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Assessment of oxygen sequestration on effectiveness of Purdue Improved Crop Storage (PICS) bags in reducing carotenoid degradation during post-harvest storage of two biofortified orange maize genotypes

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ABSTRACT

Increasing adoption of biofortified orange maize in developing countries requires economical storage methods to manage product quality and carotenoid retention. This study assessed the utility of the Purdue Improved Crop Storage (PICS) bags with a specific focus on retention of provitamin A and other carotenoids in two biofortified maize genotypes (OPVI and OPVII). Grain was stored at ambient conditions for eight months in PICS bags with and without an O_2 scavenger, (PICS-oxy) and (PICS-noxy), respectively, or in common polypropylene woven bags. After 4 months of storage carotenoid content was significantly higher ($p < 0.05$) in grain stored in PICS-oxy compared to PICS-noxy or woven bags demonstrating the importance of entrapped oxygen on maize carotenoid degradation. Differences were observed in stability between the two mize genotypes. After 8 months, carotenoid stability remained dependent on storage bag and genotype with retention being greater in PICS-oxy and PICS-noxy compared to woven bags and OPVI maintained a higher carotenoid retention than OPVII maize. Oxygen content and genotype were determining factors in the effectiveness of PICS to mitigate carotenoid degradation during post-harvest storage.

1. Introduction

High carotenoid biofortified orange maize (*Zea mays* L.) is promoted in developing countries as a strategy to address vitamin A deficiency through the improvement of the micronutrient density of staple crops (Gannon et al., 2014; Palmer et al., 2016). Carotenoids are fat-soluble plant pigments that can be divided into carotenes and xanthophylls. Carotenoids commonly found in maize are lutein, zeaxanthin, *β*-carotene, *α*-carotene and *β*-cryptoxanthin (Li et al., 2007). Those with unsubstituted *β*-ring end groups (*β*-carotene, *α*-carotene and *β*-cryptoxanthin) maintain provitamin A activity and can be converted to vitamin A (retinol) in humans through the action of intestinal or hepatic *15,15′-β*-carotene oxygenase (Biesalski et al., 2007). The major xanthophylls in maize, lutein and zeaxanthin, do not have provitamin A activity, yet are also important to human health since they accumulate in the macular region of the human retina where they play a role in eye and neuronal development as well as in protection against age-related macular degeneration (Johnson, 2014). Additionally, there is a preferential accumulation of lutein, zeaxanthin and β-cryptoxanthin in human brain tissues, with lutein accounting for more than half of brain carotenoids (Vishwanathan et al., 2014). Studies assessing macular pigment optical density have found a relationship between plasma and macular carotenoids with better cognition function in adults (Johnson et al., 2013; Ajana et al., 2018).

Substantial efforts have been made in the development of new varieties of biofortified orange maize with carotenoid levels suitable to impact health in developing countries. Efficacy studies in sub-Saharan Africa have reported the effectiveness of biofortified orange maize in increasing total body vitamin A reserves (Gannon et al., 2014). Efforts to foster translation of these promising grains to at-risk populations

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have grown through cultivation and adoption of biofortified orange maize in target countries such as Zambia where at least 126,000 households had been reached in 2015 (Bouis and Saltzman, 2017; Taleon et al., 2017). Regional interest in this product has increased in various countries that have now released biofortified orange maize and evaluation of biofortified orange maize is ongoing (HarvestPlus, 2018).

Currently, the target for maize vitamin A biofortification is 15 μg provitamin A carotenoids (pVAC) per gram of maize in order to provide 50% of the estimated average daily requirement (EAR) for children and pregnant women (Taleon et al., 2017). This relatively high value accounts for expected losses in pVAC through the value chain, including post-harvest storage and cooking (Taleon et al., 2017). pVAC losses during postharvest storage was higher than in-home preparation of two biofortified orange maize genotypes released in Zambia (Mugode et al., 2014).

Carotenoids are highly unsaturated compounds that are prone to isomerization and oxidation during storage and processing. Oxidation and isomerization reactions lead to formation of *cis*-isomers, loss of provitamin A activity and altered bioavailability (Anan et al., 2005). Losses of carotenoids are greater in milled products than intact grains when stored under similar conditions (Ortiz et al., 2018; Taleon et al., 2017). These studies highlight temperature, humidity and oxygen expsoure as main drivers of carotenoid losses in biofortified orange maize.

Reduction of post-harvest carotenoid losses in biofortified orange maize is needed to facilitate success of ongoing efforts to enhance carotenoid content and positively impact health in developing nations. One approach is the implementation of appropriate storage conditions during post-harvest that can moderate key effectors of carotenoid degradation including oxygen and humidity. Purdue Improved Crop Storage (PICS) bags are currently promoted in Africa to reduce post-harvest storage losses on grains due to pests (Murdock and Baoua, 2014). PICS bags have a two high density polyethylene (HDPE) liners (80 µm) inserted inside an outer woven polypropylene sack. The mechanism of control is a bio-generated modified atmosphere based on reduction in oxygen and buildup of carbon dioxide through grain respiration and other biological activities inside the PICS bags. When the oxygen level becomes sufficiently low, insects die of desiccation (Njoroge et al., 2014).

To date, only one study (Taleon et al., 2017), has evaluated the use of PICS bags in storing biofortified orange maize. In this study, retention of pVAC was modestly yet significantly higher in PICS bags (57.2%) compared to common woven bags (51.4%). However, it was not clear from this study what drove the protective effect of PICS bags relative to oxygen and humidity, both key and modifiable factors known to affect degradation of carotenoids. Therefore, the stability of two biofortified orange maize genotyopes stored under similar conditions (relative humidity, temperature, moisture) in PICS bags and woven bags with and without oxygen scavengers was investigated. We hypothesized that maize stored in PICS bags would have higher retention of carotenoids than those stored in woven bags and that by reducing oxygen levels inside the PICS bags, carotenoid retention would be improved during postharvest storage.

2. Materials and methods

2.1. Standards and solvents

Solvents including acetone, ethyl acetate, methanol (J. T. Baker, Phillipsburg, NJ, USA), methyl *tert*-butyl ether (MTBE) (Sigma-Aldrich, St. Louis, MO, USA) were all certified HPLC grade with > 99.9% purity. Ammonium acetate (1.0 M) solution for chromatography was prepared using double distilled water and adjusted to pH 4.6 with glacial acetic acid. Authentic carotenoid standards including lutein, *ß*-carotene, *ß*-cryptoxanthin, *ß-apo-8′*-carotenal (Sigma-Aldrich), zeaxanthin (IndoFine, Hillsborough, NJ, USA), *α*-carotene, *α*-cryptoxanthin (CaroteneNature, Lupsingen, Switzerland) were used.

2.2. Study design

A 2 × 3 factorial design was used to assess the effect of two biofortified maize genotypes, named open pollinated variety 1 (OPVI) and open-pollinated variety 2 (OPVII), and three storage bag materials (PICS-oxy: with oxygen scavenger, PICS-noxy: without oxygen scaengers) and woven on stability of carotenoids during post-harvest storage. OPVI and OPVII genotypes have an orange colored flinty endosperm and their genetic nature has been previously described (Ortiz et al., 2016). Maize was grown at the Purdue Agronomy Center for Research and Education (ACRE) in West Lafayette, Indiana during the 2016 crop season.

2.3. Maize package and storage

After harvest, the maize was dried to approximately 8.5% moisture content and immediately packed into PICS bags and woven bags. Representative samples were taken for determination of initial carotenoid content. The first treatment was PICS bags with three Oxy-Sorb oxygen scavenger sachets (Silica Gel Products, Remuera, Auckland, New Zealand) enclosed (PICS-oxy). Each sachet had oxygen scavenging capacity of 2000 cc giving a scavenging capacity of 6000 cc in each bag. This scavenging capacity was enough to lower oxygen levels to below 5% in a 50 kg bag. The second treatment was PICS bags without the oxygen scavenger (PICS-noxy). The third treatment was single layer polypropylene woven bags (Woven). Sampling was done at 0, 2, 4 and 8 months. There were two replicates per genotype, for each treatment and for sampling time, giving a total of 48 bags (50 kg each). The bags were tied with zip tiers starting with the inner layer. The middle and the outer layers were separately tied in manner that helped to ensure that little to no air was trapped inside the headspace of the bags. During time of closing, data loggers (Lascar Electronics, Inc, PA, US) were enclosed inside bags. These loggers were used to record temperature and relative humidity inside the bags and were removed at the time of opening the bags. All bags were stored in the same location with controlled temperature (29 ± 1.0 °C) and humidity (30 ± 2.0%) at the time of storage.

2.4. Sampling procedures

Before the bags were opened, a Pac Check MOCON handheld Gas Analyzer needle (Mocon, Minneapolis, MN) was inserted inside the bag to measure internal carbon dioxide and oxygen. Three measurements were taken at different locations for each bag. After measurement, the bags were opened and the biofortified orange maize was thoroughly mixed before sampling. Immediately after sampling, the maize kernels were stored at −80 °C until milling using Foss Tecator 1093 Cyclotec mill (Hoganas, Sweden) and passed through < 0.5 mm sieve after which carotenoid analysis was performed. In all cases milling was performed within one week of sampling. After milling, samples were taken for carotenoid quantification by Liquid Chromatography (LC).

2.5. Carotenoid extraction

Maize carotenoids were extracted as previously reported (Ortiz et al., 2016). Briefly, ~500 mg of milled grain samples was spiked with 100 µl of *β-apo-8*-carotenal as external standard. Spiked samples were extracted with 5 mL of chilled acetone twice and 2 mL of MTBE twice. The MTBE fractions were dried under a stream of nitrogen. Prior to LC analysis, dried carotenoids were solubilized in 2 ml of 1:1 ethyl acetate:methanol filtered through a 0.45 µm PTFE filter and analyzed immediately by LC. Extraction recovery of this method was determined from recovery of the internal standard and found to be 95.3 ± 3.6%.

2.6. LC analysis

Carotenoid separation was carried out on YMC C30 3 μm 2.0 mm × 150 mm column, with a YMC carotenoid guard column (2.0 × 23 mm) (YMC, Allentown, PA, USA) in a Hewlett-Packard 1090 HPLC equipped with a Diode Array Detector scanning at 450 nm. Samples were eluted at 0.37 ml/min under the gradient conditions described by Kean et al. (2008). Carotenoids peaks were identified by co-chromatography with authentic *all-trans*-carotenoid standards and comparison with spectral information from previous separations (Kean et al., 2008). Quantitation was completed using a seven point response curve constructed with authentic carotenoid standards in the range of 0.01–8.0 μM.

2.7. Data analysis

Data were analyzed by running ANOVA on SAS 94 version (SAS Institute Inc, NC) to determine significant differences between treatment means (PICS-oxy, PICS-noxy, Woven bags) for each genotype. Each data point represents an average of 4 determinations. Retention (%) was calculated by comparing individual and total carotenoid content at each time point relative to the initial content. Interaction effects between genotypes and storage bag were determined after 4 and 8 month storage period. Significant differences were determined using the Tukey post hoc test ($p < 0.05$). Pearson's correlation coefficients were generated to quantify the level of association between carotenoid content and humidity.

3. Results and discussion

3.1. Initial carotenoid content in maize genotypes

Initial total carotenoid content was significantly higher in OPVI than OPVII while initial pVACs were similar for the two genotype (Table 1, Supplemental Fig. 1). Major carotenoids in these maize genotypes were xanthophylls (zeaxanthin > lutein > β-cryptoxanthin > α-cryptoxanthin). It has been established that lutein and zeaxanthin are the dominant carotenoids in most maize genotypes (Mugode et al., 2014; Ortiz et al., 2016). OPVI had higher content of zeaxanthin and lower content of lutein compared to OPVII with similar content reported by previous studies on same genotype (Ortiz et al., 2016). β-carotene content was higher in 2012 (4.9 μg/g DW) compared to 2016 (2.5 μg/g DW). These differences in carotenoid content for OPVI can be explained by the difference in season when the grains were grown as variations in carotenoid content of the same genotype but from different seasons have been reported (Griffiths et al., 2007; Rodriques-Amaya, 2003). All-*trans*-β-carotene was significantly higher ($p < 0.05$) in OPVII than in OPVI while *cis*-isomers of β-carotene were similar across both genotypes (Table 1).

3.2. Temperature and relative humidity in storage room and storage bags

The temperature range during the 8 month storage study was related to normal seasonal shifts in West Lafayette, IN, USA. During the first four months (December 2016–April 2017), the average temperature inside the storage room was 29.3 ± 1.0 °C and this was same temperature recorded inside all the bags. Similarly, during the last four months (May 2017–August 2017) the average room and bag temperature decreased to 23.2 ± 2.0 °C. This was directly related to environmental controls in the storage site (i.e. heating in winter and cooling in summer months).

Mean relative humidity (RH) for OPVI was higher (in PICS-oxy ~36% and PICS-noxy ~35%) that RH for OPVII (PICS-oxy ~29% and PICS-noxy ~31%) during the first four months (Fig. 1A and B) suggesting that placing oxygen scavengers in PICS bags did not affect RH. RH in PICS bags remained constant because of physical barrier between inside bag and outer environment. The ability of PICS bags to maintain RH has been previously reported (Njoroge et al., 2014; Williams et al., 2017; Mutungi et al., 2014). However, report exists where RH in PICS bags increased dependent on initial moisture content of the grains (Ng'ang'a et al., 2016). Grains with higher moisture increased internal RH more than grains with low moisture. The increase was independent of atmospheric RH. No reasons were given for the increase in RH inside the bags but we speculate that increased physiological activity of grains, related to insect and molds, are likely responsible for this result. During storage of grains, insects and molds use oxygen to oxidize glucose and release carbon dioxide and vapor during respiration (Murdock and Baoua, 2014). The moisture produced may partly explain the increase in RH in PICS bags over time.

Average RH for OPVI in woven bags was 29.9%, 29.4%, 29.6% and 58.8% for 1, 2, 4 and 8 months, respectively (Fig. 1A). Thus, RH in the woven bag was the same for the first four months, but increased sharply over the last 4 months coinciding with the increase in the storage room RH at 8 months (Fig. 1C). By contrast, RH remained constant in the PICS bags for the entire storage period (Fig. 1A and B). Similar patterns were observed for RH of OPVII (Fig. 1B). Temparture and RH in woven bags were the same as those in the storage room (Fig. 1C). Although RH did not change during storage in PICS bags for both genotypes it remained higher in OPVI than for OPVII (~3.5% difference). The difference might be due to physiological or structural differences of the grain from these genotypes. For example, OPVI is flintier than OPVII, which may affect moisture gain and loss during storage. Overall, these data suggest that modification of RH inside PICS bags during storage is not uniform but depends on maize genotype. Similar observations have been reported in other crops that were stored in PICS bags. Mung beans and pigeon peas modified RH inside PICS bags differently though the bags were stored under the same conditions (Mutungi et al., 2014). In fact, mung beans maintained the RH, while pigeon peas increased RH during 6 months storage in PICS bags.

3.3. Changes in grain moisture and oxygen levels inside storage bags

The initial moisture of the grain was 8.7 ± 0.7% for OPVI and 8.4 ± 0.6% for OPVII. After 8 months storage, the moisture level did not change significantly (data not shown). Some studies have reported a decrease (Mutungi et al., 2014; Vales et al., 2014), an increase (Vales et al., 2014), or no change (Ng'ang'a et al., 2016) in moisture content after storing maize grains in PICS bags.

PICS bags containing oxygen scavengers (PICS-oxy) had significant decrease in oxygen after 15 days to 7.3% and 3.0% for OPVI and OPVII, respectively (Fig. 2A and B). The difference in reduction of oxygen between bags containing OPVI and OPVII cannot be attributed only to the presence of oxygen scavengers as we used same scavenging power in all bags. It is plausible that the difference in physiological activity between these genotypes can explain this observation with OPVII being more physiologically active than OPVI. Interestingly, oxygen in PICS-noxy did not reduce below 18% as previously reported (Njoroge et al., 2014). This inability to reduce oxygen consistently could be due to several reasons. The maize used did not contain significant levels of pests that would actively deplete oxygen inside the bags in a similar way to study by Njoroge et al. (2014). Thus, in the present study, the physiological activity (i.e. respiration) of the maize grain was likely the main driver of oxygen consumption in PICS-noxy. Grain respiration alone could not reduce the oxygen inside the bags to below 10% as reported by Njoroge et al. (2014) due to presumed low respiration rate at low moisture levels. A moisture level of 13–13.5% is recommended for long-term storage (Ng'ang'a et al., 2016), and potentially making maize more biologically active than maize used in this study. Interestingly, during a preliminary study when moisture content was 11.5%, OPVII reduced oxygen inside the PICS bag to 16.5% after 4 months storage (Supplemental Fig. 2), a level not achieved after 8 months in the current study, supporting importance of moisture to physiological

Table 1
Carotenoid content (μg/g dry weight) of OPVI and OPVII at the start of experiment and in maize stored in PICS-oxy, PICS-noxy and woven bags during 8 months storage period.[a,b]

Carotenoids	Genotype	Initial carotenoids	Month 2			Month 4			Month 8		
			PICS-Oxy	PICS-Noxy	Woven	PICS-Oxy	PICS-Noxy	Woven	PICS-Oxy	PICS-Noxy	Woven
Lutein	OPVI	7.21 ± 0.02*	6.76 ± 0.39a	6.04 ± 0.10b	5.92 ± 0.13b	6.10 ± 0.23a	5.37 ± 0.23b	5.11 ± 0.23b	4.50 ± 0.28a	3.97 ± 0.15a	4.31 ± 0.96a
	OPVII	11.45 ± 0.14*	10.61 ± 1.11a	8.88 ± 0.45a	9.17 ± 0.24a	8.72 ± 0.46a	7.80 ± 0.70b	7.20 ± 0.40b	6.26 ± 0.25a	5.88 ± 0.68a	4.66 ± 0.31b
Zeaxanthin	OPVI	36.98 ± 0.33*	35.09 ± 1.05a	31.07 ± 1.54b	30.50 ± 1.68b	34.93 ± 1.51a	29.05 ± 2.24b	27.99 ± 1.93b	24.86 ± 0.90a	22.81 ± 0.75a	19.28 ± 0.97b
	OPVII	22.66 ± 0.27*	20.85 ± 0.78a	16.69 ± 0.65b	19.02 ± 0.36c	19.02 ± 1.20a	17.69 ± 1.52a	16.29 ± 1.15a	12.57 ± 0.51a	12.13 ± 0.50 ab	10.94 ± 0.52b
α-Cryptoxanthin	OPVI	1.25 ± 0.04	1.13 ± 0.09a	0.97 ± 0.03a	0.95 ± 0.08a	0.97 ± 0.05a	0.77 ± 0.03b	0.81 ± 0.03b	0.87 ± 0.02a	0.70 ± 0.16b	0.77 ± 0.03b
	OPVII	1.52 ± 0.02	1.47 ± 0.25a	1.23 ± 0.29a	1.20 ± 0.03a	1.13 ± 0.10a	1.11 ± 0.22a	0.92 ± 0.10a	0.90 ± 0.05a	0.87 ± 0.08a	0.82 ± 0.11a
β-Cryptoxanthin	OPVI	2.76 ± 0.08	2.55 ± 0.21a	2.05 ± 0.10b	1.99 ± 0.08b	2.23 ± 0.05a	1.59 ± 0.06b	1.65 ± 0.09b	1.41 ± 0.04a	1.39 ± 0.04a	1.16 ± 0.10b
	OPVII	2.68 ± 0.02	2.58 ± 0.36a	2.01 ± 0.48a	1.87 ± 0.06a	1.77 ± 0.18a	1.44 ± 0.13a	1.38 ± 0.20a	0.97 ± 0.04a	1.07 ± 0.03a	1.08 ± 0.23a
cis-β-carotene	OPVI	3.55 ± 0.15	3.32 ± 0.20a	2.86 ± 0.13b	2.77 ± 0.12b	2.96 ± 0.12a	1.98 ± 0.09b	2.2 ± 0.11b	1.29 ± 0.03a	1.33 ± 0.07a	1.21 ± 0.13a
	OPVII	3.87 ± 0.27	3.02 ± 0.47a	2.53 ± 0.35a	2.31 ± 0.49a	2.35 ± 0.16a	2.11 ± 0.09 ab	2.05 ± 0.10b	1.21 ± 0.01a	1.21 ± 0.01a	1.22 ± 0.26a
trans-β-carotene	OPVI	2.55 ± 0.00*	2.10 ± 0.15a	1.56 ± 0.08b	1.55 ± 0.05b	1.58 ± 0.08a	0.99 ± 0.07b	1.14 ± 0.10b	1.29 ± 0.05a	1.18 ± 0.04 ab	1.06 ± 0.11b
	OPVII	3.19 ± 0.02*	2.90 ± 0.35a	2.09 ± 0.10b	1.83 ± 0.30b	1.45 ± 0.12a	1.08 ± 0.09 b	1.10 ± 0.13b	1.12 ± 0.05a	1.09 ± 0.04a	0.98 ± 0.21a
Total carotenoids	OPVI	54.30 ± 2.27*	50.80 ± 3.21a	44.58 ± 1.76b	43.56 ± 1.21b	48.77 ± 2.66a	39.75 ± 2.10b	38.90 ± 1.77b	34.22 ± 0.89a	31.38 ± 1.09bc	27.79 ± 0.35c
	OPVII	45.37 ± 1.44*	41.43 ± 2.28a	33.43 ± 1.51b	35.40 ± 1.01b	34.44 ± 1.21a	31.23 ± 1.16bc	28.94 ± 2.17c	23.03 ± 1.19a	22.25 ± 0.77a	19.70 ± 0.47b
Total pVAC	OPVI	8.86 ± 0.92	7.97 ± 0.87a	6.47 ± 0.56b	6.31 ± 0.89b	6.77 ± 0.47a	4.56 ± 0.23b	5.02 ± 0.14b	3.99 ± 0.10a	3.90 ± 0.05a	3.43 ± 0.12a
	OPVII	9.74 ± 1.01	8.50 ± 1.11a	6.63 ± 0.34b	6.03 ± 0.67b	5.57 ± 0.11a	4.68 ± 0.11b	4.53 ± 0.09b	3.30 ± 0.08a	3.37 ± 0.02a	3.28 ± 0.15a

*Initial carotenoids are significantly different between OPVI and OPVII, Tukey's test ($p < 0.05$).

[a] Data are expressed as mean ± SD (n = 4).

[b] For each storage time, means with different letters within the same row are significantly different according to the Tukey's test ($p < 0.05$). cis-β-carotene is the sum of 15-cis- β-carotene, 13-cis-β-carotene and 9-cis-β-carotene; pVAC is the sum of β-cryptoxanthin, cis-β-carotene, trans-β-carotene.

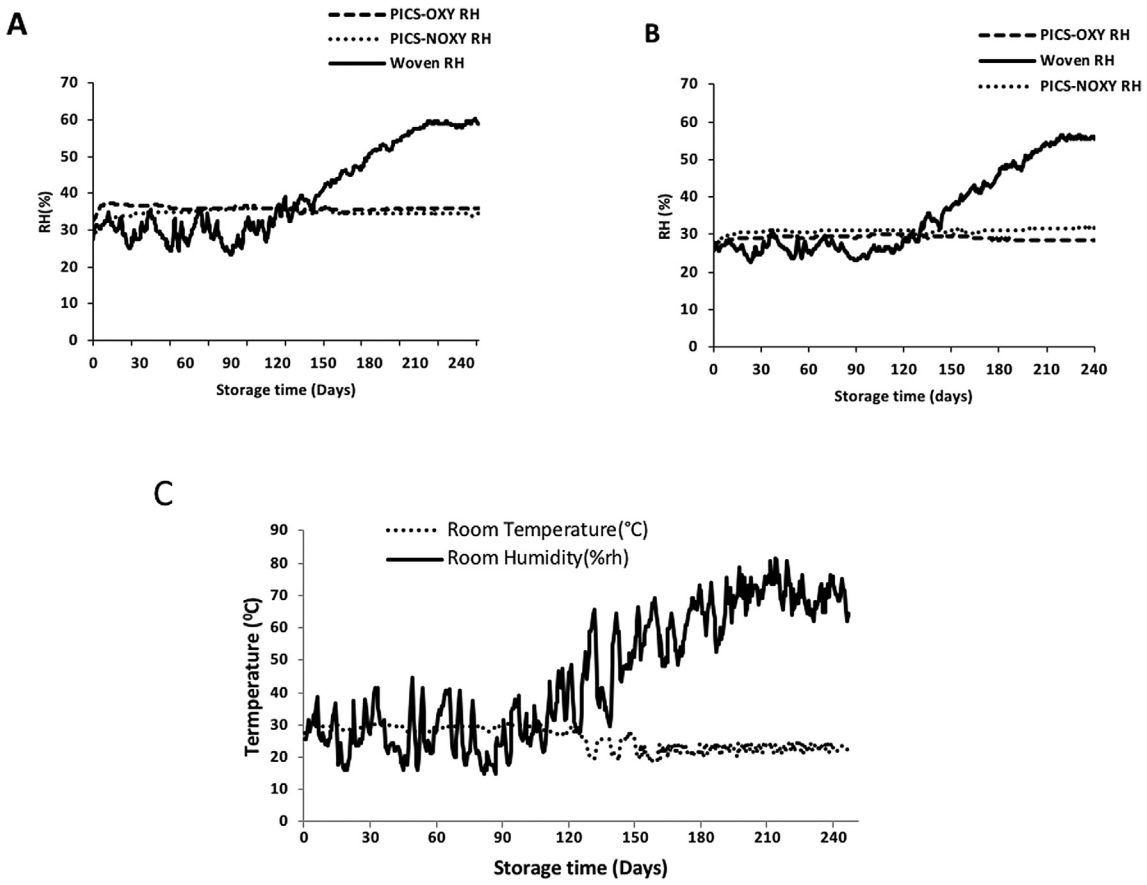

Fig. 1. Changes in relative humidity (RH) inside PICS bags with scavenger (PICS-oxy), PICS bags without scavenger (PICS-noxy) and woven bags storing OPVI (A) and OPVII (B) maize genotypes for 8 months at Purdue University. Changes in RH and temperature inside storage room (C) during the study period (December 2016–August 2017).

activity of stored grains.

As expected, oxygen remained higher in PICS-noxy and woven bags than PIC-oxy bags for the first four months (Fig. 2A and B). Despite a decrease in oxygen in PICS-oxy, we did not detect carbon dioxide inside the bags which suggests that oxygen scavengers, rather than respiration of the grains, were responsible for the decrease in oxygen. However, we detected a small proportion of carbon dioxide in the PICS-noxy. After 15 days, oxygen started to increase inside PICS-oxy until it equaled the oxygen level in PICS-noxy at 4 months. The decrease and then increase in oxygen in PICS bags during storage has been previously observed (Vales et al., 2014), as PICS bags are not a perfect hermetic seal (Mutungi et al., 2014; Ng'ang'a et al., 2016). With oxygen levels reduced by the scavenger initially a differential steep in oxygen pressure between internal and external (room) environment of the PICS bags was created and therefore oxygen likely may have diffused back into the PICS bag.

3.4. Retention of carotenoids through storage

Retention of both pVACs and total carotenoids during storage time was found to be higher in OPVI than OPVII for PICS and woven bags. Maize stored in PICS-oxy had significantly higher carotenoid content

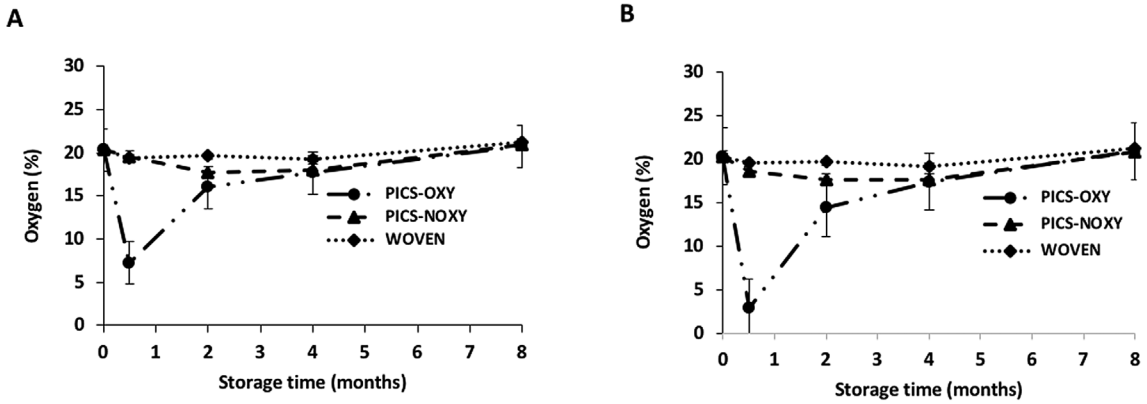

Fig. 2. Change in oxygen levels inside PICS bags with scavengers (PICS-oxy), PICS bags without scavenger (PIC-noxy) and woven bags storing OPVI (A) and OPVII (B) maize genotypes for 8 months at Purdue University.

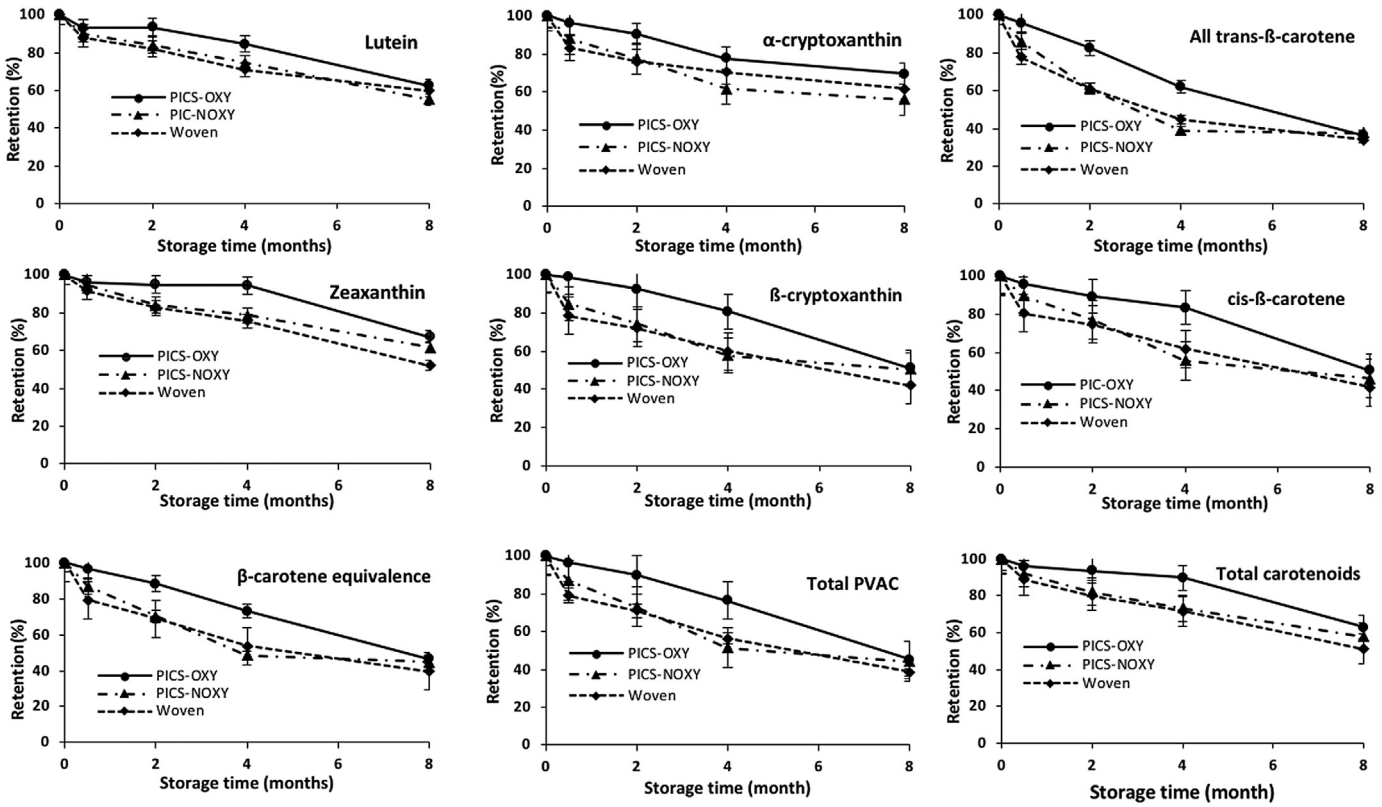

Fig. 3. Carotenoid rentention in OPVI stored for 8 months. *cis-β*-carotene = sum of 15-*cis- β*-carotene, 13-*cis-β*-carotene and 9-*cis-β*-carotene. Provitamin A in *β*-carotene equivalents = all-trans-*β*-carotene + (*β*-cryptoxanthin + *cis-β*-carotene)/2. Total PVAC = sum of all-trans-*β*-carotene, cis-*β*-carotene and *β*-cryptoxanthin). Each point represents a mean ± standard deviation of n = 4 replicates.

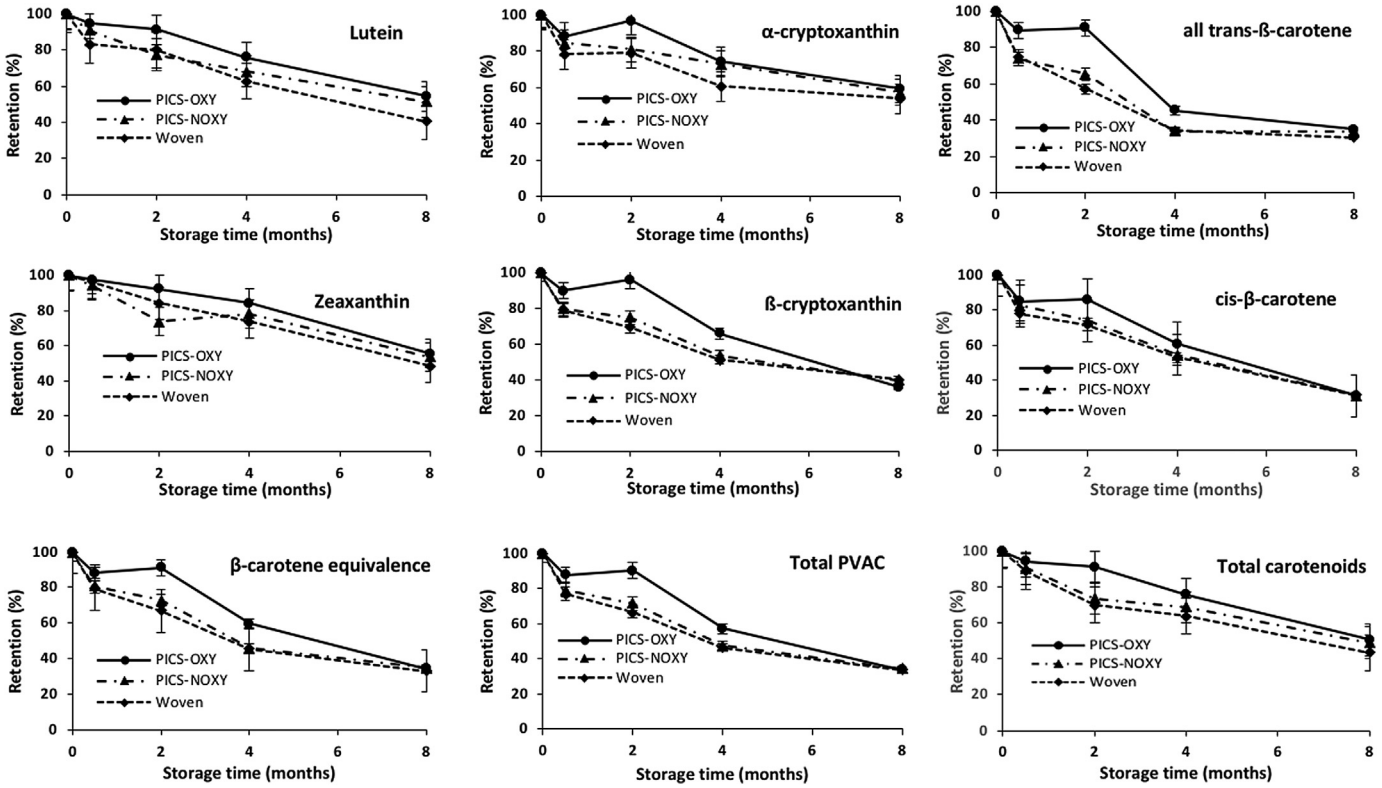

Fig. 4. Carotenoid retention in OPVII stored for 8 months. *cis-β*-carotene = sum of 15-*cis- β*-carotene, 13-*cis-β*-carotene and 9-*cis-β*-carotene. Provitamin A in *β*-carotene equivalents = all trans- *β*-carotene + (*β*-cryptoxanthin + *cis-β*-carotene)/2. Total PVAC = sum of all-trans-*β*-carotene, *cis-β*-carotene and *β*-cryptoxanthin). Each point represents a mean ± standard deviation of n = 4 replicates.

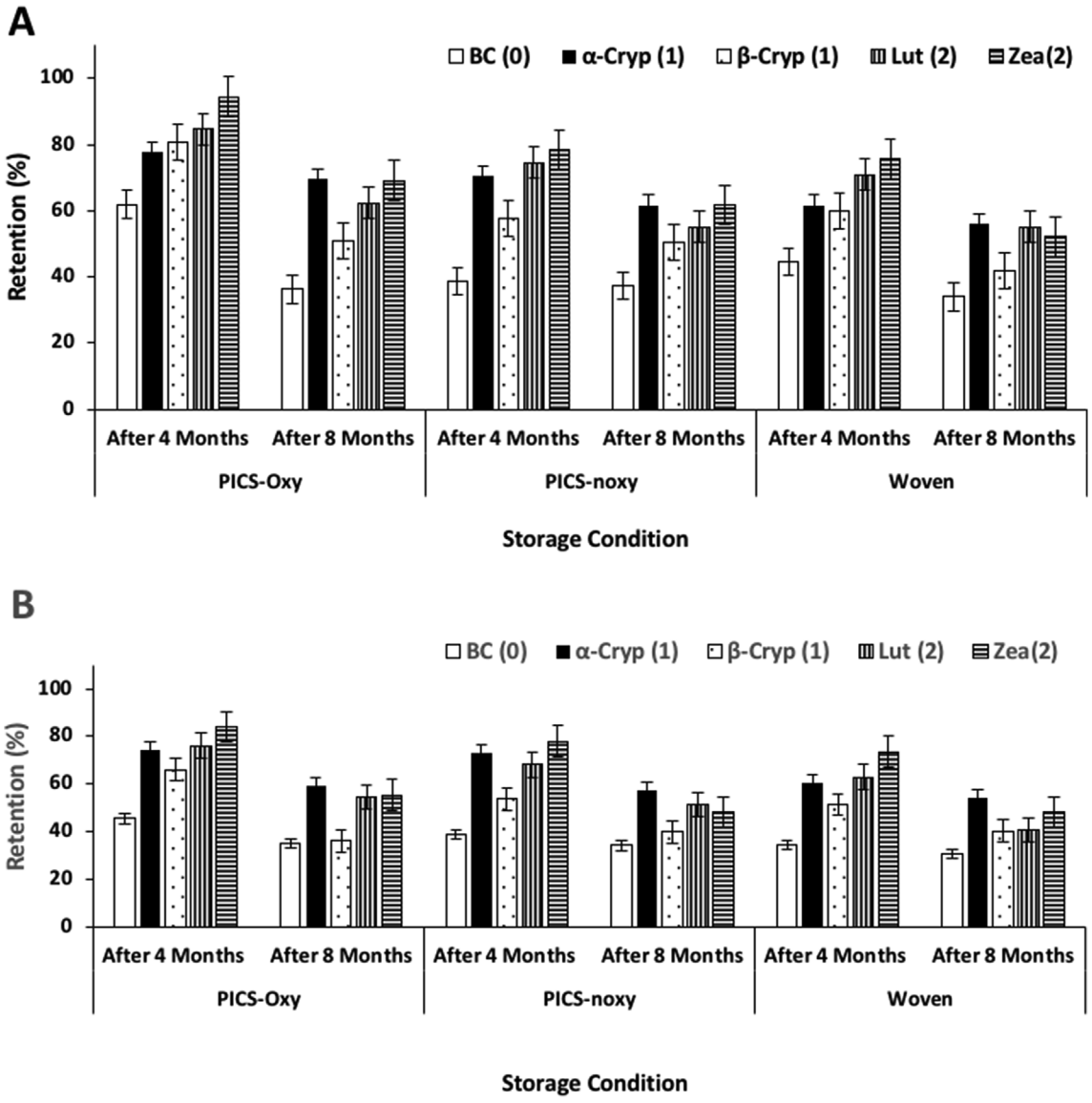

Fig. 5. Retention of individual carotenoids over 8 months storage for OPVI (A) and OPVII (B). BC = *β*-carotene, *α*-cryp = *α*-cryptoxanthin, *β*-cryp = *β*-cryptoxanthin, Lut = lutein, Zea = zeaxanthin. Numbers in parentheses indicate number of hydroxyl groups. Each point represents a mean ± standard deviation of n = 4 replicates.

($p < 0.05$) compared to PICS-noxy or woven for both genotypes. Total carotenoid content during the first four months of storage was significantly higher in PICS-oxy than PICS-noxy and woven bags for both genotypes (Table 1). After 8 months storage, total carotenoid content in PICS-oxy, PICS-noxy and woven was 34.2, 31.4 and 27.8 μg/g DW representing 63.0, 57.8 and 51.2% retention, respectively (Table 1, Fig. 3), for OPVI, while that of OPVII was 23.0, 22.3 and 19.7 μg/g DW representing 50.8, 49.0 and 43.4% retention, respectively (Table 1, Fig. 4).

For pVACs, a similar trend was observed. pVAC content after 4 months in OPVI in PICS-oxy, PICS-noxy and woven bags was 6.8, 4.6, and 5.0 μg/g DW, representing 76, 51 and 56% retention, respectively. pVAC retentions after 4 months for OPVII were 57, 47, and 46%, for PICS-oxy, PICS-noxy and woven bags, respectively (Fig. 4). After 8 months, retention of pVACs was reduced to similar levels of 39–45% and 34–35% in all bags for OPVI and OPVII, respectively, across all storage systems, suggesting little impact of PICS bags or oxygen scavenging over longer term storage (Figs. 3 and 4). However, the current work was only limited to two genotypes with somewhat similar pVAC profiles and additional efforts may be needed to determine the genotype X storage impacts that are optimal.

Retention of xanthophylls was higher in PICS-oxy than PICS-noxy and woven, but extent of enhancement was dependent on genotype (Figs. 3 and 4). Zeaxanthin was found to be more stable than lutein, *α*-cryptoxanthin and *ß*-cryptoxanthin, which is consistent with previous findings (Ortiz et al., 2016). Content of both lutein and zeaxanthin in OPVI was significantly higher in PICS-oxy than PICS-noxy and woven bags (Table 1). In OPVII, the content of lutein (μg/g DW) but not zeaxanthin was significantly higher in PICS-oxy compared to PICS-noxy and woven bags after 4 month storage. Retention of *β*-cryptoxanthin by OPVI was similar in PICS-oxy and PICS-noxy and significantly higher than in the woven bag. Both genotype ($p = 0.0024$) and bag type ($p = 0.0002$) had significant effect on retention of *β*-cryptoxanthin in both genotypes. Interestingly, there was a significant interaction effect (genotype x bag) on retention of lutein ($p = 0.0455$), zeaxanthin ($p = 0.0016$) and *β*-cryptoxanthin ($p = 0.0334$) after 8 month storage, but not after 4 months.

Maize carotenes were found to be less stable compared to xanthophylls. After 4 months, all-*trans*-*β*-carotene content (μg/g DW) in OPVI in PICS-oxy, PICS-noxy and woven bags were 1.6 ± 0.1 (62%),

1.0 ± 0.1 (39%) and 1.1 ± 0.1 (45%), respectively. OPVII showed similar contents with 1.2 ± 0.1 (45%), 1.1 ± 0.1 (34%) and 1.1 ± 0.1 (34%) in PICS-oxy, PICS-noxy and woven bags, respectively (Table 1), indicating bag ($p < 0.001$) but not genotype ($p = 0.8028$) had significant effect on *β*-carotene stability. In all cases PICS-oxy had significantly higher ($p < 0.05$) content than PICS-noxy and woven bags. After 8 months, all-*trans*-*β*-carotene content (μg/g DW) in OPVI was significantly lower in woven bags 1.1 ± 0.1 (34%) than in PICS-oxy 1.3 ± 0.1 (51%) while for OPVII did not change. Both genotype ($p = 0.0348$) and bag ($p = 0.0215$) had significant effect on content of *β*-carotene. Total *cis*-*ß*-carotene content was significantly higher in PICS-oxy than PICS-noxy or woven bags at 4 month storage but no significant differences were observed after 8 month storage in all the three bags for both genotypes (Table 1).

Total *cis*-*β*-carotene appears to have higher retention than all *trans*-*β*-carotene (Figs. 3 and 4). During storage all-*trans*-*β*-carotene isomerizes into 15-*cis*-*ß*-carotene, 13-*cis*-*ß*-carotene and 9-*cis*-*ß*-carotene (Rodriguez-Amaya et al., 2011), therefore *β*-carotene isomers increase while all *trans*-*β*-carotene fraction decrease. This could partly explain why retention of *cis*-*β*-carotene appeared to be higher than all-*trans*-*β*-carotene. When the rate of conversion from all-*trans*-*β*-carotene to its *cis*-isomers is higher than the rate of degradation, *cis*-isomers can accumulate prior to further degradation into epoxides, apocarotenals and apocarotenone (Penicaud et al., 2010) which may as well explain why retention of *cis*-isomers may have decreased.

Higher retention of xanthophylls compared to carotenes was expected as stability of these carotenoids to oxidative degradation in maize has been reported to be related to the number of hydroxyl groups in the carotenoid structure with carotenoids having more hydroxyl groups being more stable than those with less or without hydroxyl groups (Ortiz et al., 2016). The presence of additional hydroxyl groups is considered to decrease xanthophyll's reactivity as a radical scavenger and as such would make them less susceptible to oxidative reactions (Xiao et al., 2018), which is consistent with our results (Fig. 5).

Pearson's correlation coefficients (*r*) were calculated between carotenoid content, bag RH (bRH) and storage room RH (rRH). bRH and rRH were negatively correlated with carotenoid content (Table 2). The correlation coefficients were lower for bRH than rRH. Correlation coefficients for rRH were high and significant for pVACs (r = -0.897, $p = 0.039$), lutein ($r = -0.905$, $p = 0.035$) zeaxanthin ($r = -0.966$, $p = 0.008$) and total carotenoids ($r = -0.967$, $p = 0.015$) for OPVI and zeaxanthin (r = -0.922, $p = 0.026$) for OPVII in PICS-oxy bags. rRH was highly and significantly correlated ($r = 0.999$, $p < 0.001$) with woven bag RH but not PICS bag RH. Generally, this observation may suggest that rRH had more effect on carotenoid degradation than bRH and that rRH might have determined the RH in woven bags but not in PICS bags.

The differences in stability of carotenoids between the two genotypes might be explained, among other reasons, by differences in kernel porosity, surface area, kernel density and initial total carotenoid content (Ortiz et al., 2016; Weber, 1987). OPVI has a flintier kernel type than OPVII and that may have contributed to the higher stability of carotenoids in OPVI. Dent genotypes, or a little less flinty genotypes like OPVII, are more porous while flinty genotypes are compact and less porous. The porosity increases oxygen circulation and therefore makes carotenoids less stable in dent genotypes (Ortiz et al., 2016). Our findings are consistent with the role of oxygen in degradation of carotenoids and that PICS bags could potentially help reduce the rate of carotenoid degradation. Initial total carotenoid content and level of individual carotenoid species determine carotenoid stability during storage (Weber, 1987). In sweet potatoes, genotypes with lower initial total carotenoid content lost less during storage compared to genotypes with higher contents (Bechoff et al., 2010), while carotenoid species that are more abundant tend to have higher losses than those that are less abundant (Weber, 1987). This observation is supported by our results which shows that OPVII which had higher lutein and all-trans-*β*-

Table 2
Pearson correlation coefficients between carotenoid content and storage humidity during storage in PICS-oxy, PICS-noxy and woven bags during 8 months storage period.[a,b]

Factor		pVAC			Lutein			Zeaxanthin			Total Carotenoid		
		PICS-oxy	PICS-noxy	Woven	PICS-oxy	PICS-noxy	Woven	PICS-oxy	PICS-noxy	Woven	PICS-oxy	PICS-noxy	Woven
bRH	OPVI	−0.281 (0.647)	−0.859 (0.062)	−0.640 (0.245)	−0.332 (0.585)	−0.696 (0.192)	−0.645 (0.240)	−0.236 (0.703)	−0.742 (0.151)	(-0.795 (0.108)	−0.281 (0.647)	−0.774 (0.124)	−0.745 (0.148)
	OPVII	0.450 (0.391)	0.289 (0.636)	−0.519 (0.370)	0.517 (0.372)	0.170 (0.785)	−0.687 (0.120)	0.437 (0.462)	0.133 (0.831)	−0.759 (0.137)	0.437 (0.462)	0.133 (0.831)	−0.759 (0.137)
rRH	OPVI	**−0.897 (0.039)**	−0.604 (0.281)	−0.682 (0.204)	**−0.904 (0.035)**	−0.801 (0.104)	−0.688 (0.199)	**−0.966 (0.008)**	−0.787 (0.113)	−0.828 (0.084)	**−0.967 (0.015)**	−0.750 (0.144)	−0.781 (0.118)
	OPVII	−0.792 (0.110)	−0.644 (0.241)	−0.617 (0.268)	−0.854 (0.065)	−0.727 (−.164)	−0.772 (0.126)	**−0.922 (0.026)**	−0.757 (0.138)	−0.823 (0.087)	−0.873 (0.053)	−0.727 (0.164)	−0.758 (0.138)

[a] Numbers in parethesis reresent p-values for correlations made. Significant differences ($p < 0.05$) are designated by bold text.
[b] Abbreviations: bRH; relative humidity inside storage bag, rRH; relative humididty inside storage room.

carotene content than OPVI had higher losses of lutein (5.2 μg/DW (45%)) and b-carotene (2.07 μg/g DW (65%)) than OPVI that had lutein and β-carotene losses of 2.7μg/g DW (30%) and 0.57 μg/g DW (22%), respectively. This trend was similar for zeaxanthin. OPVI had higher initial content of zeaxanthin and higher absolute loss of zeaxanthin than OPVII. Unlike individual carotenoids, total carotenoids did not follow the '*high initial-high loss*' trend. OPVI had higher initial total carotenoid content (54.3 μg/g DW) than OPVII (45.4 μg/g DW). However, carotenoid loss was higher in OPVII (22.3 μg/g DW, 49%) than OPVI (20.1 μg/g DW, 37%) in maize stored in PICS-oxy after 8 months. This trend was similar in all other bags (Table 1). This discrepancy indicates that carotenoids degradation is a multifactorial process and cannot be predicted simply based on one factor.

Moth infestation was observed in grain stored in woven bags, while grain in PICS-oxy and PICS-noxy were not damaged or infested by insects (Supplemental Fig. 3). This observation is in agreement with the known protective effect of PICS bags from post-harvest pest attack as previously reported (Murdock and Baoua, 2014) and underscores the point that the protective effect of PICS bags against pest does not depend entirely on reducing oxygen inside the bags, but that a physical barrier against pests might be as equally important. Interestingly, insects in the woven bags were found to preferentially consume the germ, which is relatively higher in lipids than the endosperm, leaving the majority of the endosperm intact (Supplemental Fig. 3). This observation is of relevance as carotenoids accumulate predominantly in the endosperm (Kean et al., 2008), and any damage to the germ may have only a modest effect on carotenoid content of stored grain.

PICS bags appear to be a viable alternative for storing biofortified maize in order to maintain more pVAC nutritional quality, and at the same time provide protection from insects. While PICS bags without scavengers have generally shown modest effects in reducing carotenoid degradation, sequestering oxygen inside PICS bags through use of an oxygen scavenger seem to have a profound effect. However, the applicability of oxygen scavengers may be limited for most households in developing countries. Nevertheless, enhancing physiological activity of the grains by maintaining the recommended moisture level may be a viable alternative to using oxygen scavengers. Infact, reducing oxygen levels during packing and closing of the bags improved carotenoid stability even after the oxygen level rose again. Unfortunately, this protective effect is lost during longer-term storage (6–8 months) as oxygen diffuses inside the bag. Therefore, PICS bags effectiveness in reducing carotenoid degradation was lost during long-term storage including in bags where oxygen was reduced by scavengers at the beginning of storage. Depending on the nature of the grain, level of insect pest (for initial oxygen drop), duration of storage and extent of grain moisture, PICS bag storage of biofortified maize could help in maintaining the nutritional quality of high carotenoid biofortified orange maize more than woven bags. While this research is limited by number of genotypes that were used it offers insights on the most economic way todate to store biofortified maize, while maintaining its nutritional quality, for developing countries. Therefore, there is a need to do similar studies using many biofortified maize genotypes with different carotenoid profiles at different storage conditions than those used in this study.

Conflict of interest statement

Authors have no conflicts to declare related to this work.

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Appendix A. Supplementary data

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