

Original Research Article

Proximate composition, Microbiological evaluation and Optimization of cookies produced from whole wheat flour, unripe plantain flour (*R. markham*) and *Moringa oleifera* leaf powder.

Comment [aikh1]: optimization of the proximate composition of composite cookies using whole wheat, plantain and moringa oleifera leaf flours and examination of their impact on the microbiological status of the cookies.

ABSTRACT

This study evaluated the use of some underutilized local crops, whole wheat (*aestivum*), unripe plantain (*R. markham*) and *Moringa oleifera* leaf powder to produce cookies and assessed the proximate composition, microbial properties and optimization. The blend ratio was generated through D- optimal mixture design using statistical software (Design Expert). The proximate analysis showed that sample WPM9 had the highest moisture content, crude fibre. This study revealed that cookies produced from composite flours of whole wheat, unripe plantain

Comment [aikh2]: wrongly positioned

Comment [aikh3]: indicate sample composition

Comment [aikh4]: where's mineral table?

Comment [aikh5]: optimization process and optimal ratio was not captured in the abstract

Keywords: Whole Wheat flour, Unripe plantain (*R. markham*), Mixture design, Optimization.

1. INTRODUCTION

The World Health Organization (WHO) has recently recommended reducing the overall consumption of sugars and foods that promote high glucose responses due to the growing advocacy on the consumption of functional foods due to various health problems related to food consumption such as celiac diseases (lifelong tolerance to wheat gluten is characterized by inflammation of the proximal small intestine) [1].

Today's cookies are usually produced with soft wheat, a cereal that is grown all over the world but imported by developing nations like Nigeria due to harsh climatic circumstances [2], necessitating the search for a local wheat substitute. However, initiatives have been undertaken to encourage the use of composite flour, which substitutes flour from locally grown crops for some of the wheat used in cookies, reducing the need for imported wheat and creating enriched cookies [3]. These initiatives to increase the consumption of local agricultural goods have not been successful [4]. Despite the fact that there is currently a lot of technology for composite cookies accessible, such cookies still need at least 70% of wheat flour to be able to rise [5].

The production of snacks and confectioneries suffers from a serious underutilization of local agricultural raw materials, which drives up the cost of importing wheat, which is frequently used as a basic ingredient and is linked to a number of nutrition-related illnesses. To achieve sustainable development, we must employ more of the agricultural raw resources produced nearby. Combining whole wheat with unripe plantains (*R. markham*) and moringa oleifera leaf powder will reduce the cost of importing wheat, increase the amount of essential nutrients, improve nutrition, increase the variety of cookies, and reduce the risk of developing serious nutrition-related illnesses like cancer, diabetes, and cardiovascular diseases. It will also aid in lowering the products gluten content, as its consumption typically leads to celiac disease in people with a genetic predisposition.

The product development using wheat, unripe plantain (*R. markham*) and moringa oleifera leaf powder is not yet customized and commercialized in developing countries like Nigeria even though the product is likely to provide multi-benefit like solving malnutrition and food insecurity, which are visible in Nigeria as a developing country [6].

Gluten, a protein that is abundant in wheat (*Triticum aestivum*), is responsible for dough's ability to form during baking [7]. Along with the overall look and crump structure of baked items, it also affects the elasticity of dough [8]. Many baking ingredients are manufactured using wheat (*Triticum aestivum*) flour, which has been associated with several health problems in certain individuals. A large portion of these nutrients are lost during the milling and refining of the wheat grains to create flour, even though wheat is an excellent natural source of proteins (8–12%), vitamins like Vitamin E, minerals like iron and zinc, and dietary fibers [9]. A portion of the wheat's outer layer is removed during excessive processing and milling, resulting in loss of considerable amount of fiber is [10]. In addition, wheat lacks crucial amino acids like lysine, just like many cereals [11].

Unripe plantains (*R. markham*) have a strong nutritional profile and a high content of dietary fiber, both of which lower serum cholesterol and lessen the risk of heart attacks, colon cancer, obesity, blood pressure, and a variety of other disorders when consumed by humans [12]. On the other hand, unripe plantains contain resistant starch as well, which helps manage and prevent type 2 diabetes and pre-diabetes [13]. In contrast to foods rich in conventional fiber, resistant starches have fascinating functional qualities for use in food, such as the ability to make products with high fiber content, low volume, and better sensory aspects such as texture and appearance [11].

Moringa oleifera thrives under adverse climatic conditions [14]. Due to its superior nutritional and antioxidant profile, it is attracting growing amounts of interest from throughout the world. Minerals, amino acids, vitamins, and beta-carotene are abundant in *moringa oleifera* leaf. It also contains a rare combination of health-promoting antioxidants: zeatin, quercetin, sitosterol, caffeoylquinic acid and kaempferol [15]. Currently, there is growing interest in the use of *Moringa oleifera* leaf as an ingredient in the preparation of cookies [15].

This research work covered areas such as acquisition of raw materials for the cookies production, the operation process involved in producing the composite flour and the cookies, laboratory analysis of the flour and the cookies produced which includes the functional analysis, proximate analysis, mineral analysis, vitamin analysis, phyto-chemical determination, physical properties microbiological examination and sensory evaluation of the cookies. The research will also cover statistical evaluations of the responses involved in cookies production.

The study on the quality evaluation of cookies produced from whole wheat flour, unripe plantain flours (*R. markham*) and *Moringa oleifera* leaf powder is very necessary as it resulted in the development of biscuit with higher nutrition value, and which can be sold at a better cost than the commonly known 100 % wheat flour biscuit.

2. MATERIAL AND METHODS

2.1. Source of raw materials

Whole wheat grains and other ingredients were obtained from Eke-Awka market, unripe plantain (*R. markham*) specie was sourced from Nnamdi Azikiwe University farmers association, Awka, Anambra State and *Moringa oleifera* leaf was obtained from the Science Village farm in Nnamdi Azikiwe University, Awka, Anambra state.

2.2. Production of whole wheat flour

The whole wheat grains were sorted, cleaned, and washed with water. It was oven-dried for 15 minutes at 60 °C. Disc attrition mill was used to fine mill the whole wheat grains and it was sieved using a 200 µm sieve [16].

2.3. Production of unripe plantain flour

The unripe plantain fingers were sorted, cleaned, peeled, and sliced into smaller pieces. The sliced plantain was soaked in sodium bicarbonate solution contained in a bowl for 10 minutes and then oven dried for 30 minutes at 60°C, after which it was milled using a disc attrition mill and sieved afterwards with a 200 µm sieve [15].

2.4. Production of *Moringa oleifera* Leaf powder

Fresh *Moringa oleifera* leaf was collected and washed in running tap water till all dirt are removed. The leaf was soaked in 1 % brine solution for 5 minutes to remove microbes. The leaf was further be washed with distilled water. The leaf was put in a tray and dried under sunlight to remove moisture completely. The dried leaf was ground using electric blender and

sieved using a 200 µm sieve to obtain fine powder. The fine powder was stored in zip-lock bags and kept at room temperature [17].

2.5 Research Design

The design is mixture Optimal design. The various flours were mixed at different ratios to give 100g.

Table.1: Design of experiment for composite flour

Run	Component 1 A: Wheat flour (g)			Component 2 B: Unripe Plantain flour (g)			Component 3 C: <i>Moringa oleifera</i> leaf (g)						
1	5	4	9	7	3	5	0	2	1	0			
2	6			0	3			0	1	0			
3	5	4	9	7	3	5	0	2	1	0			
4	5	0	0	8	3	4	9	2	1	4	9	9	
5	5	6	7	8	3	1	7	4	1	1	4	7	
6	5	4	9	7	3	5	0	2	1			0	
7	5	3	4	1	3	3	3	4	1	3	2	4	
8	5	1	7	1	3	6	6	5	1	1	6	4	
9	5			0	3			0	2			0	
1	0	5		0	4			0	1			0	
1	1	5	5	0	2	3	0	0	3	1	4	9	4
1	2	5	0	0	8	3	4	9	2	1	4	9	9
1	3	5	0		2	3	2	4	1	1	7	3	9
1	4	5			0	4			0	1			0
1	5	5	5	0	2	3	0	0	3	1	4	9	4
16	5	2	6	1	3			0	1	7	3	9	

2.6. Production of cookies

The method described by [17] was used to produce cookies. The flour blends were weighed according to varying % inclusions of the whole wheat flour, unripe plantain flour and *Moringa oleifera* leaf powder and labeled respectively. One of the samples served as control produced with 100% whole wheat flour. The dry component was first mixed (composite flour, sugar, baking powder) then the wet components was mixed also (eggs, butter, milk, flavor).

The wet components were added to the dried ones and kneaded for 12 minutes into a consistent dough. The resulting dough was cut into uniform sizes and passed through series of molding, shaping and stamping. The stamped dough was baked in the oven for 45 minutes at 260°C. The product was allowed to cool and subsequently packaged with a cellophane wrapper. All cookies produced was stored at room temperature, during the period of analytical investigation.

2.7 Proximate analysis of cookies

The moisture, crude protein, fat, ash and crude fibre contents of the cookies were determined in triplicate according to standard analytical methods. Carbohydrate was obtained by difference of moisture, protein, fat and ash from 100% according to the method of [18].

2.8 Microbiological examination of the cookies

The bacteria count, coliform count and fungal count and was determined using the method described by Association of Official Analytical Chemists, AOAC [18].

one gram of the sample was macerated into 9 ml of Ringers solution and mixed thoroughly by shaking. This was further diluted to obtain 10^{-2} and 10^{-3} concentration. Then 0.1 ml dilution was transferred from each dilution bottle into the corresponding plate and 15 ml of sterile nutrient agar medium was incubated at 37 °C for 24 hours after which the colonies formed was counted using a colony counter (digital) RI AN ISO 9001-2008 and expressed as colony forming units per gram (cfu/g).

Nine (9) ml of sterilized violet, red bile agar was put into each plate containing 1 ml of inoculum from 10^{-3} dilution. The plate was shaken gently to mix the content properly, and then it was allowed to set and subsequently incubated for at 37 °C for 48 hours. After incubation, the number of colonies which may appear with dark red or pink centers was counted. This was expressed as colony forming units per gram (cfu /g).

Five (5) ml was transferred from each dilution into corresponding plates and (15 ml) of sterile Sabouraud dextrose Agar (SDA) medium was poured and mixed thoroughly with the inoculum by rocking the plates. The plates were incubated at the ambient temperature for three days after which colonies formed was counted and expressed as colony forming units per gram (cfu / g). Visible colonies were counted using a colony counter, and the count was expressed as log CFU/g of the original sample.

2.9 Optimization of process variables

The process variables were set as follows, carbohydrate (maximum), Crude fiber (in range), iron (minimum), energy value (in range).

Comment [aikh6]: No mineral was determined

2.10. Statistical Analysis

The mean of all parameters was evaluated for significance ($P \leq 0.05$) by analysis of variance (ANOVA) and the mean separation, and the significant effect tested by Duncan's multiple range of test using SPSS version 23.0

3. RESULTS AND DISCUSSION

3.1 Proximate composition (%) of cookies produced from composite flours.

The proximate composition of the cookies samples is presented in Table 1 The result showed that the proximate composition of the cookies varied with the proportion of the three flours in the formulated blend. The variations could be attributed to differences in the chemical constituents of individual flours used in composite flour formulation.

The moisture content ranged from 4.53 to 8.97%, fiber ranged from 4.14 to 7.26%, fat ranged from 19.30 to 26.53%, Ash ranged from 1.23 to 2.20%, protein ranged from 6.56 to 10.57%, carbohydrate content ranged from 48.73 to 63.32% and the energy values ranged from 454.87 Kcal/J to 459.03 Kcal/J. As observed in Table1, incorporation of unripe plantain flour and *Moringa oleifera* leaf powder in the cookies samples slightly increased their moisture, ash, fiber, fat, and protein content while the carbohydrate content of the cookie's samples decreased. The moisture content of sample WFC (100% whole wheat flour cookies) was significantly the lowest. The influence of the WPM (whole wheat flour, unripe plantain flour and *Moringa oleifera* leaf powder) blends on the moisture content of the cookies samples was clear as all the composite samples had higher moisture compared to the control sample. Smaller values ranging from 3.90 – 5.03% was reported as the moisture content of short bread biscuits produced from blends of wheat flour and water yam flours [19]. However, [16] reported higher moisture content ranging from 7.72 – 10.83% for spaghetti produced from wheat flour blend and *Moringa oleifera* leaf powder. The variation in moisture content can be attributed to differences in the raw materials used as well as environmental and experimental factors [20]. The low moisture content obtained in this study is desirable for the prevention of microbial activities and extension of the shelf-life of the cookies if protected from absorbing moisture through proper packaging.

The ash content of a food material could be used as an index of mineral constituents of the food because ash is the inorganic residue remaining, after the water and organic matter have been removed by heating in the presence of an oxidizing [7]. The values increased from 1.23% in sample WFC (100% whole wheat flour) to 2.20% in sample WPM16 (52.61% whole wheat flour, 30% unripe plantain flour and 17.39 % *Moringa oleifera* leaf powder). This agrees with the study of [21] who reported similar increase (0.52 to 1.25%) in Ash content of short bread biscuits produced from wheat flour and whitewater yam flour. [10] also reported increase in Ash content (0.48 to 1.03%) of wheat cookies fortified with pineapple peel flour although their values are lower than the ones obtained in this study. The result showed that composite flours of whole wheat, unripe plantain and *Moringa oleifera* leaf powder slightly increased the ash content of the product, this might be due to the specie of unripe plantain used and the presence of the *Moringa oleifera* leaf powder which have high ash content [17].

Crude fiber composition is a measure of the quality of indigestible cellulose, pentose, lignin and other components present in food [22]. Crude fiber has little food value, but it plays a vital role in increased utilization of nitrogen and absorption of some other micronutrients and provides bulk necessary for peristaltic action in the intestinal tract [23]. There was significant increase in the fiber content of the cookies samples as they differed significantly ($p < 0.05$). The crude fiber contents of the WPM (whole wheat flour, plantain flour and *Moringa oleifera* powder) cookies are higher than that of the control, WFC (whole wheat flour) cookies product. Sample WPM9 made with 50% whole wheat flour, 30 % unripe plantain and 20 % *Moringa oleifera* leaf powder; had a value of 7.26% followed by sample WPM11(7.17%), made with 55.02% whole wheat flour, 30.03% unripe plantain and 14.94 % *Moringa oleifera* leaf powder and sample WFC (whole wheat flour) cookies made with 100% whole wheat flour had the lowest value (4.14%). The result obtained in this study is higher than that reported in the study of [24] on evaluation of quality and sensory characteristics of spaghetti made from plantain and wheat flour blends which recorded values within the range of 2.41% to 2.98%.

The fat content of all samples differed significantly ($p > 0.05$) as shown in Table 2. The fat content of the cookies increased greatly when compared to the control sample. The percentage fat content of the cookies samples ranged from 19.20% in sample WFC (100% whole wheat flour) to 26.53% in sample WPM15 (Cookies made with 55.02% whole wheat flour, 30.03% unripe plantain flour and 14.94% *Moringa oleifera* leaf powder). The percentage fat content obtained in this study is above the range (9.95-20.45%) reported by [25] for cookies produced from wheat, defatted peanut and avocado flour blends and also higher than 3.84 -4.63% reported by [28] for Wheat-African yam bean composite cookies. The varied results could be attributed to the differences in the raw materials used. It could also be because of the function of the butter used in the cookies [23] and because of the use of n-hexane for fat extraction as this chemical has been noted to be effective in total fat extraction from products. High-fat content in food especially baked products can create the challenge of rancidity during storage, although fat facilitates absorption of fat-soluble vitamins, provides essential fatty acids and important volatile compounds for flavor and sensory qualities [26]

The protein content of sample WFC was the lowest (6.56%) as shown in Table 2, while those with unripe plantain flour and *Moringa oleifera* leaf powder substitutions had higher protein contents. This showed that the addition of unripe plantain flour and *Moringa oleifera* leaf powder resulted in increase in protein content of the cookies, sample WPM9 with 50% whole wheat flour, 30% unripe plantain flour and 20% *Moringa oleifera* leaf powder was the highest (10.57%). This observation is not in doubt because *Moringa oleifera* leaf powder had been reported to be a good source of protein [16] has been similar report on the increase in protein content of bakery products substituted with *Moringa oleifera* leaf powder flour [17].

The carbohydrate content of cookies was within the range of 48.73 to 63.32 % as shown in table 2. There was a significant ($p < 0.05$) difference between sample WFC and other cookies samples while sample WPM13 was significantly ($p > 0.05$) different from other samples. The carbohydrate content of the cookie samples made with whole wheat flour, unripe plantain flour and *Moringa oleifera* leaf powder were lower than the control sample. This may be because of the flour blends which led to the reduction or changes in the carbohydrate content of the cookies sample. [27] and [28] reported a decrease in carbohydrate content of cookies produced from blends of wheat and African yam bean and wheat and Bambara groundnut respectively. According to [29] carbohydrate content contributes energy value of food formulations. The carbohydrate content in these cookies makes them ideal for all age groups most especially infants since they require energy for their rapid growth.

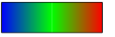
The mathematical model for the carbohydrate content of the cookies is presented in equation (1). The coefficient A, B, C are whole wheat flour, unripe plantain flour and *Moringa oleifera* leaf powder respectively.

$$\text{Carbohydrate} = 55.32A + 52.29B + 50.91C - 3.65AB - 21.27AC - 7.00BC - 177.91A^2BC + 171.58 AB^2C + 642.75 ABC^2 \quad (1)$$

From equation (1), the whole wheat flour, unripe plantain flour and *Moringa oleifera* leaf powder showed positive coefficient (+55.32, +52.29, +50.91) respectively which implies an increase in the carbohydrate content as the variables increase individually, while a negative coefficient (-3.65, -21.27, -7.00) which shows a decrease in carbohydrate content was observed for the flour blends. However, the samples with squared coefficients of unripe plantain flour and *Moringa oleifera* leaf powder showed a positive value (+171.58) and (+642.75) respectively which implies an increase in the carbohydrate content of the cookies with the increase in the unripe plantain flour and *Moringa oleifera* powder respectively and a negative value (-177.58) for the squared coefficient of whole wheat flour which indicates a decrease in the carbohydrate content with an increase in whole wheat flour in the blend. Whole wheat is a good source of dietary fiber which is a type of carbohydrate that is not digested by the **bod**, so it does not raise the sugar level the way other carbohydrates do. This reason could mean offsetting the carbohydrate content of the cookies hence the decrease in carbohydrate content of the cookies.

Carbohydrate

Color points by value of Carbohydrate:

48.73  57.51

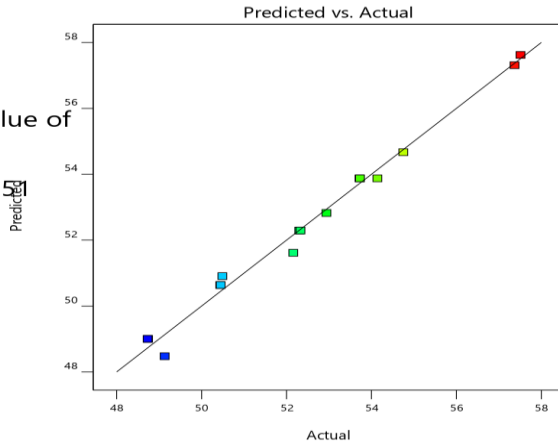



Fig 1: Graph of the Predicted vs Actual Value of the carbohydrate content of cookies

Figure 1 shows the graph of predicted and actual values which can be used to understand the accuracy of the model's predictions. The model prediction is near accurate at point 52.94:52.83, 53.73:53.88, for the actual and predicted value respectively. This is because points that fall on the diagonal line indicate that the model's predictions are accurate for those values.

Component Coding: Actual

Carbohydrate (g)

 48.73 57.51
 X1 = A: Wheat Flour
 X2 = B: Plantain Flour
 X3 = C: Moringa Flour

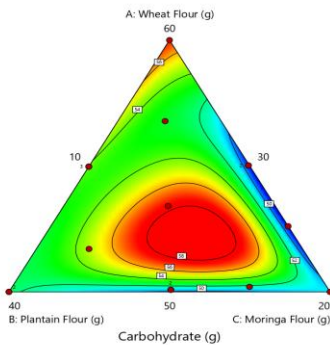


Fig 2: Contour plot of effect of composite flour of whole wheat, unripe plantain and *Moringa oleifera* leaf powder on the carbohydrate content of cookies.

From Fig 2, it shows that an increase in whole wheat flour slightly increased the carbohydrate content (54 -56 g) of the cookies and an increase in the unripe plantain flour increased the carbohydrate content of the cookies from (50 -52 g). This is because whole wheat and unripe plantain are starchy foods. However, there is no significant change in the carbohydrate content of the cookies with an increase in *Moringa oleifera* leaf powder because it is not a starchy food though packed with nutrients. Finally, a combination of the three raw materials contributed to overall carbohydrate content (54-58 g) in the cookies.

The total soluble solids (TSS) of the cookies ranged from 18.01 to 19.50%. Sample WFC had the highest value of 19.50% while sample WPM7 (53.41 whole wheat flour, 33.34 unripe plantain flour, 13.24 *Moringa oleifera* leaf powder) had the

lowest value (18.01%). This implies that the samples contain 19.50% and 18.01% of soluble solids like sugar, starch that can be dissolved in a liquid. The TSS is a way to measure the quality or purity of a food product [30]. Higher TSS means that the cookie is likely to be sweeter and denser. However composite flours of whole wheat, unripe plantain and *Moringa oleifera* leaf powder influenced the total soluble solid of the cookie as Whole wheat flour has a higher fiber content, unripe plantain is high in starch and *Moringa oleifera* leaf powder has a lot of different nutrients and compounds [31].

The energy value of the cookies as shown in Table 3 was relatively high and ranged from 437.79 – 459kcal/J. Cookies produced from sample WFC (100% wheat flour) recorded the lowest the energy value while sample WPM5 (56.78% whole wheat flour, 31.74% unripe plantain flour and 11.47% *Moringa oleifera* leaf powder). The samples were significantly different ($p>0.05$). It was observed that the cookies that have the high proportion of whole wheat flour and *Moringa oleifera* leaf powder possess high energy value. This could probably be because whole wheat flour and *Moringa oleifera* leaf powder recorded the highest fat content comparable to the unripe plantain flour sample in the study. However, the energy value recorded in this study were higher than the value (421.03kcal) reported [19] for short bread biscuits produced from water yam-wheat flour blends.

Table 2: Proximate Composition of the cookies samples.

Samples	Moisture content	Fat content	Crude Fiber	A s h	P r o t e i n	Carbohydrate	Energy Value
W F C	4.53 ^l ±0.00	19.30 ⁿ ±0.00	4.14 ^h ±0.02	1.23 ^a ±0.00	6.56 ^m ±0.11	63.32 ^a ±0.02	437.79 ⁱ ±0.59
W P M 1	7.10 ^c ±0.00	24.10 ^h ±0.10	5.36 ^g ±0.11	1.80 ^f ±0.00	7.67 ^l ±0.01	53.73 ^f ±0.00	450.65 ^f ±0.43
W P M 2	6.27 ^f ±0.05	20.10 ^m ±0.00	5.73 ^f ±0.05	1.94 ^d ±0.00	8.56 ^k ±0.01	57.37 ^c ±0.02	431.07 ^k ±0.43
W P M 3	7.10 ^c ±0.01	24.13 ^h ±0.12	5.36 ^g ±0.11	1.79 ^{ef} ±0.02	7.63 ^l ±0.01	53.72 ^f ±0.01	450.66 ^f ±0.41
W P M 4	6.77 ^d ±0.01	25.23 ^d ±0.15	6.23 ^{cde} ±0.00	1.87 ^e ±0.01	9.26 ^e ±0.01	50.44 ^j ±0.02	455.55 ^c ±0.53
W P M 5	5.24 ^k ±0.00	24.83 ^f ±0.12	6.10 ^d ±0.11	1.99 ^c ±0.01	8.92 ^g ±0.01	52.94 ^g ±0.01	459.03 ^a ±0.40
W P M 6	7.11 ^c ±0.01	24.27 ^h ±0.06	5.10 ^h ±0.10	1.80 ^f ±0.01	7.73 ^l ±0.01	54.14 ^e ±0.01	452.47 ^e ±0.43
W P M 7	5.82 ^g ±0.01	20.50 ^l ±0.00	5.99 ^c ±0.01	1.53 ^h ±0.06	8.66 ^j ±0.01	57.51 ^b ±0.02	453.52 ^j ±0.42
W P M 8	5.47 ^j ±0.01	23.43 ⁱ ±0.06	5.43 ^g ±0.06	1.77 ^f ±0.01	9.03 ^f ±0.01	54.75 ^d ±0.00	453.62 ^d ±0.52
W P M 9	8.97 ^a ±0.00	21.33 ^k ±0.11	7.26 ^a ±0.25	1.58 ^g ±0.02	10.57 ^a ±0.02	50.45 ^j ±0.02	424.87 ^l ±0.43
WPM10	5.54 ⁱ ±0.03	25.03 ^c ±0.06	6.36 ^{cd} ±0.33	1.87 ^e ±0.00	8.80 ^h ±0.10	52.33 ^h ±0.03	457.62 ^b ±0.51
WPM11	6.67 ^e ±0.02	26.20 ^b ±0.10	7.17 ^a ±0.32	1.86 ^e ±0.02	9.67 ^d ±0.01	48.74 ^l ±0.01	458.80 ^a ±0.43
WPM12	6.76 ^d ±0.01	25.43 ^c ±0.06	6.25 ^{cde} ±0.02	1.85 ^e ±0.01	9.27 ^e ±0.00	50.45 ^j ±0.03	455.66 ^c ±0.43
WPM 13	7.11 ^c ±0.01	22.91 ^j ±0.01	5.53 ^{fg} ±0.06	2.18 ^b ±0.01	10.10 ^c ±0.01	52.16 ⁱ ±0.06	442.97 ^h ±0.41
WPM 14	5.63 ^h ±0.11	25.06 ^{de} ±0.06	6.70 ^b ±0.17	1.87 ^e ±0.01	8.73 ⁱ ±0.01	52.30 ^h ±0.00	457.63 ^b ±0.43
WPM15	6.65 ^e ±0.01	26.53 ^a ±0.25	6.83 ^b ±0.06	1.87 ^e ±0.02	9.68 ^d ±0.01	48.73 ^l ±0.02	458.80 ^a ±0.41
WPM 16	7.23 ^b ±0.01	24.57 ^g ±0.23	6.37 ^c ±0.00	2.20 ^b ±0.00	10.33 ^b ±0.01	49.13 ^k ±0.03	448.69 ^g ±0.43

Values are mean ± standard deviation of triplicate determinations. Means in the same column with different superscripts are significantly different ($p = 0.05$).

WFC – Whole Flour Cookies, WPM – Wheat, Plantain, *Moringa oleifera*

4.8: Result of the microbiological examination of the cookies samples

The results of the microbial load of the cookies are shown in Table 3. The total bacteria load of cookies ranged from 1.00 to 8.11×10^5 cfu/g. Cookies made from 100 % of wheat flour had lower bacteria count as compared to cookies (8.11×10^5 cfu/g) made with the composite flour of whole wheat, unripe plantain and *Moringa oleifera* leaf powder. The study showed that the total bacteria count of cookies significantly differed ($p > 0:05$). The higher total bacteria count in cookies made with the composite flour might be due to the moisture content of the *Moringa oleifera* leaf and environmental conditions [32].

Both fungal and coliform count are also shown in table 3. The mean value for fungal and coliform count of cookies varied from 0.00 to 7.00×10^5 cfu/g and 0.00 to 1.63×10^5 cfu/g respectively. The study revealed that cookies made with the composite flour had higher fungal and coliform count as compared to 100 % of whole wheat flour. However, sample WPM11 (55.02 whole wheat flour, 30.03 unripe plantain flour, 14.94 *Moringa oleifera* leaf powder) had the highest value for bacterial, Fungal and Coliform count. Therefore, total bacteria, fungal and coliform count decreased as *Moringa oleifera* concentration increased.

This could be due to antimicrobial activity of *Moringa oleifera* as reported in the work of [16]. The source of these microbes in flour blends of whole wheat, unripe plantain flour and *Moringa oleifera* leaf could be inherent microflora in the air, environment, and handling of the samples. Nevertheless, the microbiological standard of blended foods from 103 to 105 cfu/g is an acceptable value. The total bacterial count for cereal and legume-based products exceeding 106 cfu/g is considered microbiologically unsafe [33]. With reference to this, cookies produced from composite flour of whole wheat, unripe plantain and *Moringa oleifera* leaf powder is safe for consumption.

Table 3: Microbiological Examination of the cookies samples (cfu/g)

S a m p l e s				Bacterial Count	Fungal Count	C o l i f o r m		
W	F	C	1	$1.06^{hi} \times 10^5$	$1.00^e \times 10^5$	N	i	l
W	P	M	1	$1.11^{hi} \times 10^5$	$1.00^e \pm 0.00$	N	i	l
W	P	M	2	$1.15^{ghi} \times 10^5$	N	i	l	N
W	P	M	3	$1.39^g \times 10^5$	$1.00^e \times 10^5$	N	i	l
W	P	M	4	$2.02^f \times 10^5$	N	i	l	N
W	P	M	5	$7.25^b \times 10^5$	$2.00^d \times 10^5$	$0.37^c \times 10^5$		
W	P	M	6	$1.10^{hi} \times 10^5$	$1.00^e \times 10^5$	N	i	l
W	P	M	7	$1.00^i \times 10^5$	N	i	l	N
W	P	M	8	$1.30^{gh} \times 10^5$	N	i	l	N
W	P	M	9	$1.21^{ghi} \times 10^5$	$1.00^e \times 10^5$	N	i	l
W	P	M	10	$2.32^e \times 10^5$	$4.08^c \times 10^5$	$1.10^b \times 10^5$		
W	P	M	11	$8.11^a \times 10^5$	$7.00^a \times 10^5$	$1.63^a \times 10^5$		
W	P	M	12	$2.02^f \times 10^5$	N	i	l	N
W	P	M	13	$6.03^c \times 10^5$	N	i	l	N
W	P	M	14	$2.32^e \times 10^5$	$4.08^c \times 10^5$	$1.10^b \times 10^5$		
W	P	M	15	$8.11^a \times 10^5$	$6.82^b \times 10^5$	$1.63^a \times 10^5$		
W	P	M	16	$4.25^d \times 10^5$	$1.00^e \times 10^5$	N	i	l

Values of are mean \pm standard deviation of three (3) replicates.

Values in the same column bearing different superscript differed significantly ($p=0.05$)

WFC – Whole Flour Cookies, WPM – Wheat, Plantain, *Moringa oleifera*

4.9 Optimization of process Variable.

The optimization plot for the process variables is shown on figure 3, optimization refers to improving the performance of a system, a process, or a product to obtain maximum benefit from it [34].

The process variables were set as follows, carbohydrate (maximum), Crude fiber (in range), iron (minimum), energy value (in range). The set up generated one solution for numerical optimization at maximum desirability of 0.956. from the optimization plot, cookies produced from whole wheat flour, unripe plantain flour (*R.markham*) and *Moringa oleifera* leaf powder with the blend ratios of 60: 30: 10 of the flour samples respectively was recommended.

Comment [aikh7]: you did not indicate in the methods how long the cookies were stored or what condition they were after exiting the oven before microbiological analysis. the microbiological loads obtained indicate something

Comment [aikh8]: how did you obtain optimal values of the variables you set up from the beginning

Component Coding: Actual

Overlay Plot

- Carbohydrate
- Crude fibre
- Iron
- Overall Acceptability
- Energy Value
- Design Points

X1 = A: Wheat Flour
X2 = B: Plantain Flour
X3 = C: Moringa Flour

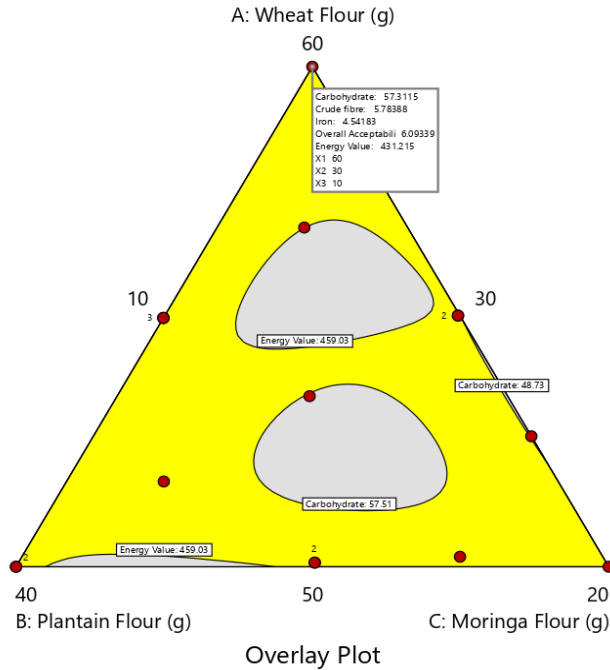


Fig 3. Optimization plot for cookies.

4. CONCLUSION

This study showed that cookies can be produced from whole wheat, unripe plantain flour with an addition of *Moringa oleifera* leaf powder up to 10 % which showed greater acceptability in the sensory evaluation. Cookies produced from composite flours of whole wheat, unripe plantain flour (*R.markham*) and *Moringa oleifera* leaf powder can compete favorably with cookies produced from wheat flour only as the latter has low mineral contents. Analytically, this combination had a significant effect on the proximate, mineral, vitamin, physical, microbiological, sensory properties, and the general acceptability of the cookies. Therefore, production of cookies from blends of whole wheat flour, unripe plantain flour and *Moringa oleifera* leaf powder should be encouraged as whole wheat, unripe plantain flour and *Moringa oleifera* leaf is readily available and affordable.

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Comment [aikh9]: numerical and graphical optimization were scantily treated, make it more explicit

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