

## Research Article

### Chemical, Lutein and Zeaxanthin Composition of Wheat-yellow Maize Bread for the Prevention of Atherosclerosis

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**Abstract:** The study examined the effect of substitution of wheat flour with yellow maize flour on the Lutein, Zeaxanthin and proximate composition as well as sensory properties of their bread samples. Standard procedures were used in the production of yellow maize flour. The blend ratios of wheat-yellow maize composite flour used in the production of the bread samples were 100:0 (control), 90:10, 80:20, 70:30, 60:40 and 50:50%, respectively. Results revealed that there were significant ( $p \leq 0.05$ ) increases in the lutein 92.4  $\mu\text{g}$  (100:0%) to 132.4  $\mu\text{g}$  (50:50%) and zeaxanthin 225.0  $\mu\text{g}$  (100:0%) to 580.3  $\mu\text{g}$  (50:50%) content of the bread samples. The proximate composition of the bread samples however, revealed that the carbohydrate, protein and ash content of the bread samples decreased with increase in yellow maize flour. The increase in yellow maize flour however, significantly ( $p \leq 0.05$ ) increased the fiber, fat and moisture content of the bread samples. Bread sample B (90:10) was generally the most accepted with an organoleptic score of 8.11, however, sample F (50:50) was the most preferred on the basis of color (7.78), flavor (7.78) and texture (7.58). It would be concluded that substitution of wheat flour with yellow maize flour could be employed to improve on the carotenoids composition of bread, increase their intake and confer protection on consumers against atherosclerosis. The low carbohydrate content of the bread samples could be employed to control spike in blood glucose levels, weight gain and in the management of diabetes. The high fibre content could be harnessed in the prevention and management of obesity and hyperlipidemia.

**Keywords:** Carotenoids, composite flour, disease burden, low density lipoprotein, phytochemicals

## INTRODUCTION

Bread is an important staple food in both developed and developing countries (Abdelghafar *et al.*, 2011). There is a growing interest in the use of composite flour for bread making owing to economic, social and health reasons (George Mateljan Foundation, 2013). The prevalence of chronic, Non-Communicable Diseases (NCDs) such as Cardiovascular Diseases (CVDs), diabetes, cancers, obesity and respiratory diseases has been reported in several literatures and is shown to be increasing at an alarming rate globally and are the leading causes of death worldwide (WHO, 2010). Atherosclerosis is a condition where the arteries become narrowed and hardened due to an excessive built up of plaque around the artery wall, disrupting the flow of blood around the body posing serious cardiovascular complications (Moore, 2012). Arteries contain a thin layer of cells called endothelium which keeps the arteries smooth and allow blood to flow easily. Atherosclerosis starts when the endothelium becomes damaged by, first being physically stressed by high blood pressure then chemically damaged by nicotine and circulating substances such as Low-

Density Lipoprotein cholesterol (LDL-c), homocysteine and free radicals, allowing LDL-c to accumulate in the artery wall forming bumps (NYU Langone Health Patient Connection, 2013). Early development of atherosclerosis is closely associated with lipoprotein metabolism via oxidative modification of LDL. The presence of oxidative modified LDL in the sub-endothelium of arteries affect monocyte differentiation to macrophages leading to the formation of foam cells and increased thickness of the artery wall (Karppi *et al.*, 2013). The immune system sends macrophage white blood cells to clean up the cholesterol pockets but when they are filled with the detrimental form of cholesterol, they get trapped, provoke an inflammation response and become a component of the plaque found in the artery walls (Moore, 2012). Over time this results in plaque being built up the artery disrupting the flow of blood around the body and potentially causing blood clots that can result in life threatening conditions such as heart attack, stroke and other cardiovascular diseases (Moore, 2012), which are the leading causes of death in men over age 35 years and all people over age 45 years (NYU Langone Health Patient Connection, 2013). A study demonstrated that in guinea pigs, dietary lutein

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was beneficial in preventing early atherosclerosis development by lowering circulating atherogenic lipoprotein and Ox LDL as well as decreasing cytokine production in aortas without changing plasma lipid profiles while aortas from control guinea pigs presented thickened intima plaque with the presence of foam cells, which was not present in the majority of guinea pigs from the lutein group (Jung *et al.*, 2011). Chronic non-communicative diseases like the cardiovascular diseases are found to be associated with the consumption of diets poor in phytochemicals, antioxidant, dietary fiber and certain micro-nutrients (Akah and Onweluzo, 2012). Phytochemicals are non-nutritive, bioactive plant chemicals that have protective or disease preventive properties, contributing to the health benefits of fruits, vegetables, legumes and grains. Though it is well known that plants produce these chemicals for their protection, recent research has demonstrated that they can protect humans against diseases (Ibrahim *et al.*, 2012). Phytochemicals, especially carotenoids, chlorogenics, phyto-estrogens and phytosterols, have been found to promote healthy hearts, protect arteries and the body against high cholesterol deposition (Rao and Rao, 2007). Research has shown that lutein and zeaxanthin can inhibit thickening of the walls of carotid arteries and LDL-induced migration of monocytes to human artery cell walls, they inhibit lipid peroxidation, prevent cellular damage by quenching singlet oxygen or neutralizing photosensitizers (Alternative Medicine Review, 2005). Lutein provides a significant degree of protection against the formation of arterial plaque and atherosclerosis; it exhibits health-promoting, anti-inflammatory and anti-oxidant benefits that help to lower plaque forming oxidized LDL-c particles, which promote arterial hardening and heart disease (Johy, 2011). Lutein and zeaxanthin are the major carotenoids in corn milled fractions and account for about 70% of the total carotenoids (Kean *et al.*, 2008). Among the five corn varieties yellow corn has the highest (406 µg/100 g) while white corn has the lowest (5.7 µg/100 g) carotenoids content (DeOliveira and Redriguez-Amaye, 2007). Maize is of the kingdom *Plantae*, Order *Poales*, Family *Poaceae*, Genus *Zea* and Specie *Mays* has a rich reservoir of carotenoids, saponins and monoterpenes of which wheat is deficient in (Awika and Rooney, 2004) but lacks the protein gluten of wheat which makes baked goods have good rising capability. Research over the past decade has focused on the development of carotenoid-rich-food to boost their intake (El-Sayed *et al.*, 2013). This study determined the chemical, lutein and zeaxanthin composition of wheat-yellow maize bread samples and their potential in the prevention of atherosclerosis.

## MATERIALS AND METHODS

**Sample collection and preparation:** Wheat flour, yellow maize (*Zea mays*) grains, granulated white sugar, refined salt, instant dry yeast, blue band

margarine and hen eggs were purchased from commercial stores at Wurukum market, Makurdi, Benue State, Nigeria. The yellow maize grains were sorted, washed with clean tap water, sun dried, milled, sieved into fine flour using 150 µm sieve and stored in polyethylene bags at ambient temperature (37±2°C) pending further use.

**Blend formulation:** The wheat and yellow maize flours were blended according to Etudaiye and Aniedu (2008) method into five samples on weight: weight or percentage: percentage basis of: A (500:0 g) (100:0%), i.e., (control), B (450:50 g) (90:10%), C (400:100 g) (80:20%), D (350:150 g) (70:30%), E (300:200 g) (60:40%), F (250:250 g) (50:50%). The quantities of the other ingredients used in baking each of the bread samples were 50 g each of sugar and margarine, 15 g yeast, 75 mL warm water, 2 eggs and salt to taste.

**Bread production:** The bread samples were prepared according to the method of Eduardo *et al.* (2013). The 15 g yeast was dissolved in 45 mL of the warm water. All the dry ingredients were mixed together (i.e., flour, sugar and salt) thereafter the margarine was rubbed in. The dissolved yeast, remaining warm water and eggs were then added and thoroughly mixed into dough. The dough was covered and left to ferment at room temperature for 45 min. After the first fermentation, the dough was gently kneaded, cut into 250 g weight, rolled into round shapes and placed into bread pans to proof for another 45 min at ambient temperature and baked for 45 min in an oven heated to 190°C. The bread loafs were cooled at ambient temperature after baking and used for proximate, Lutein and zeaxanthin composition determinations as well as sensory evaluation.

**Proximate composition determination:** The crude Protein (kjeidahl method), fat (solvent extraction), ash, moisture and crude fiber content were determined according to AOAC (1990) method. Total carbohydrate was by difference (100% - % moisture + % crude protein + % ash + % crude fiber + % fat). The energy value of the bread was calculated using Atwater factor (4×protein, 9×fat and 4×carbohydrate).

**Phytochemical analysis:** One hundred grams of the pulverized bread samples were weighed and placed in 500 mL conical flasks, into which 200 mL of 90% ethanol was added and thoroughly shaken and allowed to stand for 72 h, with intermittent shaking at interval. At the end of 72 h the extracts were filtered using Whiteman number 1 filter paper (Size 24). The extracts were thereafter vacuo concentrated using water bath at 40°C. The concentrated extract was used in the determination of carotenoids (lutein and zeaxanthin) according to the method of Harbourne (1983).

**Organoleptic evaluation:** Fifteen semi-trained properties of the bread samples based on color, flavor, texture and general acceptability. A 7-point hedonic scale (7 = like extremely and 1 = dislike extremely) was employed to elicit responses from the panelists (Iwe, 2002). Water was provided to clean and rinse the mouth after testing each coded sample to avoid carry over effect.

**Data analysis:** Data entry was performed using Microsoft EXCEL 2007 while Analysis of Variance (ANOVA) was employed in the separation and comparison of means at 5% degree of freedom. Steel and Torrie (1980) method was used to establish the Least Significant Difference (LSD) between the means at  $p<0.05$ .

## RESULTS AND DISCUSSION

Table 1 shows the effect of substitution of yellow maize flour on the proximate composition of the bread samples. Substitution of wheat flour with 10% yellow maize flour significantly ( $p\leq 0.05$ ) increased the moisture, fat and fiber content of the bread samples. The moisture content of any food is an index of its water activity (Frazier and Westhoff, 1978), which is used as a measure of stability and susceptibility to microbial contamination (Scott, 1980). The increase in moisture content of the bread samples could be attributed to the increase in their fibre content, as dietary fiber bind water molecules and promote retention of water but prevent evaporation during baking (Nasar-Abbas and Jayasera, 2012). Similar increment in moisture content has been reported for breadnuts flour (Malomo *et al.*, 2011) and rice bran flour (Marerat *et al.*, 2011). The increase in the fat content of the bread samples could be attributed to the higher fat content of maize grain (4.74/100 g) as against 1.54/100 g fat content of wheat gain (Wan Rosli and Che Anis, 2012). This may also be as a result of the increase in the fiber content of the bread samples, as

panelists were used in evaluating the organoleptic fiber had been reported to bind with fat. The fiber from fruits, vegetables and grains had been reported to serve important functions in lowering blood cholesterol concentrations, slowing glucose absorption, weight control and reducing the risk of colon cancer (Garrow and James, 1998; Whitney *et al.*, 1996). The substitution however, significantly ( $p\leq 0.05$ ) decreased the carbohydrate, protein and ash content of the bread samples. The findings of this study were in conformity with those of Lim and Wan Rosh (2013) and Sanful (2011) who had earlier reported decreases in the carbohydrate and protein content of bread samples produced from composite flours of wheat-young maize and taro flours. The decrease in protein content of the bread samples were expected due to the lower protein content of maize grain (9.4/100 g) when compared to that of wheat grain (12.6/100 g) (Wan Rosli and Che Anis, 2012). The low carbohydrate content of the bread samples can be used in carbohydrate restricted diets for the regulation of blood glucose spikes and would go a long way in creating food variety for diabetics and help them maintain healthy blood sugar levels.

The effect of substitution of wheat flour with 10% yellow maize flour on the lutein and zeaxanthin content of the bread samples are presented in Table 2. The result showed that the lutein and zeaxanthin content of the bread samples produced from the composite flours increased with increase in the level of substitution and were significantly ( $p\leq 0.05$ ) higher than that of the control. However, no significant increase in the lutein content was observed among the bread samples baked using the composite flours. Lutein and Zeaxanthin are the major carotenoids in corn milled fractions and account for about 70% of the total carotenoids making yellow corn a promising blending flour ingredient in the development of high-lutein functional foods (Kean *et al.*, 2008). The intake of 100 g of any of the bread samples produced from wheat-yellow maize blends would provide an individual with the values shown in Table 2. This would go a long way in conferring

Table 1: Effect of yellow maize addition on the proximate composition of the bread samples

Parameters (%)	Sample A (100:0)	Sample B (90:10)	Sample C (80:20)	Sample D (70:30)	Sample E (60:40)	Sample F (50:50)
Carbohydrate	65.03 <sup>a</sup> ±0.003	64.27 <sup>a</sup> ±0.008	63.20 <sup>a</sup> ±0.008	61.91 <sup>c</sup> ±0.003	60.39 <sup>cd</sup> ±0.008	59.71 <sup>d</sup> ±0.010
Crude protein	13.93 <sup>a</sup> ±0.002	12.59 <sup>b</sup> ±0.002	12.43 <sup>b</sup> ±0.003	12.36 <sup>b</sup> ±0.003	12.01 <sup>c</sup> ±0.002	11.98 <sup>cd</sup> ±0.004
Crude fiber	1.24 <sup>a</sup> ±0.002	1.36 <sup>a</sup> ±0.002	1.37 <sup>a</sup> ±0.002	1.36 <sup>a</sup> ±0.004	1.37 <sup>a</sup> ±0.002	1.37 <sup>a</sup> ±0.002
Fat	5.03 <sup>bc</sup> ±0.001	5.43 <sup>b</sup> ±0.002	5.76 <sup>a</sup> ±0.002	6.07 <sup>a</sup> ±0.002	6.39 <sup>a</sup> ±0.002	6.66 <sup>a</sup> ±0.002
Ash	2.38 <sup>a</sup> ±0.008	2.23 <sup>a</sup> ±0.002	2.38 <sup>a</sup> ±0.001	2.26 <sup>a</sup> ±0.002	2.22 <sup>a</sup> ±0.003	2.17 <sup>a</sup> ±0.001
Moisture	13.15 <sup>a</sup> ±0.002	14.32 <sup>cd</sup> ±0.002	14.89 <sup>c</sup> ±0.003	17.95 <sup>b</sup> ±0.002	18.31 <sup>a</sup> ±0.003	18.73 <sup>a</sup> ±0.001

Mean±S.D. of 3 replications; Means in a row with the same superscript are significantly not different ( $p>0.05$ )

Table 2: Effect of addition of yellow maize flour on the lutein and zeaxanthin content of the bread samples ( $\mu\text{g}/100 \text{ g}$ )

Samples	Lutein	Zeaxanthin	Lutein plus zeaxanthin
A (100%)	92.4 <sup>b</sup> ±0.003	225.0 <sup>a</sup> ±0.020	317.4 <sup>a</sup> ±0.001
B (90:10)	131.2 <sup>a</sup> ±0.001	529.9 <sup>c</sup> ±0.004	661.2 <sup>a</sup> ±0.002
C (80:20)	131.3 <sup>a</sup> ±0.004	561.0 <sup>bd</sup> ±0.002	692.3 <sup>bd</sup> ±0.003
D (70:30)	131.4 <sup>a</sup> ±0.002	567.0 <sup>bc</sup> ±0.002	698.4 <sup>bc</sup> ±0.001
E (60:40)	132.3 <sup>a</sup> ±0.002	570.3 <sup>a</sup> ±0.002	702.6 <sup>b</sup> ±0.003
F (50:50)	132.4 <sup>a</sup> ±0.002	580.6 <sup>a</sup> ±0.010	713.0 <sup>a</sup> ±0.002

Mean±S.D.; Values with the same superscript in a column are significantly not different ( $p\leq 0.05$ )

Table 3: Mean sensory scores of the bread samples

Attributes	Sample A (100%)	Sample B (90:10)	Sample C (80:20)	Sample D (70:30)	Sample E (60:40)	Sample F (50:50)
Color	7.44 <sup>a</sup> ±0.05	7.56 <sup>a</sup> ±0.07	6.78 <sup>c</sup> ±0.13	7.78 <sup>a</sup> ±0.20	7.22 <sup>b</sup> ±0.02	7.78 <sup>a</sup> ±0.12
Flavor	6.11 <sup>cd</sup> ±0.11	7.56 <sup>a</sup> ±0.07	7.56 <sup>a</sup> ±0.06	6.56 <sup>bc</sup> ±0.06	7.00 <sup>b</sup> ±0.13	7.78 <sup>a</sup> ±0.20
Texture	6.89 <sup>bc</sup> ±0.02	7.00 <sup>b</sup> ±0.07	7.33 <sup>a</sup> ±0.06	6.78 <sup>bc</sup> ±0.15	7.00 <sup>b</sup> ±0.20	7.56 <sup>a</sup> ±0.14
General acceptability	7.44 <sup>bc</sup> ±0.01	8.11 <sup>a</sup> ±0.12	7.67 <sup>b</sup> ±0.11	7.22 <sup>d</sup> ±0.02	7.11 <sup>e</sup> ±0.06	7.44 <sup>bc</sup> ±0.06

Values are Mean±S.D.; Values with the same superscript in a row are significantly not different (p>0.05)

protection against atherosclerosis. A Los Angeles Atherosclerosis study that investigated the progression of intima-media thickness of common carotid artery revealed that subjects with the highest serum lutein level (0.42 µmol/L) had 80% less arterial wall thickening relative to those at the lowest quintile of serum lutein (0.15 µmol/L). Also animal studies had revealed that lutein supplementation reduced the size of atherosclerotic lesion in susceptible mice by 45% and inhibited LDL oxidation in a dose-dependent manner up to almost 80% (Alves-Rodrigues, 2002).

Table 3 contains the result of the effect of substitution of wheat flour with yellow maize flour on the organoleptic properties of the bread samples. The result revealed that samples D (70:30) and F (50:50) were rated higher by the panelist on the basis of color appeal (7.78). The flavor (7.78) and texture (7.56) preference of the panelist revealed that sample F (50:50) was the most preferred. Sample B (90:10) was however, the most generally accepted.

## CONCLUSION

Yellow maize flour contain appreciable quantities of lutein and zeaxanthin that could be harnessed in the production of healthy baked products that could prevent and control the onset of atherosclerosis and other cardiovascular diseases. The high fiber content of the bread samples could be employed in the prevention and management of obesity and diabetes mellitus diseases among population groups.

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