Assessment of carotenoid degradation of grits from orange corn packaged in high barrier thermosealed pouches

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ABSTRACT

Genetically improved maize varieties with high carotenoid levels and dark orange color have been developed to increase dietary consumption of macular carotenoids. However, postharvest and food processing conditions can cause isomerization and oxidation of carotenoids, reducing their potential impact on consumers' health. The purpose of this study was to assess the effectiveness of high barrier pouches in reducing carotenoid losses during the storage of dry-milled corn products. Orange corn grits were packaged in paper pouch bags, and three types of low-oxygen and low-moisture permeable (LOMP) pouches. Grits were packaged in each type of LOMP pouch with (LOMP-oxy) and without (LOMP-noxy) an oxygen scavenger. For six months, all pouches were stored at semi-controlled environmental conditions (22.5 ± 1.3°C, 32 ± 18% RH). After the storage period, orange corn grits stored in paper pouch bags lost 55% of total xanthophylls, whereas grits packaged in LOMP pouches only lost 8% of total xanthophylls. Orange Corn grits packaged in LOMP-oxy pouches had slightly higher carotenoid content than in LOMP-noxy pouches. Relative humidity fluctuation in the storeroom could have caused fluctuation in moisture content in the orange corn grits packaged in paper pouches, which may affect the rate of carotenoid degradation in the orange corn grits. Therefore, an effective control of the moisture content of the packaged dry-milled product and effective control of the temperature of pouches during storage conditions is essential to maximize carotenoid retention during the storage of dry-milled high carotenoid orange corn grits.

Keywords: Biofortified orange corn, carotenoids, post-harvest storage, thermosealed pouches

INTRODUCTION

Xanthophylls are natural amphipathic oxygenated carotenoids responsible for yellow color in some fruits and vegetables.[1] Among the hundreds of xanthophylls found in nature, only lutein and zeaxanthin consumed in human diets can cross the blood-retina barrier to form macular pigments in the eye.[2–4] Lutein and zeaxanthin are the dominant carotenoids in the adult central nervous system and preferentially accumulate in the human brain.[5,6] Humans do not synthesize lutein and zeaxanthin *de novo* and therefore the only source is through dietary supply. Current epidemiological and clinical evidence supports a role of dietary lutein and zeaxanthin in reducing the risk of age-related macular degeneration (AMD).[7–10] Emerging evidence suggests a role of lutein and zeaxanthin in early neurodevelopment and certain levels of intakes are associated with improving human offspring verbal intelligence.[11] Lutein and zeaxanthin intake is also associated with improving visual and cognitive function in young, healthy adults, as well as in aged adults with and without AMD, and for patients with Alzheimer's disease.[11–14] Although lutein and zeaxanthin are the common xanthophylls present in leafy green vegetables and yellow and orange fruits. Unfortunately, most Americans only consume a fraction of the levels required to receive this protective effect (1-2mg/day vs 6-12mg/day recommended).[15,22]

Corn (*Zea mays*) has natural variation in carotenoid levels, and thus has been targeted for genetic improvement of levels of provitamin A carotenoids (pVACs) to help alleviate vitamin A deficiency in developing countries. Plant breeding has developed open pollinated varieties and hybrids with dark orange color kernels with approximately 15 μg of pVACs per dry weight to consumers in developing countries as a cost-effective approach for alleviating vitamin A deficiency in at-risk populations.[16]

Carotenoids that naturally accumulate in corn endosperm have a backbone of alternating single and double carbon bonds that form a conjugated π-electron system. These conjugated bonds are susceptible to free radical attacks primarily because of the delocalization of π-electrons, making these regions of carotenoid molecules electron-rich.[17] Additional factors can also trigger carotenoid degradation, such as heat, light, acidity, as well as enzymatic or nonenzymatic oxidation, which can contribute to carotenoid losses during the processing and storage of high-carotenoid food products. Oxidative stress has been previously reported to be a significant contributor to the deterioration of seeds during postharvest storage.[18–20] In deteriorating seeds, radical species can be produced in the food matrix during the peroxidation process, where fatty acids and other components of the lipid fraction, such as carotenoids, are degraded, resulting in reduced nutritional quality.[21] Water has an important role in lipid oxidation: the water activity of a food system influences lipid oxidation rates, exerts a protective effect on carotenoids, and controls carotenoid oxidation.[22,23] Water decreases efficiency of trace metals acting as catalysts, and hydrogen bonds between water and hydroperoxide molecules result in delayed chain propagation reaction in lipid oxidation.[24] We previously reported that corn moisture content is strongly associated with the rate of carotenoid degradation.[20] Carotenoid degradation rate was significantly lower ($P < 0.0001$) in corn kernels stored at low relative humidity (11.2 ± 0.6%), than in kernels observed at 57.1 ± 1.1% RH and 75.1 ± 0.4% RH.[20]

Carotenoids in corn kernels are prone to degrade under traditional food processing conditions[25,26] due to their susceptibility to oxidation, which may limit the potential impact of these health-promoting compounds after food processing. The traditional industrial dry milling process removes the germ and pericarp of the corn kernel and separates the endosperm into grits (600-1400 μm), meal (300-600 μm), fine meal (212-600 μm), and flour (< 212 μm).[27–30]

Notably, grits, meal, fine meal, and flour had on average, a rate of carotenoid degradation three times higher than intact whole kernels.[25] Furthermore, xanthophyll losses in dry-milled corn products range from 25% to 32% after 90 days of storage in cotton bags under controlled storage conditions (11% RH, 22.5 °C).[25] Another study[32] reported comparable results, with a 40% xanthophyll loss (sum of lutein and zeaxanthin) after storing biofortified corn flours at 22 °C for 90 days packaged in vacuum-sealed plastic bags.

Dry-milled corn products might remain acceptable for human consumption after six months and even after a year of storage. Therefore, efforts focused on reducing carotenoid losses in dry-milled corn products are needed to facilitate ongoing efforts to enhance carotenoid content in new corn varieties and thus positively impact consumers' health. Nkhata et al.[33] assessed the effectiveness of oxygen sequestration in Purdue Improved Crop Storage (PICS) bags in reducing carotenoid degradation during postharvest storage of intact corn kernels. They reported that after four months of storage at ambient conditions in PICS bags, the carotenoid content was significantly higher ($P < 0.05$) in grain stored in PICS bags with oxygen scavengers in comparison to grain stored in PICS bags without oxygen scavengers or in woven pouches. This demonstrates the importance of entrapped oxygen on corn carotenoid degradation. To our knowledge, oxygen scavengers have not yet been evaluated in reducing carotenoid losses in dry-milled orange corn products during storage in high barrier thermosealed pouches. Therefore, the objective of this study was to determine carotenoid degradation during storage of orange corn grits in high barrier thermosealed pouches made with different barrier films with and without oxygen scavengers.

MATERIAL AND METHODS

Chemicals and Standards. Solvents for extraction of carotenoids and HPLC analysis, acetone, ethyl acetate, methanol, petroleum ether (JT Baker, Phillipsburg, NJ) and methyl *tert*-butyl ether (Sigma-Aldrich St. Louis MO), were all certified HPLC and ACS-grade. A 1.0 mol L^{-1} solution of ammonium acetate (Sigma-Aldrich) was made using double distilled water and adjusted to pH 4.6 with glacial acetic acid. All-*trans* standards of lutein, β-carotene, β-cryptoxanthin, β-apo-8'-carotenal (Sigma-Aldrich, St. Louis MO), and zeaxanthin (IndoFine, Hillsborough, NJ) were obtained and used for carotenoid identification and calibration of the HPLC method.

Corn Dry-Milling Processing. A biofortified corn genotype was selected for obtaining grits for the storage experiment. Detailed information on pedigree and growing conditions of the corn genotype used in this study was reported previously.[20] The grits were made using the procedure detailed in Ortiz et al.[25] Grits were made from orange colored flinty endosperm, and only the grits corn fraction (1400-600 μm) was used in this experiment.

Corn packaging and storage. The stability of carotenoids for high-carotenoid orange corn grits stored for 6 months (January to June, 2019) was evaluated by packaging 300 g of orange corn grits in three distinct types of low-oxygen and low-moisture permeable (LOMP) pouches with and without oxygen scavengers inside (300 cm^3 capacity). The packages' physical properties are listed in Table 1. LOMP1 pouches were made by a layer of Polyester (PET, 48 ga) followed by ink, adhesive, aluminum foil (AF, 28 ga), adhesive and a Linear Low Density Polyethylene layer (LLDPE, 4.0 mil). LOMP2 pouches were made with a layer of Biotrē® Natural Kraft Paper (NKP, 50 g) followed by adhesive, Wood Pulp Based Barrier Film (WPBBF, 80 ga), adhesive, and 2 mil of a Bio-LLDPE layer. Finally, LOMP3 pouches were made with a layer of NKP followed by extruded polyethylene (PE), metallized polyester (MET PET, 48 ga), adhesive, and

2 mil of LLDPE layer. Orange corn grits were also stored in recycled Kraft brown paper pouch bags (CPB) as a control packaging material, and stapled closed. Pouches were stored on a lab shelf with temperature-controlled conditions, similar to a consumer's pantry. Temperature and relative humidity in the storage room was monitored during the 6 months of the experiment. Pouches were stored on a lab shelf with semi-controlled conditions,

Sampling procedure. A total of 24 pouches for each type of packaging (LOMP1, LOMP2, and LOMP3) were filled with corn grits and sealed with one 8mm thick heat-sealed line with a 580W ULINE H-190 12" Impulse Poly Bag Sealer on setting 6, where half of these pouches contained an oxygen scavenger. Two pouches for each treatment were randomly chosen every month, two samples of corn grits were taken from each pouch, and the samples were stored at -80 ºC until carotenoid analysis. Six CPB pouches were filled with corn grits and stapled closed without an oxygen scavenger inside. Two samples of corn grits were taken from each pouch every month and stored at -80 ºC until carotenoid analysis. Corn grits were milled using a Foss Tecator 1093 Cyclotec mill (Hoganas, Sweden) and passed through <0.5 mm sieve. Carotenoids were then extracted from each sample and analyzed. Sampling continued for 6 months. Before opening the heat-sealed packages, O_2 and CO_2 content were measured with a Pac Check MOCON handheld Gas Analyzer needle (Mocon, Minneapolis, MN).

Carotenoid analysis. Extractions and quantification of carotenoids was performed following the procedure described by Ortiz & Ferruzzi[34] with minor modifications. For every pouch drawn, two representative samples of orange corn grits were taken. In short, ground corn flours (0.6 g) were spiked with 80 µL of internal standards (30 µM β-apo-8′-carotenal in ethyl acetate) and hydrated with 1 mL of distilled water on ice for 10 minutes. Carotenoids were extracted twice with 5 mL of cold acetone and once with 2 mL of methyl *tert*-butyl ether. Extracts were dried

159 under a stream of nitrogen, resolubilized in 1:1 methanol:ethyl acetate, filtered through a 0.45
160 mm PTFE syringe filter (Macherey-Nagel, Düren, Germany) and then analyzed by HPLC.
161 Carotenoids were separated using a YMC C30, 3 μm ,2.0 mm × 150 mm column, with a YMC
162 carotenoid guard column (2.0 x 23 mm) in a Shimadzu HPLC Prominence UFLC XR series
163 system coupled with a diode array detector at 450 nm as previously described by Ortiz &
164 Ferruzzi.[34] Peaks were identified by comparing spectral information in the literature and
165 retention times with authentic all-*trans*-carotenoid standards. Quantification was based on seven-
166 point calibration curves prepared spectrophotometrically with authentic all-*trans*-standards with
167 a concentration range between 0.01 to 7.67 μM.

168 **Data analysis**. Data were analyzed using JMP Pro v15.1 (SAS Institute Inc, 2019). Results are
169 presented as mean ± standard error. Carotenoid concentrations and retentions were analyzed by
170 three-way ANOVA, posthoc comparisons between treatments were made by Tukey's test.
171 Statistical significance was considered at $P < 0.05$. Total xanthophyll content was calculated by
172 summing all-trans-lutein, all-trans-zeaxanthin, α-cryptoxanthin and β-cryptoxanthin
173 concentrations.

RESULTS

Changes in moisture and oxygen levels of grits inside storage pouches

The initial moisture of the grits stored in LOMP pouches and paper pouch bags (CPB) was 8.7 ± 1.1%. After six months of storage in semi-controlled conditions (22.5 ± 1.3°C, 32 ± 18% RH) (Figure 1A), the moisture of the grits stored in pouches did not change significantly (data not shown). All pouches containing oxygen scavengers (LOMP-oxy) had a significantly lower initial oxygen level than atmospheric oxygen levels ($P < 0.01$). After a day of storage, the oxygen scavenger reduced the oxygen levels to a mean of 0.4 ± 0.2% inside all LOMP-oxy pouches (Figure 1B). Then, after the first three months of storage, the oxygen levels across all three types of LOMP-oxy pouches increased to a mean of 10.7 ± 1.5%, and after six months of storage, reached a mean of 15 ± 1.5%. In LOMP-noxy pouches, oxygen levels did not change significantly during storage (19.1 ± 0.1%), except in LOMP1-noxy pouches. Oxygen levels decreased in LOMP1-noxy pouches in month four and month six to 8.6 ± 0.1 % and 11.4 ± 0.6%, respectively.

Retention of carotenoids throughout storage

Carotenoid concentration in the corn grits fraction was affected by the duration of the storage period ($P < 0.05$). Carotenoid levels were significantly lower after four months of storage (Table 2). Corn grits packaged in CPB pouches and stored at the semi-controlled storage conditions lost 25% and 55% of total xanthophylls content after three and six months of storage, respectively (Figure 2). Grits stored in LOMP-oxy had significantly higher carotenoid retention ($P < 0.01$) compared to LOMP-noxy, and all LOMP pouches had higher carotenoid retention than CPB pouches (Table 2). The carotenoid levels in grits were not significantly different across the three types of LOMP pouches ($P > 0.05$), indicating a similar protective effect, despite the

thermosealed pouches having a different number of layers, material compositions, and thicknesses. Packaging corn grits in LOMP pouches had 15% less carotenoid degradation than the CPB pouch control after the first three months of storage, and 47% less after six months of storage. At the end of the study, xanthophyll levels in LOMP and CPB pouches were 39.6 µg/g DW and 21.2 µg/g DW, respectively.

DISCUSSION

The goal of this study was to investigate the impact of oxygen scavengers and different packaging materials on the retention of carotenoids in orange corn grits. All LOMP pouches, with and without oxygen scavengers, had significantly more total carotenoid retention than the CPB pouches after six months of storage, 46.2 µg/g DW LOMP pouches vs. and 25.1 µg/g DW CPB pouches, respectively. Temperature was kept constant for all types of pouches, and LOMP-noxy pouches and CPB pouches had comparable levels of oxygen throughout the six months. Despite a higher oxygen concentration, the LOMP-noxy pouches still had better carotenoid retention than the CPB pouches, close to that of the LOMP-oxy pouches. This might be explained by the low moisture permeability of the LOMP pouches. Relative humidity in the storage room fluctuated (Figure 1A), which may have changed the moisture content of the orange corn grits in the CPB pouches and yet had a lesser effect on the moisture content of the grits in LOMP pouches, perhaps contributing to the significant differences between the control and the treatments. If the carotenoid degradation can be attributed to the relative humidity in the pouch and therefore the moisture content of the corn, it may be reasonable to hypothesize that moisture content has a larger effect on carotenoid degradation than oxygen concentration.

LOMP-oxy pouch oxygen levels increased more rapidly than was expected based on the specifications of the pouches used. Using the reported oxygen transmission rates, dimensions, and surface areas (Table 1), an expected oxygen level increase over time was determined for each of the three pouch types (Figure 1B). Assuming the oxygen scavenger was able to fully absorb up to 300 cm^3 of permeating oxygen in each pouch, only the LOMP2-oxy pouch was expected to accumulate any oxygen in the pouch over the span of six months due to a fully saturated oxygen scavenger. Less than 300 cm^3 of oxygen was expected to permeate LOMP1-oxy and LOMP3-oxy pouches, so no significant oxygen was expected to remain unabsorbed in the pouches. Even though each type of LOMP-oxy pouch used in this study was made of multiple layers of barrier materials with different thicknesses and oxygen transmission rates, oxygen levels across LOMP-oxy pouches in the same month of storage were not significantly different ($P > 0.05$), and oxygen levels in all three LOMP-oxy pouches increased more rapidly than expected. Thus, given that LOMP-oxy pouches with different oxygen transmission rates increased to a similar oxygen level every month, these results suggest that there was another flow of oxygen into the pouches, where the most likely source is the pouches' heat seals as opposed to the pouches' materials. Packaging material specifications have been shown to not account for the seal in reporting pouches' oxygen permeability, resulting in permeabilities not accurately representing storage conditions.[35] The impulse sealer may not have adequately sealed the pouches, allowing a faster and similar entry of oxygen into each LOMP-oxy pouch, which explains the higher-than-expected amount of oxygen permeation, and similar oxygen permeation across different pouches. A weak seal is even more plausible when considering the reduction in oxygen levels in LOMP1-noxy pouches in month four and six. This might have been because of microbial contamination, since the CO_2 levels inside the pouches increased, suggesting a

metabolic reaction. Contamination could be a further indication of a poor seal, which would further support the significance of the seal's contribution to the movement of oxygen in and out of the pouches. Results from this study showed that packaging high carotenoid milled products in low-oxygen and low-moisture permeable pouches and avoiding temperature fluctuation of the pouches is a viable option to help maintain the nutritional value of the product. More experimentation is necessary to determine if an oxygen scavenger has a protective effect on carotenoids in orange corn grits long-term. The effect of different methods of sealing pouches on carotenoid content and oxygen concentration in low-oxygen and low-moisture permeable pouches should be tested. However, the price of low-oxygen and low-moisture permeable pouches in tandem with the additional price of oxygen scavengers may make the addition of oxygen scavengers a challenge in the commercialization of orange corn grit products.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

Abbreviations used

pVACs, provitamin A carotenoids; TCC, total carotenoid content; DW, dry weight basis; RH, relative humidity; SEM, standard error of the mean; LOMP-noxy, low-oxygen and low-moisture permeable pouch without oxygen scavenger; LOMP-oxy, low-oxygen and low-moisture permeable pouch with oxygen scavenger; CPB, paper pouch bags.

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387

388 **Table 1**. Packaging characteristics [a,b,c]

Bag Code	Catalogue No.	Name	Layers composition[a]	Total Thickness (cm)	Dimensions (cm)	O_2 Transmission Rate (65% RH, 20°C, $\mu m^3/cm^2$-24h)	Moisture Vapor Transmission Rate (mg/ cm^2-24hr)
LOMP1	400-420BZ	PBI PET/AF/LLDPE bag	48 ga PET, 28 ga AF, 4.0 mil LLDPE	0.01270 ± 10%	16.5 x29.2x10.2	<0.002	0.03
LOMP2	460-420NKZ	PBI Biotrē® Natural Kraft Paper bag	50 g NKP, 80 ga WPBBF, 2 mil Bio-LLDPE	>0.008 ± 10% [b]	20.1x26.7x8.9	<0.3	0.03
LOMP3	425-420NKZ	PBI Natural Kraft Paper bag	30# NKP [c], 14# PE [c], 48 ga MET PET, 2 mil LLDPE	>0.0064 ± 10% [b]	16.5x29.2x8.9	0.09	0.03
CPB	18409	Brown Paper bag [Control]	Recycled Kraft paper	0.01270± 10%	13.3x8.7x27.8	No data	No data

389 [a] Layers composition: PET= Polyester; AF= Aluminum Foil; LLDPE= Linear Low-Density Polyethylene;
390 NKP=Natural Kraft paper; WPBBF= Wood Pulp Based Barrier Film; #14 PE=Extruded Polyester; MET
391 PET=Metallized Polyester. [b] NKP thickness not listed since it varies from point to point. [c] Thickness not indicated
392 by the manufacturer.
393

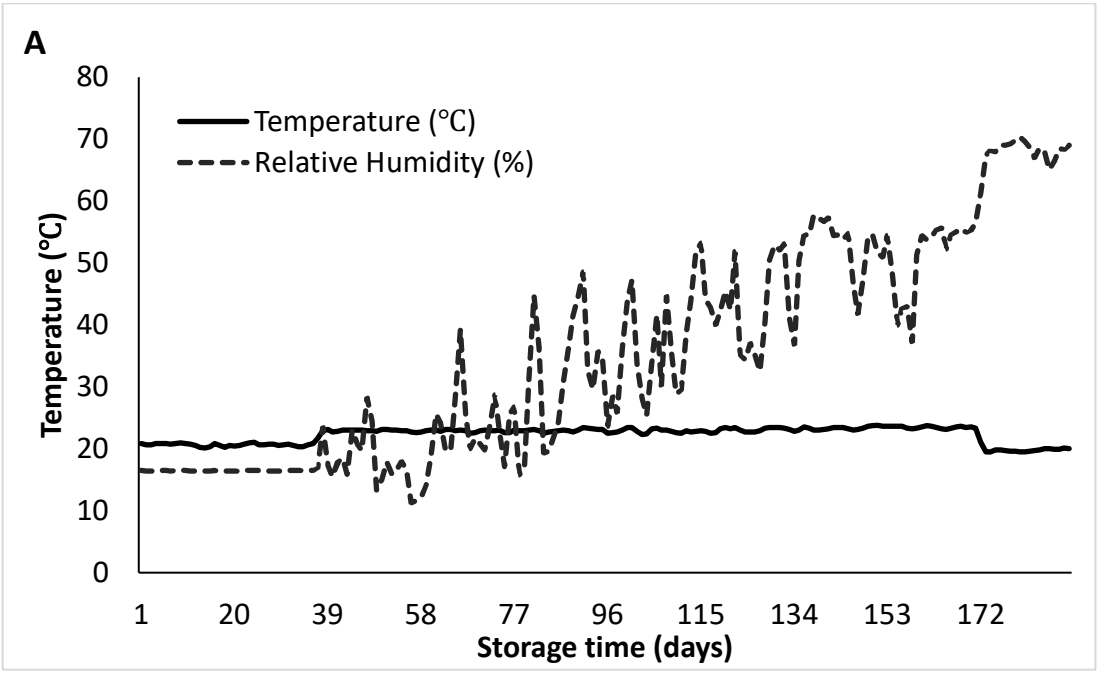
Table 2. Carotenoid content (µg/g DW) in orange corn grits during six months of storage using three different types of store pouches with (LOMP-oxy) and without (LOMP-noxy) oxygen scavenger inside, using a paper pouch bag (CPB) as a control. [a,b,c,d,e,f]

Month	LOMP1[b]		LOMP2[b]		LOMP3[b]		CPB
	oxy	noxy	oxy	Noxy	oxy	noxy	noxy
	Lutein (µg/g DW)						
1	6.0 ± 0.2a	5.7 ± 0.2a	5.6 ± 0.4a	5.3 ± 0.3a	6.3 ± 0.1a	5.6 ± 0.4a	6.5 ± 0.1a
2	6.0 ± 0.1a	5.2 ± 0.4a	6.1 ± 0.2a	4.4 ± 0.5a	6.2 ± 0.3a	5.1 ± 0.4a	5.6 ± 0.3ab
3	6.2 ± 0.3a	4.9 ± 0.6a	4.6 ± 0.9a	5.6 ± 0.1a	5.3 ± 0.6a	4.9 ± 0.5a	5.1 ± 0.4bc
4	5.9 ± 0.1a	5.8 ± 0.0a	5.4 ± 0.4a	4.7 ± 0.5a	5.4 ± 0.5a	5.8 ± 0.1a	4.7 ± 0.3bc
5	5.6 ± 0.7a	5.2 ± 0.1a	6.0 ± 0.1a	5.4 ± 0.2a	5.6 ± 0.1a	5.0 ± 0.6a	4.2 ± 0.3c
6	4.9 ± 0.5a	5.2 ± 0.6a	5.5 ± 0.8a	5.1 ± 0.5a	5.3 ± 0.5a	4.7 ± 0.5a	3.7 ± 0.3c
	Zeaxanthin (µg/g DW)						
1	36.1 ± 0.8a	35.1 ± 1.3a	36.5 ± 0.8a	33.9 ± 0.5a	39.0 ± 1.1a	35.1 ± 0.6a	39.5 ± 0.7a
2	36.5 ± 1.1a	33.8 ± 3.0a	37.6 ± 1.3a	29.7 ± 2.1a	37.4 ± 3.5a	33.4 ± 2.3a	33.3 ± 1.1b
3	36.3 ± 1.3a	35.8 ± 1.0a	32.4 ± 1.8a	33.4 ± 0.7a	34.3 ± 0.6a	32.2 ± 0.2a	28.3 ± 0.7c
4	35.1 ± 0.4a	34.2 ± 0.2a	34.4 ± 2.0a	31.2 ± 0.5a	34.4 ± 1.0a	33.0 ± 0.5a	23.3 ± 0.8d
5	33.8 ± 0.7ab	30.9 ± 0.4a	34.6 ± 0.6a	31.9 ± 1.1a	33.5 ± 0.2a	31.1 ± 2.1a	19.3 ± 0.4e
6	30.1 ± 1.6b	33.8 ± 0.9a	34.2 ± 2.0a	31.3 ± 0.4a	33.2 ± 1.1a	29.8 ± 0.5a	15.9 ± 0.8f
	Xanthophylls (µg/g DW)[c]						
1	43.6 ± 1.0ab	42.2 ± 1.5a	43.6 ± 1.1a	40.5 ± 0.6a	46.8 ± 1.2a	42.2 ± 1.0a	47.6 ± 0.9a
2	44.2 ± 1.2ab	40.4 ± 3.3a	45.3 ± 1.5a	34.6 ± 3.0a	45.2 ± 4.0a	39.8 ± 2.4a	40.2 ± 1.4b
3	45.3 ± 1.8a	42.6 ± 0.8a	39.0 ± 3.1a	41.5 ± 0.9a	41.9 ± 1.6a	39.2 ± 0.8a	35.5 ± 0.9c
4	42.5 ± 1.3ab	42.5 ± 0.3a	42.4 ± 2.5a	38.9 ± 1.1a	42.4 ± 1.5a	41.2 ± 0.6a	30.2 ± 1.0d
5	41.8 ± 1.7ab	38.3 ± 0.5a	43.2 ± 0.7a	39.3 ± 1.6a	41.6 ± 0.3a	38.5 ± 2.7b	25.5 ± 0.6e
6	37.5 ± 1.8b	41.3 ± 1.8a	42.4 ± 2.9a	38.7 ± 1.1a	41.1 ± 1.5a	36.6 ± 0.9b	21.2 ± 1.1e
	PVAC (µg/g DW)[d]						
1	5.9 ± 0.1a	5.2 ± 0.2ab	5.9 ± 0.1a	5.1 ± 0.0ab	6.3 ± 0.2a	5.3 ± 0.1a	5.7 ± 0.1a
2	6.2 ± 0.2a	5.1 ± 0.4ab	6.0 ± 0.1a	4.8 ± 0.1b	6.1 ± 0.4a	4.9 ± 0.3a	5.0 ± 0.2b
3	6.0 ± 0.3a	5.4 ± 0.3a	5.7 ± 0.2a	4.8 ± 0.1b	5.6 ± 0.2a	4.8 ± 0.1a	4.6 ± 0.1bc
4	6.0 ± 0.0a	5.0 ± 0.0ab	5.8 ± 0.4a	5.2 ± 0.6ab	6.0 ± 0.2a	4.9 ± 0.0a	4.4 ± 0.2c
5	6.2 ± 0.1a	4.4 ± 0.1b	5.9 ± 0.1a	4.3 ± 0.1b	5.2 ± 0.2a	4.6 ± 0.3a	3.7 ± 0.1d
6	5.3 ± 0.6a	5.7 ± 0.2a	6.4 ± 0.6a	6.5 ± 0.5a	6.3 ± 0.6a	4.5 ± 0.1a	3.1 ± 0.1e
	TCC (µg/g DW)[e]						
1	50.3 ± 1.2ab	48.2 ± 1.7a	50.2 ± 1.3a	46.3 ± 0.6a	53.9 ± 1.4a	48.3 ± 1.1a	54.1 ± 1.0a
2	51.3 ± 1.5ab	46.3 ± 3.7a	52.0 ± 1.6a	40.1 ± 3.1a	52.1 ± 4.4a	45.5 ± 2.8a	46.0 ± 1.6b
3	52.4 ± 2.1a	49.0 ± 0.8a	45.7 ± 3.3a	47.0 ± 1.1a	48.5 ± 1.8a	44.9 ± 0.7a	41.0 ± 1.0c
4	49.6 ± 1.4ab	48.6 ± 0.3a	49.1 ± 2.9a	44.5 ± 1.1a	49.3 ± 1.5a	47.0 ± 0.6a	35.6 ± 1.2d
5	49.0 ± 1.8ab	43.3 ± 0.5a	50.1 ± 0.8a	44.5 ± 1.8a	47.7 ± 0.5a	43.8 ± 3.0a	30.0 ± 0.7e
6	43.7 ± 2.5b	47.7 ± 1.9a	49.4 ± 3.5a	46.1 ± 1.4a	48.4 ± 2.0a	41.9 ± 0.9a	25.1 ± 1.2f

[a] Data represents a mean ± SEM, n=4 observations. [b] Low-oxygen and low-moisture permeable (LOMP) pouches: 1) bag made of polyester & aluminum foil film, 2) bag made of wood pulp-based barrier film, 3) bag made of metallized polyester film. All pouches included a linear low density polyethylene layer. [c] Xanthophylls: sum of lutein + zeaxanthin + β-cryptoxanthin + α-cryptoxanthin. [d] PVAC: pro-vitamin A carotenoids is the sum of *all-E*-β-carotene + 1/2 (*Z's*-β-carotenes + β-cryptoxanthin + α-carotene) [e] TCC: total carotenoid content, sum of all quantified carotenoid species. [f] Presence of different letters indicate significant differences in carotenoid content ($p<0.05$) among storage times for each column (bag types).

FIGURES

Figure 1. Temperature and relative humidity fluctuations (**A**) in the storage room during the six-month (January to June) span of storage at Purdue University, and actual vs expected oxygen volumes (**B**) inside pouches with oxygen scavenger during the same six months.



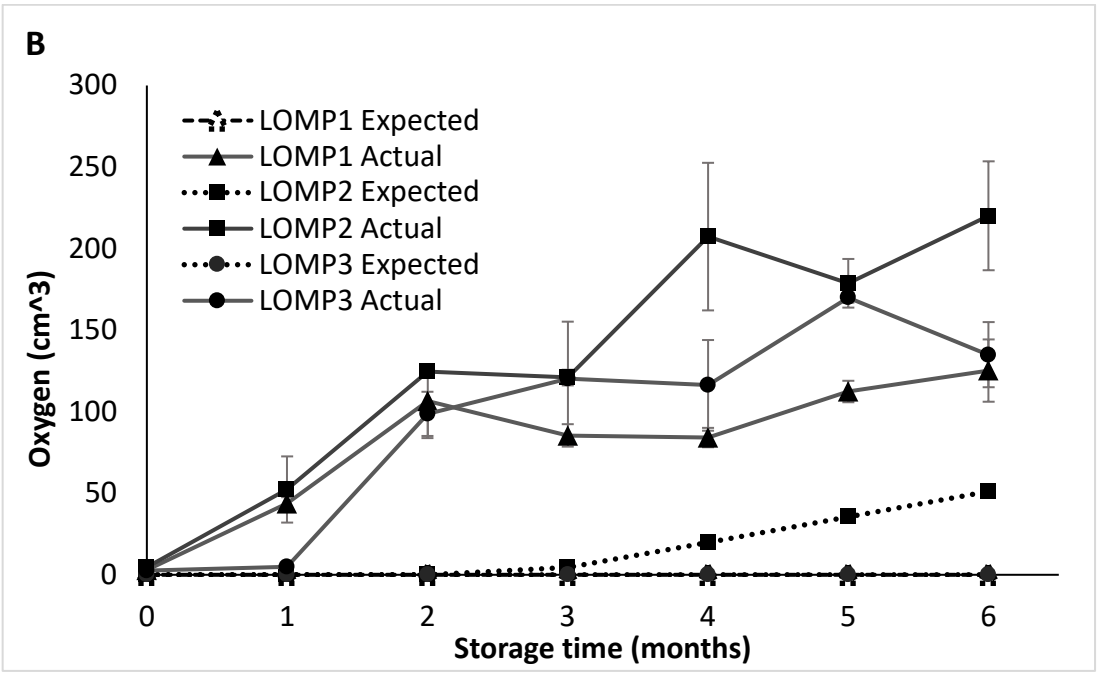
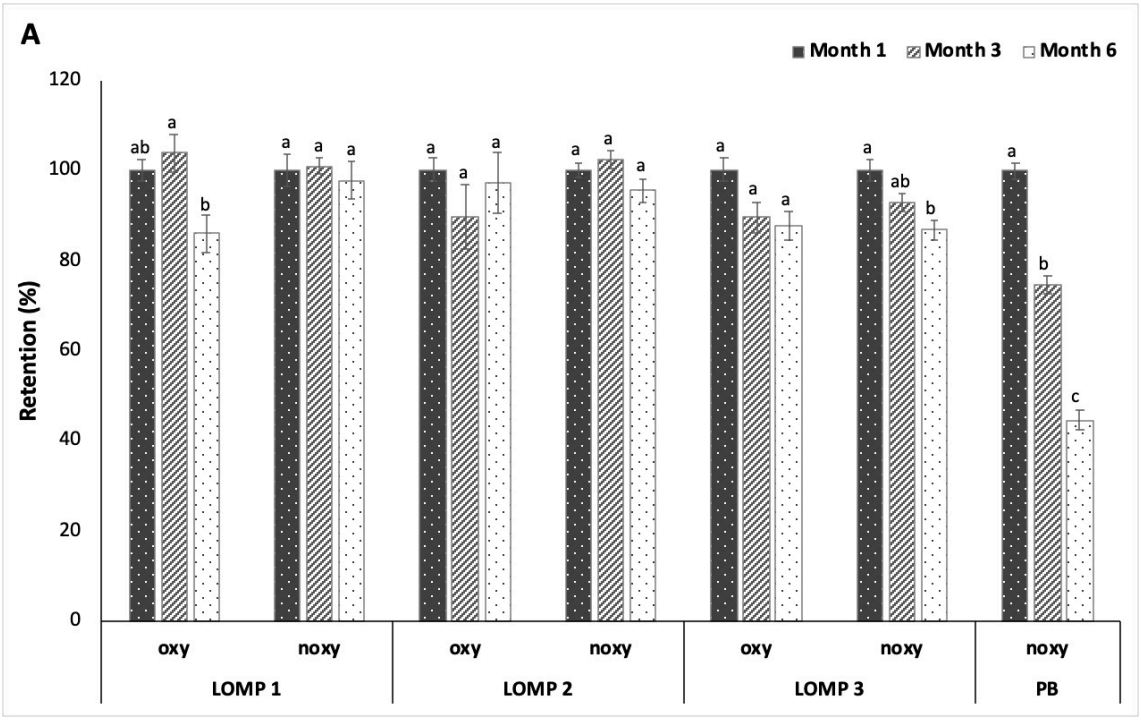
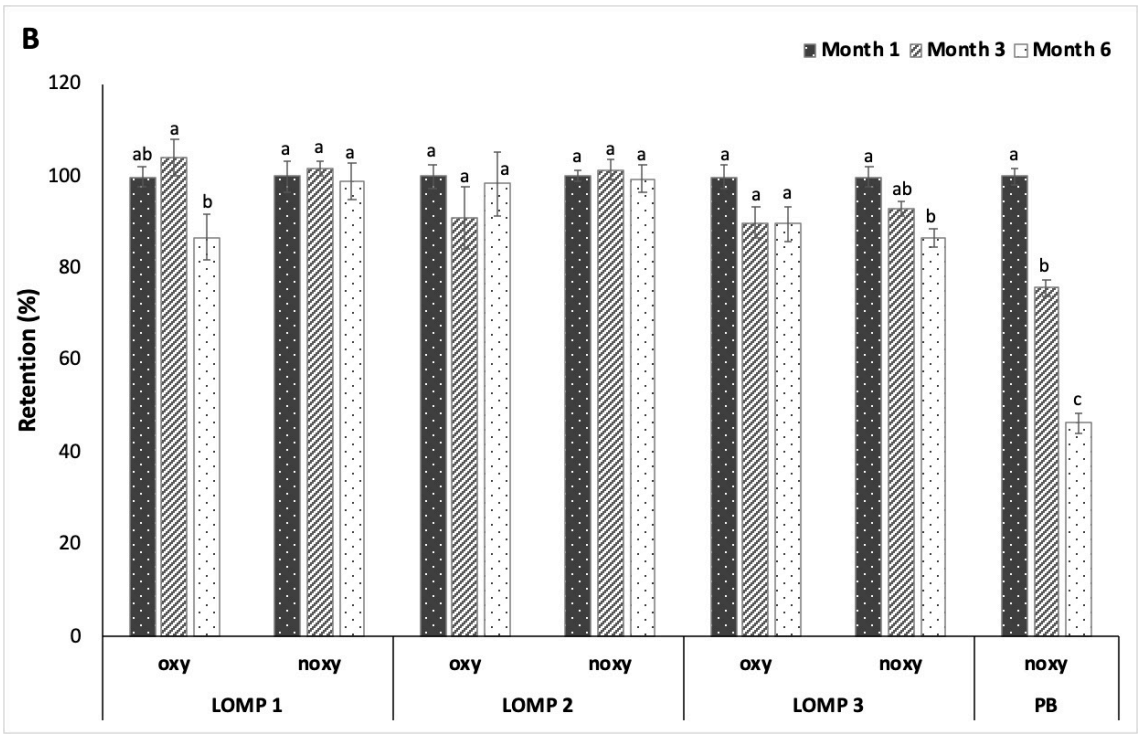


Figure 2. Retention (%) of xanthophylls (**A**) and total carotenoids (**B**) in orange corn grits during six months of storage packaged in paper pouches and low-oxygen and low-moisture permeable (LOMP) pouches with (LOMP-oxy) and without (LOMP-noxy) an oxygen scavenger.





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