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Research paper

Evaluation of Antioxidant Activity and Total Phenolic Content in Bread Incorporated with Green Coffee Beans (GCB)

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Abstract

Bioactive components in coffee had been scientifically proven to have a preventive role against various degenerative diseases. Green coffee is characterized by its unique composition and properties such as total phenolic content and antioxidant activity. The objective of this work was to investigate the effect of baking condition on the total phenolic content (TPC) and antioxidant activity (AA) of the bread incorporated with green coffee bean (GCB). Wheat flour was replaced with GCB flour at 1 to 5 wt% GCB flour over total of 200 g flour at varying baking conditions (baking temperature of 180, 200 and 220°C and baking time of 20, 30 and 40 minutes). TPC and AA of incorporated bread were determined using Follin-Ciocalteu reagent and DPPH assay, respectively. The results showed that the addition of GCB had significantly improved the total phenolic content and antioxidant activity of bread. The ideal baking condition that results in the highest of TPC and AA values was achieved at 180.1°C of baking temperature, 38.88 minutes of baking time and 5 wt% of GCB concentration.

Keywords: *antioxidant activity (AA); bread; green coffee beans (GCB); optimization; total phenolic content (TPC)*

1. Introduction

The increasing demand by consumer for health food has prompted the food industry to develop food infused with functional ingredients. One of the most popular strategies is by increased the content of natural antioxidant in existing food. The strategy employs addition of one or more functional ingredients to a food, thus preventing the deficiency or providing the food with an additional benefit [1]. The enrichment of food strategy enables large number of consumers that able to accesses to health food especially when it is being apply in staple food [2,3].

Coffee is considering the most consumed beverages worldwide and ranked second as the most traded global commodity after petroleum [4]. Green coffee refers to raw and unroasted seed of *Coffea* fruits. The coffee that is being drink largely by consumers today are the product by processing the green coffee beans in several stage. The genus *Coffea* (*Rubiaceae* family) has over 90 different species. Only *C. arabica* and *robusta* from the family that are widely cultivated and have high economic value. *Coffea* arabica that take almost 60% of the world productions and *C. canephora* or *robusta* take up almost 40% of world productions [5]. The major component for green coffee beans is insoluble polysaccharides such as cellulose and hemicellulose. These two components contribute to 50% of the beans weight. Other than that, there are also presence of soluble carbohydrates like fructose, galactose, arabinose, and sucrose as well as non-volatile aliphatic acids, volatile acids, phenolic species, oils and waxes, protein and free amino acids and minerals. The in vitro and in vivo study indicates that the derivatives of coffee products have high antioxidant capacity [6]. A diversity of bioactive compounds from coffee beans can interact with human body in a complex way that can increase the feasible outcome. This includes the improvement of antioxidant and scavenging properties, neural system simulation and weigh management by increasing the body metabolism activity [7]. It has been acknowledged that chlorogenic acid and caffeic acid are the most prominent markers in a coffee sample and along with ferrulic acid and coumaric acids, they contribute to high content of antioxidant in green coffee beans [7].

Bread that is a staple food in most part of the world is made mainly of wheat flour, salt, sugar and yeast. In conjunction of using commercial refined wheat flour, the bread lack antioxidant rich compound due to the milling process of the wheat flour. The bread making process however, is believed to compromise the nutrient content, leading with the loss of 30% vitamin E and it also shown that non-nutrient substances, phenolic acids are labile to high temperature [8]. Moreover, the bread making process with infused functional ingredient is proven to have better effect on its antioxidant and phenolic content properties [6]. Thus, the objectives of this study are to evaluate the effect of bioactive rich green coffee powder on antioxidant properties and total phenolic content of the bread. The type of coffee beans use is *liberica coffea* with concentration of 1% w/w to 5% w/w green coffee beans over 200g flour. The changes in antioxidant activity and total phenolic content at different baking conditions of the bread incorporated with green coffee were also studied and evaluated. The baking condition evaluated for this study is temperature range from 170 to 230°C and time varies from 20 minutes to 40 minutes.

2. Materials and Methods

2.1. Materials

Commercial wheat flour, dried yeast, salt, butter and yeast procured from the local market in Shah Alam, Selangor were used in the study. *Liberica* honey processed green coffee derived from Johor procured from MyLiberica, Malaysia. The harvesting period is around July to September 2017 and the moisture content is 9%. Distilled water was used for the experiment procured from the laboratory. α, α-diphenyl-β-picrylhydrazyl (DPPH) assay, methanol, Follin-Ciocalteu reagent, sodium carbonate, gallic acid were purchased from Sigma-Aldrich. All chemicals were analytical grade.

2.2. Green coffee flour preparation

The green coffee beans (GCB) flour were prepared by grinding the beans using Waring commercial blender (7011 HS, Waring Commercial Inc., Stamford CT 06902-7901, U.S.A) equipped with stainless steel blade and heavy-duty motor. The GCB powder produces was sieved to ensure size particle between 480μm to 680μm.

2.3. Bread sample preparation

The dough was prepared by replacing wheat flour with GCB flour at concentration of 1 wt%, 2% wt%, 3 wt%, 4 wt% and 5 wt% of GCB powder over total flour weight of 200g. Straight dough method was used to prepare the loaf. The GCB infused flour was mixed with 4g of yeast, 3g of salt, 8g butter, and 6g of sugar to prepare the dough. Then, water was added to the mixture at 120g before the mixture was knead for 10 minutes. The dough was then proving for 30 minutes covered with clean cloth. Next, the dough was kneaded again for second time for 10 minutes and left to prove again for 30 minutes. After second proving, the dough was ready to bake and put into baking tin, then baked in the oven at temperature varies from 180°C, 200°C and 220°C and the baking time interval is 20, 30 and 40 minutes. Lastly, the bread was left at room temperature to cool before being put into the universal oven for 24 hours to dry upon extraction.

2.4. Bread sample extraction

The bread sample was dried in the universal oven at 40°C for 24 hours before grinding. The grinded bread was passed through 0.5 mm sieve when milled. The extraction process was conducted with methanol as solvent according to the method by Yu, et al. [8] with some modification. The 2g ground bread sample mixed with 10 mL methanol solvent in a beaker. Then the mixture was stirred with magnetic stirrer for 45 minutes. The resulting mixture was then centrifuged at 10,000 rpm at 5°C for 15 minutes. The supernatant produced was collected and stored in the dark at 20°C until they need to be used for determination of total phenolic compound and DPPH radical scavenging activity.

2.5. Determination of total phenolic content

The total phenolic content of each extract was determine using the modified version of Folin-Ciocalteu method as describe in Yu, et al. [8] with slight modification. 10 v/v% Follin-Ciocalteu reagent was prepared by diluting the reagent with distilled water at room temperature with 10 mL reagent and 90 mL distilled water. Sodium carbonate solution at 7 w/v% concentration was prepared by dissolving 7g sodium carbonate solid into 100 mL distilled water. These reagents were prepared ahead of the experiment and stored in amber bottle for further used. 2 mL of bread extract was added with 0.5 mL of the Folin-Ciocalteau reagent. After 5 minutes, 1 mL of 7 w/v% sodium carbonate was added and the contents mixed thoroughly. The final volume should be adjusted to 10 mL with distilled water and left to stand for 30 minutes at room temperature. The absorbance was measured at 765 nm in spectrophotometer against the blank. The standard curve was prepared using gallic acid as a standard. Calculation of content of total phenolic content, is based on gallic acid equivalent (GAE) as presented by equation (1):

$$\text{Total phenolic content (mg GAE/ g dry weight)} = c \times V/m \quad (1)$$

where c is concentration of gallic acid obtained from calibration curve (mg/mL), V is volume of extract (mL) and m is mass of extract (g).

2.6. Determination of antioxidant activity

The antioxidant activity for the bread sample was measured using DPPH method according to Genwali, et al. [9] with slight modification. DPPH solution was prepared at 0.1mM concentration by dissolving 3.9mg of DPPH in 100mL methanol and to be stirred at room temperature. A control solution was prepared by mixing 4mL methanol and 1mL 0.1mM DPPH solution. The solution was shaken well and kept in the dark for 30 minutes at room temperature. For sample analysis, each 1mL of the extracted sample was added with 4mL of methanol. Then, 1mL of 0.1mM of DPPH solution was added to the solution and the mixture was shake vigorously. The mixture was kept in the dark for 30 minutes at room temperature. The absorbance was measure at 517nm against blank. The radical scavenging activity is expressed as the radical scavenging percentage using equation (2):

$$\% \text{ scavenging} = [(Ac-As)/Ac] \times 100 \quad (2)$$

where, As is absorbance of the sample solution and Ac is absorbance of the control.

2.7. Statistical analysis

All experimental results were evaluated by analysis of variance (ANOVA). The statistical analysis between the samples were estimated using the Design Expert version 6.0.8 statistical software (Stat-Ease, Inc. Minneapolis, MN). All the statistical analysis was carried out at significant level of $\alpha \leq 0.01$. A central composite design (CCD) with three coded levels for all the three factors that are baking temperature (A), baking time (B) and GCB concentration (C) were used. The experimental results for the CCD can be fitted into a second order polynomial equation for predicting total phenolic content and antioxidant activity in bread incorporated with GCB.

3. Results and Discussion

3.1. Total phenolic content (TPC)

The initial study conducted shows that total phenolic content in GCB contains 9.75 mg GAE/ g dry extract. This finding confirms with the results of previous study conducted by Şemen, et al. [5]. It shows that GCB is suitable as functional ingredient to be applied in bread, due to the total phenolic content present. The control bread was prepared without incorporation with green coffee powder. The amount of total phenolic content in the control bread without GCB compared to bread with addition of 1 wt% GCB at various baking conditions are represented in Fig. 1. It can be observed, addition of GCB flour into the bread has more prominent effect on the amount of total phenolic content. It clearly shows that at the same baking conditions, the amount of TPC in incorporated bread is higher from the control bread for all samples. The results obtained shows the amount of total phenolic content is affected by the heat treatment. It was found the TPC is slightly decreased in sample 4 (220°C, 20 minutes), however increased in sample 5 (220°C, 40 minutes). This could be due to the Maillard

reaction which also contributed to the formation of TPC compound [10, 11].

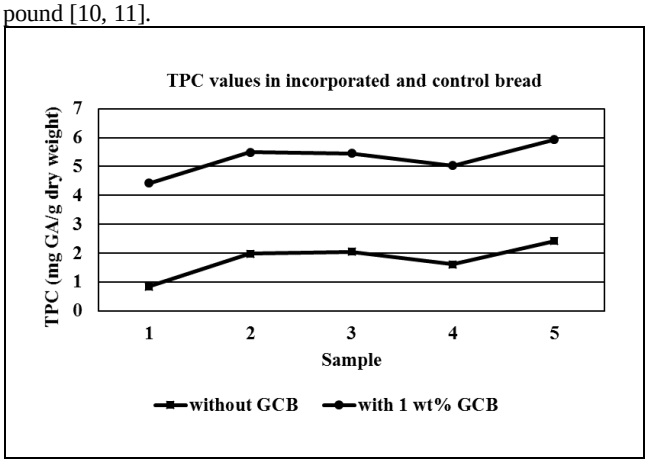

Fig. 1: TPC in incorporated and control bread

*sample 1 (180°C, 20 minutes); sample 2 (180°C, 40 minutes); sample 3 (200°C, 30 minutes); sample 4 (220°C, 20 minutes); sample 5 (220°C, 40 minutes)

The analysis of bread incorporated with green coffee beans were conducted by using analysis of variance (ANOVA). The response from ANOVA for response surface quadratic model are significant for the model as of for the total phenolic content. The final model equation is represented as equation (3):

$$\text{Total phenolic content} = -0.015C + 0.044\,C^2 \quad (3)$$

C = concentration of green coffee bean

Fig. 2 represent the experimental design and the results obtained for total phenolic content. The actual and predicted values of TPC shows that the values are in good agreement for most samples. The gap of difference for the actual value and predicted value are significant with percentage error of most sample is less than 15%. This verified the validity of the model and the existence of the optimal point.

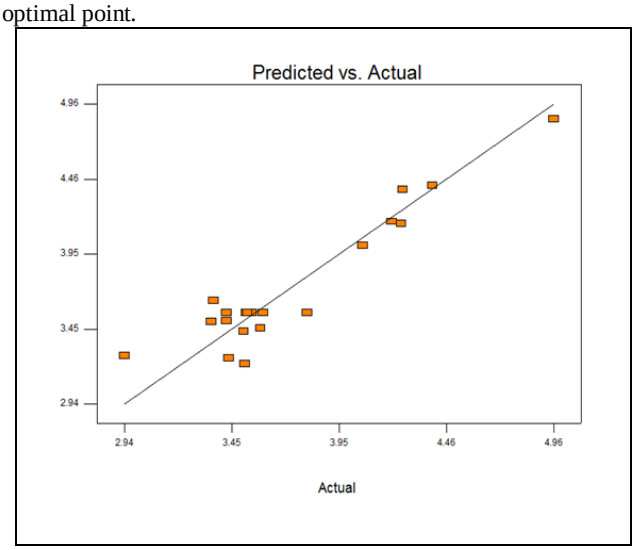

Fig. 2: The experimental and predicted results for TPC

Table 1 represent the ANOVA response for total phenolic content. The ANOVA for the response has significant model F-value of 8.92. This implies that the model is significant and there is 0.10% chance that a "model F-Value" this large is occurred due to noise. Table 1 also shows that the lack of fit and residual values is in range for total phenolic content. The sum of squares value is 0.47 and 0.39 for residual and lack of fit respectively. This suggest the validity of the model with P value less than 0.01 and R^2 value of 0.8892.

Table 1: ANOVA for TPC

Source	Sum of Squares	F Value	Prob > F	
Model	3.8	8.92	0.001	significant
C	3.05	64.5	< 0.0001	
C^2	0.46	9.7	0.011	
Residual	0.47			
Lack of Fit	0.39	4.75	0.0563	not significant
R^2	0.8892			

Fig. 3, 4 and 5 shows the response surface based on the final model, holding one variables constant at their optimum level while varying the other two within their experimental range. As from Fig. 3, the amount of total phenolic content is increasing with decreasing in baking temperature and time at constant concentration of GCB. This suggest that baking time and temperature have a very significant effect on the total phenolic content. Fig. 4 presents the effect of GCB concentration and baking time for total phenolic content at constant baking temperature. GCB concentration has more prominent effect on TPC compared to baking time. It also shows that baking time has no significant effect in TPC values.

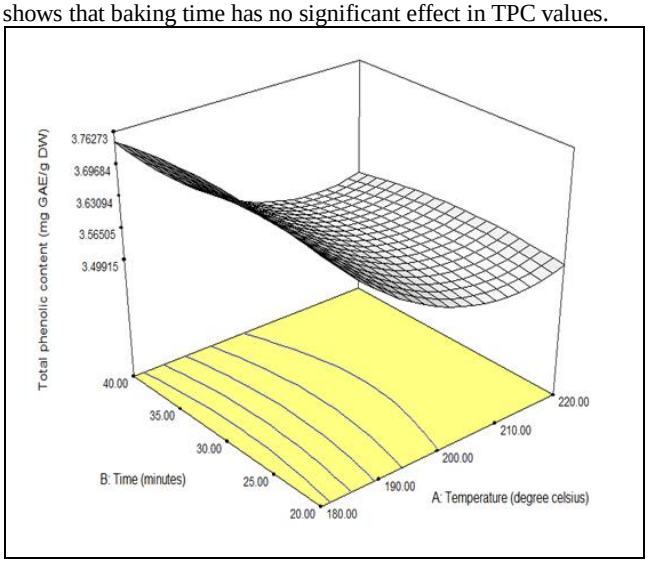

Fig. 3: Effect of baking time and temperature on TPC

On the other hand, Fig. 5 shows the effect of baking temperature and GCB concentration at constant baking time towards the amount of total phenolic content. It shows that both parameters have significant effect on TPC values. Overall, it can be observed that GCB concentration was found give more significant effect than baking temperature. The concentration of GCB also plays an important role in determining the total phenolic content of bread incorporated with GCB. The findings obtained were like the previous study conducted by Mukkundur Vasudevaiah, et al. [12].

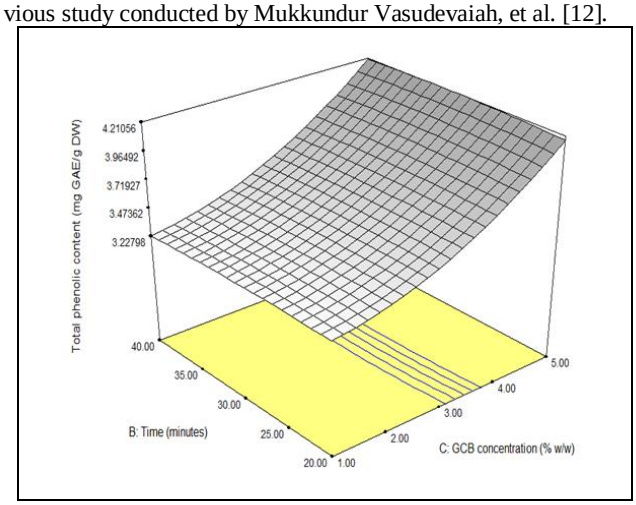

Fig. 4: Effect of baking time and GCB concentration on TPC

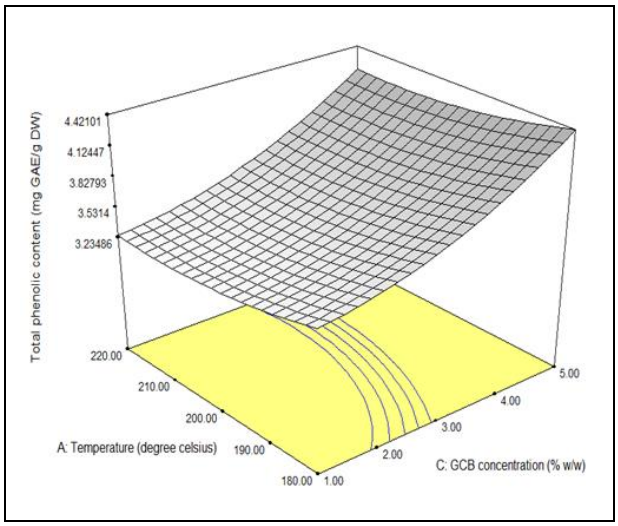

Fig. 5: Effect of baking temperature and GCB concentration on TPC

3.2. Antioxidant Activity (AA)

The amount of antioxidant activity in green coffee beans powder was initially found at 86.3%. Similar finding was also found by Dziki et al. [6] which confirms the compatibility of green coffee beans to be incorporated with bread due to the high of antioxidant activity. Fig. 6 shows the antioxidant activity in bread incorporated with 1% GCB and control bread at different baking conditions. The amount of antioxidant activity of control bread without GCB is lower than incorporated bread. The addition of 1 wt% GCB increased the antioxidant activity up to 95%. The higher antioxidant activity shows a good agreement with previous study dia by Budryn, et al. [13] that shown similar observation in fried pastry. As for the bread incorporated with various concentration of green coffee flour, the result shows that the ANOVA for response surface quadratic model are significant for the model as of the antioxidant activity. The experimental results of the CCD are fitted to the second order polynomial equation for predicting antioxidant activity for bread incorporated with green coffee beans. The equation is represented as:

$$AA = 0.045C^2 - 1.812e^{-5}AB \quad (4)$$

where A is baking temperature, B is baking time and C is GCB concentration

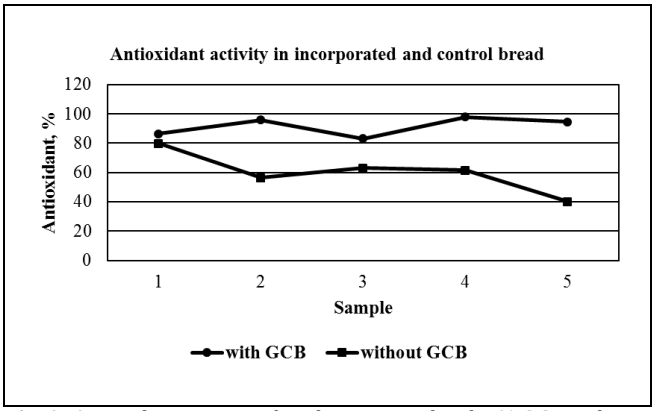

Fig. 6: Antioxidant activity in bread incorporated with 1% GCB and control bread

*sample 1 (180°C, 20 minutes); sample 2 (180°C, 40 minutes); sample 3 (200°C, 30 minutes); sample 4 (220°C, 20 minutes); sample 5 (220°C, 40 minutes)

Based on Table 2, the ANOVA for the response has significant model F-value of 5.89. This implies that the model is significant and there is 0.53% chance that a "model F-Value" this large is occurred due to noise. The lack of fit and residual values is in range for antioxidant activity. The sum of squares value is 47.12 and 38.10 for residual and lack of fit respectively. The lack of fit F-value of 4.23 implies there is a 6.98% chance that a lack of fit F-value this large could occur due to noise. The lack of fit is not significant. This suggests the validity of the model. Fig. 7 shows the experimental and predicted values for antioxidant activity. It demonstrates that the values of antioxidant activity have good agreement for most samples. This verified the validity of the model with p value less than 0.01 and R^2 equal to 0.8412. The values of antioxidant activity between the predicted and actual have percentage error less than 10%.

Table 2: ANOVA for antioxidant activity

Source	Sum of Squares	F Value	Prob > F	
Model	249.59	5.89	0.0053	significant
C^2	100	21.31	0.001	
AB	48.27	10.24	0.0095	
Residual	47.12			
Lack of Fit	38.10	4.23	0.0698	not significant
R^2	0.8412			

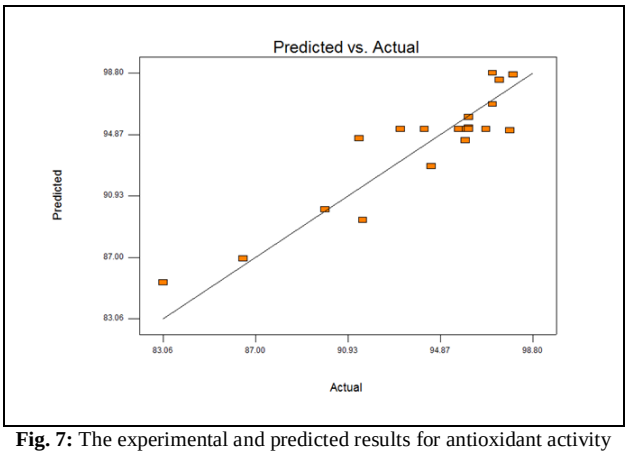

Fig. 7: The experimental and predicted results for antioxidant activity

Fig. 8, 9 and 10 illustrate the response surface that were based on the final model, holding one variables constant at their optimum level while varying the other two within their experimental range. As from Fig. 8, the value of antioxidant activity is decreasing with increasing temperature and time at constant GCB concentration. This suggest that time and temperature have a very significant effect on the antioxidant activity, thus as temperature and time increased, the antioxidant activity decreased until it reaches the optimum point and the value gradually increased. This might be due to the formation of Maillard reaction product which has positive impact towards antioxidant activity. Fig. 9 demonstrates the effect of GCB concentration and baking time for antioxidant activity at constant baking time while Fig. 10 shows the effect of baking temperature and GCB concentration towards amount of antioxidant activity at constant baking time. Both graphs have similar characteristics where antioxidant activity increased with increasing CGB concentration. Thus, the concentration of GCB plays an important role in determining the antioxidant activity of bread incorporated with green coffee beans.

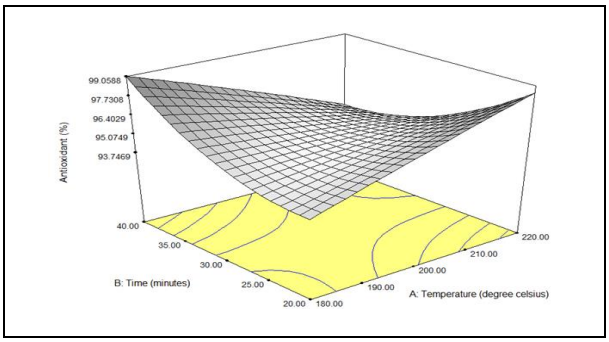

Fig. 8: Effect of baking time and temperature on AA

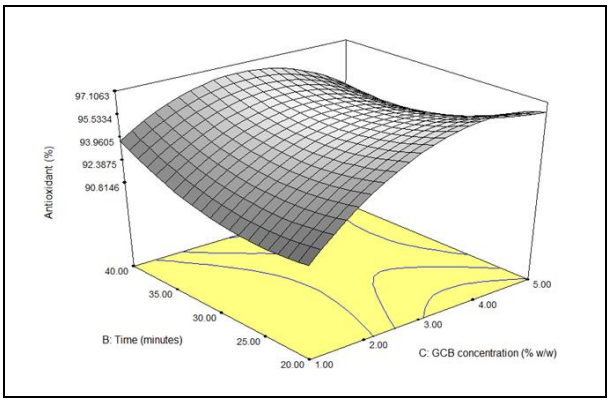

Fig. 9: Effect of baking time and GCB concentration on AA

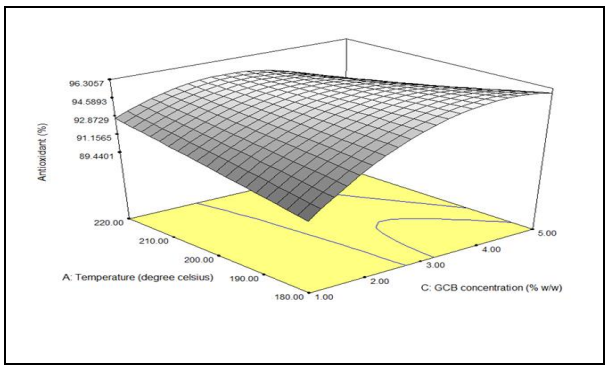

Fig. 10: Effect of baking temperature and GCB concentration on AA

3.3. Optimization process

The total phenolic content and antioxidant activity, as predicted by the final quadratic model along with the corresponding observed values, are given in Table 3. Comparison of these values indicated that there was a positive agreement between the predicted and experimental data. The location of optimum, obtained by the differentiation of the quadratic model, for achieving highest amount total phenolic content and antioxidant activity was at baking temperature of 180.1°C, baking time of 38.8 minutes and concentration of GCB at 5.00 %w/w. The calculated total phenolic content and antioxidant activity corresponding to these values were 4.42 mg GAE/g dry weight and 97.96%, respectively. Experimental validation was conducted in triplicates to verify the optimized condition. The results yielded a total phenolic content of 4.52 mg GAE/g dry weight and antioxidant activity of 97.09%. The percentage error obtained for TPC is 2.26% and for antioxidant is 0.9%. These values obtained have low error percentage thus ensuring the positive agreement between predicted and experimental results. The positive agreement between the predicted and experimental results verified the validity of the model and the existence of the optimal point.

Table 3: Predicted and experimental values of TPC and AA in bread incorporated with GCB at optimum condition

	Predicted	Experimental	Percentage error
TPC (mg GAE/g)	4.42	4.52	2.26
AA (%)	97.96	97.09	0.9

4. Conclusion

The addition of green coffee bean (GCB) flour into wheat bread significantly improved the phenolic content and antioxidant activity. Other than that, both total phenolic content and antioxidant for bread is influenced by the heat exposure that comes in term of baking condition. The optimum condition was found baking temperature of 180.1°C, baking time of 38.88 minutes and GCB concentration of 5 wt%. The calculated total phenolic content and antioxidant activity corresponding to this optimum condition was 4.42 mg GAE/g dry weight and 97.96% respectively. The validation experiments yielded a total phenolic content of 4.52 mg GAE /g dry weight and antioxidant activity of 97.09%. Confirmation of this optimization model is conducted, and the results show a positive agreement between the predicted and experimental results. The objective to evaluate the effect of green coffee on antioxidant activity and total phenolic content is successful as the results shows positive effect on both parameters analysed.

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