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Carotenoid profile and functional properties of flour blends from biofortified maize and improved soybean varieties for product developments

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Abstract: Biofortified maize has received increased attention from a nutraceutical perspective because of its bioactive phytochemical components, including carotenoids. However, biofortified maize is limiting in some amino acids which are present in soybeans; hence both crops are used as blends in food products. Thus, this study aimed to evaluate the carotenoids and functional properties of maize-soybean flour blends as influenced by biofortified maize variety. Flour blends were prepared from each maize flour by substituting with 0–30% soybean flour. The flour samples were analysed for the carotenoid profile, proximate composition, colour, functional and pasting profile using standard methods. Carotenoids varied between the biofortified maize flours with xanthophylls (10.93–12.61 µg/g) being the most abundant, especially zeaxanthin (6.31–6.75 µg/g). Biofortified maize-soybean flour blends had lower carotenoid profiles with lower pro-vitamin A (3.79–6.99 µg/g) and xanthophylls (2.94–10.59 µg/g). The blends had higher protein, fat and ash contents with lower crude fibre and total carbohydrate than 100% biofortified maize flours. The blends also had lower bulk density, dispersibility, swelling power and pasting viscosities but increased solubility for both maize varieties. Maize flour from Sammaz 39 variety had higher pasting viscosities than Sammaz 40 variety. Trough, setback and final viscosities of maize-soybean flour blends made with S39 maize variety indicate pasting properties that will produce desirable properties in food products. The results showed that the blends could provide the raw material for the production of food products with improved carotenoid and protein contents as well as desirable functional qualities.

PUBLIC INTEREST STATEMENT

Recently, breeders have developed some nutrient-dense maize and soybean varieties that need to be evaluated for product development applications. An approach to avoid nutritional problems in populations consuming large amounts of maize products is protein enrichment of maize flour used in the preparation of such products. Composite flours have been reported to have improved functional properties and, consequently, the quality of food products. The carotenoid and functional characterization of flour blends made from these improved varieties is vital for appropriate product development applications. Therefore, the objective of this study was to determine the carotenoid profile and functional properties of composite flour produced from improved orange-maize and soybean varieties. The results showed that the blends could provide the raw material to produce food products with improved carotenoid and protein contents as well as desirable functional qualities. The biofortified maize-soybean flours may be used in the management of protein-energy malnutrition due to their improved protein contents relative to the 100% maize flours.

Subjects: Agriculture and Food; Food Additives & Ingredients; Food Chemistry

Keywords: Biofortified maize; soy flour; carotenoid profile; functional properties; pasting properties

1. Introduction

Carotenoids are natural pigments responsible for the red, orange, and yellow hues of plant parts (Mellado-Ortega & Hornero-Méndez, 2015). More than 600 carotenoid compounds have been characterized, with β-carotene being the most prominent (Olson & Krinsky, 1995). They are synthesized by only plants, bacteria, fungi, and algae, while animals acquire them from their diet (Mellado-Ortega & Hornero-Méndez, 2015). Most of the carotenoids in the diet and the human body is represented by β-carotene, α-carotene, lycopene, lutein, and cryptoxanthin (Rao & Rao, 2007). Increased consumption of a diet rich in carotenoids leads to diminished risk for several degenerative disorders, including various types of cancer, cardiovascular or ophthalmological diseases (Mayne, 1996), hence the increased interest in carotenoids. They are part of the antioxidant defense system in the human organism and are essential dietary sources of vitamin A (Paiva & Russel, 1999; Sies & Stahl, 1995).

Maize (*Zea Mays*) is an essential staple cereal crop that naturally accumulates carotenoids in the edible seed endosperm, which makes it an obvious target for biofortification projects. Yellow maize naturally contains the xanthophylls, lutein and zeaxanthin, as major carotenoids, with much lower levels of the provitamin A carotenoids β-carotene and β-cryptoxanthin (Rodriguez-Amaya et al., 2011). Maize has a great significance as human food, animal feed, and diversified uses in a large number of industrial products (Gupta, 2011). Biofortified maize has received increased attention from a nutraceutical perspective because it contains several bioactive photochemical such as carotenoids, tocopherols, phytic acid and phenolic compounds (Hu & Xu, 2011; Zilić et al., 2012).

Biofortification refers to micronutrient enrichment of staple crops through plant breeding to address the negative economic and health consequences of vitamin and mineral deficiencies in humans (Nestel et al., 2006). The goal of biofortification strategies is to increase the natural content of pro-vitamin A carotenoids through conventional breeding and transgenic approaches in staple crops. Biofortification has made significant progress towards increasing provitamin A carotenoid content in maize varieties and other crops (Ortiz et al., 2016). Golden Rice producing β—carotene (Paine et al., 2005) and Multivitamin Corn producing β -carotene, zeaxanthin, lutein, lycopene, ascorbic acid, and folate (Naqvi et al., 2009) are examples of biofortification of cereals. Such crops have the potential to improve human health directly in areas predominantly reliant on cereals for nutrition (Farré et al., 2011, 2015), they can also improve animal health when used as feed (Nogareda et al., 2016) and could pass those benefits on to humans who consume meat and other products from these animals. However, despite the success of maize biofortification to improve its carotenoid profile, maize is limiting in lysine and tryptophan but rich in methionine (Enwere, 1998). An approach to avoid nutritional problems in populations consuming large amounts of maize products is protein enrichment of maize flour used in the preparation of such products.

Soybeans have been used in various foods to mitigate the shortage of protein (Ene-Obong & Izuchukwu, 1991; Sanni & Akinlua, 1996). Soybeans are rich in lysine, which is deficient in cereals, while cereals have sufficient sulphur-containing amino acids, which are limiting in legumes. Hence, maize and soybeans complement one another in providing limiting amino-acids; therefore, both crops are used as blends or composites in food products.

Composite flours may be blends of wheat and other flours for the production of baked products, kinds of pasta, porridges, and snack foods; or non-wheat blends of flours or meals, for the same purpose (Chandra et al., 2015). Composite flours have been reported to have improved functional properties and, consequently, the quality of food products (Chandra et al., 2015).

Functional properties are the fundamental physico-chemical properties that reflect the complex interaction between the composition, structure, molecular conformation, and physico-chemical properties of food components together with the nature of the environment in which these are associated and measured (Kinsella & Melachouris, 1976).

Recently, breeders at the International Institute for Tropical Agriculture in Ibadan, Nigeria, developed some biofortified maize and improved soybean varieties, such as the improved orange maize varieties SAMMAZ 39 and SAMMAZ 40 and improved soybean TGx 1989–19 F. The carotenoid and functional characterization of flour blends made from these improved varieties is vital for appropriate product development applications. Therefore, the objective of this study was to determine the carotenoid profile and functional properties of composite flour produced from improved orange-maize and soybean varieties.

2. Materials and methods

2.1. Materials

Two biofortified orange-maize varieties (SAMMAZ 39 and SAMMAZ 40) and improved soybean variety (TGx 1989–19 F) were obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria.

2.2. Preparation of biofortified maize flour

Maize flour was produced as described by Okoruwa (Okoruwa, 1995). Orange-maize kernels were sorted to remove stones, dirt, and other foreign materials. Water was sprinkled on cleaned maize to allow absorption of water by the grains, toughening the pericarp and germ, so they do not splinter during milling. The grain was left for about 10 min before dehulling and milling. The flour was sieved (250 µm) and sealed in polyethylene for further use.

2.3. Preparation of soybean flour

Soybean flour was produced as described by Oluwamukomi and Oluwalana (Oluwamukomi & Oluwalana, 2005). Soybean grains (*Glycine max L Merrill*) were sorted, washed and boiled in water at 100 °C for 30 min. The grains were dehulled manually, oven-dried at 100 °C for 4 h, milled to obtain the flour, sieved (300 µm) and packaged until ready for further use.

2.4. Preparation of biofortified maize-soybean flour blends

Flour blends were prepared on a weight percent ratio by substituting maize flour with soybean flour at 0, 10, 20, 30 and 100% levels; the samples were labelled $M_{100}S_0$, $M_{90}S_{10}$, $M_{80}S_{20}$, $M_{70}S_{30}$, M_0S_{100} respectively.

3. Analysis of flours

3.1. Carotenoid profile

Flour sample (0.6 g) was weighed in a screw cap tube. Ethanol (6 ml) containing 0.1% butylhydroxytoluene (BHT) was added, vortexed for 1 min and allowed to stand for 5 min in a water bath at 85 °C. The tube was taken out of the water bath, and 0.5 ml of 80% KOH was added. The mixture was vortexed for 30 s, allowed to stand for 5 min in a water bath at 85 °C, vortexed for another 30 s and returned to the water bath (85 °C) for another 5 min. The tube was removed from the water bath and placed into ice, cold H_20 (3 ml) and hexane (3 ml) were added. The mixture was vortexed for 10 s and centrifuged for 10 s at 1000 rpm. The upper phase was pipetted into a 50 ml concentrator tube. Another 3 ml of hexane was added, vortexed, centrifuged and pipetted three times repeatedly. The extract was concentrated in the evaporator at 40 °C using N_2 gas for 25 min. Immediately before the injection, the sample was dissolved in 1 ml of 50:50 DCE: MeOH and vortexed for 10 sec. It was transferred into HPLC vials, introduced into the HPLC equipment, and ran for major carotenoids. The major carotenoids were identified from the chromatogram produced, and the value of each carotenoid was calculated (Kimura & Rodriguez-Amaya, 2004).

3.1.1. Calculation of carotenoids

$$Cs = \frac{AxXCxXtotalvolumeofextract(ml)}{AsXsampleweight(g)}$$

Where C_x = concentration of carotenoid X (µg/ml)

A_x = peak area of carotenoid X

C_s = concentration of the standard (µg/ml)

A_s = peak area of the standard

3.1.2. Estimation of provitamin A carotenoids

Total provitamin carotenoids (pVAC) was calculated as described in Awoyale et al. (2018) using the formula below:

Total pVAC = βC + (½)(9-cis-βC) + (½)(13-cis-βC) + (½)(βCX)

where βC = β-carotene, 9-cis- βC = 9-cis-β-carotene, 13-cis- βC = 13-cis-β-carotene, βCX = β-cryptoxanthin

3.2. Proximate composition

Moisture, protein, crude fat, crude fibre and ash contents were determined according to AOAC (2005). Total carbohydrate content was calculated by the difference between 100 and sum of moisture, protein, crude fat, crude fibre and ash contents.

3.3. Functional properties

3.3.1. Bulk density

This was determined by the method of Wang & Kinsella (1976). About 10 g of flour blends were weighed into a 50 ml graduated measuring cylinder. The flour blends were packed by gently tapping the cylinder on the benchtop. The volume of the flour blends was recorded.

$$Bulkdensity\left(\frac{g}{ml}\right) = \frac{Weightofflour(g)}{Volumeofflouraftertapping(ml)}$$

3.3.2. Water absorption capacity

The method used is as described by Beuchatt (Beuchat, 1977). One gram of flour sample was mixed with 10 ml of distilled water in a centrifuge tube and allowed to stand at room temperature (30 ± 2 °C) for 1 h. The mixture was centrifuged at 2000 rpm for 30 min, and the volume of water or the sediment-water was measured. Water absorption capacity was calculated as volume (ml) of water absorbed per gram of flour.

3.3.3. Swelling power and solubility index

The swelling power and solubility index was determined, as described by Oladele and Aina (Oladele & Aina, 2007). One gram of the flour was mixed with 10 ml of distilled water in a centrifuge tube and heated at 80 °C for 30 min while shaking continuously. The tube was removed from the bath, wiped dry, cooled to room temperature and centrifuged for 15 min at 2200 rpm. The supernatant was evaporated, and the dried residue weighed to determine the solubility using the formula:

$$Solubility(\%) = \frac{Weightofdriedsampleinsupernatant}{Weightoforiginalsample}x100$$

The swollen sample (paste) obtained from decanting the supernatant was also weighed to determine the swelling power. Swelling power was calculated using the formula:

$$Swellingpower = \frac{Weightofwetmasssediment}{Weightofdrymatterinpaste}$$

3.3.4. Dispersibility

This was determined by the method described by Kulkarni et al. (Kulkarni et al., 1991). Ten grams of flour sample was suspended in 100 ml measuring cylinder, and distilled water was added to reach a volume of 100 ml. The mixture was stirred vigorously and allowed to settle for 3 h. The volume of settled particles was recorded and subtracted from 100. The difference was taken as percentage dispersibility.

3.3.5. Colour properties

The colour of the flour was measured using a Konica Minolta CR-210 chromameter. The colorimeter was calibrated using a standard white plate. Samples were placed in the sample holder for measurement. Colour values were recorded as L*(lightness), a* (redness), and b* (yellowness).

3.3.6. Pasting properties

It was determined using the Rapid Visco Analyzer (RVA Series 4, Newport Scientific P.T.V., Warriewood, Australia). About 3.5 g of the flour was weighed to the nearest 0.01 g into a weighing vessel. About 2.5 ml of distilled water was dispensed into a test canister. The sample was transferred onto the water surface in the canister. A paddle was placed into the canister, and its blade was rigorously jogged through the sample up and down 10 times. Jogging was repeated to ensure that the sample remaining on the paddle was dissolved. The paddle and canister assembly were inserted firmly into the paddle coupling so that the paddle is appropriately centred. The measurement cycle was initiated by depressing after initiation and terminated automatically. From the recorded viscosity, the following parameters were read: peak viscosity, trough, breakdown, setback, final viscosity, peak time, and pasting temperature (IITA, 2001).

4. Statistical analysis

Data were analyzed with Statistical Package for Social Science (SPSS) v.18, using one-way analysis of variance (ANOVA) at $p < 0.05$. Where there is a significant difference, Duncan's multiple range test was used to separate the means.

5. Results and discussion

5.1. Carotenoid profile of biofortified maize-soybean flour blends

Tables 1 and 2 show the carotenoid profiles of maize-soybean flour blends. Xanthophylls (lutein and zeaxanthin) (Table 2) were the most abundant carotenoids in 100% maize flour (10.93–12.61 µg/g) and 100% soybean flour (11.23 µg/g). Sammaz 40 (S40) maize variety had higher xanthophylls (12.61 µg/g) than Sammaz 39 (S39) (10.93 µg/g). Flours from 100% maize and 100% soybean were richer in zeaxanthin (6.08–6.75 µg/g) than in lutein (4.62–5.85 µg/g).

Beta and Hwang (Beta & Hwang, 2017) reported a similar trend of relative abundance of xanthophylls in flour from traditional orange maize from Malawi; however, the flour contained more lutein than zeaxanthin. S39 maize flour had higher β-cryptoxanthin, α-carotene and 9-cis-β-carotene; however, S40 flour was higher in 13-cis-β-carotene, trans-β-carotene, total β-carotene and total provitamin A carotenoids (Table 1). The values for carotenoids in this study were higher than for yellow maize grain flour (Awoyale et al., 2018); the higher values suggest evidence of improved carotenoids content as a result of biofortification. On the other hand, lower carotenoid values in this study relative to orange maize flour (Beta & Hwang, 2017) may be due to varietal and environmental factors. Meanwhile, the variation in carotenoid profiles between S39 and S40 maize flours may be due to variation in the bio-engineering approach and procedures involved during the biofortification of this golden crop (Giuliano, 2017). Carotenoids play significant roles in human health by preventing several diseases (Fraser & Bramley, 2004). The primary function of dietary carotenoids is their provitamin A activity. Since it has been suggested that 21 µg instead of 12 µg of β-carotene have the same vitamin A activity as 1 µg (Fraser & Bramley, 2004), consumption of

Table 1. Provitamin A carotenoids (pVAC) profile (µg/g) of maize-soybean flour blends as influenced by bio-fortified maize variety

Flour blend	**β-cryptoxanthin**		**α-carotene**		**9-cis-β-carotene**		**13-cis-β-carotene**		**trans β-carotene**		**Total β-carotene**		**Total pVAC**	
	S39	S40	S39	S40	S39	S40	S39	S40	S39	S40	S39	S40	S39	S40
$M_{100}S_0$	3.01^{d}	2.32^{e}	0.82^{a}	0.61^{b}	1.12 ^{c}	0.46^{b}	0.71^{d}	2.15^{a}	1.11^{b}	3.63^{d}	2.94e	6.24d	5.36e	8.71e
$M_{90}S_{10}$	2.89 ^{c}	1.52^{a}	1.43 ^{c}	0.58^{b}	0.68^{b}	0.42^{ab}	0.63 ^{c}	1.82 ^{c}	0.82^{a}	2.87^{bc}	2.13b	5.11 c	4.23 c	6.99 c
$M_{80}S_{20}$	2.68^{b}	1.89 ^{c}	1.41 ^{c}	0.50^{a}	0.66^{b}	0.39^{ab}	0.51^{b}	1.78 ^{c}	1.43 ^{c}	2.98 ^{c}	2.63d	5.15 c	4.56d	7.18d
$M_{70}S_{30}$	2.03^{a}	1.75^{b}	1.06^{b}	0.56^{ab}	0.48^{a}	0.36^{a}	0.43^{a}	1.67^{b}	1.41 ^{c}	2.78^{b}	2.32 c	4.81b	3.79b	6.72b
M_0S_{100}	2.07^{a}	2.07^{d}	1.11^{b}	1.11 ^{c}	0.44^{a}	0.44^{ab}	0.44^{a}	0.44^{a}	1.06^{b}	1.06^{a}	1.94a	1.94a	3.42a	3.42a

M_x:S_y Maize:Soybean, S39: Sammaz 39 maize variety, S40: Sammaz 40 maize variety

Values are means of triplicate analysis

Mean values followed by different alphabets within a column are significantly ($p < 0.05$) different

Table 2. Xanthophylls profile (µg/g) of maize-soybean flour blends as influenced by bio-fortified maize variety

Flour blend	Lutein		Zeaxanthin		Total xanthophylls	
	S39	S40	S39	S40	S39	S40
$M_{100}S_0$	4.62^{b}	5.85^{e}	6.31^{d}	6.75^{e}	10.93^{b}	12.61^{e}
$M_{90}S_{10}$	4.68^{b}	1.51^{a}	5.89^{b}	1.43^{a}	10.58^{b}	2.94^{a}
$M_{80}S_{20}$	4.57^{b}	3.19^{c}	6.02^{c}	3.27^{c}	10.59^{b}	6.47^{c}
$M_{70}S_{30}$	2.47^{a}	2.61^{b}	4.44^{a}	2.60^{b}	6.91^{a}	5.22^{b}
M_0S_{100}	5.15^{c}	5.15^{d}	6.08^{c}	6.08^{d}	11.23^{b}	11.23^{d}

M_x:S_y Maize:Soybean, S39: Sammaz 39 maize variety, S40: Sammaz 40 maize variety
Values are means of triplicate analysis
Mean values followed by different alphabets within a column are significantly ($p < 0.05$) different

products made with biofortified maize flour in this study may contribute to controlling vitamin A deficiency as a food-based approach. Despite the high xanthophyll carotenoids in the soybean used in this study, generally, for both varieties of maize, substitution with soybean flour significantly ($p < 0.05$) reduced the carotenoid profiles of the maize-soybean flour blends.

5.2. Proximate composition of biofortified maize-soybean flour blends

The proximate composition of maize-soybean flour blends is presented in Table 3. The range of moisture content (MC) for 100% maize flour was 7.33% (S40) to 9.13% (S39), while 100% soybean

Table 3. Proximate composition of maize-soybean flour blends as influenced by bio-fortified maize variety

Flour blend	Moisture content (%)		Crude protein (%)		Crude fat (%)		Crude ash (%)		Crude fibre (%)		Total carbohydrate (%)	
	S 39	S40	S 39	S40	S 39	S40	S 39	S40	S 39	S40	S 39	S40
$M_{100}S_0$							9.13^{d}	7.33^{c}	5.62^{a}		3.21^{a}	
4.87^{a}							9.61^{a}	1.36^{a}	1.45^{a}		1.68^{e}	
1.18^{c}							77.34^{e}	77.22^{e}				
$M_{90}S_{10}$							8.75^{c}	4.35^{b}	18.12^{b}		10.00^{b}	
8.36^{b}							13.58^{c}	1.72^{b}	1.58^{b}		1.13^{d}	
1.13^{c}							61.92^{d}	69.25^{d}				
$M_{80}S_{20}$							8.10^{a}	4.01^{ab}	20.00^{c}		12.16^{c}	
10.00^{c}							14.05^{d}	1.99^{d}	1.62^{bc}		0.98^{c}	
1.09^{bc}							58.39^{c}	66.02^{c}				
$M_{70}S_{30}$							8.00^{a}	3.70^{a}	20.40^{d}		14.34^{d}	
10.55^{c}							14.15^{d}	2.00^{d}	1.65^{c}		0.85^{b}	
1.02^{b}							41.80^{a}	55.14^{b}				
M_0S_{100}							8.47^{b}	8.47^{d}	34.28^{e}		34.28^{e}	
12.18^{d}							12.18^{b}	1.92^{c}	1.92^{d}		0.45^{a}	
0.45^{a}							42.70^{b}	42.70^{a}				

M_x:S_y Maize:Soybean, S39: Sammaz 39 maize variety, S40: Sammaz 40 maize variety
Values are means of triplicate analysis
Mean values followed by different alphabets within a column are significantly ($p < 0.05$) different

flour had 8.47% moisture. There was a significant ($p < 0.05$) decrease in MC with an increase in substitution of maize flour with soybean flour. The lowest (3.70%) and highest (9.13%) MC were found in $M(S40)_{70}S_{30}$ and $M(S39)_{100}S_{0,}$ respectively. The MC of the flour samples were within the FAO recommended limit for the preservation of powdery foods. The values were also within the range reported by Akubor and Onimawo (Akubor & Onimawo, 2003). The low MC of foods will enhance their storage stability by avoiding mould growth and other unfavourable biochemical reactions (Singh et al., 2005).

Generally, there was a significant ($p < 0.05$) increase in protein, fat and ash contents while crude fibre and total carbohydrate decreased as the level of soybean substitution increased in the blends. Consequently, maize flour substituted with 30% soybean flour had the highest protein, fat and ash contents, while blends containing 10% soybean flour had the highest fibre and total carbohydrate. However, the protein content (PC) in S40 blends were higher than in the 100% soybean flour. Expectedly, the highest PC (34.28%) was found in 100% soybean flour, while the lowest was found in 100% maize flour [3.21% (S40)-5.62% (S39)]. The protein content of the blends indicated that incorporation of biofortified maize with soybean flour improved the protein content of the flour samples; therefore, it is expected that products made with these blends may be used in the management of protein-energy malnutrition which is prevalent in developing countries of the world (Akinola et al., 2015). Akubor and Onimawo (Akubor & Onimawo, 2003) also reported an increase in fat content as soybean flour was included. Although raw soybeans contain about 20% fat, the fatty acid composition is considered to be healthy (Wang et al., 2003).

The ash contents of the blends indicate an improvement in mineral contents and hence nutrient due to substitution with soybean flour. Edema et al. (Edema et al., 2005) reported a similar trend for maize-soybean flour blends. The crude fibre content of maize-soybean flour blends in this study was lower than reported by Edema et al. (Edema et al., 2005). The presence of fibre in food contributes bulk to food, facilitates bowel movement and prevention of many gastrointestinal diseases in humans (Satinder et al., 2011).

5.3. Functional properties of biofortified maize-soybean flour blends

Table 4 shows the functional properties of biofortified maize-soybean flours. For both varieties of biofortified maize, 100% maize flour had the highest bulk density (0.82–1.13 g/ml), dispersibility (31.62–32.04%), and swelling powers (12.69–23.39 °C) while 100% soybean flour had the lowest values. The highest solubility (3.16%) was observed in 100% soybean flour. The increase in substitution of biofortified maize flour with soybean flour significantly ($p < 0.05$) reduced the bulk density, dispersibility, and swelling power but increased the solubility for both maize varieties. The functionality of foods is the characteristics of food ingredients other than nutritional quality, which influences its utilization (Mahajan & Dua, 2002). The result in this study was similar to that of Akubor and Onimawo (Akubor & Onimawo, 2003), who recorded a decrease in bulk density as soybean flour was added. Bulk density is influenced by particle size and starch polymers structure (Plaami, 1997). Lower bulk density of maize-soybean blends relative to each of maize and soybean flour is desirable; this is expected to contribute to lower dietary bulk, ease of packaging and transportation (Aluge et al., 2016). Dispersibility measures how individual molecules of flours, disperse and homogenize within a medium of dispersion (Olapade et al., 2014). The reduced dispersibility of the blends suggests lower reconstitution in water (Oladele & Aina, 2007).

The trend in water absorption capacity (WAC) of the flours differed between the maize varieties; the values reduced with soybean substitution in S39 variety while the values increased for S40 variety. Water absorption capacity is the ability of a product to associate with water under a water limiting condition (Ajatta et al., 2016). Akubor and Onimawo (Akubor & Onimawo, 2003) observed an increase in WAC, while Edema et al. (Edema et al., 2005) reported a decrease in WAC with increased proportions of soybean flour. The variations in trend suggest that the influence of soybean flour on the WAC depended on the variety of maize used in each of the studies. The

Table 4. Functional properties of maize-soybean flour blends as influenced by bio-fortified maize variety

Flour blend	Bulk density (g/ml)		Dispersibility (%)		Water Absorption capacity (%)		Solubility (%)		Swelling power							
									70 °C		80 °C		90 °C		100 °C	
	S39	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**
$M_{100}S_0$	1.13^{b}	0.82^{b}	32.04^{b}	31.62^{e}	193.31^{a}	172.82^{b}	1.67^{a}	1.06^{a}	12.69^{e}	13.21^{e}	17.31^{d}	16.94^{e}	20.11^{d}	18.93^{e}	23.39^{e}	21.67^{e}
$M_{90}S_{10}$	0.90^{a}	0.81^{ab}	31.68^{ab}	24.09^{a}	188.45^{d}	184.45^{c}	1.69^{a}	1.18^{b}	11.21^{b}	12.17^{d}	14.68^{b}	15.72^{d}	16.21^{b}	18.22^{d}	20.48^{b}	20.87^{d}
$M_{80}S_{20}$	0.85^{a}	0.83^{b}	31.12^{a}	28.11^{b}	184.32^{c}	189.92^{d}	1.87^{b}	1.51^{c}	11.67^{c}	9.11^{c}	14.82^{c}	13.12^{c}	16.75^{c}	15.21^{c}	21.67^{c}	18.45^{c}
$M_{70}S_{30}$	0.80^{a}	0.84^{b}	31.00^{a}	29.36^{c}	183.72^{b}	191.73^{e}	1.90^{b}	1.68^{d}	11.89^{d}	8.93^{b}	14.90^{c}	10.45^{b}	16.80^{c}	13.45^{b}	22.00^{d}	17.67^{b}
M_0S_{100}	0.74^{a}	0.74^{a}	30.87^{a}	30.87^{d}	169.46^{a}	169.46^{a}	3.16^{c}	3.16^{e}	6.21^{a}	6.21^{a}	7.38^{a}	7.38^{a}	8.71^{a}	8.71^{a}	9.85^{a}	9.85^{a}

M_x:S_y Maize:Soybean, S39: Sammaz 39 maize variety, S40: Sammaz 40 maize variety

Values are means of triplicate analysis

Mean values followed by different alphabets within a column are significantly ($p < 0.05$) different

observed variations between the maize varieties in the present study may be due to different protein concentrations (Table 3), their degree of interaction with water and conformational characteristics (Butt & Batool, 2010). The relatively high WAC of all the flours (169.46–193.31%), particularly the blends (183.72–191.73%), suggests that they can be used in the formulation of dough-based and baked food products.

Generally, the solubility of the flours except for 100% soybean flour (3.16%) was low (1.06–1.90%). Water solubility measures the number of free molecules leached out from the starch granules on the addition of excess water and thus reflects the extent of starch degradation (Onwuka, 2005). Lower values of solubility imply the existence of strong bonding forces within the flour granules arising from coagulated protein or fat that form complexes with amylose preventing it from leaching the granules (Sung & Stone, 2003). Factors capable of influencing the solubility of flours include flour composition and particle size, density and pH, processing conditions, and storage conditions (Mirhosseini & Amid, 2013).

The swelling power of the maize and soybean flours (single and blends) increased with an increase in temperature for both maize varieties. However, there was a decrease in swelling power with an increased proportion of soybean flour. The results compared favourably with that of maize-soybean flour blends reported by Edema et al. (Edema et al., 2005). The swelling power of flour granules is an indication of the extent of associative forces within the granules (Moorthy & Ramanujam, 1986). The swelling power of flours depends on the size of particles, variety, and processing methods, or unit operations (Chandra et al., 2015). It also depends on the temperature, availability of water, species of starch, and other carbohydrates and proteins (Sui et al., 2006).

5.3.1. Colour properties

Maize flours have lower lightness (L*) (84.53–85.39) but higher yellowness (b*) (21.61–24.77) than soybean flour (L*:86.39, b*:14.72) (Table 5). Maize flours substituted with 10% soybean flour had the highest L* and b* values. There was a significant ($p < 0.05$) decrease in L* and b* with an increase in soybean flour of the blends. Hwang et al. (Hwang et al., 2016) reported lower L* (79.66–82.17) but higher b* (35.51–43.05) values for orange maize kernels from different locations in Malawi. In addition to differences in the varietal composition of orange maize used in both studies, the differences in colour values may be due to the differences in the orange maize form used. For instance, orange maize flour used in the present study has a lower moisture content (7.33–9.13%) (Table 3), compared to fresh maize kernels with higher moisture content (12.06–17.77%) studied by Hwang et al. (Hwang et al., 2016).

Table 5. Colour properties of maize-soybean flour blends as influenced by bio-fortified maize variety

Flour blend	**L***		**a***		**b***	
	S39	**S40**	**S39**	**S40**	**S39**	**S40**
$M_{100}S_0$	85.39^{b}	84.53^{b}	0.83^{d}	−0.56^{a}	24.77^{e}	21.61^{d}
$M_{90}S_{10}$	86.41^{c}	85.69^{c}	0.56^{c}	−0.51^{a}	22.26^{d}	20.90^{c}
$M_{80}S_{20}$	86.77^{d}	83.65^{b}	0.39^{b}	−0.24^{b}	20.73^{c}	17.95^{b}
$M_{70}S_{30}$	84.08^{a}	82.43^{a}	0.52^{c}	0.13^{c}	20.54^{b}	17.84^{b}
M_0S_{100}	86.39^{c}	86.39^{c}	0.23^{a}	0.23^{d}	14.72^{a}	14.72^{a}

M_x:S_y Maize:Soybean, S39: Sammaz 39 maize variety, S40: Sammaz 40 maize variety

Values are means of triplicate analysis

Mean values followed by different alphabets within a column are significantly ($p < 0.05$) different

Table 6. Pasting properties of maize-soybean flour blends as influenced by bio-fortified maize variety

Flour blend	Peak viscosity (RVU)		Trough viscosity (RVU)		Breakdown viscosity (RVU)		Final viscosity (RVU)		Setback viscosity (RVU)		Peak time (min)		Pasting temperature (°C)	
	S39	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**	**S39**	**S40**
$M_{100}S_0$	623.00^e	64.75^e	504.00^e	57.33^d	120.00^e	7.41 ^{c}	745.00^e	143.66^e	215.00^e	86.33^e	7.00^b	7.03^b	86.95^e	83.65^e
$M_{90}S_{10}$	284.00^d	49.91^d	258.00^d	44.08 ^{c}	108.00^d	5.83^b	540.00^d	103.73^d	148.00^d	59.66^d	7.00^b	7.00^b	83.55^d	82.30^d
$M_{80}S_{20}$	205.00 ^{c}	28.91 ^{c}	180.00 ^{c}	24.75^b	82.00 ^{c}	4.16^a	224.00 ^{c}	60.58 ^{c}	126.00 ^{c}	35.83 ^{c}	7.00^b	7.00^b	78.50 ^{c}	71.79^b
$M_{70}S_{30}$	183.00^b	27.58^b	126.00^b	23.58^b	38.00^b	4.00^a	195.00^b	54.50^b	96.00^b	30.91^b	7.00^b	7.00^b	75.63^b	78.02 ^{c}
M_0S_{100}	26.00^a	26.00^a	17.00^a	17.00^a	9.00^a	9.00^d	27.00^a	27.00^a	10.00^a	10.00^a	2.47^a	2.47^a	0.00^a	0.00^a

M_x:S_y Maize:Soybean, S39: Sammaz 39 maize variety, S40: Sammaz 40 maize variety

Values are means of triplicate analysis

Mean values followed by different alphabets within a column are significantly ($p < 0.05$) different

5.3.2. Pasting properties

Maize flour from S39 variety had higher pasting viscosities (120–745 RVU) than S40 variety (7.41–143 RVU) (Table 6). Maize flour from each biofortified variety had higher pasting viscosities than soybean flour (9.00–27.00 RVU). The lower pasting viscosities of soybean flour, a richer source of protein than maize, is expected because the presence of protein restricts starch swelling and pasting. The addition of soybean flour to maize flour significantly ($p < 0.05$) reduced the pasting viscosities of the flour blends.

Lower peak viscosities (PV) of maize-soybean flour blends indicate lower strength of pastes formed during gelatinization (Maziya-Dixon et al., 2004; Sanni et al., 2004). The difference in PV of the flours from the two maize varieties indicates that there were differences in the rate of water absorption and starch granule swelling during heating (Ragaee & Abdel-Aal, 2006). Higher trough viscosity (TV) of maize-soybean flour blends made with S39 maize variety suggests a higher ability of their paste to withstand breakdown during cooling (Tharise et al., 2014). S39 maize-soybean flour blends may be less resistant to heat and shearing during cooking due to their high breakdown viscosity values (Adebowale et al., 2005). High final viscosity (FV) of flours from S39 maize and its soybean blends indicates their ability to form a viscous paste upon cooling.

In contrast, lower FV of flours and soybean blends from S40 maize indicates the lower resistance of their paste to shear stress during stirring (Liu, 1997). Formulations made with S39 maize flour and its soybean blends have higher setback viscosities. Consequently, they may produce meals with less retrogradation; this is desirable because of the adverse effects of retrogradation on the sensory properties of food products (Asaam et al., 2018)).

The range of peak time and pasting temperature for maize flours was 7.00–7.03 min and 83.65–86.95 °C, respectively, while the values for soybean flour were 2.47 min and 0.00 °C respectively.

There was no significant ($p > 0.05$) difference in the peak time of maize flours and their soybean blends, while the peak time of soybean flour was significantly ($p < 0.05$) lower. Maize-soybean flour blends with significantly ($p < 0.05$) lower pasting temperatures may require lower energy costs and also ensure the stability of food components in their products (Kaur & Singh, 2005; Shamelis et al., 2006). The pasting properties of biofortified maize-soybean flour blends were generally higher than the values reported for maize-soybean flour blends by Edema et al. (Edema et al., 2005).

6. Conclusions

Carotenoid profiles varied between the flours from Sammaz 39 and Sammaz 40 biofortified maize. Xanthophylls were the most abundant carotenoids in 100% maize flour, with zeaxanthin more abundant than lutein. Despite the high xanthophyll carotenoids in the soybean, substitution with soybean flour significantly reduced the carotenoid profiles of the maize-soybean flour blends. Nevertheless, the consumption of products made with biofortified maize flours and their soybean blends in this study may contribute to controlling vitamin A deficiency as a food-based approach.

Biofortified maize-soybean blends had higher protein, fat and ash contents with lower crude fibre and total carbohydrate than 100% biofortified maize flours. The biofortified maize-soybean flours may be used in the management of protein-energy malnutrition due to their improved protein contents relative to the 100% maize flours.

Substitution of biofortified maize flour with soybean flour significantly reduced the bulk density, dispersibility, and swelling power but increased the solubility for both maize varieties. Maize flour from Sammaz 39 variety had higher pasting viscosities than Sammaz 40 variety. The pasting viscosities of biofortified maize-soybean flour blends were lower than for the maize flours. Trough, setback and final viscosities of maize-soybean flour blends made with S39 maize variety indicate pasting properties that will produce desirable properties in food products.

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Conflicts of interests
The authors declare no competing interests

Data availability statement
The data is available on request from the corresponding author.

Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Emmanuel Oladeji Alamu, Mojisola Olanike Adegunwa, Ganiyat Olatunde, Adebanjo Lukman, Awoyinfa Oluwashola, and Soyoye Joshua. The first draft of the manuscript was written by Ganiyat Olatunde, Emmanuel Oladeji Alamu and Mojisola Olanike Adegunwa, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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