

QUANTIFICATION OF CAROTENOIDS FROM PUMPKIN JUICE BY HPLC-DAD

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Abstract

*The carotenoid content of the juice obtained from fruits of *Cucurbita maxima* Duch were quantified by high performance liquid chromatography with diode array detection (HPLC-DAD). The concentration of carotenoids in the extracted juice is 12.45 µg/ g, the major carotenoid being β-carotene (5.80 µg/g), followed by lutein (1.52 µg/g). HPLC analysis revealed that the bigger amount of carotenoid in juice is in a free, unesterified form. Sterilization lead to a decrease of the carotenoids' concentration at 4.18 µg/; after storage for seven months, the final concentration of the total carotenoids was 2.72 µg/g juice. Sterilization destroyed totally the carotenoids lactucaxanthin, α-cryptoxanthin, β-cryptoxanthin and α-carotene, while storage leads to the total degradation of neoxanthin, cucurbitaxanthin A and β-caroten-5, 6-epoxide. The most stable carotenoid proved to be β-carotene.*

Keywords: *HPLC, analysis, juice, chromatography, stability, food composition, storage, *Cucurbita maxima* Duch.*

Introduction

Fruit and vegetable juices have become a focus of people's attention due to their nutrition value; essentially, juices are solutions of sugars together with organic acids, proteins, pectins, tannins, minerals, pigments, vitamins and essential oils. Freshly extracted juices contain suspended matter and the above-mentioned substances; the type and the quality alter according to fruit variety, time of harvesting, age of fruit and method of extraction. Juicing opens up the indigestible cellulose walls of plants and exposes the intracellular ingredients in

greater concentrations that can benefit health. Juicing also can increase the total dietary benefit of specific foods (it is much easier to drink the juice of 10 carrots than it is to eat them!); elderly persons with poor chewing ability can certainly benefit from juicing.

Processing may result in dramatic decreases in antioxidant compounds, depending on time/temperature conditions utilized (Chen, 1995); the antioxidant levels may continue to decline during storage (Chen, 1995). However, some degree of heat is desirable for inactivation of oxidative enzymes; blanching or mild pasteurization treatments focused on enzyme inactivation may result in higher degrees of antioxidant activity.

Pumpkin juice can be consumed as it is, being a rich source of carotenoids, but because of its intense orange color originating from the contained carotenoids, it can also be utilized for food coloring. Fruit juices usually used to color foods are those obtained from black currant, blueberry, elderberry, raspberry and tomato; pumpkin juice can be included in this list, as it can provide a pleasing yellow to orange color to various foods, without contributing any undesirable flavors. Pumpkin juice can color cheese products orange, as well as provide a creamy yellow hue to milk beverages flavored banana and lemon. The benefit to using such colorants is they add value by contributing to a natural image of a food product, as they are listed on ingredient panels as, for example, carrot and beet juice. Consuming pumpkin juice is beneficial, as it is rich in carotenoids. Besides serving as precursors of vitamin A, carotenoids possess antioxidant capacity and are thought to afford protection against atherosclerosis and age-related macular degeneration.

Experimental

Pumpkin juice was obtained from ripe fruits of *Cucurbita maxima* Duch. harvested from the experimental field of the University of Agricultural Sciences and Veterinary Medicine Cluj Napoca. Fruits were cut in pieces, than the seeds, the placental tissue and the rind were removed. The resulting mesocarp was cut in smaller pieces, from which juice was extracted using a Moulinex juicer. From 1647 g mesocarp, 731 ml juice resulted, leading to a juice yield of 443.8 ml

juice/ kg mesocarp. This juice has a sweet taste and a melon flavor; its color is dark orange and the appearance is turbid, due to the small particles in suspension. The preservation procedure consisted in addition of 1g sodium benzoate to 400 ml juice, followed by sterilization at 105°C for 20 minutes in an oven. Juice was kept in closed bottles for seven months at room temperature. Samples of 20 ml were collected in triplicates after each processing stage (immediately after extraction, after sterilization and after storage), and then the carotenoid content was assessed by HPLC.

Carotenoid extraction: an appropriate amount of echinenone solution (internal standard) was added to each sample of 20 ml juice, then the mixture was filtered under reduced pressure on a sintered – glass funnel. The residue was extracted successively with 25 ml acetone and 25 ml diethyl ether; the extracts were combined in a separatory funnel, in which 50 ml diethyl ether was added, then the extract was washed ten times with volumes of 200 ml distilled water. The epiphase was transferred in a 50 ml round-bottom flask, being then evaporated to dryness in a rotary evaporator. The residue was transferred to a 100 ml flat-bottom flask using 25 ml diethyl ether; an equal volume of 30% KOH in methanol was also added in the flask, then saponification was carried out overnight at room temperature, on a magnetic stirrer. The reaction mixture was transferred in a separatory funnel, being washed repeatedly with distilled water until free of alkali. The epiphase was transferred in a 50 ml round-bottom flask, being evaporated to dryness in a rotary evaporator. The residue was transferred to a 5 ml flat-bottom flask using ethyl acetate, for HPLC analysis; prior to injection, samples were filtered through 0.5 µm filters (Whatman).

HPLC analysis was selected in order to quantify carotenoids, this being considered the method of choice for the separation, identification and quantification of carotenoid available to date (Britton, 1995 and 1996; Cortes, 2004; Muntean, 2003). HPLC separations were performed on a system consisting of: a Kontron Instruments pumping system 322, a Rheodyne 7125 injection valve with 20 µl loop, a Waters 990 photodiode array detector and a 80486 computer running a WATERS 990 software for data analysis. Separations were carried out by using a Nucleosil 120 - 5C₁₈ column (250 x 4.6 mm, 5 µm particle size), at room temperature, at a flow rate of 1 ml/min, under the following

gradient: initial conditions were 90% A, 10%B, then from 0 – 15 min. 30%A, 70% B, from 16 to 20 min. 90%A, 10%B (A is a mixture of acetonitrile: water (9:1) and B ethyl acetate, both A and B containing 0.5% EPA). Identification of carotenoids was made on the basis of visible spectral characteristics, retention times, HPLC co-chromatography with standards; quantification was achieved using the internal standard method, with echinenone as internal standard.

Total carotenoids were determined by VIS-spectrophotometry (Britton, 1996).

Results and discussion

The concentration of carotenoids in the extracted juice is 12.45 µg/g, the major carotenoid being β-carotene, followed by lutein (table 1, figure 1). The comparative analysis of HPLC chromatograms of the unsaponified extract and that of the saponified extract (figures 1 and 2) revealed that the bigger amount of carotenoid in juice is in a free, unesterified form.

Table 1. Average concentrations of carotenoids from the juice of *Cucurbita maxima Duch.* fruits recorded during experiments [µg/ g]

| Peak index | Carotenoids | Initial juice | Sterilized juice | Juice after 7 months |
|------------|----------------------------|---------------|------------------|----------------------|
| 1 | Neoxanthin | 0.28 | 0.09 | 0 |
| 2 | Violaxanthin | 0.80 | 0.23 | 0.11 |
| 3 | Cucurbitaxanthin A | 0.34 | 0.11 | traces |
| 4 | Lactucaxanthin | 0.15 | traces | 0 |
| 5 | Lutein | 1.52 | 0.48 | 0.27 |
| 6 | α-cryptoxanthin | traces | 0 | 0 |
| 7 | β-cryptoxanthin | traces | 0 | 0 |
| 8 | β-caroten-5, 6 - epoxide | 0.40 | 0.12 | 0 |
| 9 | α-carotene | 0.33 | 0 | 0 |
| 10 | β-carotene | 5.80 | 2.23 | 1.53 |
| 11 | 15, 15' Z.-β, β - carotene | 1.47 | 0.51 | 0.41 |

After sterilization, the concentration of carotenoids decreases at 4.18 µg/ g; the seven months of storage lead to a final concentration of

2.72 μg total carotenoids/ g juice. The behavior of the involved carotenoids is summarized in table 1.

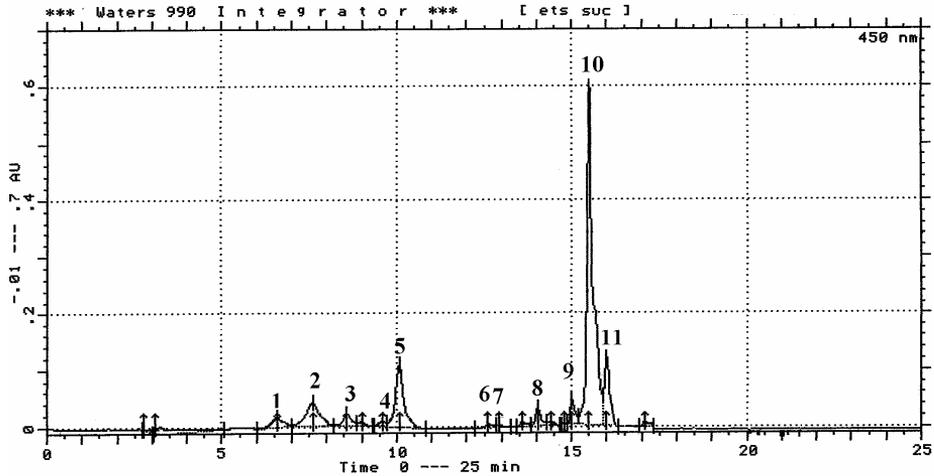


Fig. 1. HPLC chromatogram of the saponified extract obtained from the juice of *Cucurbita maxima Duch.* (peak identities are in table 1).

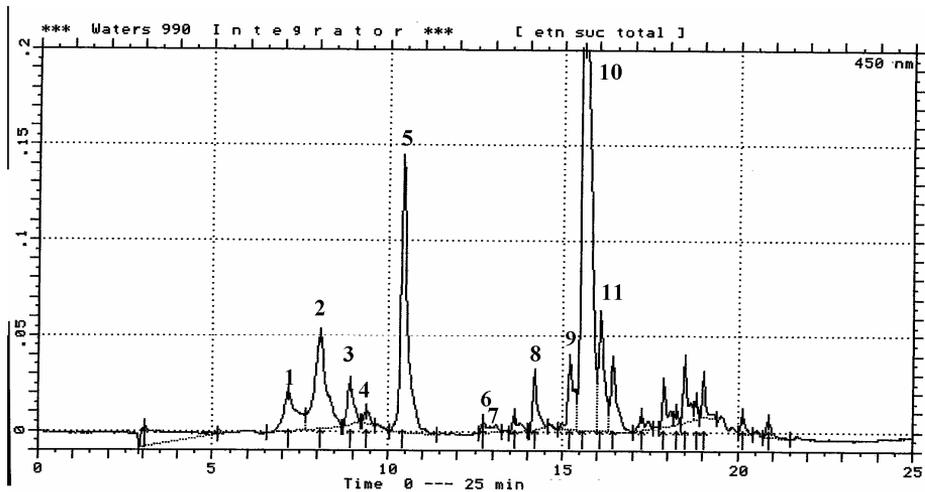


Fig. 2. HPLC chromatogram of the unsaponified extract obtained from the juice of *Cucurbita maxima Duch.* (peak identities are in table 1). Peaks without indexes correspond to carotenoids' esters.

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Analysis of data from table 1 reveals the low stability of lactucaxanthin, α -cryptoxanthin, β -cryptoxanthin and α -carotene which were destroyed in an early processing stage; storage affects afterwards strongly neoxanthin, cucurbitaxanthin A and β -caroten-5, 6-epoxide. The most stable carotenoid proved to be β -carotene; the apparently low degradation of 15, 15' Z- β , β -carotene is not an indication of stability in its case, as this carotenoid is an isomer of β -carotene and isomerization usually occurs during heat treatment and storage (Britton, 1995).

Conclusions

Even though it is not produced on a large scale, pumpkin juice can be considered among other juices both as a valuable source of carotenoids and as a possible food-coloring agent.

Quantitative data presented in this paper can be helpful for those interested in food applications.

References

- Britton, G., Liaaen-Jensen, S., Pfander, H. (1995). *Carotenoids*. Birkhauser Verlag, vol.1A, 210 - 214, Basel.
- Britton, G., Liaaen-Jensen, S., Pfander, H. (1996). *Carotenoids*. Birkhauser Verlag, vol.1B, 13 - 62, Basel.
- Chen, B.H., Peng, H.Y., Chen, H.E. (1995). Changes of carotenoids, color and vitamin A contents during processing of carrot juice. *J.Agric.Food Chem.*, 43 (7), 1912 - 1918.
- Chen, G., Zheng, W., Wu, X. (1995). Stability of carotene in carrot juice system. *Shipin Gongye Keji*, 1, 20 - 23.
- Cortes, C., Esteve, M.J., Frigiola, A., Torregrosa, F. (2004). Identification and quantification of carotenoids including geometrical isomers in fruit and vegetable juices by liquid chromatography with ultraviolet-diode array detection. *J.Agric.Food Chem.*, 52, 2203-2212.
- Muntean, E. (2003). HPLC analysis in establishing fruit juices authenticity. *Proceedings of the International Symposium "Euro-Aliment 2003"*, (Galați, 23-25.10.2003), 414 - 418.
- Muntean, E., Socaciu, C. (2003). Carotenoid analysis in quality assessment of natural vs. natural identical juices. *Proceedings of the "1st International Symposium on Food Quality Management for East European Countries"*, 42-47.