HPLC ASSESMENT OF PROVITAMIN A CAROTENOIDS FROM *CUCURBITA MAXIMA DUCH. EX. LAM.* (MARIȚA CULTIVAR) FRUITS

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Abstract: *Carotenoids are biologically active compounds widely distributed in plants. The fruits of Cucurbita maxima Duch. ex.Lam. - Mariţa cultivar are used as forage, being valuable sources of carbohydrates and carotenoids, including provitamin A ones. This study emerged from the need for reliable data on the carotenoid content of feeds, knowing the demonstrated correlation between carotenoid intake (especially of those with provitamin A activity) and health. Meanwhile, knowing the carotenoid levels is an important task for rationally using this fruits in animal feeding. Provitamin A were completely extracted from ripe fruits of Cucurbita maxima Duch. ex.Lam. - Mariţa cultivar using methanol and acetone. High performance liquid chromatography was accomplished using a Nucleosil 120-5 C_{18} column, detection, detection being made using a Waters 990 photodiode array detector. Carotenoid identification was completed based on HPLC co - chromatography with authentic standards and by comparison of the visible absorption spectra with those of reference carotenoids; quantification was based on the external standard method. The major identified provitamin A in the fruits' mesocarp is β,β - carotene (15.36 μg/ g dry weight); minor provitamins A are the hydrocarbons β,ε-carotene and 15, 15' - Z - β,β - carotene and the xanthophylls 5,6–epoxy-β-carotene, α-cryptoxanthin and β–cryptoxanthin, all with concentrations less than 1.00 μg/ g dry weight. The fruit epicarp showed a similar provitamin A pattern, with lower amounts of β,β - carotene (9.47 μg/ g dry weight), while from the minor provitamins A, 5,6–epoxy-β-carotene is missing.*

Key words: *carotenoids, provitamins A, chromatography, HPLC, feed, Cucurbita maxima Duch. ex.Lam. - Mariţa cultivar*

INTRODUCTION

Carotenoids are biologically active compounds widely distributed in plants, being responsible for the yellow to orange or red color of different plant tissues. Besides natural carotenoids, synthetic ones are used as additives for animal feed, the main purposes being either to provide adequate supplies of provitamin A or the desired color to animal tissues or to derived products, such as astaxanthin to salmon, lutein and zeaxanthin to chicken (for egg yolk and skin coloration), β - carotene to cattle (for cream coloration), etc. However, natural carotenoids are preferred in their original matrix, these acting in a synergic way with other nutrients and being better accepted by animals.

The increased interest in carotenoid-rich plant matrix has been mainly generated by their health-related properties. Hence, carotenoids with β-ring end groups taken from the diet act as precursors for the production of retinoids in animal cells [DEMMIG-ADAMS – 2002; Fraser - 2004]. Only a small number of carotenoids have provitamin A activity; from these, β - carotene have the highest provitamin A activity potential. Carotenoids acts as free radical scavengers and antioxidants in vivo, this biological function being independent of the provitamin A activity; an inverse relationship exists between the dietary intake of carotenoid-rich foods such as fruit and vegetables and the incidence of lung, breast, colon, and prostate cancers, UV-induced skin damage, coronary heart disease, cataracts, and macular degeneration [DEMMIG-ADAMS – 2002; FRASER – 2004; STAHL - 2005].

This study emerged from the need for reliable data on the carotenoid content of feeds, knowing the demonstrated correlation between carotenoid intake (especially of those with provitamin A activity) and health. Meanwhile, knowing the carotenoid levels is an important task for rationally using this fruits in animal feeding. The above-mentioned plant matrix was selected for this study knoving that plants belonging to the genus *Cucurbita* are valuable sources of carbohydrates and carotenoids, besides their considerable dietary and feeding value, host notable amounts of carotenoids in their fruits [GROSS – 1991; MUNTEAN – 2000]. Provitamin A analysis was accomplished using the best analytical technique available to date: high performance liquid chromatography.

MATERIAL AND METHODS

The carotenoid references were provided by F. HOFFMAN - LA ROCHE, Basel, Switzerland. All solvents for chromatography were HPLC grade purity (ROMIL Chemicals) and they were filtered through Whatman glass microfibre filters (0.45 μm), then degassed in an ultrasonic bath, under vacuum, before use. Solvents for extraction were p.a. quality, freshly distilled. All operations were carried out in reduced light, avoiding samples heating at more than 40°C. Prior to injection in HPLC systems, carotenoid solutions were filtered through 0.45 μm Whatman filters.

Ripe fruits of *Cucurbita maxima Duch. ex. Lam. - Marita cultivar* were harvested from the experimental field of the University of Agricultural Sciences and Veterinary Medicine Cluj Napoca; the seeds and the placental tissue were removed, then the rind was peeled. The epicarp and the mesocarp were therefore cut in small pieces, which were mixed and packed in sealed polyethylene bags, which were weighed and stored at -25°C, until analysis.

Carotenoids from both epicarp and mesocarp (samples between 10 - 15 g) were processed separately, being extracted in a blender, using 100 mL methanol; 0.2 g butylated hydroxytoluene (BHT) and 2 g CaCO3 were added for avoiding oxidation and acidic isomerization during the extraction procedure. The resulting mixture was filtered under suction with a sintered - glass funnel and the solid material was re-extracted repeatedly with acetone (150 mL), until the resulting filtrate was colorless. The resulting extract was washed ten times with distilled water, concentrated at 40°C under reduced pressure in a Buchi rotary evaporator and dissolved in 25 mL diethyl ether; the remainder extract was saponified with 25 mL solution 30% KOH in methanol at room temperature for 16 hours. The unsaponifiable fraction was next extracted with petroleum ether and washed repeatedly with distilled water until free of alkali; the aqueous layers were re-extracted with small volumes of diethyl ether until colorless, then the organic layers were combined, washed several times with distilled water and evaporated to dryness under reduced pressure. The saponified extract was dissolved in 10 mL ethyl acetate and an aliquot was used for HPLC.

HPLC analysis was performed on a system consisting of a Kontron Instruments pumping system 322, a Rheodyne 7125 injection valve with 20 μl loop, a Nucleosil 120 - $5C_{18}$ column (250 mm length and 4.6 mm i.d., 5 μm particle size), a WATERS 990 photodiode array detector connected with a computer running a WATERS 990 software for data analysis. Separation was achieved using the following mobile phases: A - acetonitrile : water = 9 : 1 and B - ethyl acetate (both with 0.1% EPA); the flow rate was 1 mL/ min., the solvent gradient being: from 0-10 min 0 to 60% A, then from 10 to 15 min 60 to 0% B next isocratic with 0%B from 15 to 20 min. Carotenoid identification was based on HPLC co - chromatography with authentic standards and by comparison of the visible absorption spectra with those of reference carotenoids. Quantification of the carotenoids was achieved by the external standard method.

The provitamin concentrations were expressed in retinol equivalents (RE), according to the requirements of FAO/WHO [FAO/WHO - 1998].

RESULTS AND DISCUSSIONS

In fruit mesocarp, one major provitamin A was identified as β, β - carotene (peak nr. 5 in figure 1); besides, five minor provitamins A were determined, two belonging to hydrocarbons (β, ε - carotene and 15, 15' Z - β, β - carotene) and three to xantophylls (α-cryptoxanthin, β-cryptoxanthin and 5,6-epoxy-β-carotene). The chromatographic pattern of carotenoids from fruit epicarp is similar, the peak nr. 3 being absent.

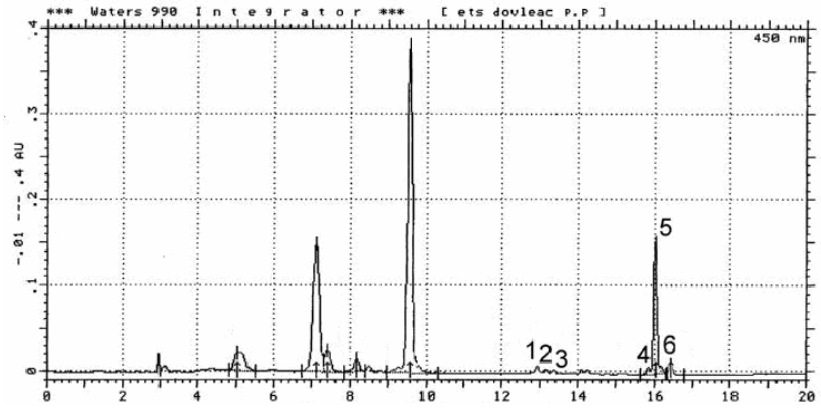

Figure 1. Chromatogram of the total saponified extract from fruit mesocarp of *Cucurbita maxima Duch. ex. Lam.* - Marita cultivar (peak identities in table 1)

Table 1 summarizes the quantitative recorded, together with the carotenoids' distribution between mesocarp and epicarp: the provitamin A value, expressed in retinol equivalents, is bigger for the fruit epicarp than for the mesocarp, but reported on dry weight basis the situation is reversed, due to the higher water content of the mesocarp.

Table 1

Distribution of provitamin A carotenoids in fruits of *Cucurbita maxima Duch. ex. Lam.* - Marita cultivar

Peak Nr.	Carotenoid identity	Carotenoids in mesocarp		Carotenoids in epicarp	
		[μg/ g]	[μg/ g dry w]	[μg/ g]	[μg/ g dry w.]
1	α-Cryptoxanthin	0.01	0.21	0.06	0.42
2	β-Cryptoxanthin	0.01	0.11	0.03	0.21
3	β-Carotene 5, 6 - epoxide	0.01	0.11	0.00	0.00
4	β, ε - Carotene	0.02	0.32	0.06	0.39
5	β, β - Carotene	0.96	15.36	1.38	9.47
6	15, 15' Z - β, β - carotene	0.06	0.94	0.06	0.38
	RE	0.17	2.70	0.25	1.71

CONCLUSIONS

This study allowed an overview of the chromatographic profile of the provitamins A from fruits of *Cucurbita maxima Duch. ex. Lam.* - Marita cultivar, being the first modern investigation on this cultivar from this point of view.

HPLC analysis revealed that the major provitamin A carotenoid is β, β-carotene; besides, smaller amounts of β, ε– carotene, 15, 15' Z - β, β - carotene, α- and β-cryptoxanthin, β-carotene 5, 6 - epoxide were also detected.

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