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Functional and Pasting Properties of Composite Flours from *Triticum durum*, *Digitaria exilis*, *Vigna unguiculata* and *Moringa oleifera* Powder

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Authors' contributions

This work was carried out in collaboration between both authors. Author CAO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SUU managed the analyses of the study. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The objective of this study was to determine the functional and pasting properties of composite flours from *Triticum durum* (wheat), *Digitaria exilis* (acha), *Vigna unguiculata* (cowpea) flours and *Moringa oleifera* leaf powder. The flour samples were mixed in a four by four factorial, in complete randomized design (CRD) to formulate the composite blends at four different levels (25, 50, 75 and 100) which gave 16 samples. The statistical analysis of data collected was used to select five (5) generally accepted composite flour samples (wheat, acha, cowpea and *moringa oleifera* leaf powder flours) with ratio of 100:0:00, 75:25:00, 0:50:50:0, 50:23:25:2 and 75:25:00, respectively. The flour samples were analyzed for functional and pasting properties using standard methods. Results of the functional properties showed that water absorption capacity of the composite flour blend ranged from 0.87-1.11g/g, bulk density 0.39-0.42 g/ml, least gelation concentration 2.00-4.00%, solubility 19.46-25.35%, wettability 2.57-4.02min, oil absorption 1.61-1.79g/g and least

gelation temperature 62.00-68.50°C. The functionality of the composite flours such as water and oil absorption capacities, least gelation concentration and bulk density were improved when cowpea was incoporated into the blends than for *moringa oleifera* leaf powder and acha flour. On the other hand, wettability and solubility of the flour blends were improved when acha was incorporated into the blend. Results of pasting properties showed that peak viscosity ranged from 73.04-385.79RVU, trough viscosity 57.96-341.42RVU, break down viscosity 15.08-44.38RVU, final viscosity 109.54-581.58RVU, set back viscosity 51.58-240.17RVU, pasting time 5.70-6.40min and pasting temperature 50.08°C-50.35°C. These properties were shown to be higher when cowpea was incorporated into the flour blends than for *moringa oleifera* leaf powder and acha flour. However, pasting properties of the composite flour blends were higher than 100% wheat flour. This result therefore showed that composite flour from wheat, acha, cowpea and *Moringa oleifera* leaf powder has improved functionality and high pasting properties than the individual wheat flour and will serve as a useful ingredient in food formulations such as in dough, soups and baked products.

Keywords: Acha; cowpea; wheat; Moringa leaf powder; functional; pasting.

1. INTRODUCTION

The use of composite flour for the production of baked and pastry products are increasing in Nigeria. This is advantageous as it reduces the importation of wheat flour thereby promoting the use of locally grown crops in the country. Recently the use of composite flour in which flour from locally grown crops replaces a portion of wheat flour is common so as to meet the high demand for functional foods as well as decreasing demand for imported wheat and encouraging the production and use of locally grown non-wheat agricultural products [1]. Composite flour according to Julianti et al. [2] is a mixture of several flours obtained from legumes, tubers, cereals, roots and other ingredients with the intend to replace wheat flour totally or partially in bakery and pastry products. The use of composite flour has been reported to improve the functionality and physicochemical properties of the final product [3]. Several researches have also been reported on the functionality of composite flour from cereals, tubers and legume combination and it was deduced that the composite flour showed good functionality than the individual flour [4,5,6,7].

Cowpea (Viana unquiculata) is a tropical legume crop originating from Africa particularly in the semi-arid West Africa Savannah [8]. It is the second most important food grain legume constituting a cheap source of protein for humans [9]. It is reported by llesanmi and Gungula [9] to contain 19.58% protein and 59.25% carbohydrate. Cowpea be can processed and consumed into "moi-moi", a steamed paste, or "akara", a deep fat fried paste product or cooked plain and mixed with other foods.

Acha (*Digitaria exilis*) is an annual cereal crop indigenous to West Africa [10]. It is a cheap source of carbohydrate for man and considered as a healthy grain since they are consumed whole. According to Chukwu and Abdul-Kadir [11], acha can be recommended as a dietary supplement for diabetic patients due to its protein quality (methionine) and its high fibre content. They are reported to contain 7.9% protein, 1.8% fat, 71% carbohydrate and 6.8% fibre [12].

Moringa oleifera belongs to the family *Moringaceae*, native to India, Pakistan, Bangladesh and Afghanistan. It is a promising plant which could aid better intake of some essential nutrients and beneficial phytochemicals in the human diet [13]. The *moringa* plant is beneficial and almost all parts can be used as food. The leaves from *Moringa oleifera* plant is a power house of nutrients which can be consumed fresh, cooked or processed to powder [14].

For composite flours to be applied in the production of food systems, a foreknowledge of their performance is required [15] as Improper knowledge of these functionalities have resulted in products with varying consumer acceptability [15]. Functional properties are fundamental physicochemical properties which reflect how food ingredients behave during preparation and cooking. These properties such as bulk density, oil and water absorption, solubility and swelling power that influences the utilization of flours for product development. For efficient utilization and acceptance of cowpea, acha, moringa oleifera leaves and wheat flour composites, studies on its desirable functional properties are important as their application for the production of baked goods is primarily governed by these properties. The success of replacing wheat flour with cowpea, acha and Moringa oleifera leaves for product development could be better achieved if the composite flours are adequately characterized in terms of its pasting and This functional behaviour. study was therefore carried out in an effort to encourage the use of composite flour from locally grown crops such as cowpea, acha and Moringa oleifera leaves. The objective of this work was also to provide information on the functional and pasting properties of composite flours from wheat, cowpea, acha and Moringa oleifera leaf powder with a view of establishing the full industrial potential of these composite flours for utilization in bakery and pastry products.

2. MATERIALS AND METHODS

2.1 Sample Collection

Wheat (Triticum durum) flour was obtained from Dufil foods Choba in Obi-akpor I ocal Government Area of Rivers State. Digitaria exilis and Vigna unguiculata were purchased from town market in Port Harcourt Local Government Area of Rivers State while Moringa oleifera (matured and newly developed leaves) was harvested from a Moringa oleifera farm in Sakpenwa, Tai Local Government Area of Rivers State. Identification of the plant materials was done by a Plant Taxonomist in the Department of Botany and Ecological Studies, University of Uyo to ascertain that the right materials were used for the research. All chemicals used for this study were of analytical grade.

2.2 Preparation of Acha (*Digitaria exilis*) Flour

Acha flour was prepared according to the method of Adekunle and Abiodun [16]. Five kilograms (5 kg) of Digitaria exilis grains were manually cleaned and sorted by hand-picking of the chaff and foreign materials. Dust and sands were removed by washing severally with plastic bowls using clean tap water. The washed grains were sun dried for 6 hours in a sunny day, after which the dried grains were finely milled into flour with а Kenwood Manual Milling Machine (Model AT941A). sieved through a size 14 mm mesh sieve to remove coarse and fibrous materials. The flour was stored in Ziplock bags at room temperature (37°C) for further use.

2.3 Preparation of Cowpea (*Vigna Unguiculata*) Flour

This was produced according to the method of Aremu et al. [17]. Five kilograms (5 kg) of *Vigna unguiculata* was cleaned and sorted by hand, washed and manually dehulled. The dehulled seeds were oven dried at 60°C for 6 hrs and then milled into flour with a Kenwood Manual Milling Machine (Model AT941A). The flour was sieved using size 14 mm mesh sieve and stored in Ziplock bags at room temperature (37°C) for further use.

2.4 Preparation of *Moringa oleifera* Leaf Powder

Moringa oleifera leaf powder was prepared according to the method of Emelike et al. [18]. Moringa oleifera was manually selected by hand to get healthy leaves while diseased and damaged leaves were discarded. Three kilograms (3 kg) of selected leaves were washed in running tap water for 10 minutes to remove dirt and dust. The washed leaves weresoaked in 1% saline solution (NaCl) for 5 minutes and rinsed twice with 1.5 litres of distilled water. The excess water on the leaves was removed by spreading the leaves in a colander for a brief period till the water present on the leaf surface was drained. The leaves were then dried thoroughly using a clean muslin cloth in a shaded area until it was well dried. The dried leaves were milled to powder using a Kenwood Milling Machine (Model AT941A). The resulting powder was stored in air tight labelled plastic bottles at room temperature protected from light and humidity for further use.

2.5. Formulation of Composite Blends

Digitaria exilis flour, *Vigna* unguiculata flour, *Triticum* durum flour and *Moringa* oleifera leaf powder were mixed in four by four factorial in complete randomized design (CRD) to formulate the composite blends at four different levels (25, 50, 75 and 100) that gave 16 treatments . The statistical analysis of data collected was used to select five (5) generally accepted composite flour samples (wheat, acha, cowpea and *Moringa* oleifera leaf powder flours) with ratio of 100:0:0:0, 75:25:0:0, 0:50:50:0, 50:23:25:2 and 75:25:0:0, respectively (Table 1).

2.6 Functional Properties of the Flour Blends

Bulk density, wettability, least gelation temperature, least gelation concentration (LCG),

Treatments	WF (%)	AF (%)	CF (%)	MLP (%)
100% WF	100	-	-	-
75% WF, 25% CF	75	-	25	-
50% WF, 50% CF	50	-	50	-
50% AF, 50% CF	-	50	50	-
50% WF, 23% AF, 25% CF, 2% MLP	50	23	25	2
75% WF, 25% AF	75	25	-	-

 Table 1. Formulation of composite flour blends

Keys: WF= Wheat (Triticum durum) flour, CF= Cowpea flour (Vigna unguiculata) flour, AF= Acha (Digitaria exilis) flour, MLP= Moringa oleifera leaf powder

oil absorption and water absorption capacities (WAC) were determined using the method described by Onwuka [19]. Solubility and swelling power were determined using the methods of Adegunwa et al. [20].

2.7 Pasting Properties of the Flour Blends

This was determined using the rapid visco analyzer (RVA) Tecmaster, Perten instrumented) as described by Newport scientific (1998) as reported by Ohizua et al. [21]. The sample was turned into slurry by mixing 3g of the sample with 25ml of water inside the RVA can. The can was inserted into the tower which was then lowered into the system. The slurry was heated from 50°C within 14min-12mins parameters to estimate was peak trough, final breakdown and set back viscosities, pasting and time to reach peak viscosity.

2.8 Statistical Analysis

All data obtained from the functional and pasting properties were subjected to analysis of variance (ANOVA) using the Statistical Analysis System [22]. Means were compared using Least Significance Difference (LSD) with significant difference determined at 5% probability level (p < 0.05). Determinations were carried out in triplicates.

3. RESULTS AND DISCUSSION

3.1 Functional Properties of Composite Flours from Wheat, Cowpea, Acha and *Moringa oleifera* Leaf Powder

Table 2 shows the functional properties of composite flours from wheat, cowpea, acha and *Moringa oleifera* leaf powder. Water absorption capacity of the composite flours ranged from 0.80 g/g in sample E (75% WF: 25% AF) to 1.56 g/g in sample B (50% AF: 50%CF). There was an increase in the water absorption capacity of the flours with increase in the substitution of

cowpea flour and on the inclusion of Moringa oleifera leaf powder. There was also a significant difference (p<0.05) in the water absorption capacity of the flour blends. Composite flours with low water absorption produce thin meals which are also desirable for infant formulations [23]. Water absorption is required in food formulations especially those involving dough formation. It is determined by the ability of protein in flours to physically bind with water [24]. This explains why water absorption capacity increased with increase substitution of cowpea flour. Cowpea with a better protein quality tended to absorb more water than acha and wheat flour. Flours with high water absorption capacity have been reported to be good ingredients in bakery products as they improve handling characteristics [5].

Oil absorption capacity (OAC) of the flour blends ranged from 1.59g/g in sample e to 1.88g/g in sample B. OAC of sample B (75% WF: 25%CF) and sample D (50% WF: 23%AF: 25%CF and 2% MLP) were significantly (p<0.05) similar while the remaining samples (A, C and E) followed this same trend. OAC was significantly (p<0.05) higher as wheat flour was substituted with cowpea flour at ratio of 75:25% and on inclusion of Moringa oleifera leaf powder at 2%. Oil absorption capacity is an important property in food formulation because fats enhances mouth feel while retaining the flavour of foods [25]. The high OAC of the composite flour on substitution of wheat with cowpea flour could be due to the protein in cowpea which is lipohilic and also has strong affinity to hold fat globules [26]. Higher oil absorption of the composite flour is needed in food systems such as sausages production and meat analogues where optimum fat retentions are desired [27].

Bulk density of the flour blends ranged from 0.39 g/ml to 0.42 g/ml. Bulk density of the composite flours were significantly (p<0.05) similar. The

bulk density of the composite flours were found to be lower than the value of 0.54-0.72 g/ml for acha, defatted soybean and groundnut flour blends as reported by Eke-Ejiofor et al. [5]. It was also lower than 0.61-0.67g/ml for maize-soyapumpkin flour formulations reported by Asaam et al. [28]. Bulk density of flours is very important in determining packaging requirement and material handling. Low bulk density of the flour blends is desired as it contributes to lower dietary bulk, ease of packaging and transportation [29].

Swelling power of the composite flours ranged from 6.63g/g in sample D (50% WF: 23%AF: 25%CF: 2%MLP) to 7.31g/g in sample C (50% AF: 50% CF). Swelling power of sample C was significantly higher (p<0.05) than all other samples while samples D and E (75% wheat flour: 25% acha flour) were significantly (p<0.05) similar. Swelling power is related to protein and starch content as well as to the amyloseamylopectin ratio of the starch where low amylose content results to a high swelling power [24]. The inclusion of Moringa oleifera leaf powder and substitution of cowpea at low level produced low swelling powers of the flour blends. However, an equal proportion of acha and cowpea flours resulted to a higher swelling power more than the control wheat flour. The variations in swelling power observed could be due to differences in molecular organization within the starch granules [24].

Solubility of the flour blends ranged from 18.56% in sample D to 25.35% in sample F. Solubility of flour sample with 75% wheat and 25% acha was significantly (p<0.05) that all other samples. Flour samples without wheat flour inclusion and samples with moringa oleifera leaf powder had reduced solubilities. This may be due to the absence of wheat flour in the composites and low level of wheat flour inclusion. Solubility is the ability of solids to dissolve or disperse in an aqueous solution mostly in water [30]. Lower values of flour solubility indicates the existence of strong bonding forces within the flour granules while an increase in solubility values could be attribute to increase leaching of solubilized amylose molecules from swelled starch granules thereby promoting the destruction of the starches [28].

Wettability of the flour samples ranged from 0.52min/sec in sample C to 4.02min/sec in sample E. Wettability of the wheat flour substituted with 25% acha flour was significantly (p<0.05) higher than all other flour samples while those blended with cowpea and *moringa leaf*

flour were low. The high value of wettability could be due to the substitution with acha flour while the low value of wettability is attributed to the inclusion of *moringa leaf powder* and cowpea into the blends. Wettability is a function of ease of dispersing flour samples in water and the sample with low wettability values dissolves faster in water than those with high wettability values [31]. This indicates that acha flour blended with 50% cowpea flour and composite flour blends of wheat, moringa leaf powder, cowpea and acha will wet faster compared to sample E (75 %wheat flour: 25% acha flour).

Least gelation concentration of the flour blends rangeD from 2.00-4.00% with samples B and C showing significantly higher (p<0.05) values than samples A, D and E. This is due to the substitution of cowpea flour into the blends. Least gelation concentration of flour measures the least level of flour or blends needed for a gel to be formed in a given volume of water [26]. Flour blends with low least gelation concentration has been reported to be used in food systems where thickening and gel-forming agents are required [32].

Least gelation temperature of all the flours were in the range of 62.00-68.50°C. There is a significant variation in the least gelation temperature of the flour blends with sample C having the lowest while sample D had the highest value. Flour samples with cowpea substitution levels of 25 and 50% had significantly (p<0.05) low least gelation temperature. This could be due to the presence of protein in cowpea. Similar observations have been reported previously by Falade and Okafor [30] who stated that high gelation temperature could be due to the presence of other components such as proteins and lipids that would obstruct the swelling of granules and thus increase the amount of heat required to reach the final swelling. Least gelation temperature is the temperature at which starch molecules in a food substance lose their structure and leach out from the granules as swollen amylose. This in turn affects the time required for the cooking of food substances [33].

3.2 Pasting Properties of Composite Flours from Wheat, Cowpea, Acha and *Moringa oleifera* Leaf Powder

Pasting properties of composite flours from wheat, cowpea, acha and *Moringa oleifera* leaf powder is shown in Table 3. Peak viscosity of the flour samples were found to be in the range

73.04-385.79RVU with sample A (100% wheat flour) having the lowest PV and was significantly different (p<0.05) from all other flour samples. Formulation C (50%AF: 50% CF) had the highest PV value and was also significantly different (p<0.05) from the remaining formulations. Peak viscosity of the flour blends with cowpea flour substitution had significantly (p<0.05) higher peak viscosity than 100% wheat flour. The relatively high peak viscosity of the composite flours as compared to 100% wheat flour indicates that the flours will form a very thick paste hence, may be suitable for products requiring low gel strength and elasticity [34]. Sanni et al. [35] reported that peak viscosity is the ability of starches to swell freely before their physical breakdown and it indicates the strength of the pastes formed during gelatinization.

Trough viscosity also known as holding period is the point at which viscosity reaches its minimum during heating or cooling process [5]. Trough viscosity of the flour samples ranged from 57.96-341.42RVU with sample A showing the lowest and formulation C the highest. Trough viscosity of the composite fours were significantly (p<0.05)higher than 100% wheat flour. High trough viscosity of the composite flours may be attributed to substitution with cowpea flour. The high trough viscosity indicates that the composite flours can withstand high-heat treatments during processing than 100% wheat flour which had the lowest trough viscosity. These values were higher than values 39.60-59.19RVU reported for wheat and walnut flour blends [36].

Breakdown viscosity of the flours ranged from 9.33-44.38RVU with formulation D and C showing the lowest and highest values, respectively. There was a significant (p<0.05) difference in the break down viscosity of the flour samples. Similarly, break down viscosity of the composite flours was observed to be higher when cowpea flour was included into the blend. The low break down viscosity of sample D could be attributed to the inclusion of moringa oleifera leaf powder. The lower the break down viscosity of flours, the higher its stability under hot conditions [37]. The result from this study suggests that the flour blend with moringa leaf powder will be more stable and able to withstand heating as compared to other samples.

Final viscosity of the flours ranged from 109.54-581.58RVU. Final viscosity of formulation C (50% AF: 50% CF) was significantly higher (p<0.05) than all other flour samples while that of formulations B, D and E were significantly (p<0.05) similar. The high final viscosity of sample C could be attributed to cowpea substitution. Liang and King [38] stated that final viscosity of flours is important in determining their ability to form gel during processing. Thus, the thickening and gelling property of the flours will increase with substitution with increased levels of cowpea in the blends.

Set back viscosity indicates resistance to retrogradation [39]. Set back viscosities of the flour samples ranged from 51.58RVU in 100% wheat flour to 240.17RVU in formulation C. It was observed that set back viscosity of the composite flours were significantly higher (p<0.05) than 100% wheat flour owing to the substitution with cowpea flour. This indicates that 100% wheat flour had a higher possibility of retrogradation than those formulations with cowpea, acha and Moringa oleifera leaf powder. The high set back viscosity in the composite flours with 50% cowpea: 50% acha could be attributed to high fibre content fibre is a good water absorption as component that gives good stabilizing effects on foods [25].

Peak time is a measure of the cooking time [5]. The result showed that the pasting time of the flour blends ranged from 5.70-6.50min with 100% wheat flour showing the lowest while formulation D was highest. Peak time of the composite flours were significantly (p<0.05) similar and higher than that of 100% wheat flour. This is mainly attributed to the substitution with cowpea. Similar observation was also noted by Eke-Ejiofor et al. [5] who reported an increase in peak time of acha-defatted soybean and groundnut flour blends (5.33-6.71min) as substitution with groundnut flours defatted soybean and increased.

Pasting temperature gives an indication of the minimum temperature required to cook or gelatinize flour. The highest pasting temperature was recorded for formulation E (50.35°C) while the lowest was for formulation B (49.23°C). Pasting temperature of the flour samples were (p<0.05) significantly similar. Pasting temperature of the flours from this study was lower than 81.30-94.32°C for acha-defatted soybean and groundnut flour blends reported by Eke-Ejiofor et al. [5] and is below the boiling temperature of water. This indicates that they can form a paste in hot water below the boiling point.

Samples	Bulk density (g/ml)	Water Absorption	Oil Absorption (g/g)	Swelling Power	Solubility (%)	Wettability (min.sec.)	Least Gelatinization Concentration (%)	Least Gelatinization
		(g/g)		(g/g)				Temp. (^o C)
А	0.42 ^a	0.87 ^d	1.61 ^b	7.25 ^{ab}	19.46 ^b	2.57 ^c	2.00 ^b	68.25 ^{ab}
В	0.41 ^a	0.97 ^c	1.88 ^a	6.90 ^{bc}	21.40 ^{ab}	2.75 [°]	4.00 ^a	66.80 ^c
С	0.40 ^a	1.56 ^ª	1.58 ^b	7.31 ^ª	18.61 ^b	0.52 ^d	4.00 ^a	62.00 ^d
D	0.39 ^a	1.11 ^b	1.79 ^a	6.63 ^c	18.56 ^b	1.17 ^b	2.00 ^b	68.50 ^a
Е	0.39 ^a	0.80 ^e	1.59 ^b	6.83 ^c	25.35 ^ª	4.02 ^a	2.00 ^b	67.90 ^b
LSD	0.03	0.05	0.12	0.38	5.19	0.39	1.00	0.36

Table 2. Functional properties of composite flours from wheat, cowpea, acha and Moringa oleifera leaf powder

Means with the same letters along the same column are not significantly different (p>0.05).

KEYS: A= 100% WF, B= 75% WF: 25% CF, C=50% AF: 50% CF, D=50% WF: 23% AF: 25% CF: 2% MLP, É= 75% WF: 25% AF.

WF= Wheat (Triticum durum) flour, CF= Cowpea flour (Vigna unguiculata) flour, AF= Acha (Digitaria exilis) flour, MLP= Moringa oleifera leaf powder

Table 3. Pasting	a properties a	of composite flo	ur from wheat, cov	wpea, acha and <i>Mori</i>	nga oleifera leaf powder
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Treatments	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Set back viscosity (RVU)	Pasting time (MIN)	Pasting temp (°C)
Α	73.04 ^d	57.96 [°]	15.08 ^c	109.54 ^c	51.58 ^c	5.70 ^b	50.08 ^a
В	178.42 ^b	151.29 ^b	32.13 ^{ab}	236.54 ^b	85.25 ^b	6.27 ^a	49.23 ^a
С	385.79 ^a	341.42 ^ª	44.38 ^a	581.58 ^a	240.17 ^a	6.40 ^a	49.78 ^a
D	178.42 ^b	163.96 ^b	9.333 [°]	253.46 ^b	89.50 ^b	6.50 ^a	50.28 ^ª
E	111.87 [°]	136.00 ^b	20.88 ^{bc}	232.04 ^b	96.04 ^b	6.27 ^a	50.35 ^a
LSD(0.05)	37.74	42.93	14.29	72.49	30.17	0.28	1.06

Means with the same letters along the same column are not significantly different (p>0.05).

KEYS:

A= 100% Wheat flour; B= 75% Wheat flour: 25% Cowpea flour, C=50% Acha flour: 50% Cowpea flour, D=50% Wheat flour: 23% Acha flour: 25% Cowpea flour: 2% Moringa leaf powder, E= 75% Wheat flour: 25% Acha flour;

4. CONCLUSION

The result obtained from this study has shown that supplementation with cowpea flour resulted in considerable improvement in pasting properties. The result of the functional properties showed that flour blends substituted with high levels of cowpea had higher water absorption, oil absorption capacity, wettability and least gelation concentration. Acha and cowpea flour blends had higher water absorption capacity, swelling power and least gelation concentration while wheat and acha flour blends had higher solubility. The functionality of the composite flours (except for wettability, least gelation temperature and solubility) was more enhanced when cowpea was included into the blends than for Moringa oleifera leaf powder and acha flour. This study therefore encourages the use of composite flour from wheat, acha, cowpea and Moringa oleifera leaf powder as useful ingredient in food formulations such as in dough, soups, gravies and baked products.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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