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Carotenoids in *Cucurbita* fruits

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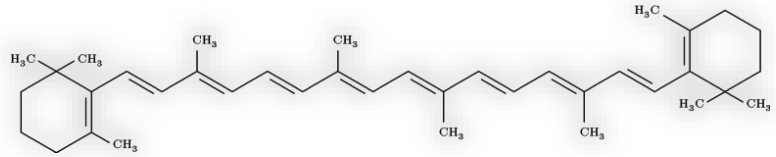
Introduction



Carotenoids are important isoprenoids including more than 700 yellow, orange and red pigments. The need for reliable data on the carotenoid content from food has become increasingly important since the enhanced interest in the link between carotenoid intake and health [2, 3, 6].:

- they can act as free radical scavengers and antioxidants
- an inverse relationship exists between the dietary intake of carotenoid-rich foods and the incidence of certain cancers, UV-induced skin damage, coronary heart disease, cataracts and macular degeneration
- carotenoids with β-ring end groups are precursors for the production of retinoids in animal cells, hence they can prevent xerophthalmia or blindness

Introduction

Pumpkins and squashes are cultivated in almost all areas with an appropriate climate in the world, some of them having a high nutritional value and hosting notable amounts of carotenoids.

In many regions, these fruits are important dietary sources of provitamins A in human nutrition especially during winter season, being consumed either raw or processed.

Carotenoids from *Cucurbita* fruits were the subject of many researches and the reported data were highly variable, since they depend on numerous factors, such as genotype, environmental conditions, fertilization, degree of maturation, etc. [1, 4].

Research objectives

The aims of this work were:

- to develop a reversed-phase high performance liquid chromatographic method for the determination of carotenoids in *Cucurbita* fruits in a minimum separation time and to use it
- to provide data on the content of these antioxidants from several cultivars available on the Romanian market

Material and methods

Plant material:

- fruits belonging to six *Cucurbita* cultivars, harvested from the experimental field of the University of Agricultural Sciences and Veterinary Medicine Cluj Napoca.

Sample preparation:

- the fruit rind was peeled, seeds and the placental tissue were removed, then the epicarp and the mesocarp were cut in small pieces;
- representative samples of ~10 g were weighed and extracted by blending with ethanol : acetone (1:1)
- the extracts were filtered under vacuum then saponified with a KOH/ CH_3OH, being afterwards washed and evaporated to dryness in a rotary evaporator.
- the obtained residues were dissolved in acetonitrile, filtered through 0.47 μm membrane filter and subjected to high performance liquid chromatography (HPLC).

Material and methods

HPLC analysis was accomplished on an system equipped with a Waters diode array detector using a C_{18} column, with a gradient based on ethyl acetate and a mixture acetonitrile : water (9:1), using a flowrate of 1 mL/ min and an injection volume of 1 μL. The separations were accomplished at room temperature, being monitored at 450 nm; carotenoids' identification was based both on comparison of peak retention times with that of authentic standards, and on the spectral characteristics (VIS), while the quantification was based on the external standard method. Method validation was accomplished for neoxanthin, violaxanthin, lutein, α-cryptoxanthin and β-carotene (table 1).

Table 1: A summary of validation parameters

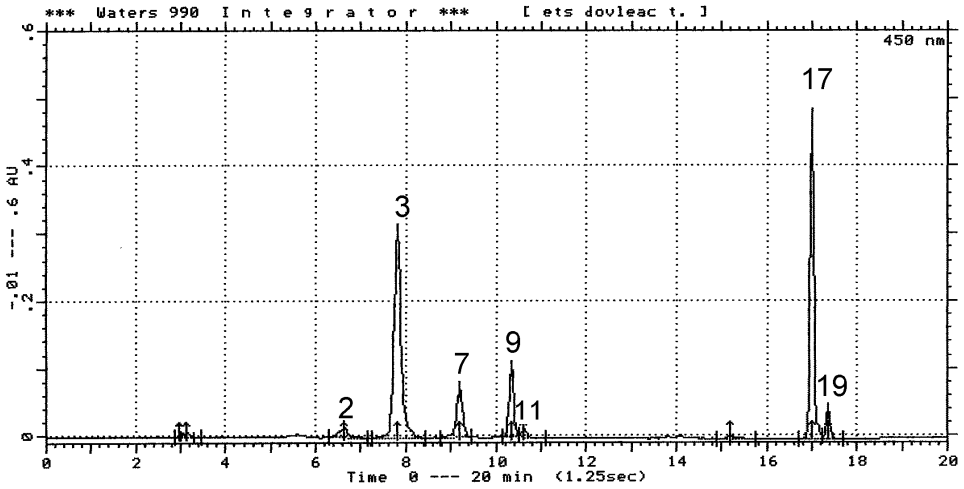
	Neoxanthin	Violaxanthin	Lutein	α-cryptoxanthin	β-carotene
Concentration range [mg/L]	1.2 – 11.6	1.5 – 12.7	1.8 – 31.2	1. 3 - 19.5	1.1 - 27.3
Linearity (R^2)	0.9993	0.9991	0.9997	0.9995	0.9989
Limit of detection [mg/L]	0.005	0.007	0.006	0.011	0.018
Limit of quantification [mg/L]	0.015	0.021	0.018	0.033	0.054
Recovery [%]	95.02	93.18	95.17	89.45	83.29

Besides, total carotenoid content was assessed by visible spectrophotometry, as well as the dry weight (by drying at 105^0C)

Results

HPLC analysis revealed different chromatographic fingerprints of carotenoids in the analyzed fruits. The major carotenoids in *Cucurbita maxima* cultivars were beta-carotene and violaxanthin, while in *Cucurbita pepo* cultivars were lutein and neoxanthin (table 2), these being followed by smaller amounts of zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, β-carotene 5,6-epoxide, α-carotene, 9Z-β-carotene and 15Z-β-carotene.

Figure 1. Representative HPLC chromatogram for carotenoids from C.maxima Duch. Peak IDs are: 2 – neoxanthin, 3 – violaxanthin, 7 – cucurbitaxanthin A, 9 – lutein, 11- zeaxanthin, 17 – β-carotene, 19 – 15Z -β-carotene



Results

Table 2. Concentration ranges for carotenoids in Cucurbita fruits [mg/ Kg dry weight]

Carotenoid	Range	Cultivar with maximum content
Neoxanthin	0.23 - 129.7	*C.pepo L. giromontia* (epicarp)
Violaxanthin	0.1 - 36.4	*C.Maxima Duch* (mesocarp)
Lutein 5, 6 - epoxide	0.13 - 2.4	*C.Maxima Duch* (mesocarp)
Cucurbitaxanthin A	0.52 - 10.1	*C.Maxima Duch* (mesocarp)
Lutein	0.10 – 234.8	*C.pepo L* (epicarp)
α-cryptoxanhtin	0.42 – 2.9	*C.pepo L* (epicarp)
β– carotene (all-E)	0.05 - 61.7	*C.pepo L. giromontia* (epicarp)
15Z-β-carotene	0.02 - 11.8	*C.Maxima Duch* (mesocarp)
Total carotenoids	0.18 – 366.8	*C.pepo L* (epicarp)

Conclusions

- The developed HPLC-DAD method proved to be fast (less than 20 minutes run-time), sensitive, reproducible, accurate and suitable for the analysis of fruits containing both xanthophylls and carotenes.
- The results extended the current knowledge, showing the content of individual carotenoids in the studied cultivars.
- The reported values can support future nutrition studies involving carotenoids from plant sources, as well as their use in different functional products.

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Many thanks for your attention!

Please send your questions to
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