Antimicrobial Properties and Sensory Evaluation of Bread Enriched with Green Coffee Beans (GCB)

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Abstract. Recently, the trend of enhancing daily consumed foods with functional ingredients has been increasing. Bread, being a food product, which is consumed regularly, contains most nutrients but lacks antioxidant rich polyphenolic compounds due to the usage of refined wheat flour. Green coffee beans (GCB) are known for their green colour, which is the colour of coffee beans prior to roasting. The aroma is described as mild, green, and bean-like, which deters many from trying to consume the product. This experiment is conducted to determine antimicrobial properties and the sensory response to study the physiological effect of caffeine in bread incorporated with GCB. The bread was baked with three (3) variable parameters which were: the concentration of GCB powder, baking time, and baking temperature. The effect of concentration of GCB powder, baking time, and baking temperature on bacteria growth were also observed. It is concluded that to control the bacteria growth, the optimum condition of incorporated bread was determined at baking temperature of 180.3°C, baking time of 39.9 minutes, and GCB powder concentration of 4.85 wt%. Sensory evaluation analysis for the optimised bread (enriched with GCB) and control bread (without GCB) were conducted by a series of sensory judges. The sensory evaluation determined the differences in all aspects of appearance, aroma, taste, and texture between the enriched and normal bread. The most significant differences are that the bread incorporated with green coffee bean was darker in colour, has more distinctive wood, caramel, and smoke smell, more pungent and less salty. In addition, it is also more bitter, and harder with a stronger aftertaste when compared to normal bread. In conclusion, bread enriched with GCB exhibits strong antimicrobial properties and acceptable differences in appearance, aroma, taste, and texture compared to normal bread.

1. Introduction

Bread is known to be a growingly popular bakery product and is even a staple food in some parts of the world. Bread loses a large amount of nutrients during the milling process of wheat. To counter this, added ingredients, called functional ingredients which have various numbers of health benefits are added to the bread to increase the amount of nutrients contained in bread. The study of enrichment of bread has been done by various researches by using numerous amounts of functional ingredients. Dziki, et al. [1] conducted a study by using several ingredients as functional ingredients to be added to bread. For example, wholegrain flour from wheat, rye, and barley were observed to increase the phenolic contents in bread, resulting in higher antioxidant activity. Beetroot juice, when added to bread induce a blood pressure lowering effect on subjects [2].

Coffee has been consumed for hundreds of years and is known for having a positive impact on health with moderate consumption. Epidemiological studies have also shown that there is a strong
relationship between coffee consumption and the prevention of several chronic diseases including type 2 diabetes, Parkinson’s, Alzheimer’s, and liver malfunction [3]. Green coffee beans (GCB) are coffee beans that have not been roasted, being green in colour, they are also called raw coffee beans. GCB is known to have a mild, green, bean-like aroma instead of the well-known aroma of coffee which is only developed during the roasting process. GCB are rich in chlorogenic acid (CGA) and caffeic acids, which are the main phenolic acids in green coffee. Chlorogenic and caffeic acids exhibit antimutagenic, anticarcinogenic, and antioxidant activities in vitro [4]. During the roasting process of coffee beans, it is found that there is progressive destruction and transformation of CGA, with 8-10% lost for every 1% loss of dry matter [5]. Like other dietary polyphenols, CGA is an antioxidant which scavenges radicals, effectively decreases cellular levels of reactive oxygen species, increases the resistance of low-density lipoproteins to lipid peroxidation, and inhibits DNA damage.

Bread, being a food product, which is consumed regularly, contains most nutrients but lacks antioxidant rich polyphenolic compounds due to the usage of refined wheat flour. Therefore, the act of adding functional ingredients into bread has been an increasingly popular trend in food sciences. The technology to improve the quality, taste, functionality, and bioavailability of bread products are headed by researchers involved in optimizing the products [6]. The addition of functional ingredients in bread is also a common practice. Although various studies on the fortification of bread have been conducted, the number of these studies are limited [7]. Various studies by researchers on the enrichment of bread by addition of functional ingredients has been done. The findings focus mainly on empowering bakery products with only natural raw materials and not synthetic ones. Raw materials such as green tea, ginger powder, coriander leaf powder, and turmeric have been added into bakery products to induce a variety of enriching properties available in bakery products [6]. Since GCB contains high amount of chlorogenic acid which takes various benefit mainly antioxidant activity [8, 9], thus this study is investigating the potential of GCB as functional ingredient focusing on the sensory and antimicrobial properties of the enriched bread.

2. Methodology

2.1. Materials
All-purpose flour, yeast, salt, sugar, and butter were bought from the local market. Green coffee beans (Liberica) were attained from My Liberica Coffee Sdn. Bhd. Nutrient agar, nutrient broth, and parafilm were used for the antimicrobial analysis. The colonies on the agar plate were counted using a colony counter.

2.2. Preparation of GCB Powder
The beans were dried by local farmers by semi-washed drying process, where the outer skin is removed but the mucilage remains on the parchment and is sun-dried. The parchment is later removed after drying. The beans were then ground using a heavy-duty grinder and sieved with 500µm sized sieve.

2.3. Preparation of Bread
The parameters for bread baking (baking temperature, baking time and GCB concentration) were inserted into the Design Expert software. Twenty (20) run of experiment were required for the process. Wheat flour was used to bake the bread (200g flour) was purchased locally at the local market. GCB powder replaced the wheat flour at concentrations of 1% w/w (2g), 2% w/w (4g), 3% w/w (6g), 4% w/w (8g), and 5% w/w (10g) of total wheat flour by weight. 4g of instant yeast, 8g of softened butter, 3g of salt and 6g of sugar were used for dough preparations. 120g of water were added to the mixture of salt, yeast, flour, sugar, butter, and GCB powder in a large bowl for mixing and kneading process. Then, the dough was left to proof and covered with a clean cloth for 30 minutes before being kneaded again. After the second knead, the dough was proofed again for 30 minutes before transferring into a baking tin which was greased with a small amount of butter to prevent sticking of the dough. The dough was baked in the oven at baking temperature and time of 180 °C, 200 °C, 220 °C and 20, 30, 40
minutes, respectively according to experimental design provided by Design Expert software. After baking, the bread was left on a wire rack for 15 minutes to cool before serving to the sensory panel for the evaluation process.

2.4. Antimicrobial Analysis
The baked bread was stored in a cool dark place for 48 hours after baking. Post 48 hours, a 1cm x 1cm x 1cm sample of the bread, cut from the crumb, was placed in a universal bottle containing nutrient broth. The broth was incubated at 37°C for 24 hours. After 24 hours, the universal bottle was shaken and 100µL of the broth was dropped using a micropipette onto a petri dish containing nutrient agar and spread evenly. The petri dish was incubated for 24 hours at 37°C. After 24 hours, the number of colonies on the agar was observed and recorded. The turbidity of bacterial suspension was adjusted to 0.5 McFarland’s standard giving a bacterial load of about $1 \times 10^8$ CFU/ml.

2.5. Experimental Validation
The experimental results were validated at optimum baking temperature, time, and GCB concentration. The bread at optimum condition was baked again together with control bread (at 0% w/w GCB) and sensory evaluation was commenced on both breads.

2.6. Sensory Analysis (Appearance, Aroma, Taste, and Texture)
A sensory analysis was carried out by using a modified Elia’s method [11]. The evaluation was performed by testing four (4) major groups of sensory depictions which are appearance, aroma, taste, and texture.

For the appearance evaluation, the sensory panel was given a sensory evaluation form to evaluate the appearance of the whole bread before slicing. The evaluation form includes rating towards colour and cracks on the surface and the base of the bread. Next, the bread was sliced, and the inner crust and crumbs were evaluated based on the same parameters as the whole bread with several additions such as the thickness of the crumbs, pore size, and pore regularity.

After the evaluation of appearance, the sliced bread was cut into several smaller pieces, separating the crumb from the crust for aroma evaluation. The sample bread pieces were placed into a cup and covered. The sensory panel lifted the cup up to their nose and evaluates the aroma of bread, separately for both crumb and crust. The sensory panel then marked the rating for aroma in the sensory evaluation form.

The texture of the samples was evaluated by giving three (3) samples of each crust and bread to the sensory panel. The bread was consumed by sensory the panel and evaluated based on the crustiness, elasticity, hardness, friability, graininess, doughy, chewiness, and mouth residue. The sensory panel marked the rating for texture in the sensory evaluation form.

The taste of bread was evaluated at the same time as texture, with parameters covering aftertaste, sweetness, saltiness, sourness, pungent, straw, and toasted notes. Samples were evaluated separately for crust and crumb. The sensory panel marked the rating for taste in the sensory evaluation form.

3. Results and Discussion

3.1. Antimicrobial Analysis
The significance of the addition of GCB to induce antimicrobial properties of bread is noticeable. When compared to regular bread, the number of colonies formed are significantly less in the incorporated bread. With regards to baking time and temperatures, the roles played by these parameters also exhibited significance as the higher baking temperature, the more colonies were formed. On a side note, the baking time slightly showed the formation of colonies. The difference in antimicrobial properties are shown in Figure 1, where there is a clear difference in the growth of colonies. Bread with GCB showed the strongest antimicrobial activity with minimum inhibitory concentrations of 0.2 mg/ml.
Figure 1. Bread with (a) and without (b) GCB concentration

3.2. Statistical Analysis
The experimental results were evaluated by the analysis of variance (ANOVA). The statistical analysis between the samples were estimated using the Design Expert version 6.0.8 statistical soft-ware (StatEase, Inc. Minneapolis, MN). The statistical analysis was accepted 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 ($X_1$), baking time ($X_2$) and GCB concentration ($X_3$) were used. The experiment was done in triplicate for accuracy. The three values are averaged to obtain a single number to input into the analysis. Statistical data for antimicrobial analysis is presented in Table 1.

| Source  | Sum of squares | DF | Mean square | F value | P value |
|---------|----------------|----|-------------|---------|---------|
| Model   | 61441.85       | 3  | 20480.62    | 15.67   | <0.0001 |
| $X_1$   | 41894.61       | 1  | 41894.61    | 32.06   | <0.0001 |
| $X_2$   | 1241.78        | 1  | 1241.78     | 0.95    | 0.3442  |
| $X_3$   | 18305.46       | 1  | 18305.46    | 14.01   | 0.0018  |
| Lack of fit | 14269.45     | 11 | 1297.22    | 0.98    | 0.5504  |

($X_1$ = baking temperature, $X_2$ = baking time, $X_3$ = GCB powder concentration)

The Model F-value of 15.67 and Prob>F value of less than 0.0001 implies that both the model and model terms are significant. There is only a 0.01% chance that a “Model F-Value” this large could occur due to noise. The Lack of Fit F-Value of 0.98 implies that the Lack of Fit is not significant relative to the pure error. This is desirable as the model must fit, hence its insignificance. Although, there is a 55.04% chance that a Lack of Fit Value this large could occur due to noise.

The regression analysis, as shown in Table 1, indicates that the R-squared value is 0.746, and 26% is not explained by the model due to the values not revolving around the mean. An equation was then formed to estimate the number of colonies in the sample of bread. The equation accounts for the relationship between baking temperature ($X_1$), baking time ($X_2$), GCB concentration ($X_3$). The number of colonies can be estimated by using equation (1).

$$\text{Number of colonies} = -305.4 + 2.71X_1 - 1.07X_2 - 18.89X_3$$  \hspace{1cm} (1)

The optimized conditions for baking obtained is 180.3°C of baking temperature, 39.9 minutes of baking time, and 4.85 wt% GCB powder concentration. The experiment is then repeated with a control
to compare the antimicrobial properties exhibited and its’ effect on sensory evaluation by a series of sensory judges.

3.3. Effect of Baking Parameters on Antimicrobial Properties

Figure 2 shows the response for baking time and temperature on antimicrobial properties. It was observed that the higher the baking temperature, the more colonies formed, which indicated fewer antimicrobial properties shown for the sample. Higher baking temperatures disrupts the antimicrobial properties of GCB, thus making it less efficient in preventing the growth of colonies. However, baking time shows little effect on the number of colonies formed.

Figure 2. Effect of baking time and baking temperature on antimicrobial properties

Figure 3 shows the response for baking time and GCB concentration on antimicrobial properties. The graph shows that when increasing amounts of GCB was added into the bread, less colonies were formed. This proves that GCB exhibits antimicrobial properties and its antimicrobial properties increases with the amount added into the bread. Although, baking time depicts little to no effect when compared to the number of colonies formed.

Figure 4 shows the response for baking temperature and GCB concentration on antimicrobial properties. It can be observed from the graph that when increasing amounts of GCB was added into the bread, less colonies were formed. This proves that GCB has antimicrobial properties and can be incorporated into bread. The baking temperature also affects the number of colonies formed, but directly proportional. The higher the baking temperature, the more colonies were formed.

Figure 3. Effect of baking time and GCB concentration on antimicrobial properties
Figure 4. Effect of baking temperature and GCB concentration on antimicrobial properties

Figure 5 shows the graph of predicted and actual values. The trend line shows the prediction by analysis and the orange boxes show the actual values. Several outliers are observed from this graph. Despite that, R-squared value is known to be 0.746 thus most of the actual results are explained by the model.

Figure 5. Predicted vs actual graph for antimicrobial analysis

3.4. Optimisation Result
The optimised conditions for baking was found at 180.3°C, 39.9 minutes, and 4.85 wt% of green coffee bean. The experiment was repeated and validated with the optimised conditions. Table 2 shows the number of colonies between the experimental and predicted values. A comparison of these values indicates that there is a positive agreement between the predicted and experimental data. The results yielded 48.9 and 53 number of colonies for predicted and experimental at optimised condition, respectively. These values obtained have low percentage error (± 10%), thus ensuring the positive agreement between predicted and experimental results. Sensory evaluation analysis for the optimised bread (enriched with GCB) and control bread (without GCB) were conducted by a series of sensory judges.
Table 2. Comparison between Predicted and Experimental Values on the Number of Colonies

| Baking conditions                                      | Number of colonies | Percentage error, % |
|--------------------------------------------------------|--------------------|---------------------|
| 180.3°C, 39.9 minutes, and 4.85 wt% of green coffee bean | Predicted value: 48.9 | Experimental value: 53.0 | 7.5 |

3.5. Sensory Analysis

Table 3 shows the averaged values of response by the sensory panels. The sensory form used scales to measure the intensity of sensations. The scales varied from 0 to 10 for each group of sensory depictions. At a glance, there are minute differences, but at certain aspects some differences were observed.

The physical differences were the first to be noticed. Although most parameters remained approximately the same, some differences were observed. The sensory judges noted that the bread with GCB powder added had a darker bottom crust colour with a rating of 3.5 on average compared to the 1.6 scored by the bread without GCB added. The appearance of the bread is as shown in Figure 1. Also, the convexity of the top crust was deemed to be less on the bread with GCB added with the score on convexity being 5.5 in accordance to the bread without GCB, scoring a 7.8. It is also worth noting that the sensory panel judged the colour of the crumb to be darker in the bread with GCB added compared to the bread without, the scores being 3.4 and 1.0 respectively.

The aroma difference for the bread with GCB has been noted by the sensory panel to be more significant with more hints of wood, caramel, and smoke. Where these three parameters scored higher by a margin compared to the bread without GCB. Less hints of lactic acid and mouldy scents were detected after GCB is added into the bread.

On the taste and flavour profiles, the addition of GCB has rendered the bread to be less salty with a rating of 2.0 for the crust and 2.2 for its crumb compared to the 4.4 for the crust and 3.9 for the crumb scored by bread without GCB. GCB had made the bread’s taste more bitter compared to its counterpart. GCB had also increased the notes of straw and toasty flavours in the bread scoring 5.0 and 6.2 respectively compared to bread without GCB having a score of 3.8 and 4.8 respectively for the parameters. It is also noted that bread with GCB is more pungent compare to bread without GCB. Overall, the global intensity decreased after adding GCB into the bread but left a more noticeable aftertaste.

In determining the texture of the bread, the bread with GCB added was found to be harder and crustier compared to bread without GCB. Also, bread with GCB was deemed chewier than bread without GCB. Other than that, most parameters ratings stayed approximately the same as the texture differences are mainly affected by the recipe for the bread itself.

Overall, the incorporation of GCB in bread resulted in increased antimicrobial properties in bread. Thus, the presence of CGA in the green coffee bean and its antimicrobial activity have potential to increase the shelf life of bread and to be one of the good preservatives [10]. However, the addition of GCB lead in reducing the sensory properties score of overall characteristics especially for aroma and taste which agrees with [8].

Figure 6. Bread with (a) and without (b) GCB concentration
### Table 3. Sensory Evaluation Rating Score (Based on Lexicon for Sensory Evaluation of Bread)

| BREAD SAMPLES | Control | Incorporated with GCB | Definition | Reference |
|---------------|---------|-----------------------|------------|-----------|
| **APPEARANCE** |         |                       |            |           |
| Whole bread   |         |                       |            |           |
| Top crust     | Colour  | 5.9                   | Intensity of colour | white (0) to black (10) |
|               | Shine   | 2.0                   | Reflection of light | null (0) to pure bright (10) |
|               | Cracks  | 2.2                   | Presence of cracks on the surface | Value 5 = 50% of surface with cracks |
| Bottom crust  | Colour  | 1.6                   | Intensity of colour | white (0) to black (10) |
|               |         |                       |            |           |
| Cut bread     | Convexity | 7.8                  | Distance from the surface where the bread lies to the horizontal axis which divides superior crust from inferior crust to the bottom, measured on a cross section of bread, when there is convexity | Value = 0, flat bread (Figure 2) |
|               | Concavity | 1.2                 | Distance from the horizontal axis which divides superior crust from inferior crust to the bottom, measured on a cross-section of bread, when there is concavity | Value = 0, flat bread (Figure 3) |
|               | Thickness | 3.2                 | Width of the crust | Intensity = 10 for PGI Pan de Pagès Català (Figure 4) |
|               | Convexity | 3.7                 | Those described for the top crust |           |
|               | Concavity | 3.1                 | Intensity of colour | white (0) to black (10) |
|               | Thickness | 2.5                 |                |           |
| Crumb         | Colour   | 1.0                   | Intensity of colour | white (0) to black (10) |
|               | Pore size | 3.7                  | Size of the holes in the crumb | Value = 10 Pan de Cristal (Figure 5) |
|               | Pore regularity | 5.3              | Homogenity of the pores in the crumb | Value = 10 Pan Candeal (Figure 6) |
|               |         |                       |            |           |
| **AROMA**     | Acetic acid | 2.6                  | The sour aroma associated with vinegar |           |
|               | Lactic acid | 4.4                  | The aroma associated with soured milk |           |
|               | Butter    | 2.9                   | The aroma associated with butter |           |
|               | Wood      | 2.0                   | The aroma associated with dry wood |           |
|               | Caramel   | 1.5                   | The aroma associated with toasted sugar |           |
|               | Smoky     | 1.3                   | The aroma associated with dust and fire |           |
|               | Mouldy    | 4.3                   | The aroma associated with damp closed air spaces |           |
| Crumb         | Acetic acid | 3.4                  | Those described for the crust |           |
|               | Lactic acid | 5.3                  |                |           |
|               | Butter    | 4.3                   |                |           |
|               | Wood      | 1.7                   |                |           |
|               | Caramel   | 1.6                   |                |           |
|               | Smoky     | 1.5                   |                |           |
|               | Mouldy    | 3.3                   |                |           |
| **TASTE AND FLAVOUR** | Sweet | 2.5                   | Sweet basic taste |           |
|               | Salty     | 4.4                   | Salty basic taste |           |
|               | Sour      | 3.3                   | Sour basic taste |           |
|               | Bitter    | 3.4                   | Bitter basic taste |           |
|               | Pungent   | 2.3                   | An itchy trigeminal sensation on the tip of the tongue |           |
|               | Straw     | 3.8                   | A general taste associated with fields of ripe cereals |           |
|               | Toasted   | 4.8                   | The aromatic associated with toasted notes |           |
|               | Aftertaste | 5.4                   |                |           |
|               | Global intensity | 5.7       | The general impression of the whole flavour of the sample |           |
## BREAD SAMPLES

| BREAD SAMPLES | Control | Incorporated with GCB | Definition | Reference |
|---------------|---------|-----------------------|------------|-----------|
| Crumb | Sweet | 3.2 | 3.2 | Those described for the crust |
| | Salty | 3.9 | 2.2 | |
| | Sour | 3.7 | 2.9 | |
| | Bitter | 2.0 | 2.8 | |
| | Pungent | 2.8 | 3.2 | |
| | Straw | 3.5 | 5.1 | |
| | Toasted | 1.7 | 4.4 | |
| | Aftertaste | 4.7 | 5.8 | |
| | Global intensity | 5.8 | 4.3 | |
| | Crust | Crustiness | 3.4 | 4.2 | Noise made in the first bite of the sample between the molars (auditory assessment) |
| | Hardness | 3.7 | 5.2 | Force required to first bite through the sample with the molars |
| | Elasticity | 4.0 | 4.4 | Sample recovery after the first bite |
| | Friability | 3.9 | 3.9 | Ease with which the sample is broken into smaller particles during chewing (crumbly) |
| | Graininess | 4.0 | 4.5 | Size of the particles once the sample has been masticated until disintegrated and then formed a homogenous bolus |
| | Doughy | 4.7 | 3.8 | Pasty feeling (flour and water) which is perceived in the mouth during chewing |
| | Chewiness | 3.4 | 5.1 | Toughness of the sample perceived during mastication |
| | Mouth residue | 3.4 | 3.7 | Number of residual particles attached to the mouth after chewing |
| | Crumb | Crustiness | 0.9 | 1.2 | Those described for the crust |
| | Hardness | 1.0 | 1.6 | |
| | Elasticity | 4.4 | 3.7 | |
| | Friability | 3.8 | 4.5 | |
| | Graininess | 3.5 | 2.9 | |
| | Doughy | 5.4 | 4.3 | |
| | Chewiness | 3.6 | 3.2 | |
| | Mouth residue | 3.4 | 3.2 | |

### Figure 7. Convexity

### Figure 8. Concavity

### Figure 9. Pan de Pagès Català

### Figure 10. Pan de Cristal

### Figure 11. Pan Candeal

#### 4. Conclusion
The objective of this research was to observe the antimicrobial properties and sensory exhibited by bread incorporated with GCB powder. It is observed that there is a significant difference between...
regular bread and incorporated bread as the colony count is lower in the incorporated bread compared to the regular bread. As it exhibits antimicrobial properties, it is believed to be able to slightly prolong the shelf life of bread besides giving additional benefits to the consumer. The sensory evaluation of the bread determined that there are indeed some noticeable differences in all aspects of appearance, aroma, taste, and texture. Where the most significant differences would be that the bread with GCB was darker in colour, has a more distinctive wood, caramel, and smoke smell, more pungent, less salty but also more bitter, and harder with a stronger aftertaste when compared to bread without GCB.

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