

## **CAROTENOID ANALYSIS IN SEEDS OF MAIZE LOCAL VARIETIES FROM SOUTHERN BRAZIL BY LIQUID CHROMATOGRAPHY**

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The analysis of carotenoids in maize local varieties correlates to the economical importance of that cereal to small farmers in some Southern regions of Brazil, such as Santa Catarina State. Considering the fundamental relevance of to keep the genetic variability of this species, studies aiming at to add value to that biomass might stimulate the local farmers to continue the investment to preserve the maize germplasms. Carotenoids are among the pigments responsible for the maize's seed coloration and are well known by their pro-A vitamin activity, antioxidant properties and prevention of degenerative diseases. The aim of the present study was to determine the majoritary carotenoids in seed samples of local maize varieties cultured typically using agro-ecological management by small farmers, at far West region of Santa Catarina State, Brazil. For that, seeds of five local varieties were selected: Roxo do Valdecir (purple maize), Mato Grosso-Palha Roxa (red maize), MPA 01 (yellow maize), Rosado (light pink maize) and Branco (white maize). Carotenoids were extracted (1g - dry weight, 1h, R.T.) with hexane:acetone (v/v), containing butylated hydroxy-toluene (BHT - 100mg/L). The samples were vacuum-filtered to remove particles and the solvent was evaporated by using N<sub>2</sub> flow. The residue was dissolved in hexane and washed three times with distilled-deionized water. Analysis of the carotenoids was performed in RP-HPLC by injecting concentrated sample (10µL) into liquid chromatograph (Shimzadu LC-10A) equipped with a C<sub>18</sub> reversed phase column (Vydac 218TP54, 25cm x 4.6mm Ø, 30°C) and UV-vis detector (450nm). The elution consisted in MeOH:CH<sub>3</sub>CN (90:10, v/v), with flow rate at 1 ml/min. The identification and quantification of the compounds of interest, i.e.,  $\beta$ -carotene, lutein and zeaxanthin, was performed using the retention times of standard compounds (Sigma-Aldrich,

MO-USA) and external standard curves (0.5ug/mL – 45ug/mL, lutein and zeaxanthin and 0.01µg /mL – 12 µg /mL to  $\beta$ -carotene) analyzed with the same experimental conditions.

The main carotenoids present in the samples were zeaxanthin, lutein and  $\beta$ -carotene, but some unknown and minor compounds remain to be identified. The majoritary carotenoid found in the seed extracts was zeaxanthin, followed by lutein and  $\beta$ -carotene (Table 1). The ratio lutein/zeaxanthin was highly rated in some varieties such as Mato Grosso-Palha Roxa (MGPR), Rosado, and Branco, probably indicating the high genetic variability among the germoplasms.

**Table 1.** Carotenoid content (µg/g dry weight) in seeds of maize local varieties cultured in Southern Brazil.

<i>Carotenoid/variety</i>	<i>BRS 1030</i>	<i>MPA 01</i>	<i>MGPR</i>	<i>RV*</i>	<i>Rosado</i>	<i>Branco</i>
Lutein	1.33*	4.54	2.82	1.96	0.56	0.19
Zeaxanthin	13.70	9.62	4.86	5.68	0.95	0.26
$\beta$ -carotene	0.06	0.09	0.06	0.04	n.d.	0.00
Lutein/zeaxanthin	0.09	0.47	0.58	0.34	0.58	0.75

\* Values are presented as mean of two extractions, **RV**: Roxo do Valdecir, **n.d.** not detected

Distinct carotenoid contents for the varieties were found, emphasizing the higher content of zeaxanthin (9.62µg/g) and lutein (4.54µg/g) for the yellow maize MPA 01. For purpose of comparison, a quantitation analysis of the carotenoids was performed using a commercial yellow maize variety sample (BRS 1030 – Embrapa). Similar total carotenoid contents were found for the local and commercial varieties (14.25µg/g and 15.09µg/g, respectively). However, lower lutein/zeaxanthin ratio was detected for the later variety, indicating a negative effect of conventional genetic breeding programs that usually pursue mostly higher yields. A superior content of lutein in maize seeds might be an interesting approach as one aims at to add value to them emphasizing their nutritional properties. The results herein described reveal that typical liquid chromatography analysis of carotenoid pigments might be an useful tool in order to get more insights regarding to the genetic variability present in maize local varieties. These findings suggest the potential of application of local varieties in breeding programs.