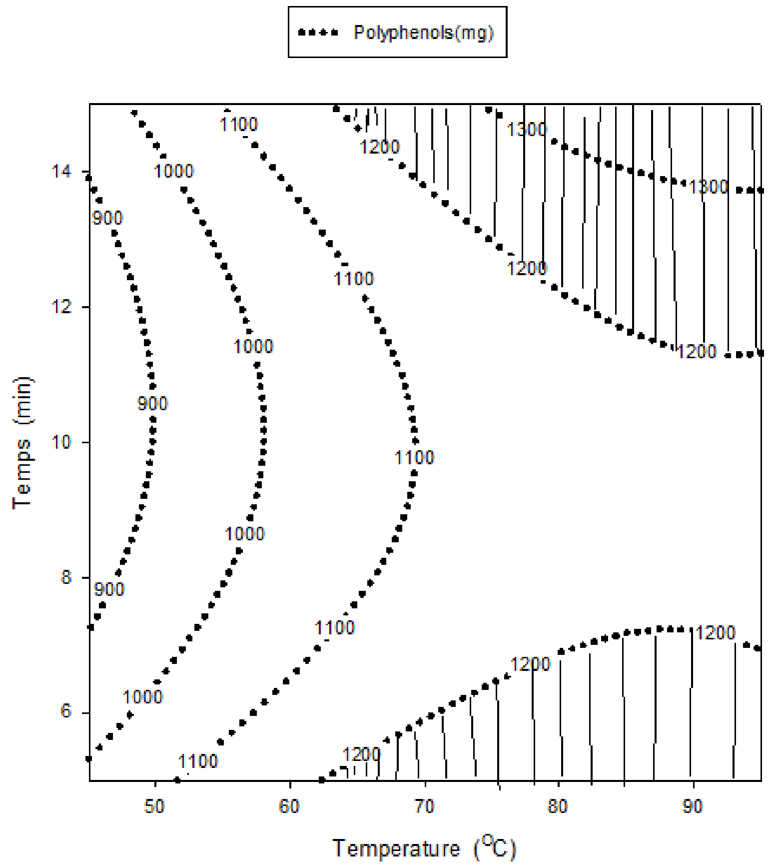


Figure 2: Influence of temperature and time for extraction of polyphénols for V=150mL.

3.2.2 ANOVA ANALYSIS FOR TANNINS

As summarized in Table 4, the ANOVA analysis of response 2: tannins, there is only a 0.01% chance that a “Model F-Value” could be large which may occur due to noise. Values of Probability > F less than 0.0500 indicate model terms are significant. In this case five effects: A, B, C, AA, BB and BC are significant model terms. The insignificant model terms can be eliminated to improve the model. In this study, backward elimination procedure was used to reduce the insignificant terms. The predicted R^2 of 0.9605 is in reasonable agreement with the adjusted R^2 of 0.9610. The adjusted R^2 value corrects the R^2 value for the sample size and for number of terms used in the model. The high adjusted R^2 value (0.9609) obtained from ANOVA analysis indicating that the developed model is highly significant (Akhnazarova and Kafarov, 1982; Box et al., 1978). This model shows standard deviation (SD), mean, absolute standard’s mean, and standard’s estimation value of 36.2450, 52.2933, 2.5183and 5.1951 respectively. Here, the calculated value of residual autocorrelation (R.A) of 1 order is 0. 1763and this lower value of R.A designates a better reliability of the model. A correlation coefficient (R^2) of 0.9861was obtained indicating high degree of correlation between the experimental parameters and response [9].

Table 4: ANOVA table for response surface quadratic model (Response: Tannins of Khaya Tea)

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	p-value Prob > F	Decision
Model	9834.2829	9	1092.6981			
A:	1203,52	1	1203,52	44,59	0,0011	S
B:	239,203	1	239,203	8,86	0,0309	S
C:	1912,22	1	1912,22	70,85	0,0004	S
AA	3958,47	1	3958,47	146,67	0,0001	S
AB	0,883935	1	0,883935	0,03	0,8635	NS
AC	51,204	1	51,204	1,90	0,2268	NS
BB	437,22	1	437,22	16,20	0,0101	S
BC	1883,31	1	1883,31	69,78	0,0004	S
CC	148,252	1	148,252	5,49	0,0661	NS
Total error	134.9400	5	26.9881	-	-	
Total (corr.)	9682.2700	14				
Mean =52.2933			R- Squared = 0.9861			
Standard Deviation = 36.2450			Adj R- Squared = 0.9609			
Mean Absolute standard = 2.5183			Pred. R-Squared = 0.9605			
Estimation of standard = 5.19501			Residual Autocor. of 1 order = 0. 1763			

S: significant ; **NS**: Non Significant

The normal probability described in Fig. 3 which shows some scatters along the line which indicates that the residuals follow a normal distribution. This designates that the model satisfies the assumptions of the ANOVA which depicting the accuracy and applicability of RSM in optimizing all three parameters to maximize the extraction of tannins.

The experimental results of the CCD design were fitted with a second order polynomial equation. The Eq. 9 depicts the empirical relationship between tannins of tea and the three independent variables in coded units obtained by applying RSM.

The source AA that the regression coefficient is (-1.68861), influenced significantly and negatively the general model equation and can be eliminated for optimized extraction of tannins like others insignificant source (AB, AC, CC).

Tannin = 37,8073 + 12,2654*A + 5,46813*B + 15,4605*C + 32,7427*A^2 - 0,470089*A*B + 3,57785*A*C + 10,8818*B^2 + 21,6986*B*C + 6,33653*C^2 (i).

In this case six effects A, B, C, AA, BB, BC are significant model terms. The insignificant model terms AB, AC, CC can be eliminated to improve the model. And the relationship between significant model terms and polyphenol obtained by the application of RSM is given by equation (j):

Tannin contents = 37,8073 + 12,2654*A + 5,46813*B + 15,4605*C + 32,7427*A^2 + 10,8818*B^2 + 21,6986*B*C (j).

The quadratic equations used were delivered when others factors were kept at the center of design (coded value equal to zero).

Tannin contents = 37,8073+5,46813*B+15,4605*C+10,8818*B*B+21,6986*B*C (k).

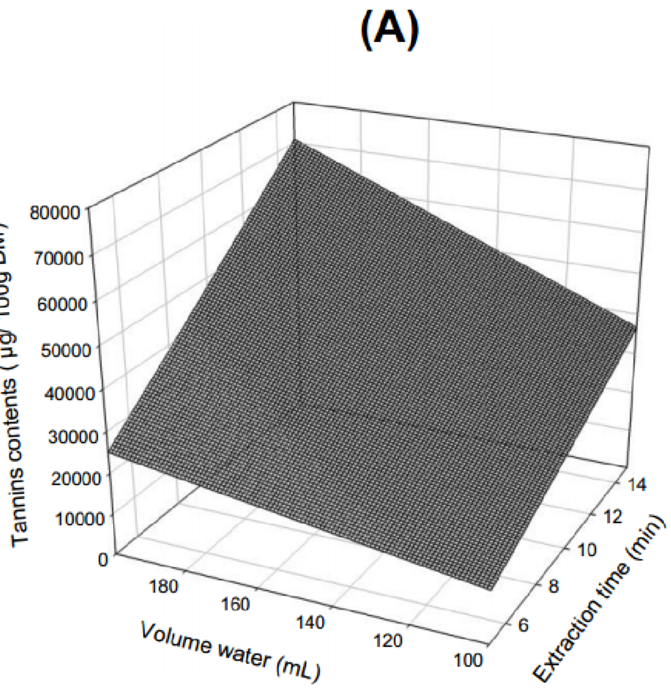


Figure 3 (A): Response surface plots for the effects of extraction time and water volume on tannins of Khaya tea extracts

For fig.3 (A), the optimum zone of tannins contents (60000-80000 µg) are obtained for the parameters zone between 150-200 mL (water volume) and 11-15 minutes (time). It shows the effect of the interaction of extraction time and water volume on the tannins contents at a fixed extraction temperature of 0 level. Minimum tannins value was obtained at the lowest extraction time and reached the maximum value at 15 min of extraction time similarly with water volume.

Tannin contents = 37,8073 + 12,2654*A + 15,4605*C + 32,7427*A*A (I)

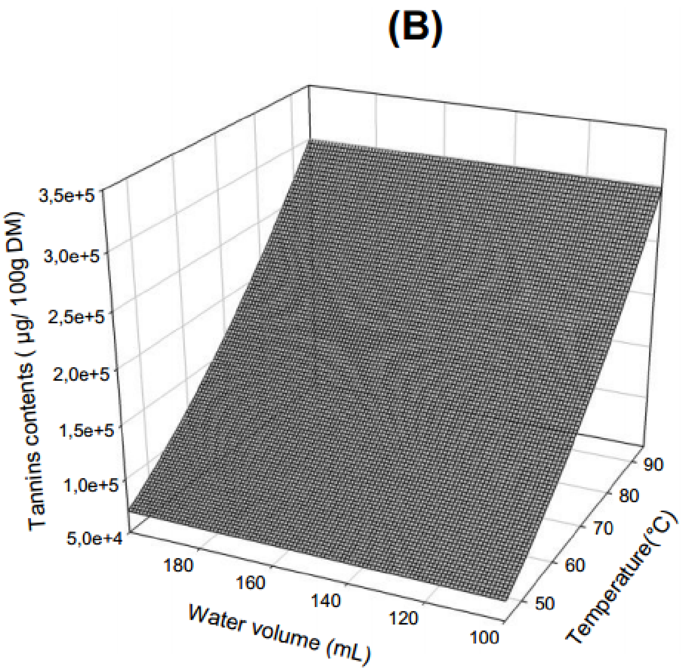


Figure 3 (B): Response surface plots for the effects of water volume and extraction temperature on tannins of Khaya tea extracts

For fig.3 (B), the optimum zone of tannins contents are obtained for the parameters zone between 100-200 mL (water volume) and 45-95°C (temperature). And it shows the effect of the interaction of water volume and extraction temperature on the polyphenols contents at a fixed extraction time of 0 level. Minimum tannin value was also obtained at the lowest of temperature and reached the maximum value at 95°C of temperature in the varied extraction water volume (100-200 mL).

Tannin contents= 37,8073+12,2654*A+5,46813*B+32,7427*A*A+10,8818*A*A (m)

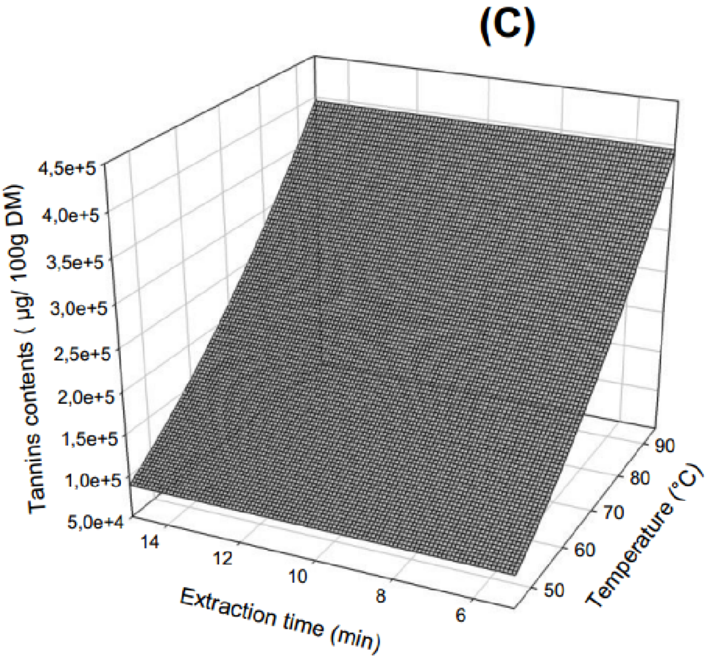


Figure 3 (C): Response surface plots for the effects of extraction time and extraction temperature on tannins of Khaya tea extracts.

For fig.3 (C), the optimum zone of tannins contents (800-2200 mg) are obtained for the parameters zone between 65-95°C (temperature) and 5-15minutes (time). It shows the effect of the interaction of extraction time and extraction temperature on the tannins contents at a fixed water volume of 0 level. Minimum tannins value was obtained at the lowest extraction temperature and reached the maximum value at 95°C of extraction temperature in the varied extraction temperature. Moreover, we have found that temperature (C) was the most significant factor affecting the responses at the level of $p < 0.05$. Significant increase in tannins content is observed in first with the increase of both extraction time and volume water (Fig. 3 (A)), in second with the increase of both water volume and extraction temperature (Fig. 3 (B)), and in third with the increase of both extraction time and temperature (Fig. 3 (C)).

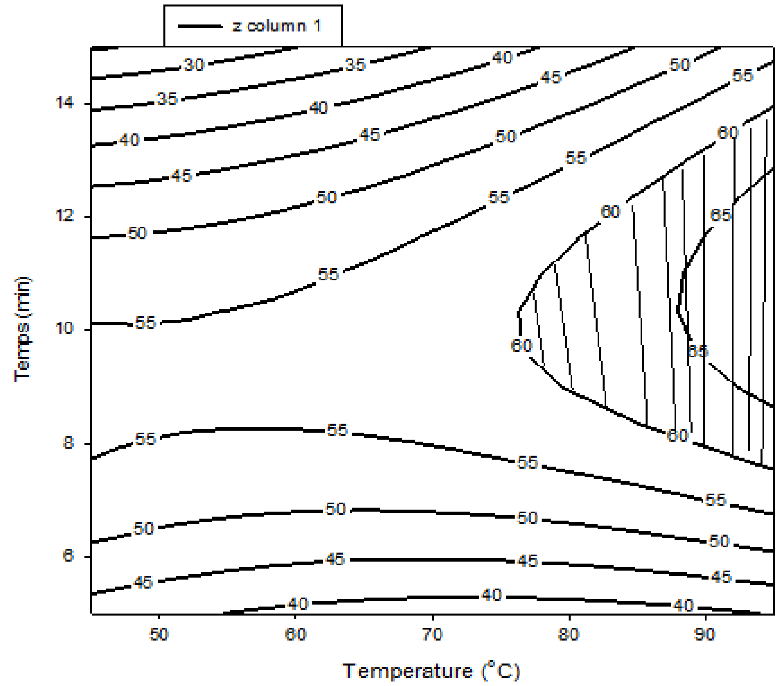


Figure 4 : Influence of temperature and time for extraction of polyphénols for V=150mL.

3.3 Validation Of Developed Model

3.3.1 For polyphenols

As represented in figure 5, experimental values were reasonably close to the predicted values confirming the validity and adequacy of the proposed model with $R^2 = 0.9786$.

Plot of predicted values vs. experimental or actual value of total polyphenols (given in Fig. 5) represents a degree of similarity which designates the accuracy of the developed method. It also describes that the developed model satisfy the variance requirement and these also reflect applicability and accuracy of RSM for improved extraction of total polyphenols.

The developed model was further validated by performing three additional experiments which constitutes the experimental combinations from the design. Moreover, the validation experiments also proved that the predicted values of polyphenols could be satisfactorily achieved within 2.14% of predicted error of experimental values.

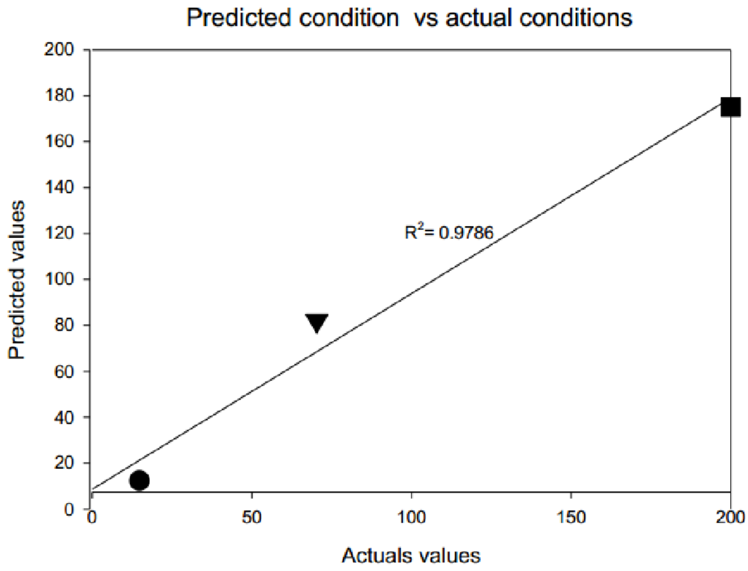


Figure 5: Plot of predicted versus actual values of total polyphenols

3.3.2 FOR TANNINS

As represented in figure 6, experimental values were reasonably close to the predicted values confirming the validity and adequacy of the proposed model with $R^2 = 0.9897$.

Plot of predicted values vs. experimental or actual value of total polyphenols (given in Fig. 6) represents a degree of similarity which designates the accuracy of the developed method. It also describes that the developed model satisfy the variance requirement and these also reflect applicability and accuracy of RSM for improved extraction of tannins.

The developed model was further validated by performing three additional experiments which constitutes the experimental combinations from the design. Moreover, the validation experiments also proved that the predicted values of tannins could be satisfactorily achieved within 1.03% of predicted error of experimental values.

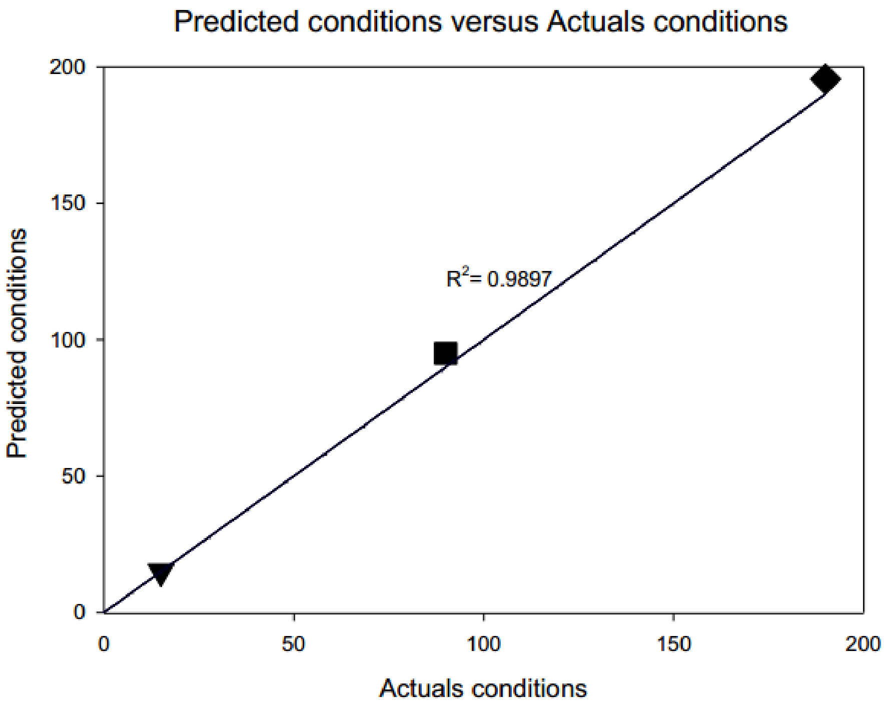
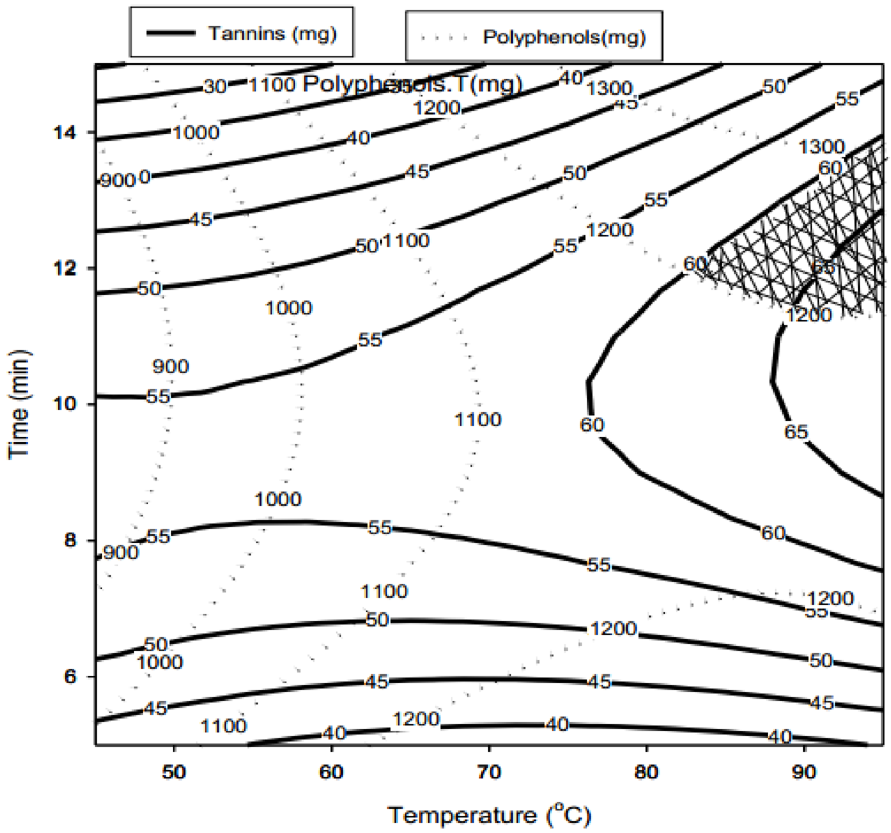


Figure 5: Plot of predicted versus actual values of tannins



4 Discussion

Extraction of polyphenol is greatly influenced by extraction time and water volume Fig. 1(A) because increase of extraction time prolongs the interaction between powder of khaya tea and extraction media. But, longer extraction period increases process economics and also chances of proteolysis. So, the magnitude of all these parameters should be optimized to maximize the extraction of Polyphenols. High extraction temperatures can increase the yield of tea catechins because the cell walls of the green tea leaves become more permeable to the solvent and to the constituents [10], [11]. However, the catechins can also be subject to degradation and epimerization when the extraction is conducted at too high temperatures [12]. This epimerization is undesirable because a large amount of the most important catechin, EGCg, is transformed into GCg [13]. Conversely, extraction at low temperatures is desirable to avoid these changes, while the efficiency of the extraction is low. Thus, it was necessary to add some others parameters like time of extraction and solvent volume (water) to improve the efficiency of extraction of catechins from tea. The results from this study about time and temperature of tea preparation concur with the findings reported by Reference [14], who reported that Polyphenol content at 0 and 120 min were similar (3.4 and 3.7/100mg) and suggest that high polyphenol contents can be produced at fermentation time between 60 and 90 min. However, reference [15], reported that fermentation duration of 90 min resulted in black tea with higher levels of the aflavins but lower the arubigins and colour than fermentation for 110 min. Honey bush tea fermented at 70°C for 24 h showed a significant increase in polyphenol content (129.2 g kg-1ss) than honey bush tea fermented for 36 -72 h (117.5 and 95.6 g kg-1ss). These results suggest that increasing fermentation time in honey bush tea would lead to a decline in measured polyphenol concentration as complex colour and flavour compounds are formed. Furthermore, tea quality such as taste and astringent will be enhanced in bush tea as it showed an optimistic increase in polyphenol content when fermented between 60 and 90 min.

5 Conclusion

The aim of this optimization process was to find the optimum values of extraction parameters in order to maximize the value of totals polyphenols and tannins in the extract of khaya tea. In the present study, response surface methodology was used to optimized the extraction of polyphenols and tannins contents from khaya tea. A central composite design was used to determine the optimum process parameters and the second order polynomial models for predicting responses were obtained. Temperature extraction was the most significant factor affecting extraction of polyphenols contents and the optimal extraction conditions were 89±6° C (temperature) for 12±1min (time) at 150 ml (water volume). The optimal extraction polyphenols and tannins are respectively 1250±50 mg/100g DM and 64±4 mg/100g DM. Under optimized conditions the experimental values were very close to the predicted values. Compared to the conventional shaking extraction methods, incubator extraction requires less extraction time, lower water volume. As such, it may be said that incubator extraction is an effective and practical method for obtaining a high polyphenols content from khaya tea.

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