Impact of packaging material and storage condition on retention of provitamin A carotenoids and xanthophylls in yellow-seeded maize flour

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ABSTRACT

Background: Pro-vitamin A carotenoid (pVAC) rich foods are those foods that contain substance which can be converted within the human body into retinol, and which contribute to the reduction of vitamin A deficiency diseases. Yellow-seeded maize flour is an example of such pVAC rich food. Identifying the right packaging materials and storage conditions that retain pVAC in this food is essential for their health benefits. Traditionally, maize flour is stored in different packaging materials to increase its shelf life. For instance, previous studies have shown that during storage in different food matrices, including maize grains, carotenoids are highly susceptible to degradation by temperature, light, and oxygen. The aim of this study is to investigate the effect of storage packaging materials (polypropylene woven sacks-PWS, high-density polyethylene bags-HDPE, and polyvinyl plastic containers-PPC) and storage condition (temperature and relative humidity) on retaining pVAC in yellow-seed maize flour.

Methods: The yellow-seeded maize grains were collected and ground into flour. The maize flour was divided into portions (200 g) and each portions was packed and sealed in PWS, HDPE, and PPC. The control samples (12 pieces) were stored on top of the storage box. The packed samples were stored in both the upper (12 samples) and lower (12 samples) compartment of a storage wooden box. The interior of the upper part was lightened with aluminum foil and fitted with fluorescent tube to increase the light intensity, while the lower compartment was darkened with gloss black painting. The flour samples were stored for 28 days, with samples collected for pVAC and xanthophylls analyses at 7 days interval using standard methods.

Results: The results showed that packaging in PPC and storing in dark compartment gave the highest total pVAC (92.39%) and total xanthophylls (89.44%) retention, and retinol equivalent (RE) (0.40 μg/g); whereas packaging in HDPE and storing in lighted compartment resulted in the lowest pVAC (44.92%) and total xanthophylls (46.76%) retention, and RE (0.19 μg/g).

Conclusions: Packaging yellow-seeded maize flour in PPC and storing in the dark may be recommended for maximum retention of carotenoids in yellow-seeded maize flour since this packaging material and storage condition gave the highest pVAC retention and retinol equivalent.

Keywords: Yellow-seeded maize, Carotenoids, Packaging materials, Storage conditions, Retinol equivalent

BACKGROUND

Carotenoids, the lipid-soluble plants secondary metabolites with 30 and 40 carbon atoms, are important in human nutrition and health due to the various benefits they provide. They are prominent for their antioxidative properties, protecting the cells against free radicals and reactive oxygen species [1], and the oxidative damage they cause to cellular biomolecules such as deoxyribonucleic acid, proteins and lipids [2]. In addition to this, carotenoids possess several other health benefits including provitamin A and immunostimulatory [3], anti-hepatocellular cancer [4], anti-inflammatory and antiapoptotic activities [5], and prevention of cataracts and age-related macular degeneration [6]. Carotenoids also contribute to both organoleptic and nutritional properties of food and food by-products providing red, yellow or orange colors for fruits and vegetables. They are used as food colorants in foods and animal products such as egg yolk, butter, crustaceans, trout, salmon, and shrimp [7]. Through their structure, carotenoids are classified into two main groups: (i) carotenes also called carotenoid hydrocarbons, which only contain carbon and hydrogen; and (ii) xanthophylls or oxygenated carotenoids that may contain different functional groups (epoxy, methoxy, hydroxy, carbonyl and carboxyl acid groups) [8]. However, humans, as well as other animals, are unable to synthesize carotenoids. They, therefore, depend on consumption of plant dietary products to meet their carotenoids demands for the various physiological and health benefits they provide [9].

Among the various cereal dietary sources of carotenoids, maize (Zea mays L.), especially the yellow-seeded type, is notable as the cereal that accumulates the highest level of carotenoids, having as high as 80 μg total carotenoids per gram dry weight [10]. The yellow-seeded maize contains both provitamin A carotenoids (pVAC - β-carotene, α-carotene, and β-cryptoxanthin) and xanthophylls (lutein and zeaxanthin) without provitamin A activity [11]. However, the provitamin A content of maize varies naturally, with yellow maize varieties having 0.5 to 1.5 μg/g provitamin A [12], which is insufficient to prevent vitamin A deficiency (VAD) in areas where diets are dominated by maize and maize products. For this reason, breeding programmes have targeted to increase the level of pVAC in yellow maize varieties from below 2 μg to 15 μg/g pVAC/g in orange grain varieties through biofortification [10]. However, processing of the maize grain into floury products may reduce the quantity of the pro-vitamin A carotenoid (pVAC).

Traditionally, maize flour is stored in different packaging materials to increase its shelf life. The research carried out by De Moura et al [13] and Rodriguez-Amaya [14] revealed that carotenoids in stored maize grain foods are highly susceptible to degradation by temperature, light, and oxygen during storage. Bechoff et al. [15] and Alves et al. [16] further demonstrated that oxygen contributed more to the degradation of carotenoids than the temperature in food matrixes having high exposure to oxygen. Other studies have also indicated that depending on the genotype when the grains of maize are stored for a period of six months using traditional methods, the degradation of pVAC in the grains may reach 60% [13, 17]. Also, previous studies reported 78–100% pVAC retention when cooking freshly ground biofortified orange maize meal to prepare Nshima [18, 19]. Stability of carotenoids during storage of biofortified maize has been studied by Mugode et al. [19]. These researchers reported that most of the carotenoid degradation occurred in the first weeks of storage and the degradation rate then lowered. This makes it imperative to identify storage conditions and packaging materials that can maximally retain both pVAC and xanthophylls in flour of yellow-seeded maize during storage.

This study was therefore designed to investigate the impact of different packaging materials and storage conditions on the retention of pVAC and xanthophylls in yellow-seeded maize flour.

MATERIALS AND METHODS

Materials

Packaging materials: The various packaging materials, including polypropylene woven sacks (PWS), high-density polyethylene bags (HDPE), and polyvinyl plastic containers (PPC), were produced by Afriplast Industries Ltd, Oke-Bola, Ibadan, Nigeria. The properties of the packaging materials as specified by the manufacturer (Afriplast Industries Ltd, Sw7/8, Obafemi Awolowo Way, Oke-Bola, Ibadan, Nigeria) are shown in Table 1 [20].

Table 1. Properties of the packaging materials

Packaging material	Thickness (μm)	Oxygen permeability at 25 °C/24 h (mm/100 cm^2)	Water vapor permeability at 37.8 °C/24 h (mm/100 cm^2)	Relative humidity (%)
PWS	0.75	160.00	0.27	90.00
HDPE	1.30	500.00	1.40	90.00
PPC	0.45	80.00	8.00	90.00

PWS-polypropylene woven sacks, HDPE-high-density polyethylene bags, PPC-polyvinyl plastic containers

Chemicals and reagents: Chemicals, reagents, and solvents used for the carotenoids profiling were all HPLC grade. Carotenoids standards (9-cis-β-carotene, 13-cis-β-carotene, all-trans-β-carotene, β-cryptoxanthin, lutein, and zeaxanthin) were bought from CaroteNature, GmbH (Lupsingen, Switzerland). All other chemicals, reagents, and solvents were of analytical grade.

Sample collection and preparation: A sample of yellow maize (DMR-LSRY) grains were collected from the Maize Improvement Programme of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The grains were sorted and subsequently ground into flour using a laboratory hammer grinding machine fitted with 0.5 mm mesh sieve. The flour was immediately used for the storage study.

Storage study: The storage study was conducted in a cubic wooden box of 2.6 ft length, width, and height, having upper and lower compartments. The interior part of the upper compartment was lined with aluminum foil and fitted with a T8 LongLast™ fluorescent tube (2 ft, 18 W, & 3000 K warm white), to increase light intensity. Gloss black paint was used to paint the lower compartment to maintain a dark enclosure for the samples. Each compartment had a separate door, for the ease of sample collection. The packaging materials, specifically the HDPE and PWS bags were prepared to be used for packaging by cutting them into required sizes using a scissor and shaped using electric sealing and stitching machines respectively. The sizes of the packaging materials are PWS (25 cm height × 13 cm breadth) and HDPE (23 cm height × 16 cm breadth). The PPC size was measured to be 6 cm height ×13 cm breadth. A portion (200 g) of the flour was properly weighed using a weighing scale, packed and sealed in each of the three different packaging materials as follows: PWS sealed with a stitching machine; HDPE sealed with an electric sealer; and PPC covered hermetically with a lid [21]. The packaged flour samples replicated four times were stored in both the upper (lighted) and lower (dark) compartments of the storage box. The control flour samples also replicated four times were stored on top of the storage box. The flour samples (36 pieces) were stored for 28 days, during which samples were collected from each package at 7 days interval for carotenoids profiling. The ambient temperature and relative humidity of each storage condition were measured with thermo-hygrometer (Max-Min) prior to each batch of sample collection (Table 2).

Table 2. Temperature and Relative humidity of each of the storage compartment

Compartment	3weeks		6weeks		9weeks		12weeks	
	Temp.(ºC)	R.H (%)	Temp.(ºC)	R.H (%)	Temp.(ºC)	R.H (%)	Temp.(ºC)	R.H (%)
Light	33.7	54.0	33.8	50.0	34.2	52.0	35.2	50.0
Dark	27.4	66.0	27.2	58.0	28.0	63.0	30.0	58.0
Outside box	26.6	75.0	26.1	63.0	27.5	68.0	27.9	73.0

Temp- Temperature, R.H-Relative humidity

Analysis of provitamin A carotenoids (pVAC) and xanthophylls using HPLC-PDAD

The pVAC and xanthophylls profiles of the flour samples were quantified according to the method described by Howe and Tanumihardjo [22], using high performance liquid chromatography system coupled to photodiode array detector (HPLC-PDAD). The HPLC instrumentation comprised Waters HPLC system (Water Corporation, Milford, MA), binary HPLC pump (Waters 626), auto-sampler (Waters 717), PDAD (Waters 2996) and Empower 1 software (Waters Corporation).

Carotenoids were extracted from the flours by mixing 0.6 g of sample with 10 mL of ethanol containing 0.1% butylated hydroxyl toluene (BHT) and allowing the mixture to stand in a water bath at 85° C for 5 min [22]. Subsequently, interfering oil in the mixture was saponified by adding 500 µL of potassium hydroxide (80% w/v). Afterward, the mixture was returned to the water bath (at 85 °C for 5 min) for two successive cycles, with thorough mixing after each cycle. Thereafter, the samples were placed in an ice bath and cold deionized water (3 mL) was added to it. Then, the carotenoids in the samples mixture were separated for three consecutive times by adding 3 mL of hexane and then centrifuging at 1200 g for 5 min. The collected hexane fraction was washed three times with deionized water, centrifuged at 1200 g for 5 min and dried under nitrogen gas using TurboVap LIV concentrator. Subsequently, the dried extract was redissolved in 1 mL methanol/dichloromethane (50:50 v/v), out of which an aliquot of 100 µL was injected into the HPLC system for the quantification of the carotenoids. The mobile phase consisted of solvent A [methanol: water (92:8 v/v), with 10 mmol/L ammonium acetate] and solvent B (100% methyl tertiary-butyl ether). Gradient elution programme, set at 1 mL/min, was as follows: linear gradient from 83% to 59% A for 29 min; linear gradient from 59% to 30% A for 6 min; 1 min hold at 30% A; linear gradient from 30% to 83% A for 4 min; and 4 min hold at 83%. Chromatographic separation of carotenoids was carried out using a C30 YMC guard column (4.6 9 250 mm, 3 µm). Identifications and quantifications of carotenoids were done at 450 nm using an external standard method by comparing their retention times and spectra with those of reference standards. Samples were analyzed in triplicate.

Estimation of total provitamin A carotenoid content: Total pVAC was calculated using the formula reported by Taleon et al. [23]: Total pVAC = βC + (1/2) (13-cis-βC) + (1/2) (9-cis-βC) + (1/2) (βCX); where βC is β-carotene, 13-cis-βC is 13-cis-β-carotene, 9-cis-βC is 9-cis-β-carotene, and βCX is β-cryptoxanthin.

Retention of provitamin A carotenoid: The percentage apparent retention (%AR) of total pVAC of the samples was calculated using the formula reported by Li et al. [24] as follows:
%AR = (pVAC per g of maize after storage x 100) / (pVAC per g of maize before storage)

Calculation of retinol equivalent of the provitamin A carotenoid: Retinol equivalent (RE) of the pVAC, expressed in µg per g dry weight, was calculated according to the in-vivo conversion factor proposed by the world health organization, and reported by Djuikwo et al. [25], where 6 µg of β-carotene and 12 µg of α-carotene correspond to 1 RE. The RE of 13-cis-β-carotene and 9-cis-β-carotene was computed at the same strength equivalence as β-carotene.

Statistical analysis: Result data, expressed as the mean ± standard deviation, were analyzed statistically using analysis of variance (ANOVA) and least significant difference tests using SPSS statistical software package (version 17) at 95% confidence level.

RESULTS AND DISCUSSION

Carotenoids are unstable compounds, and due to their polyene structure, they act as antioxidants; a property linked to their own destruction and raised by abiotic factors such as temperature, light, metal catalysts, and water content. Although, the same structural attributes of carotenoids that make them beneficial to human health also subject them to oxidation. Oxidizing environments leading to non-enzymatic cleavage can also be initiated by cellular activities. Co-oxidation of carotenoids as a consequence of lipoxygenase activities is a long-known example of the non-enzymatic cleavage of carotenoids in which the fatty acyl peroxy radicals generated cleave carotenoids by a random attack of the polyene chromophore [26, 27].

In this study, the pVAC (β-cryptoxanthin; α-carotene; 13-cis-β-carotene; 9-cis-β-carotene, all-trans-β-carotene, total β-carotene and total pVAC) profiles (μg/g dry weight) of the yellow-seeded maize grain flour as affected by storage period is presented in Table 3. The result shows that β-cryptoxanthin and all-trans-β-carotene were the predominant pVAC in the flour before and at the 28th day of storage (2.22, 1.63 μg/g; and 0.77, 0.56 μg/g, respectively). Generally, the levels of all the pVAC decreased as the storage period increased. However, whereas the decrease of the carotenoids during storage was significant ($p < 0.05$) for α-carotene, all-trans-β-carotene, 9-cis-β-carotene, total β-carotene and total pVAC, it was not significant for β-cryptoxanthin and 13-cis-β-carotene ($p > 0.05$). Similarly, the various packaging materials influenced the pVAC profile of the flour during the storage period. The effect of the packaging materials on the carotenoids was significant ($p<0.05$) for β-cryptoxanthin, 13-cis-β-carotene, all-trans-β-carotene, total β-carotene, the total pVAC and α-carotene, but not significant ($p > 0.05$) for 9-cis-β-carotene. Overall, storage period and packaging material combined only had significant ($p<0.05$) effect on the level of all-trans-β-carotene (Table 3).

The range of the various pVAC observed in this study agrees with values reported by some previous studies for the pVAC content of grains of yellow maize varieties [19, 17, 23]. The preponderance of β-cryptoxanthin and all-trans-β-carotene over other pVAC both before and during storage is also in agreement with an earlier report by Mugode et al. [19], which indicated that these two carotenoids are the major pVAC in dry yellow maize grains, even with the use of different packaging materials and storage conditions in the present study.

Some previous studies have reported a decrease in the pVAC content of the yellow maize grain during storage [19, 23], which is attributed to exposure to high temperature, oxygen and light that cause carotenoids degradation during storage [13, 14]. Recently, Awoyale et al. [20] also reported a decrease in the pVAC content of Ogi powder made from yellow maize grain during storage. Thus, the decrease in the pVAC content of the yellow maize flour with increasing storage period observed in this study is inconsonant with the existing literature on the behavior of pVAC in both yellow maize grain and its products during storage, at different temperatures and relative humidity. It is important to add that the measurement of the temperatures and relative humidity of the storage box was used to check the effect of lighting and darkness on the storage condition/atmosphere of the samples vis-à-vis carotenoid degradation.

Table 3. Provitamin A carotenoids (pVAC) profile (µg/g dry weight) of yellow maize grain flour as affected by storage period and packaging materials

pVAC	Storage period (days)	Average (µg/g)	Range (µg/g)	P Storage period	P Packages	P Storage Period x Packages
βCX	0	2.22				
	7	1.95	1.52 - 2.74	NS	**	NS
	14	1.70	1.34 - 2.02	NS	**	NS
	21	1.71	1.43 - 2.04	NS	**	NS
	28	1.63	0.94 - 2.51	NS	**	NS
αC	0	0.34				
	7	0.30	0.22 - 0.43	*	***	NS
	14	0.25	0.19 - 0.29	*	***	NS
	21	0.23	0.17 - 0.29	*	***	NS
	28	0.16	0.03 - 0.27	*	***	NS
13-cis-βC	0	0.17				
	7	0.17	0.12 - 0.21	NS	**	NS
	14	0.15	0.10 - 0.17	NS	**	NS
	21	0.13	0.09 - 0.21	NS	**	NS
	28	0.11	0.06 - 0.27	NS	**	NS
9-cis-βC	0	0.35				
	7	0.30	0.08 – 0.44	**	NS	NS
	14	0.28	0.22 – 0.33	**	NS	NS
	21	0.28	0.23 – 0.34	**	NS	NS
	28	0.27	0.15 – 0.39	**	NS	NS
trans-βC	0	0.77				
	7	0.66	0.51 – 0.79	*	**	*
	14	0.58	0.46 – 0.66	*	**	*
	21	0.60	0.47 – 0.70	*	**	*
	28	0.56	0.33 – 0.90	*	**	*
Total βC	0	1.29				
	7	1.12	0.89 – 1.41	**	**	NS
	14	1.00	0.80 -1.17	**	**	NS
	21	1.01	0.80 – 1.25	**	**	NS
	28	0.94	0.53 – 1.56	**	**	NS
Total pVAC	0	2.57				
	7	2.24	1.76 - 2.96	**	**	NS
	14	1.98	1.59 - 2.26	**	**	NS
	21	1.98	1.61 - 2.42	**	**	NS
	28	1.84	1.04 - 2.95	**	**	NS

Data represent the results of triplicate analyses. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS - not significant ($p > 0.05$); P- p-value; βCX - β-cryptoxanthin; αC – α-carotene; 13-*cis*-βC - 13-*cis*-β-carotene; 9-*cis*-βC - 9-*cis*-β-carotene; *trans*-βC – all-*trans*-β-carotene; βC – β-carotene; pVAC - provitamin A carotenoids.

The xanthophylls (lutein, zeaxanthin and total xanthophylls) profiles (µg/g dry weight) of the yellow maize grain flour as affected by storage period is presented in Table 4. As with the pVAC, the levels of the xanthophylls decreased with increasing storage period. Lutein decreased from

5.76 µg/g to 3.75 µg/g; zeaxanthin decreased from 10.61 µg/g to 8.18 µg/g; while the total xanthophylls decreased from 16.37 µg/g to 11.94 µg/g, before storage (0 days) and at the 28th day of storage, respectively. However, whereas the decrease was significant ($p<0.05$) for lutein and the total xanthophylls, it was not significant ($p > 0.05$) for zeaxanthin. The various packaging materials had a significant ($p<0.05$) effect on the levels of lutein, zeaxanthin and total xanthophylls. When considered together, storage period and packaging material had significant ($p< 0.05$) effect on the levels of lutein and the total xanthophylls but had no significant ($p > 0.05$) effect on the level of zeaxanthin.

The levels of xanthophylls (lutein and zeaxanthin) quantified in the maize flours packaged in different materials and stored for 28th days under different conditions (Table 4) were consistently higher than the levels of their counterpart pVAC (Table 3). This observation is consistent with some previous reports that indicated that xanthophylls are the major carotenoids in yellow maize varieties [17, 23]. However, among the various carotenoids (both pVAC and xanthophylls), zeaxanthin was the most abundant carotenoid in the flours. This observation is in conformity with an earlier report by Ortiz et al. [17], who reported that zeaxanthin was the predominant carotenoid in the different maize genotypes they evaluated in their study. As noted for the pVAC, the decrease in the xanthophylls may also be attributed to carotenoids degradation during storage because of exposure to high temperature, oxygen, and light [13, 14].

Table 4. Xanthophylls profile (µg/g dry weight) of yellow maize grain flour as affected by storage period and packaging materials

Xanthophylls	Storage period (days)	Average (µg/g)	Range (µg/g)	P Storage period	P Packages	P Storage period x Packages
Lut	0	5.76				
	7	5.08	4.19 – 5.99	***	**	*
	14	4.70	3.78 - 5.59	***	**	*
	21	4.18	3.23 - 4.71	***	**	*
	28	3.75	2.08 - 5.42	***	**	*
Zeax	0	10.61				
	7	8.78	7.41 - 10.56	NS	**	NS
	14	8.12	6.34 - 9.77	NS	**	NS
	21	7.68	1.67 - 9.77	NS	**	NS
	28	8.18	4.93 - 12.91	NS	**	NS
Total xanto	0	16.37				
	7	13.64	11.65 - 16.55	*	**	*
	14	12.82	10.12 - 15.29	*	**	*
	21	11.85	6.27 - 14.47	*	**	*
	28	11.94	7.01 - 18.01	*	**	*

Data represent the results of triplicate analyses. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS - not significant ($p > 0.05$); P- p-value; Lut - lutein; Zeax - zeaxanthin; Xanto – xanthophylls.

The percentage apparent retention (%AR) of total pVAC in the yellow maize grain flour as affected by packaging materials, storage period and storage condition is depicted in Figure 1. At the end of the 28 days storage period, both packaging materials and storage period had a significant effect ($p < 0.05$) on the retention of the total pVAC of the maize flour. Generally, storage in the dark compartment of the storage box resulted in higher retention of total pVAC, when compared to storage in the lighted compartment of the box and storage outside the box, irrespective of the packing material used. Furthermore, packaging in PPC led to higher retention of the pVAC relative to HDPE and PWS, irrespective of the storage period and condition. Maize flour packaged in PPC and stored in dark compartment of the storage box (MCD) had the highest ($p < 0.05$) retention of total pVAC (92.39%) relative to the other packaging materials and storage conditions. In contrast, maize flour packaged in HDPE and stored in a lighted compartment of the storage box (MNL) had the least total pVAC retention (44.92%).

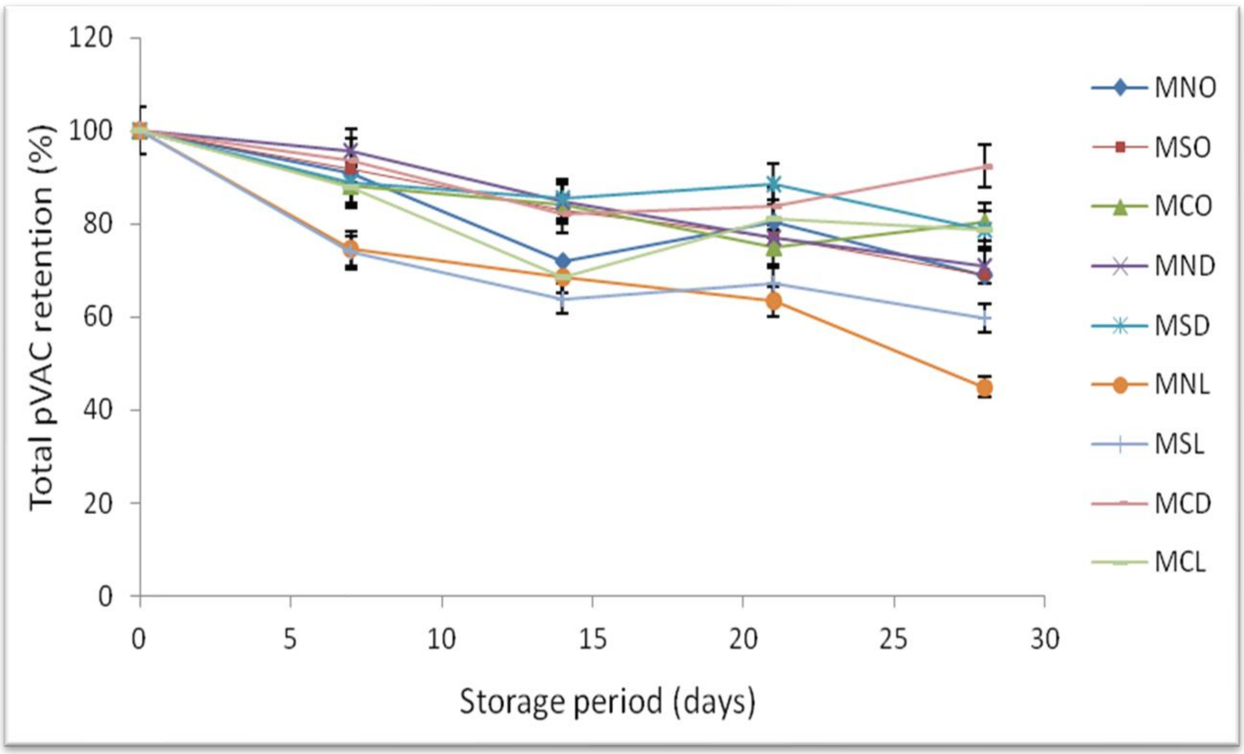

Figure 1. True retention (%TR) of total provitamin A carotenoids (pVAC) in yellow maize grain flour as affected by packaging materials, storage period and storage condition

The trend of the total pVAC retention as the storage period increased was not definite. Whereas there was a steady decrease in the retention of the total pVAC for the various packaging materials and storage conditions from the zero (0) day to the 14th day of the storage, the decrease remained progressive for packaging in PWS and HDPE until the 28th day, irrespective of the storage condition. However, packaging in the PPC resulted in an increase in the total pVAC retention at the 28th day for both storages in the dark compartment of the box, and storage outside the box.

Degradation of pVAC, leading to their low retention in yellow maize grain [23] and its product (Ogi) [20] during storage has been previously reported. The differential retention of total pVAC during the storage period by different packaging materials under different storage conditions could be explained by the nature of the packaging materials in relation to temperature, oxygen, and light. For the maize flour packaged in PPC and stored in dark compartment of the storage box, which had the highest pVAC retention, it is possible that the PPC and the dark compartment of the storage box provided an environment that excluded light and reduced exposure to heat. On the contrary, the lowest retention of pVAC in maize flour packaged in HDPE and stored in a lighted compartment of the storage box may be attributed to light and heat (ambient temperature of 35 °C) in that compartment [20, 28].

The percentage apparent retention (%AR) of total xanthophylls in yellow maize grain flour as affected by packaging materials, storage period and storage condition is shown in Figure 2. The Figure shows that total xanthophylls retention varied, depending on the storage period, packaging material and storage condition. At the end of the 28 days storage period, maize flour packaged in PPC and stored in dark compartment of the storage box (MCD) had the highest ($p < 0.05$) retention of total xanthophylls (89.44%), followed by maize flour packaged in PPC and stored outside the box (MCO) (85.85%); while maize flour packaged in HDPE and stored in lighted compartment of the storage box (MNL) had the least total xanthophylls (46.76%). Total xanthophylls retention decreased progressively from the zero (0) day to the 28th day of storage in maize flour packaged in HDPE and stored in both the lighted compartment (MNL) and the dark compartments (MND) of the storage box, and in maize flour packaged in PWS and stored in dark compartment (MNL).

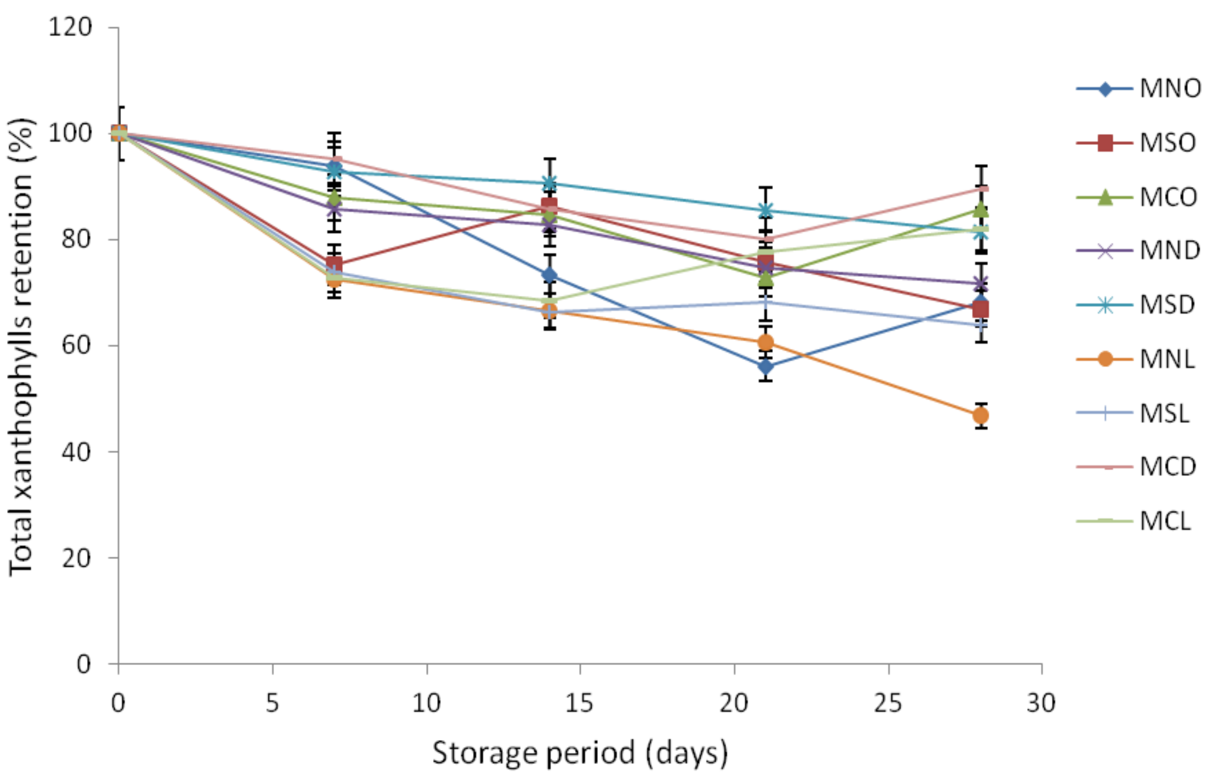

Figure 2. True retention (%TR) of total xanthophylls in yellow maize grain flour as affected by packaging materials, storage period and storage condition

In contrast, total xanthophylls retention decreased steadily until the 21st day of the storage but increased at the 28th day in maize flour packaged in PPC and stored in dark compartment of the storage box (MCD) and outside the storage box (MCO), as well as in maize flour packaged in HDPE and stored outside the box (MNO).

As explained for the pVAC, the higher retention of total xanthophylls in the maize flour packaged in PPC and stored in dark compartment of the storage box may be due to the exclusion of light and heat. This was, however, not the case for the maize flour packaged in HDPE and stored in the lighted compartment of the storage box, which had the least retention. A comparison of the retention of the total pVAC (Figure 1) and that of total xanthophylls (Figure 2) indicates that irrespective of the storage period, packaging material and storage condition, there was a higher retention of total pVAC (92.39%) than total xanthophylls (89.44%). This may be due to the unsaturated (double) bonds present in the carbon chain of the xanthophylls structure, which makes them more susceptible to degradation during processing and storage [29-31]. In fact, oxidative degradation, which is dependent on factors including the presence of oxygen, light, heat, enzymes, co-oxidation with lipid hydroperoxides and metals [32, 33], has been identified as the principal cause of more extensive degradation of carotenoids.

It was reported by Otten et al. [34] that the biological value of food materials with vitamin A activity is expressed as retinol equivalent (RE). Figure 3 shows the RE (µg/g) of total pVAC in yellow maize grain flour as influenced by packaging materials, storage period and storage condition. Maize flour (not subjected to packaging and storage) had the highest RE (0.43 µg/g). The RE decreased as the storage period increased, irrespective of the packaging material and storage condition. However, the extent of the decrease varied depending on the packaging material and storage condition. As expected, among the various packaging materials and storage conditions, MCD (with the highest total pVAC retention) had the highest RE (0.40 µg/g), whereas MNL (with the least total pVAC retention) had the lowest RE (0.19 µg/g) (Figure 1). Generally, at the 28th day of storage, maize flour packaged in PPC displayed the highest RE at the three different storage conditions used in the study. As earlier stated, due to the unsaturated (double) bonds present in the carbon chain of carotenoids, the presence of light, heat and oxygen are known to precipitate some chemical reactions such as isomerization (cis to trans) and oxidation during processing and storage of foods, leading to their degradation and biological activity reduction [29, 31]. This could explain why the maize flour packaged in PPC and stored in dark compartment of the storage box (MCD) had the highest RE (Figure 3), as there might have been retardation in the rate of oxidation and isomerization of the pVAC in the flour, due to exclusion of light and reduction in exposure to heat. In contrast, that maize flour packaged in HDPE and stored in lighted compartment of the storage box (MNL) had the lowest RE could indicate that both the light and the heat (ambient temperature of 35 °C) in that compartment may have enhanced the oxidation and isomerization of the pVAC in the flour, causing their degradation and loss of biological activity, in this case, retinol activity.

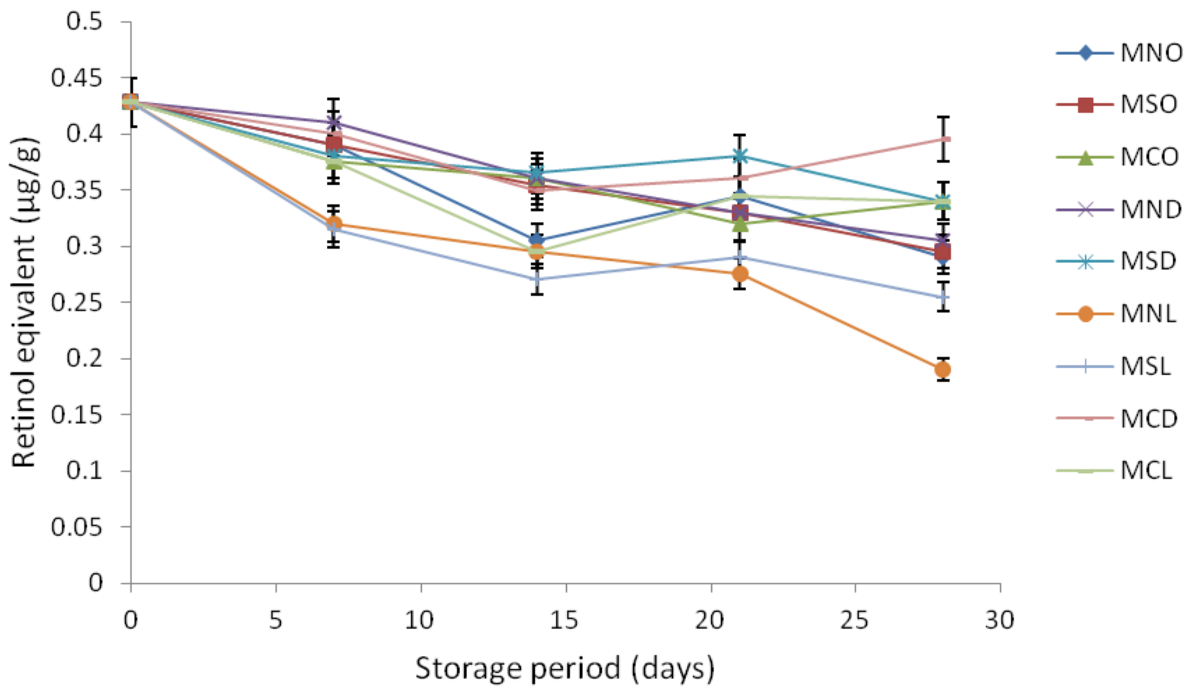

Figure legend

MNO: maize flour packaged in high-density polyethylene bag and stored outside the box;
MSO: maize flour packaged in polypropylene woven sack and stored outside the box;
MCO: maize flour packaged in polyvinyl chloride container and stored outside the box;
MND: maize flour packaged in high-density polyethylene bag and stored in the dark compartment;
MSD: maize flour packaged in polypropylene woven sack and stored in the dark compartment;
MNL: maize flour packaged in high-density polyethylene bag and stored in the lighted compartment;
MSL: maize flour packaged in polypropylene woven sack and stored in the lighted compartment;
MCD: maize flour packaged in polyvinyl chloride container and stored in the dark compartment;
MCL: maize flour packaged in polyvinyl chloride container and stored in the lighted compartment.

CONCLUSIONS

Xanthophylls were more abundant than provitamin A carotenoids (pVAC); while among the pVAC, β-cryptoxanthin and all-trans-β-carotene were predominant in the biofortified yellow maize flour, both before and during storage. Packaging materials and storage periods affected the retention of both the pVAC and xanthophylls and the retinol equivalent (RE) of the pVAC during the storage period. Packaging in polyvinyl chloride container and storing in dark compartment of the storage box resulted in the highest total pVAC and total xanthophylls retention, and RE; whereas packaging in high-density polyethylene bag and storing in a lighted compartment of the storage box resulted in the least total pVAC and total xanthophylls retention, and RE. Hence, packing in polyvinyl chloride container and storing in the dark is recommended for maximum retention of pVAC, xanthophylls and retinol activity in yellow maize flour, to ensure its optimum health benefits.

Competing Interests: The authors declare no conflict of interest.

Authors' Contributions: WA, BMD and AM designed the research, WA, EOA, BMD and AM performed the experiment, WA, EOA, EAI and BMD prepared the manuscript.

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