Indian Journal of Experimental Biology
Vol. 59, February 2021, pp. 79-87

# Time course evaluation of provitamin A carotenoids stored under different storage regimens in maize

Harmanjot Kaur[1], Abhijit Kumar Das[2], Mehak Sethi[1], Mukesh Choudhary[2], Sujay Rakshit[2] & Dharam Paul Chaudhary[2]*

[1]Department of Biochemistry, Punjab Agricultural University, Ludhiana-141 004, Punjab, India

[2]ICAR-Indian Institute of Maize Research, Ludhiana-141 004, Punjab, India

*Received 07 February 2020*; *revised 29 July 2020*

Yellow maize is natural source of provitamin A components. However, the provitamin A carotenoids are known to degrade fast as a result of oxidation and isomerization due to exposure to heat and oxygen during storage. Keeping this in view, here, we evaluated the provitamin A carotenoids in maize stored under different storage conditions. For this purpose, $F_2$ grains of six hybrids consisting of two provitamin A rich, two QPM and two normal maize were stored in earthen pot, aluminium box, cotton cloth and jute bag for a period of 6 months under ambient temperature and carotenoid components were estimated at monthly interval. Provitamin A components are found to reduce significantly within two to six months under various storage conditions. However, the samples stored in aluminium box exhibited least degradation of β-carotene (73%) and β-cryptoxanthin (81%), whereas those stored in earthen pot exhibited highest degradation of β-carotene (86%) and β-cryptoxanthin (90%), after six months of storage. The provitamin A rich hybrids especially APH27 retained highest concentration of provitamin A carotenoids after six months of storage. The least losses observed in the samples stored in aluminium box may be attributed to reduced oxidation and least light penetration.

**Keywords**: β-Carotene, β-Cryptoxanthin, Micronutrient malnutrition, Storage conditions, Yellow maize, *Zea mays*

Malnutrition has turned out to be a leading health problem affecting millions of people worldwide[1]. One out of every nine individuals in the world is suffering from malnutrition with majority of them residing in the developing nations[2]. Micronutrient malnutrition is most prevalent form of malnutrition and it is estimated that one in three persons or more than two billion individuals, globally are suffering from micronutrient deficiencies. Vitamin A plays a vital role in vision, and its deficiency causes night blindness and partial or even complete loss of eyesight in humans[3]. About 500 μg $day^{-1}$ of vitamin A is required for adult women, whereas 275 μg $day^{-1}$ of vitamin A is required for children of 4-6 years age[4]. Vitamin A deficiency (VAD) has affected about 19 million pregnant women and 190 million preschool-aged children, mostly in Southeast Asia and Africa and accounts for 70% of childhood deaths worldwide[5-6]. Keeping in view the severity of deficiency, around 255 million children were provided with two doses of vitamin A supplement (UNICEF 2018)[7]. The magnitude of clinical and subclinical VAD among young children in India is higher than the neighbouring countries in the Southeast Asia[8]. Most of the malnourished people are the ones who cannot pay for high-quality and nutrient rich foods. Studies from developing regions advise that up to 80% of the nutritional intake of vitamin A comes from food sources which are provitamin A rich. Poverty abolishment and universal food security has been the targets of world leader, specifically battling the menace of hidden hunger. Since a significant segment of human population depends upon cereals for deriving all of their nutritional requirements, the biofortification of staple crops is found to be the most sustainable and cost effective approach to overcome the micronutrient malnutrition, particularly VAD in humans[9]. Diversification, fortification and supplementation of diets with nutrients have been attempted to address the problem of malnutrition, though with little success. Fortified food products or commercially available supplements with higher costs are beyond the reach of heaps of low-wage workers in the developing countries.

Maize (*Zea Mays* L.) is the second most significant cereal crop in terms of acreage. It is reported that maize contributes 15-56% of the total daily calories and 15% of world's protein in the diet of

* Correspondence:
Phone: +91 8728900427 (Mob.), Fax: +91 161 2430038
E-Mail: chaudharydp@gmail.com

people in about 25 different developing countries[10]. Yellow maize naturally accumulates provitamin A carotenoids, including α-carotene, β-carotene and β-cryptoxanthin which can be metabolically converted to active vitamin A in the human body[11]. As human body cannot produce vitamin A, it must be obtained from diet via carotenoids. As maize is widely consumed as food in the developing world it is considered to be one of the most suitable crops for biofortification.

In the last few years, some biofortified provitamin A rich maize genotypes have been developed possesses around 10-15 µg/g of provitamin A components. However, many research reports have indicated that provitamin A in cereals is highly unstable and gets degraded within 2-6 months after harvest[12-13]. This is because carotenoids are prone to isomerization and oxidation. In case of isomerization, trans-carotenoids get converted to *cis*-carotenoids. This happens due to exposure to heat, light and contact with acids. Oxidation involves initially epoxidation, followed by formation of apo-carotenoids and hydroxylation[14]. However, the extent and rate of degradation of provitamin A carotenoids is not well understood. Moreover, to overcome this problem, an appropriate storage technology is needed to reduce the storage losses in maize carotenoids. In this context, in the present study, tried to understand the percentage loss of provitamin A components in maize stored under different storage conditions for different intervals of time.

## Materials and Methods

### Seed material

A panel of six maize hybrids consisting of two provitamin A bio-fortified hybrid (PUSA VIVEK QPM9 and APH27), two hybrids (PUSA HM4 and PUSA HM8) bio-fortified with lysine and tryptophan (Quality Protein Maize) and two normal maize hybrids (PMH1 and CMH-08-292) were selected. Selected genotypes are the high yielding hybrids released under All India Coordinated Research Project (AICRP) on Maize. Variation for kernel colour (yellow, dark yellow and orange) was evident in the experimental material. Genotypes were sown in the experimental field of ICAR-Indian Institute of Maize Research (IIMR), Ludhiana in the plot size of 42 $m^2$ during rainy season of 2017. Sufficient numbers of ear were self-pollinated to produce required quantity of $F_2$ grains in order to get pure sample of the experimental genotypes. The grain samples were dried in shade and processed for the estimation of total carotenoids and provitamin A components (β-carotene and β-cryptoxanthin).

### Experimental design

Ten kilograms of the grain samples were stored under four different storage conditions, *viz*., earthen pot, aluminium box, cotton cloth and jute bag for a period of six months. Required quantity of samples were taken from each of the storage material after 30, 60, 90, 120, 150 and 180 days of storage and total carotenoids and the provitamin A components *i.e.* β-carotene and β-cryptoxanthin were estimated.

### Extraction and estimation of Total carotenoids

Samples were analysed in duplicate as per the method described in Rivera and Canela[15]. For this purpose, the samples were ground to very fine powder using grinding mill. Fine ground seed powder of 0.5 g was quantified and transferred to 15 mL falcon tube to which 6ml of Ethanol: BHT solution was added. After thorough vortexing, the samples were incubated at 85°C for six min in a water bath followed by addition of 120 µL of KOH. The reaction was incubated for 10 min at 85°C with intermittent vortexing. The samples were cooled in ice for one hour and 4 mL of distilled water was added followed by 3 mL of PE:DE (2:1, v/v). This was followed by centrifugation at 3700 rpm for 10 min. The upper phase of PE:DE containing carotenoid was transferred to fresh 15 mL Falcon tubes and the extraction was repeated twice with 3 mL of PE:DE mixture. The organic phases were collected in the new tube and the volume was made up to 10 mL with PE:DE and the samples were mixed. The optical density was taken at 450nm in a spectrophotometer with the suitable blank containing PE:DE (2:1, v/v).

### Analysis of provitaminA components by UPLC

Very fine column material, high pressure and differentially proportioned solvent system in ultra-performance liquid chromatography (UPLC) lead to separation of individual fraction of carotenoid, which is detected by the photo diode array (PDA) 2996 detector. UPLC-PDA analysis was performed using ACQUITY Ultra performance LC™ system connected to a PDA detector (Waters, milford, MA, USA). MassLynx™ Software version 4.1 (Waters) was used to run the instrument, and for data acquisition and processing. UPLC chromatographic separation was performed on a reverse phase column

ACQUITY UPLC$^R$ BEH 130A° C18, 1.7 µm, 2.1 × 100 mm (Waters) and a gradient system with the mobile consisting of solvent A [ACN: Methanol] (7:3, v/v) and solvent B [$H_2O$ 100%]. Column and sample temperature were set at 32°C and 25°C, respectively. Before utilization, all solutions were passed through Millex 0.2 µm nylon membrane syringe filters (Millipore, Bedford, MA, USA). Injection volume of 5 µL was fixed. The concentration of β-carotene (BC) as well as β-cryptoxanthin (BCX) are analysed using UPLC as per the method described earlier[16]. Solvent Gradient was used in the chromatographic separation of carotenoids. Gradient procedures were: 0-2 min linear gradient 85% A:15%B, 2-11.6 min linear gradient 100% A, 11.6-15 min linear gradient 85% A:15%B. The standards of BC and BCX (Sigma Aldrich, USA) were used to make the regression curves and estimate the components. UPLC chromatogram of standard solution of BCX and BC are presented in Fig. 1. The proA concentration (µg/g on dry wt. basis) was estimated as sum of BC plus half the BCX concentration[17].

**Statistical analysis**

Correlation analysis was performed using SPSS (Version 16.0) software. Factorial analysis was also performed using SPSS (Version 16.0) software taking genotypes (level = 6), storage method (level=4) and storage duration (level=6) as factors and interactions (two and three factor) among the factors were also calculated.

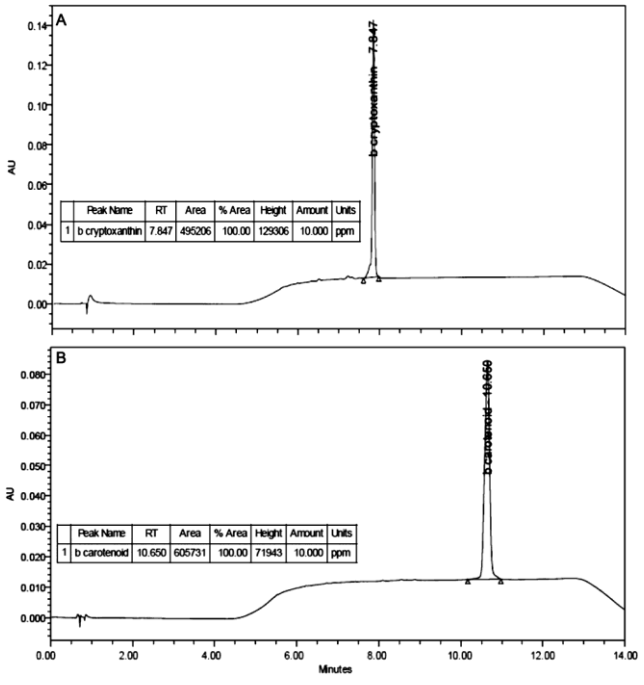


Fig. 1 — UPLC chromatogram of standard solution of (A) β-cryptoxanthin; and (B) β-carotene.

## Results

Total carotenoids and provitamin A components were analysed in the freshly harvested and stored grains for a period of six months at monthly interval to estimate the losses of carotenoids over time and under different storage condition.

**Variation among kernel carotenoid in different maize hybrids at harvest**

The concentration of total carotenoids, β-carotene and β-cryptoxanthin of the experimental hybrids is presented in Table 1. Highest concentration of total carotenoids was recorded by APH27 (44.68 µg/g) followed by PUSA HM8 (42.36 µg/g), CMH-08-292 (40.59 µg/g), PMH1 (37.82 µg/g), PUSA Vivek QPM9 (35.68 µg/g) and PUSA HM4 (32.89 µg/g). Bio-fortified hybrids, viz., APH27 and PUSA Vivek QPM9 exhibited highest amount of β-carotene (6.51 and 4.38 µg/g, respectively) as well as β-cryptoxanthin (13.37 and10.9 µg/g, respectively).

β-carotene content of PUSA HM8, CMH-08-292, PUSA HM4 and PMH1 were 4.46, 4.04, 4.29 and 3.57 µg/g, respectively whereas, the β-cryptoxanthin content of PUSA HM8, CMH-08-292, PUSA HM4 and PMH1 were 2.90, 2.83, 2.29 and 2.17 µg/g respectively. The concentration of β-cryptoxanthin was found to be approximately half the amount of β-carotene.

**Interaction effect among genotypes, duration of storage and method of storage**

Analysis of variance (ANOVA) and factorial analysis was performed to find out the significance of different factors *viz*., genotypes, duration of storage and method of storage and their interaction on retention of different carotenoid components (Table 2). Genotype and duration of storage significantly affected β-carotene, β-cryptoxanthin and total carotenoid retention, whereas storage method affected only the retention of β-cryptoxanthin and total carotenoid. Further, all the two factor and three factor interactions were significant for β-cryptoxanthin and total carotenoid, while only genotype × storage two factor interaction was significant for β-carotene.

**Rate of reduction of carotenoid components under different storage condition**

Although total carotenoids are important for various health functions, provitamin A components

Table 1 — Time course evaluation of carotenoids under different storage conditions

| Genotype | Storage Condition | Components | Freshly harvested | 30 days | 60 days | 90 days | 120 days | 150 days | 180 days |
|---|---|---|---|---|---|---|---|---|---|
| PUSA HM4 | Earthen Pot | β-car | 4.29 | 3.59 | 2.71 | 2.20 | 0.95 | 0.33 | 0.11 |
| | | β-cryp | 2.29 | 1.86 | 1.27 | 0.86 | 0.33 | 0.10 | 0.01 |
| | | TC | 32.89 | 31.22 | 30.76 | 28.65 | 26.40 | 24.16 | 23.28 |
| | Aluminium Box | β-car | 4.29 | 4.03 | 3.58 | 2.80 | 1.97 | 1.32 | 1.00 |
| | | β-cryp | 2.29 | 2.01 | 1.57 | 1.01 | 0.77 | 0.24 | 0.07 |
| | | TC | 32.89 | 32.51 | 32.26 | 31.89 | 29.20 | 26.89 | 25.68 |
| | Cotton Cloth | β-car | 4.29 | 3.80 | 3.01 | 2.59 | 1.87 | 0.82 | 0.53 |
| | | β-cryp | 2.29 | 1.97 | 1.43 | 1.00 | 0.44 | 0.14 | 0.03 |
| | | TC | 32.89 | 31.91 | 31.65 | 30.65 | 28.28 | 26.41 | 25.12 |
| | Jute Bag | β-car | 4.29 | 3.85 | 2.84 | 2.35 | 1.51 | 0.69 | 0.39 |
| | | β-cryp | 2.29 | 1.87 | 1.34 | 0.91 | 0.34 | 0.12 | 0.02 |
| | | TC | 32.89 | 31.90 | 31.55 | 29.64 | 28.20 | 28.00 | 25.60 |
| PUSA HM8 | Earthen Pot | β-car | 4.46 | 3.82 | 3.24 | 2.74 | 1.83 | 1.30 | 0.39 |
| | | β-cryp | 2.90 | 2.36 | 1.84 | 1.46 | 0.87 | 0.43 | 0.23 |
| | | TC | 42.36 | 41.23 | 40.23 | 38.66 | 36.88 | 33.26 | 31.60 |
| | Aluminium Box | β-car | 4.46 | 4.07 | 3.81 | 3.24 | 2.68 | 2.17 | 1.82 |
| | | β-cryp | 2.90 | 2.57 | 2.01 | 1.92 | 1.33 | 1.02 | 0.55 |
| | | TC | 42.36 | 41.96 | 41.55 | 40.63 | 38.50 | 35.84 | 33.52 |
| | Cotton Cloth | β-car | 4.46 | 4.00 | 3.46 | 2.85 | 2.17 | 1.82 | 0.87 |
| | | β-cryp | 2.90 | 2.45 | 1.99 | 1.85 | 1.13 | 0.88 | 0.46 |
| | | TC | 42.36 | 41.66 | 40.96 | 39.88 | 37.60 | 34.87 | 32.62 |
| | Jute Bag | β-car | 4.46 | 3.87 | 3.39 | 2.77 | 2.04 | 1.79 | 0.68 |
| | | β-cryp | 2.90 | 2.38 | 1.96 | 1.52 | 0.94 | 0.76 | 0.38 |
| | | TC | 42.36 | 41.65 | 40.59 | 39.20 | 37.10 | 34.20 | 32.48 |
| PUSA VIVEK QPM9 | Earthen Pot | β-car | 10.90 | 10.24 | 9.63 | 7.73 | 6.23 | 4.14 | 2.10 |
| | | β-cryp | 4.38 | 3.69 | 2.95 | 2.39 | 1.77 | 1.21 | 0.80 |
| | | TC | 35.68 | 34.85 | 33.50 | 31.28 | 30.58 | 28.66 | 27.12 |
| | Aluminium Box | β-car | 10.90 | 10.47 | 9.89 | 8.27 | 7.12 | 5.29 | 3.45 |
| | | β-cryp | 4.38 | 3.82 | 3.36 | 2.97 | 2.37 | 1.86 | 1.26 |
| | | TC | 35.68 | 35.26 | 34.45 | 32.09 | 31.66 | 29.69 | 28.33 |
| | Cotton Cloth | β-car | 10.90 | 10.37 | 9.80 | 7.93 | 6.60 | 4.90 | 2.25 |
| | | β-cryp | 4.38 | 2.45 | 1.99 | 1.85 | 1.13 | 0.88 | 0.46 |
| | | TC | 35.68 | 35.12 | 33.82 | 31.69 | 31.12 | 29.35 | 27.82 |
| | Jute Bag | β-car | 10.90 | 10.29 | 9.67 | 7.79 | 6.43 | 4.71 | 2.15 |
| | | β-cryp | 4.38 | 2.38 | 1.96 | 1.52 | 0.94 | 0.76 | 0.38 |
| | | TC | 35.68 | 35.00 | 33.69 | 31.42 | 31.08 | 29.20 | 27.62 |
| APH27 | Earthen Pot | β-car | 13.37 | 12.65 | 11.93 | 9.51 | 7.06 | 5.25 | 3.33 |
| | | β-cryp | 6.51 | 5.81 | 5.24 | 4.87 | 4.26 | 2.73 | 1.57 |
| | | TC | 44.68 | 42.22 | 40.36 | 37.36 | 36.66 | 35.22 | 33.29 |
| | Aluminium Box | β-car | 13.37 | 12.92 | 12.38 | 10.35 | 8.85 | 6.06 | 4.15 |
| | | β-cryp | 6.51 | 6.02 | 5.87 | 5.28 | 4.87 | 3.13 | 2.76 |
| | | TC | 44.68 | 43.62 | 41.66 | 38.82 | 37.55 | 36.82 | 34.72 |
| | Cotton Cloth | β-car | 13.37 | 12.87 | 12.02 | 9.98 | 7.65 | 5.63 | 3.64 |
| | | β-cryp | 6.51 | 5.94 | 5.50 | 5.02 | 4.57 | 2.92 | 2.14 |
| | | TC | 44.68 | 43.12 | 41.20 | 38.58 | 37.10 | 36.50 | 34.32 |
| | Jute Bag | β-car | 13.37 | 12.79 | 11.96 | 9.90 | 7.48 | 5.46 | 3.48 |
| | | β-cryp | 6.51 | 5.82 | 5.34 | 4.93 | 4.49 | 2.89 | 1.95 |
| | | TC | 44.68 | 42.82 | 40.76 | 38.25 | 36.80 | 35.79 | 33.69 |
| PMH1 | Earthen Pot | β-car | 3.57 | 3.00 | 2.62 | 2.13 | 1.71 | 1.11 | 0.52 |
| | | β-cryp | 2.17 | 1.87 | 1.57 | 1.16 | 0.74 | 0.21 | 0.08 |
| | | TC | 37.82 | 36.22 | 35.39 | 33.09 | 30.64 | 29.22 | 27.52 |
| | Aluminium Box | β-car | 3.57 | 3.19 | 2.83 | 2.36 | 1.95 | 1.39 | 0.82 |
| | | β-cryp | 2.17 | 2.01 | 1.76 | 1.52 | 0.97 | 0.41 | 0.13 |
| | | TC | 37.82 | 37.32 | 36.45 | 34.22 | 31.08 | 30.12 | 29.36 |

(Contd.)

Table 1 — Time course evaluation of carotenoids under different storage conditions (*Contd*.)

| Genotype | Storage Condition | Components | Freshly harvested | 30 days | 60 days | 90 days | 120 days | 150 days | 180 days |
|---|---|---|---|---|---|---|---|---|---|
| CMH-08-292 | Cotton Cloth | β-car | 3.57 | 3.04 | 2.69 | 2.30 | 1.85 | 1.23 | 0.67 |
| | | β-cryp | 2.17 | 1.93 | 1.66 | 1.42 | 0.96 | 0.30 | 0.11 |
| | | TC | 37.82 | 37.11 | 36.20 | 33.85 | 30.91 | 29.83 | 29.11 |
| | Jute Bag | β-car | 3.57 | 3.03 | 2.63 | 2.26 | 1.77 | 1.17 | 0.60 |
| | | β-cryp | 2.17 | 1.91 | 1.64 | 1.20 | 0.85 | 0.22 | 0.09 |
| | | TC | 37.82 | 36.82 | 35.88 | 33.69 | 30.70 | 29.32 | 27.80 |
| | Earthen Pot | β-car | 4.04 | 3.58 | 2.99 | 2.37 | 1.85 | 1.16 | 0.59 |
| | | β-cryp | 2.83 | 2.42 | 1.97 | 1.42 | 1.00 | 0.43 | 0.11 |
| | | TC | 40.59 | 39.25 | 38.63 | 36.25 | 35.16 | 33.20 | 31.44 |
| | Aluminium Box | β-car | 4.04 | 3.90 | 3.24 | 2.85 | 2.25 | 1.90 | 1.12 |
| | | β-cryp | 2.83 | 2.56 | 2.11 | 1.85 | 1.37 | 0.89 | 0.37 |
| | | TC | 40.59 | 40.20 | 39.08 | 37.80 | 36.40 | 34.52 | 32.85 |
| | Cotton Cloth | β-car | 4.04 | 3.65 | 3.08 | 2.63 | 2.08 | 1.75 | 0.96 |
| | | β-cryp | 2.83 | 2.46 | 2.01 | 1.76 | 1.23 | 0.72 | 0.22 |
| | | TC | 40.59 | 39.84 | 38.96 | 37.20 | 35.84 | 34.01 | 31.81 |
| | Jute Bag | β-car | 4.04 | 3.65 | 3.05 | 2.50 | 1.91 | 1.67 | 0.83 |
| | | β-cryp | 2.83 | 2.43 | 2.00 | 1.56 | 1.20 | 0.70 | 0.19 |
| | | TC | 40.59 | 39.60 | 38.70 | 36.80 | 35.30 | 33.85 | 31.50 |

[TC: Total carotenoid; β-car: β-carotene; β-cryp: β-cryptoxanthin]

Table 2 — Analysis of variance for main effects and 2-factor and 3-factor interactions

| Source of variation | DF | β-carotene | | β-cryptoxanthin | | Total carotenoids | |
|---|---|---|---|---|---|---|---|
| | | Mean Square | F | Mean Square | F | Mean Square | F |
| Replication | 2 | 0.921 | 0.503$^{ns}$ | 0.075 | 4.087$^{*}$ | 1.099 | 11.268$^{*}$ |
| Genotype | 5 | 615.994 | 336.364$^{*}$ | 112.730 | 6177.087$^{*}$ | 1065.181 | 10916.819$^{*}$ |
| Storage duration | 5 | 244.919 | 133.738$^{*}$ | 54.163 | 2967.879$^{*}$ | 646.057 | 6621.303$^{*}$ |
| Storage method | 3 | 4.526 | 2.471$^{NS}$ | 3.065 | 167.947$^{*}$ | 34.686 | 355.495$^{*}$ |
| Genotype × Storage duration | 25 | 16.464 | 8.990$^{*}$ | 1.192 | 65.335$^{*}$ | 3.309 | 33.914$^{*}$ |
| Duration × Storage method | 15 | 2.241 | 1.224$^{ns}$ | 0.083 | 4.528$^{*}$ | 0.773 | 7.927$^{*}$ |
| Genotype ×Storage method | 15 | 1.891 | 1.032$^{ns}$ | 0.819 | 44.887$^{*}$ | 1.536 | 15.742$^{*}$ |
| Genotype × Storage duration ×Storage method | 75 | 1.902 | 1.039$^{ns}$ | 0.045 | 2.441$^{*}$ | 0.335 | 3.431$^{*}$ |
| Error | 286 | 1.831 | - | 0.018 | - | 0.098 | - |

[*Significant at p value 0.01, **Significant at p value 0.05, and $^{ns}$Not significant]

β-carotene and β-cryptoxanthin, play an important role in human health, particularly in vision and reproduction. Rate of reduction (%) of β-carotene and β-cryptoxanthin in each of the storage material was calculated considering the value of previous month of storage as the initial value for next month and same pattern was followed for consecutive reading. In earthen pot, reduction of β-carotene and β-cryptoxanthin varied from 9.23 (30 days) to 47.03% (180 days) and 14.56 (30 days) to 45.21% (180 days), respectively whereas reduction in β-carotene and β-cryptoxanthin in aluminium box was 5.05 (30 days) to 31.83% (180 days) and 9.91 (30 days) to 31.92% (180 days), respectively. Reduction in β-carotene content in cotton cloth and jute bag ranged from 7.14 (30 days) to 44.77% (180 days) and 7.75 (30 days) to 47.51% (180 days), respectively, whereas the same for β-cryptoxanthin was 11.52 (90 days) to 41.44% (180 days) and 15.19 (60 days) to 44.77% (180 days). Across the storage condition, the reduction in β-carotene was 7.29 (30 days) to 42.78% (180 days) whereas, 15.05 (60 days) to 40.83% (180 days) in β-cryptoxanthin.

Linear regression analysis for reduction in β-carotene and β-cryptoxanthin content in different storage materials with number of days was performed and their slopes were compared to determine the rates of degradation in β-carotene and β-cryptoxanthin (Fig. 2). Under each of the storage condition significant reduction in β-carotene and β-cryptoxanthin was evident. Coefficient of determination for β-carotene and β-cryptoxanthin varied from 0.89 (jute bag) to 0.96 (aluminium box) and 0.74 (cotton cloth) to 0.87 (earthen pot), respectively. Highest reduction of both β-carotene and β-cryptoxanthin was observed in earthen pot (Slope: 0.25 and 0.23, respectively) whereas least was observed in aluminium box (Slope: 0.18 and 0.18,

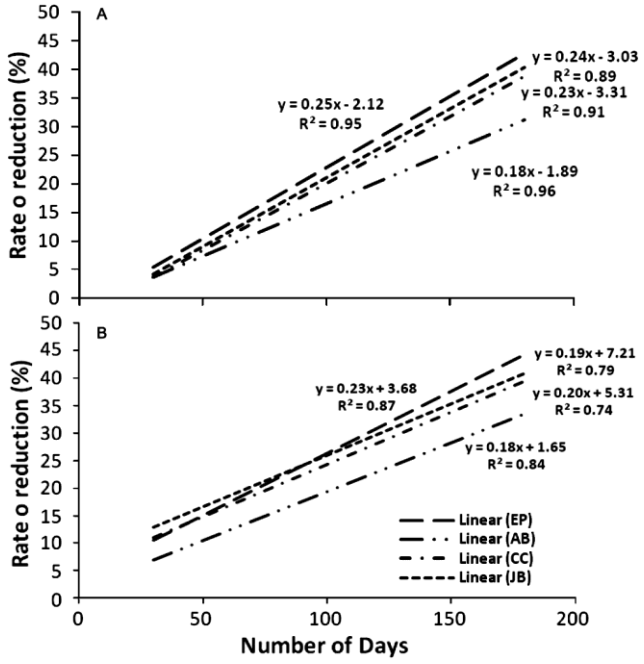


Fig. 2 — Rate of reduction of (A) β-carotene; and (B) β-cryptoxanthin under different storage condition.

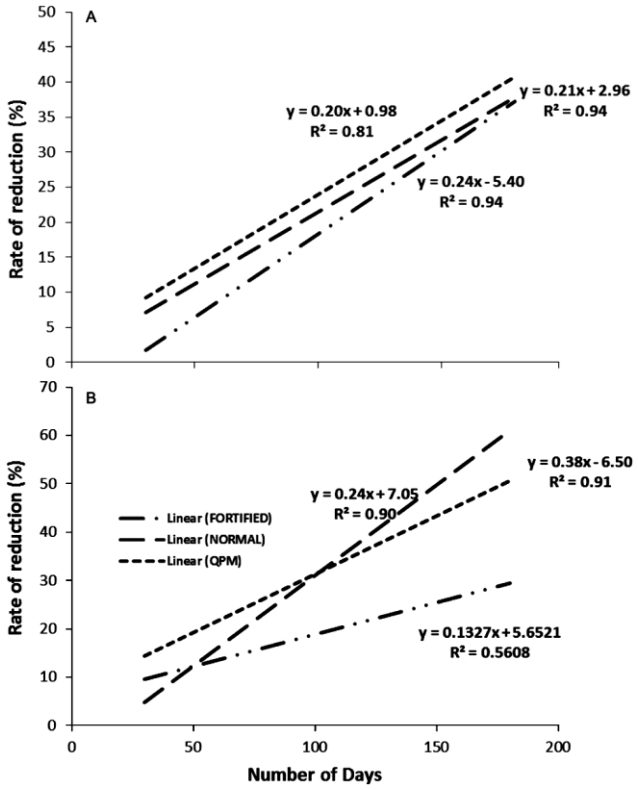


Fig. 3 — Rate of reduction of (A) β-carotene; and (B) β-cryptoxanthinin bio-fortified, normal and QPM hybrids.

respectively). Increased rate of degradation with days to storage was observed for both β-carotene and β-cryptoxanthin under each of the storage condition. In aluminium box, lowest degradation for β-carotene was observed in initial 60 days and the same for β-cryptoxanthin was in initial 90 days.

**Rate of reduction of carotenoid among different hybrids**

Reduction trend of carotenoid components in bio-fortified, QPM and normal maize hybrids were analysed separately. The % loss of β-carotene increased over time across the three groups [bio-fortified: 4.61(30 days) to 40.76% (180 days), Normal: 11.17 (30 days) to 46.31% (180 days) and QPM: 11.34 (30 days) to 43.46% (90 days)]. However, minor reduction of β-carotene was observed in bio-fortified group for initial 60 days [4.61% (30 days) and 5.75% (60 days)] compared to normal and QPM hybrids [Normal: 11.17% (30 days) and 14.46% (60 days); QPM: 11.34% (30 days) and 16.08% (60 days)]. Similar trend was also observed for β-cryptoxanthin in normal [12.05% (30 days) and 66.49% (60 days)] and QPM hybrids [15.85% (30 days) and 52.57% (60 days)], however minimum rate of reduction in bio-fortified hybrids was observed at 30 days (10.35 %) followed by 90 days (10.49%) and maximum was at 150 days (32.87%). Overall rate of reduction of β-carotene was 19.45, 22.47 and 25.04% and β-cryptoxanthin was 19.58, 32.91 and 32.46% in bio-fortified, normal and QPM hybrids, respectively.

Linear regression analysis for reduction in β-carotene and β-cryptoxanthin content was also performed to identify the suitable hybrids with minimum reduction rate (Fig. 3). For β-cryptoxanthin, highest and lowest reduction rate was observed in normal (Slope: 0.38) and bio-fortified (Slope: 0.13) hybrids, respectively. However, rate of reduction for β-carotene was exceptionally high in bio-fortified hybrids (Slope: 0.24) than normal (Slope: 0.21) and QPM hybrids (Slope: 0.20). Even with highest rate of degradation bio-fortified hybrids retained maximum β-carotene after storage period which is four times higher than normal and 4.3 times higher than QPM. In bio-fortified hybrids also the least reduction for β-carotene was observed in initial 60 days whereas for β-cryptoxanthin the same was initial 90 days.

**Retention of carotenoids after storage period**

After six month of storage, PUSA HM4 (earthern pot 23.28 µg/g, aluminium box: 25.68 µg/g, cotton

Table 3 — Correlation of rate of reduction among carotenoid components under different storage condition

| | Storage conditions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EP | | | AB | | | CC | | | JB | | |
| | TC | β-car | β-cryp | TC | β-car | β-cryp | TC | β-car | β-cryp | TC | β-car | β-cryp |
| TC | 1 | 0.55 | 0.54 | 1 | 0.84** | 0.73 | 1 | 0.78 | 0.57 | 1 | 0.83** | 0.61 |
| β-car | | 1 | 0.93** | | 1 | 0.92* | | 1 | 0.86** | | 1 | 0.91* |
| β-cryp | | | 1 | | | 1 | | | 1 | | | 1 |

[TC: Total carotenoid; β-car: β-carotene; β-cryp: β-cryptoxanthin, EP: Earthern pot; AB: Aluminium box; CC: Cotton cloth; JB: Jute bags; *Significant at p value 0.01, **Significant at p value 0.05]

Table 4 — Correlation of rate of reduction among carotenoid components in different hybrids

| | Fortified | | | Normal | | | QPM | | |
|---|---|---|---|---|---|---|---|---|---|
| | TC | β-car | β-cryp | TC | β-car | β-cryp | TC | β-car | β-cryp |
| TC | 1 | 0.42 | 0.04 | 1 | 0.45 | 0.46 | 1 | 0.82** | 0.82** |
| β-car | | 1 | 0.77 | | 1 | 0.94* | | 1 | 0.99* |
| β-cryp | | | 1 | | | 1 | | | 1 |

[TC: Total carotenoid; β-car: β-carotene; β-cryp: β-cryptoxanthin; *Significant at p value 0.01, **Significant at p value 0.05]

cloth: 25.12 µg/g and jute bags:25.6 µg/g) retained maximum total carotenoids followed by APH27, PUSA HM8, CMH-08-292, PMH1 and Pusa Vivek QPM 9 irrespective of storage condition. Least degradation for β-carotene (earthern pot: 3.33 µg/g, aluminium box: 4.15 µg/g, cotton cloth: 3.64 µg/g and jute bags:3.48 µg/g) and β-cryptoxanthin (earthern pot:1.57 µg/g, aluminium box: 2.76 µg/g, cotton cloth: 2.14 µg/g and jute bags: 1.95 µg/g) was observed in APH27 followed by PUSA Vivek QPM9. However, lowest value for β-carotene and β-cryptoxanthin was recorded by PUSA HM4 despite of showing highest total tocopherol.

#### Stability of β-carotene vs. β-cryptoxanthin

To find out which of the two provitamin A components *viz*. β-carotene and β-cryptoxanthin is more stable, retention of these two components was also compared in bio-fortified, QPM and normal hybrids separately. In normal hybrids after the storage period retention of β-carotene and β-cryptoxanthin was 20.07 and 6.50%, respectively, whereas in QPM the corresponding values were 16.54 and 8.43%. However, in bio-fortified hybrids retention of β-carotene and β-cryptoxanthin was in similar range between 25.29 and 25.99%, respectively.

#### Correlation among carotenoid components for their rate of reduction over time

Degradation of total carotenoid was associated with reduction of β-carotene only when the hybrids were stored in aluminium box (0.84; $p$=0.05) and jute bags (0.83; $p$=0.05), however association lacked for total carotenoid with β-cryptoxanthin in any of the storage condition. On the contrary, reduction of β-carotene and β-cryptoxanthin was correlated with each other in each of the storage condition (EP: 0.93; $p$=0.05, AB: 0.92; $p$=0.01, CC: 0.86; $p$=0.05 and JB: 0.91; $p$=0.01) (Table 3). Correlation of rate of reduction of carotenoid components was also calculated in different form of hybrids *viz*. biofortified, normal and QPM. In QPM hybrids, degradation of total carotenoids was related with both β-carotene (0.82; $p$=0.05) and β-cryptoxanthin (0.82*; $p$=0.05). Degradation of β-carotene and β-cryptoxanthin were also correlated in QPM (0.99**; $p$=0.05) as well as in normal (0.94**; $p$=0.01) hybrids; however, in biofortified hybrids none of the components were associated with each other (Table 4).

## Discussion

Present investigation demonstrates the effects of various storage conditions on retention of carotenoid components in grains of different types of maize i.e. bio-fortified, normal and QPM. Earthen pot, aluminium box, cotton cloth and jute bag are routinely used to store food materials in common Indian households. Hence identification of suitable storage material which can retain more provitamin A has direct benefit to rural population.

Freshly harvested bio-fortified maize genotypes (APH27 and PUSA VIVEK QPM9) recorded higher β-carotene and β-cryptoxanthin than normal as well as QPM hybrids. During storage, least degradation of kernel β-carotene was observed in the bio-fortified maize grains which also had higher concentration of β-carotene in the freshly harvested stage. Similar to β-carotene, bio-fortified hybrids, viz., PUSA VIVEK QPM9 and APH27 also showed higher retention of β-cryptoxanthin than other experimental hybrids. Degradation of total carotenoid followed similar trend

irrespective of the type of hybrids under study which indicated the involvement of other carotenoid components in addition to β-carotene and β-cryptoxanthin. Exposure to air, light and heat causes isomerization and oxidation of carotenoids in foods. Loss during storage in maize was also reported to accelerate with increasing temperature and affected by genotype as the rate of carotenoid degradation in maize grains stored at low temperature (4 and 22.5°C) was found to be significantly lower than that observed at high temperature (55°C)[18]. It has also been reported that the grains stored at high temperature lost almost all the provitamin A content after 27 months, whereas, grains stored at 4 and 22.5°C maintained around 20 to 40% of their initial provitamin A[18]. Similarly, rate of carotenoid degradation was significantly lower at low humidity than that observed at high humidity values[18]. Degradation of carotenoids occurs via two mechanisms called specific and non-specific. In specific mechanisms the carotenoid dioxygenases (enzymes that catalyse carotenoids to apocarotenoids) are involved, whereas, non-specific mechanisms include enzymatic and non-enzymatic oxidation[19]. The enzymatic oxidation is triggered by lipoxygenases that catalyse the hydro peroxidation of polyunsaturated fatty acids; the radicals produced during the intermediate steps of substrate hydro peroxidation can cause the oxidation of the carotenoid pigments. The non-enzymatic degradation is due the characteristic conjugate double-bond structure found in carotenoids, an electron-rich system susceptible to oxidizing agents thus leading to the generation of epoxy- and peroxyl-derivatives of carotenoids, which decompose into apocarotenoids[19].

Among different storage materials reduction rate of provitamin A i.e. β-carotene and β-cryptoxanthin was minimum in samples stored in aluminium box. Previous studies also reported significant variation in the retention of β-carotene between genotypes and across storage conditions[12]. Similar findings were reported in maize grains stored for 180 days in aluminium bags, Purdue Improved Crop Storage (PICS) bags, silo with candle, woven bags, silo without candle and ears in woven bags where retention of carotenoid in grains stored in aluminium bags with oxygen absorbers was the highest[13]. Limited oxygen content and non-penetration of lights inside the aluminium box could be attributed to lower degradation of β-carotene and β-cryptoxanthin in aluminium box.

β-carotene and β-cryptoxanthin were more stable in the initial 60 days and initial 90 days, respectively. It was reported that the levels of all the provitamin A components including β-carotene and β-cryptoxanthin decreased as the storage period increases[20]. Significant differences in total provitamin A retention were found between grain storage methods (48.1–57.2%) in maize after six months of storage[21].

β-carotene was found to be more stable than β-cryptoxanthin in QPM and normal hybrids whereas in bio-fortified hybrids the stability of both the components were in similar range. β-cryptoxanthin was reported to be more stable than β-carotene in grains and flour evaluated under different storage and the packaging conditions[13]. Further greater losses of carotenoids were observed in high β-carotene maize compared with high-xanthophyll maize at all the experimental storage conditions under different temperatures[12]. In light of this, it is feasible that maize grains fortified with higher proportions of β-cryptoxanthin compared to β-carotene could have higher impact on the alleviation of vitamin A deficiency. In contrast to present finding, degradation rate of β-cryptoxanthin was reported to be 51% lower than β-carotene during storage of orange maize grain and higher stability of β-cryptoxanthin than β-carotene has also been reported[13].

Association of degradation rate of β-carotene and β-cryptoxanthin was significant between normal and QPM hybrids whereas it was not related in bio-fortified hybrids. In carotenoid biosynthetic pathway, lycopene is converted to β-carotene with the help of enzyme *lycopene beta cyclase* (*lcyE*) and hydroxylation of β-carotene by *β-carotene hydroxylase1* (*crtRB1*) produce β-cryptoxanthin. Bio-fortified hybrids were developed through marker assisted introgression of mutant allele of *crtRB1* which stops the downward hydroxylation of β-carotene. Hence higher accumulation of β-carotene and lower conversion rate of β-carotene to β-cryptoxanthin in bio-fortified hybrids could be responsible for lack of association between them.

## Conclusion

The results suggest that although β-carotene and β-cryptoxanthin are unstable compounds, higher levels of them in the freshly harvested grains ensures availability of the provitamin A carotenoids to the end users. Hence, bio-fortification of maize grain to

enhance the kernel provitamin A level holds immense potential to eradicate malnutrition. Moreover, the grains should preferably be consumed within 2-3 months of harvesting to obtain maximum benefit of the biofortified grains. Further, in order to minimize the losses of these carotenoids, the grains should be stored in dark in aluminium box with tight lid so that minimum oxidation can take place.

**Acknowledgment**

The authors thank Indian Council of Agricultural Research, New Delhi for financial support and Indian Agricultural Research Institute, New Delhi, Indian Institute of Maize Research, Ludhiana and Punjab Agricultural University, Ludhiana for providing experimental lines.

**Conflict of interest**

Authors report no conflict of interest.

**References**

1 World Health Organization. Global nutrition report. 2018. Shining a light to spur action on nutrition.

2 FAO, IFAD, UNICEF, WFP, WHO, The state of food insecurity in the world 2019. Safeguarding against economic slowdowns and downturns.

3 Prasanna BM, Palacios-Rojas N, Hossain F, Muthusamy V, Menkir A, Dhliwayo T, Ndhlela T, Vicente FS, Nair SK, Vivek BS, Zhang X, Olsen M & Fan X, Molecular breeding for nutritionally enriched maize: status and prospects. *Front Genet*, 10 (2020) 1.

4 Anderson MS, Saltzman A, Virk PS & Pfeiffer WH, Progress update: Crop development of biofortified staple food crops under Harvetplus. *Afr J Food Agri Nutr Dev*,17 (2017) 11905.

5 World Health Organization report on Vitamin A supplementation in infants and children 6-59 months of age. 2017.

6 Bailey RL, West KP Jr. & Black RE, The epidemiology of global micronutrient deficiencies. *Ann Nutr Metab*, 66 (2015) 22.

7 UNICEF Annual Report 2018. For every child every right. (New York: UNICEF). https://www.unicef.org/media/55486/file/UNICEF-annual-report-2018%20revised%201.pdf, 16.

8 Vijayaraghavan K, National control programme against nutritional blindness due to vitamin A deficiency: Current status & future strategy. *Ind J Med Res*, 148 (2018) 496.

9 Govender L, Pillay K, Siwela M, Modi AT & Mabhaudhi T, Improving the dietary vitamin A content of rural communities in South Africa by replacing non-biofortified white maize and sweet potato with biofortified maize and sweet potato in traditional dishes. *Nutrients*, 11 (2019) 1198.

10 Tandzi LN, Mutengwa CS & Ngonkeu EL, Breeding for Quality Protein Maize (QPM) varieties: A review. *Agron J*, 80 (2017) 1.

11 Orlovskaya OA, Vakula SI, Khotyleva LV & Kilchevsky AV, Association between total carotenoid content of maize kernels (*Zea mays* L.) and polymorphic site INDEL1 in PSY1 gene. *Russ J Genet Appl Res* 8 (2018) 74. https://doi.org/10.1134/S2079059718010112.

12 Sowa M, Yu J, Palacios-Rojas N, Goltz SR, Howe JA, Davis CR, Rocheford T & Tanumihardjo SA, Retention of carotenoids in biofortified maize flour and β-cryptoxanthin-enhanced eggs after household cooking. *ACS Omega*, 10 (2017) 7320.

13 Taleon V, Mugode L, Cabrera-Soto L & Palacios-Rojas N, Carotenoid retention in biofortified maize using different post-harvest storage and packaging methods. *Food Chem*, 232 (2017) 60.

14 Rubio-Diaz DE, Santos A. Francis DM & Rodriguez-Saona LE, carotenoid stability during production and storage of tomato juice made from tomatoes with diverse pigment profiles measured by infrared spectroscopy. *Agric Food Chem*, 58 (2010) 8692.

15 Rivera S, Canela R, Influence of Sample Processing on the analysis of carotenoids in maize. *Molecules*, 17 (2012) 11255.

16 Rosales A, Agama-Acevedo E, Bello-Perez L, Gutiérrez-Dorado R & Palacios-Rojas N, Effect of traditional and extrusion nixtamalization on carotenoid retention in tortillas made from provitamin A-enriched maize (*Zea mays* L.). *J Agri Food Chem*, 64 (2016) 8289.

17 Zunjare RU, Hossain F, Muthusamy V, Baveja A, Chauhan HS, Bhat JS, Thirunavukkarasu N, Saha S & Gupta H. S. Development of biofortified maize hybrids through marker-assisted stacking of *β-carotene hydroxylase*, *lycopene-ε-cyclase* and *opaque2* genes. *Front Plant Sci*, 9 (2018) 178. doi: 10.3389/fpls.2018.00178.

18 Ortiz D, Rocheford T & Ferruzzi MG, Influence of temperature and humidity on the stability of carotenoids in biofortified maize (*Zea mays* L.) genotypes during controlled post-harvest storage. *J Agric Food Chem*, 64 (2016) 2727.

19 Trono D, Carotenoids in Cereal Food Crops: composition and retention throughout grain storage and food processing. *Plants*, 8 (2019) 551. doi:10.3390/plants8120551.

20 Awoyale W, Alamu EO, Irondi EZ, Maziya-Dixon B & Menkir A. Impact of packaging material and storage condition on retention of provitamin A carotenoids and xanthophylls in yellow-seeded maize flour. *Func Foods Health Dis*, 8 (2018) 462.

21 Taleon V, Mugode L, Cabrera-Soto L & Palacios-Rojas N. Carotenoid retention in biofortified maize using different post-harvest storage and packaging methods. *Food Chem*, 232 (2017) 60