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Carotenoids in Several Transylvanian Maize Hybrids †

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† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Published: 1 December 2020

Abstract: Maize is one of the most important crops due to its high productivity and its multiple uses as a food source for humans, livestock feed and as a raw material in various industries; among staple crops, it has a relatively high amount of carotenoids. The major aim of this work is to characterize the carotenoid content from several Transylvanian maize hybrids produced by the Research and Development Station for Agriculture (RDSA) Turda. Maize kernels were milled, weighed and extracted with ethanol and acetone, the resulting suspension being filtrated under vacuum, then by membrane filtration. Total carotenoids were determined by UV/VIS spectrophotometry, while major carotenoids were determined by reverse-phased high performance liquid chromatography (HPLC). HPLC analysis emphasized that lutein is the major carotenoid in most hybrids, while in a smaller number of cases β-cryptoxanthin was the major carotenoid. HPLC analysis, principal component analysis and cluster analysis revealed five HPLC fingerprint profiles. Besides being a helpful tool for future nutrition studies, this work is in the meantime a valuable tool in assisting the breeding activity for improvement the nutritional quality of maize

Keywords: carotenoids; maize; chromatography; HPLC; analysis; fingerprinting; nutrition; quality

1. Introduction

The increased interest in the biotechnology of carotenoids has been mainly generated by their health-related antioxidant properties [5]. It is well-established that dietary carotenoids fulfill essential requirements for human nutrition [1]. Carotenoids with β- ring end groups taken from the diet act as precursors for the production of retinoids in animal cells; vitamin A deficiency can lead to xerophthalmia, blindness and premature death. Besides, carotenoids have multiple beneficial effects for human health, such as: the capacity of acting as free radical scavengers and antioxidants, while an inverse relationship exists between the dietary intake of carotenoid-rich foods such as fruit and vegetables and the incidence of cancer (lung, breast, colon and prostate cancer), UV-induced skin damage, coronary heart disease, cataracts and macular degeneration [2,3]. Maize is one of the most important crops due to its high productivity and its multiple uses as a food source for humans, livestock feed and as raw material in various industries; among staple crops, it has a relatively high amount of carotenoids.

The major aim of this work is to characterize the carotenoid content from several maize hybrids produced by the Research and Development Station for Agriculture (RDSA) Turda.

2. Experiments

***Plant material*:** maize kernels from 50 hybrids originating from RDSA Turda.

***Extraction*:** kernels were milled (WZ-1 laboratory mill, Sadkiewicz Instruments, Poland); samples of ~1 g were weighed and extracted with 25 mL ethanol, then with 25 mL acetone, the

resulting suspension being filtrated each time under vacuum. A supplementary membrane filtration (0.47 mm) was accomplished before the spectrophotometric and chromatographic determinations.

Determination of total carotenoids was accomplished using a T80+ UV-VIS spectrophotometer (PG Instruments Ltd., UK).

High performance liquid chromatography (HPLC) was accomplished using a Flexar system (Perkin Elmer, USA), consisting in two UHPLC pumps, a solvent degasser, an autoinjector, an UV-VIS detector, a controller and a computer running Chromera software. Separations were monitored at 450 nm, using a Nucleosil 5-C_{18} column (Macherey Nagel), the quantitative determinations being accomplished by the external standard method.

Data analysis: principal component analysis (PCA) and cluster analysis were completed on autoscaled data using Matlab (The Mathworks Inc., USA).

3. Results and Discussion

The carotenoids' content of maize grains from the studied maize hybrids revealed significant differences between total carotenoids (Table 1). HPLC analysis highlighted that lutein is the major carotenoid in most hybrids with concentrations in the range of 2.75–10.13 mg/kg; the concentrations of zeaxanthin and β- cryptoxanthin did not differ significantly (1.42 and 1.37 mg/kg respectively). Low concentrations were observed for b-carotene in the range of 0.15–1.93 mg/kg.

Table 1. Descriptive statistics on carotenoids' content for 50 hybrids produced at ARDS Turda [mg/kg].

Parameter	Lutein	Zeaxanthin	β-cryptoxanthin	β-carotene	Total carotenoids
Mean value	5.43	1.42	1.37	0.54	10,49
Minimum value	2.75	0.48	0.21	0.15	4.13
Maximum value	10.13	4.17	4.07	1.93	24,22
Range	7.38	3.69	3.86	1.78	20.09

Based on the obtained data, a PCA model was developed using 4 variables (carotenoids' concentrations), in which: the first two principal components explain 72.61% variance (Figures 1 and 2). PCA revealed a close correlation between the recorded concentrations of lutein and zeaxanthin (Figure 2).

HPLC analysis, principal component analysis and cluster analysis revealed five HPLC fingerprint profiles (Figures 1–5), from which the most prominent ones are those having lutein as major carotenoid (Figure 2) and those with b-cryptoxanthin as major carotenoid (Figure 4)

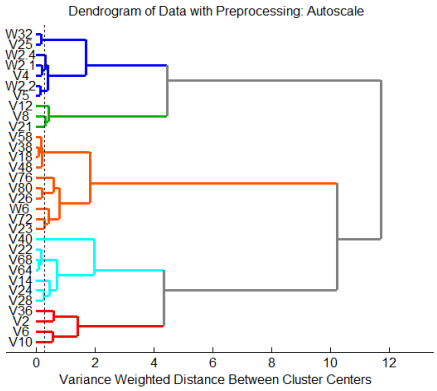

Figure 1. Cluster analysis using Ward's method.

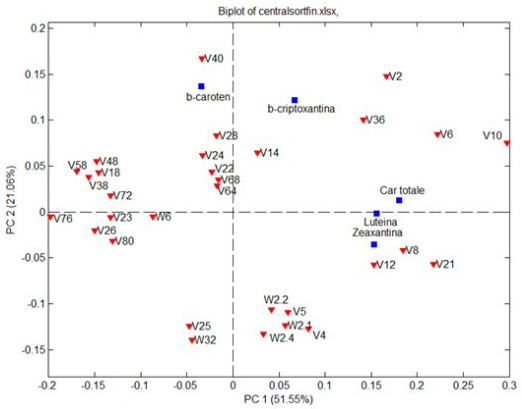

Figure 2. Biplot for experimental data Mahalanobis distance.

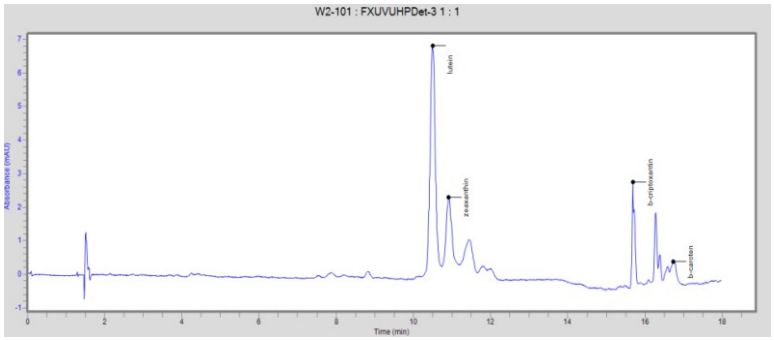

Figure 3. HPLC chromatographic fingerprint of maize kernels having lutein as major carotenoid (cluster 2–samples V8, V12, V21).

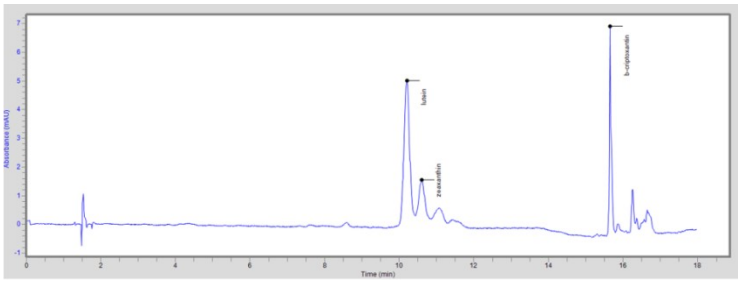

Figure 4. HPLC chromatographic fingerprint of maize kernels having β-cryptoxanthin as major carotenoid (cluster 5-samples V2, V6, V10, V36).

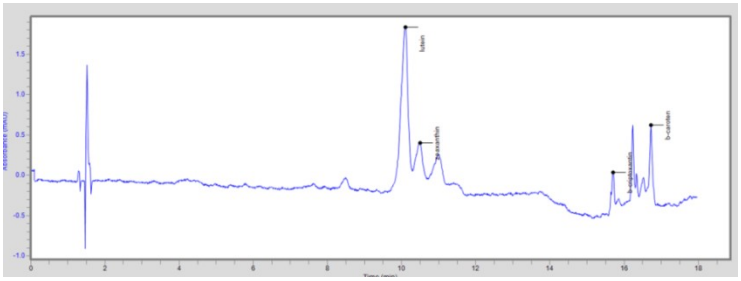

Figure 5. HPLC chromatographic fingerprint of maize kernels having β-carotene in higher amounts (cluster 4–samples V40… V28).

4. Conclusions

The total carotenoids' content of maize grains revealed significant differences between certain hybrids; HPLC analysis emphasized that lutein is the major carotenoid in most hybrids, while in a smaller number of cases β-cryptoxanthin was the major carotenoid. Zeaxanthin and β-cryptoxanthin are in most cases in the mid-level range of concentrations, while β-carotene is a minor carotenoid. HPLC analysis, principal component analysis and cluster analysis revealed five HPLC fingerprint profiles.

Because provitamin A carotenoids (β-cryptoxanthin and β-carotene) are present in small quantities, the provitamin A value of the investigated hybrids is rather modest; however, certain hybrids proved to contain significant amounts of lutein and zeaxanthin – important in many physiological processes.

Besides being a helpful tool for future nutrition studies, this work is in the meantime a valuable tool in assisting the breeding activity for improvement the nutritional quality of maize.

Acknowledgments: The analyzed samples were provided by the Maize Breeding Laboratory team from the RDSA Turda; sampling and sample processing were accomplished by R. Calugar, L. Pacurar and A. Ceclan.

Conflicts of Interest: The author declares no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

HPLC	high performance liquid chromatography
PCA	principal component analysis
RDSA	Research and Development Station for Agriculture

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