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PEPO-LUTEIN: A DIETARY SUPPLEMENT OBTAINED FROM THE EPICARP OF *CUCURBITA PEPO L. VAR. GIROMONTINA* FRUITS

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Abstract

This paper presents a laboratory-scale procedure for producing a natural dietary supplement, using as plant matrix the epicarp of Cucurbita pepo L. var. giromontina fruits. The final product contains a mean value of 104.45 μg lutein/ ml extract. HPLC analysis of the saponified extract revealed that besides the only major carotenoid, there are also minor amounts of violaxanthin, neoxanthin, lactucaxanthin and β-carotene. Traces of α-cryptoxanthin, β-cryptoxanthin, α-carotene and 15, 15' Z - β, β - carotene were also detected.
Keywords**:** *dietary supplement, carotenoids, lutein, HPLC, analysis, chromatography, food composition, Cucurbita pepo L. var. giromontina*

Introduction

Carotenoids are a large group of fat soluble pigments widely distributed in plants and animals, being responsible for the yellow to red color of fruits and vegetables. Among them, evidences showed that both in animals and in plants, lutein is involved in two important ways: as a filter of high energy blue light and as potent antioxidant that quenches and scavenges photoinduced reactive oxygen species (Krinsky, 2002).

Besides its stereoisomer zeaxanthin, lutein have been attracting particular attention due to the increasing number of scientific evidences for a link between them and a reduced risk of eye diseases, especially cataracts and age-related macular degeneration (AMD).Cataracts are the result of increasing opacification of the crystalline lens of the eye, whilst AMD has more severe consequences and is the most common cause of blind registration amongst older

people in western society. Several studies shoved that lutein consumption is inversely related with the risk for ocular diseases, including AMD (Mares-Perlman, 2001; Seddon, 1994) and cataracts (Brown, 1999; Gale, 2001). Higher levels of lutein and zeaxanthin were measured in the retina of individuals without known clinical history of AMD while lower levels were measured in the eyes of individuals with known clinical history of AMD (Bone et al., 2001).

The main focus of interest are lutein and zeaxanthin because these carotenoids are selectively deposited in the macula lutea, an area of the retina responsible for central and high acuity vision. In fact, lutein and zeaxanthin are the only carotenoids present in these tissues (Landrum, 2001; Yeum, 1995), where lutein acts both as a filter of high-energy blue light to protect the sensitive cells of the retina and as powerful antioxidant (Shao, 2001). Lutein may also protect skin from UV-induced damage (Stahl, 2000) and may help maintain heart health by reducing the risk of atherosclerosis (Dwyer, 2001; Kritchevsky, 2000; Mares-Perlman, 2002). Lutein is the second most prevalent carotenoid in human serum (Khachik, 1997).

Though lutein is deposited into many areas of the body prone to free radical damage it cannot be manufactured by the body. The only way to take advantage of lutein's antioxidant benefits is by consuming it or, as a growing number of skin care products are on the market, by applying it to the skin.

In human diet, lutein can be obtained in several different ways, usually from fruits or vegetables but also via nutritional supplements and most recently in functional foods. Lutein is abundantly present in dark, leafy green vegetables, such as spinach, broccoli, watercress, turnip greens and kale, as well as in corn, orange peppers, kiwifruit, oranges, nectarine, squashes and egg yolk (Humphries, 2003; Sommerburg, 1998). The yellow-orange fruits and vegetables proved to have a greater ratio of lutein/zeaxanthin while lutein was found to be the prominent carotenoid in greens.

In an average healthy diet, the intake of lutein ranges from 2 - 6 mg per day (Blum, 2001); it was suggested that 6 mg lutein/ day may reduce the risk of AMD by 43% (Seddon, 1994). This concentration is equivalent to consuming 1 kg of corn, 8 kg of tomatoes, 2 salad bowls of spinach or one salad bowl of kale a day. For people who fail to

consume enough lutein in their diet, dietary supplementation or lutein-enriched functional foods may be another option. As a consequence, food fortification with lutein extract is convenient and appealing to health conscious consumers. Commercial forms of lutein and zeaxanthin are on the market, being extracted from marigolds or obtained as a by-product of chlorophyll extraction; lutein enriched vitamin supplements provide up to 250 μg of lutein per tablet.

Official recommendations of dietary lutein intake have not yet been made. However, increased consumption of fruits and vegetables rich in antioxidants, and lutein and/or zeaxanthin is generally recommended to maximize good eye health. Lutein supplementation of the diet results in increased lutein levels both in the macula lutea and in serum (Bone, 2003; Johnson, 2000; Landrum, 2000) and improved the visual function in patients suffering from AMD and other ocular diseases (Olmedilla, 2003).

Experimental

Materials: 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, then degassed in an ultrasonic bath, under vacuum, before use. Solvents for extraction were p.a. quality, freshly distilled.

Cucurbita pepo L. var. giromontia (zucchini) fruits were bought from the marketplace of Cluj Napoca. Their epicarp was peeled using a special knife obtaining pieces with a thickness of 1 - 1.5 mm; these were then cut in smaller parts and sealed in polyethylene bags and stored at -20^0C until extraction.

HPLC analysis was selected for quantitative analysis of carotenoids, this being considered the method of choice for the separation, identification and quantification of carotenoid available to date (Britton, 1995 and 1996; Muntean, 2001). 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 computer running WATERS 990 software for data analysis. Separations were carried out by using a

Nucleosil 120 - 5C_{18} 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 22 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 external standard method (Britton, 1995).

Results and Discussion

Production of the dietary supplement started with an extraction, which was performed using 1054 g frozen epicarp by and 200 ml ethylic alcohol, in a blender; 1 g BHT (antioxidant) and 10 g calcium carbonate 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 twice with acetone (150 ml), until the resulting filtrate was colorless. The extraction procedure can stop here, leading to an extract rich in both carotenoids and chlorophylls; a final step in this case is evaporation to dryness under vacuum, then re-disolvation under sonication in sunflower oil. In this case the extract was transferred in a separation funnel, where 100 ml diethyl ether and 100 ml saturated solution of sodium chloride were added; after phase separation, the epiphase was transferred in another separation funnel, where the extraction with volumes of 100 ml diethyl ether was repeated until the hypophase remained colorless. All epiphases were collected in another separation funnel, where they were washed ten times with distilled water. The diethyl ether extract was transferred in a round - bottom flask, where it was concentrated to dryness under reduced pressure in a Buchi rotary evaporator. The residue was dissolved in 25 ml diethyl ether by sonication, being then subjected to saponification with 25 ml solution 30% KOH in methanol at room temperature for 16 hours. The unsaponifiable fraction was 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 until a neutral pH was reached, being then evaporated to dryness under reduced pressure.Keeping the extract under high vacuum eliminated traces of solvents and led to 1.9327 g brown residue, with a waxy appearance.This residue was dissolved finally in sunflower oil by sonication, the final solution being transferred in a 50 ml volumetric flask, where the volume was completed with sunflower oil.

Three aliquots of 1 ml from this were subjected to HPLC quantitative analysis. Each sample was diluted with 5 ml petroleum ether, being then applied on a top of a minicolumn of alumina grade 3 (50 mm x 5 mm i.d.); after elution with 10 ml petroleum ether, carotenoids were eluted with diethyl ether and the final solution was collected into a 10 ml calibrated flask, which was brought to the volume using diethyl ether.

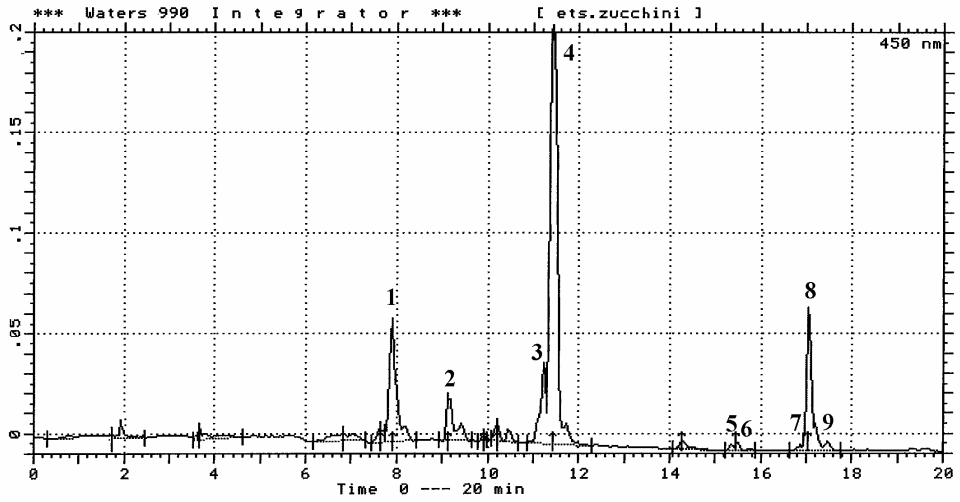

Fig. 1. The chromatogram of the dietary supplement obtained from the epicarp of *Cucurbita pepo l. var. giromontia*. Peak identities are as follows: 1-neoxanthin; 2-violaxanthin; 3-lactucaxanthin; 4-lutein, 5-α- cryptoxanthin; 6-β-cryptoxanthin; 7-α-carotene, 8-β-carotene, 9-15, 15' Z - β, β - carotene.

HPLC analysis of the saponified extract (figure 1) revealed only one major carotenoid, which is lutein, this being followed by minor

amounts of violaxanthin, neoxanthin, lactucaxanthin and β-carotene and traces of α-cryptoxanthin, β- cryptoxanthin, α-carotene and 15, 15' Z - β, β - carotene. Mean values of the carotenoids' concentrations are: lutein: 104.45 μg/ ml; neoxanthin: 12.82 μg/ ml; β-carotene: 12.44 μg/ ml; lactucaxanthin: 6.92 μg/ ml; violaxanthin: 4.82 μg/ ml, 15, 15' Z - β, β - carotene: 3.35 μg/ ml.

Conclusions

Researches directed on the effects of dietary components, especially lutein and zeaxanthin, on eye function is expected to become increasingly important over the next few years in order to address the health problems of the growing ageing population. Multivitamin supplements formulated for eye health are already available in a number of countries, and the food ingredients sector is beginning to show interest in this area.

The carotenoid content in the fruit epicarp of *Cucurbita pepo L. var. giromontia* places this plant matrix among the richest source of carotenoids in our country.

An industrial-scale production of the described dietary supplement can be profitable when the solvents used for extraction are recovered (during the evaporation to dryness procedures) and recycled and the raw material (the epicarp of zucchini) is provided by a cannery, as a by-product.

Stopping the production procedure before alkaline hydrolysis can direct to a much simpler approach (which is also much cheaper), leading to a dietary supplement which contains beside lutein high amounts of chlorophylls.

Due to its coloration power, in addition to its health effects, the product described here can be utilized as an additive without toxic effects in food coloration, being possible to use it instead of yellow synthetic colorants tartrazine (E102) or Sunset Yellow (E 110), both with well-known side-effects. This product could potentially be added to a range of foods, including breakfast cereals, cereal bars and sauces, and beverage products such as fruit juices, dairy drinks and energy drinks. Trials by lutein manufacturers suggest that lutein can be incorporated into many existing food and beverage formulations

without the need to change the types of other ingredients. Lutein has not been found to alter the flavor, odor or texture of food and drinks products, but it may produce a slight change in color in clear beverages.

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