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HPLC ASSESSMENT OF CAROTENOIDS' STABILITY IN PASTA COLOURED WITH A NATURAL EXTRACT

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Abstract

This paper presents a new plant extract together with a possible use of this as a natural food coloring agent, rich in carotenoids (especially in lutein). The plant matrix utilized for extraction was the epicarp of Cucurbita pepo L. var. Giromontia fruits (zucchini). The stability of carotenoids was assessed using high performance liquid chromatography for two cases with practical effect: thermal processing (boiling) and storage. Both lead to major degradation of major carotenoids lutein (31.08% after storage, 48.3% after boiling) and β-carotene (53,06% after storage and 74.14% after boiling); from minor carotenoids, neoxanthin was affected in the highest grade, as it disappeared completely after storage.

Keywords**:** *HPLC, analysis, pasta, chromatography, stability, food composition, storage, natural extract, food coloring*

Introduction

Food color is one of the most important features in the presentation of food products, being usually associated with the food quality; for this reason, color is frequently improved, or even changed, by the addition of synthetic or natural colorants (Tămaş, 1986; Otterstatter, 1998). Among colorants, carotenoids are often preferred when producers needs yellow, orange or red colors, since beside their coloring properties, they also own important qualities, which made them unique between other colorants (Klaui, 1981). Thus, few of them have provitamin A activity; carotenoids are potent quenchers of singlet oxygen; there is a also proven association between dietary carotenoids and cancer (Peto, 1981). Natural sources of carotenoids have been used

as food colorants for centuries (especially annatto, saffron, tomato and paprika), as well as extracts obtained from these (during the last decades). Nowadays, nature-identical carotenoids are produced on a large scale, but natural extracts are still used.

Besides other food products, pasta is often the subject of coloring, as the eggs' color used in the manufacture is usually pale, leading to a non – commercial appearance of the final product. This is the reason why we proposed a new food additive for coloring this food matrix: the extract obtained from the epicarp of *Cucurbita pepo L.var.giromontia* fruits. We also tested the stability of the carotenoids both after a normal shelf – life and after thermal processing. High performance liquid chromatography was selected in order to quantify carotenoids, this being the best method for carotenoid analysis available to date (Britton, 1995 and 1996; Muntean, 2001; E-Siong, 1991).

Experimental

Reagents and materials: All solvents for chromatography were HPLC grade purity (ROMIL Chemicals) and they were filtered through Whatman glass microfibre filters, and then degassed in an ultrasonic bath, under vacuum, before use. Water was bidistilled, then filtered and degassed. Reference carotenoids were provided by F. Hoffman-La Roche, Basel, Switzerland, being purified before use; reference solutions were prepared by dissolving carotenoids in ethyl acetate.

Special precautions. All operations required for analysis were carried out in reduced light. Prior to injection in HPLC systems, carotenoid solutions were filtered through 0.45 μm Whatman filters.

Carotenoid extract utilized for coloring pasta was obtained from 300 g epicarp of *Cucurbita pepo L. var. giromontia* fruits by extraction with ethylic alcohol in a blender; 1 g butylated hydroxitoluene and 50 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 six times with 250 ml ethylic alcohol, until the resulting filtrate was colorless. The obtained extract was concentrated under reduced pressure in a Buchi rotary evaporator and dissolved in 50 ml diethyl ether, being then saponified with 50 ml

solution 25% KOH in methanol at room temperature for 16 hours. The unsaponifiable fraction was extracted with diethyl ether and washed repeatedly with distilled water until free of alkali, then evaporated to dryness under reduced pressure. Keeping the extract under high vacuum eliminated traces of diethyl ether (a highly volatile solvent); the extract was finally dissolved in 100 ml "Floriol" sunflower oil using an ultrasonic bath.

Pasta samples were obtained from 3 eggs, 310 g "Dobrogea" flour type "000" and 15 ml carotenoid extract in sunflower oil. Reference pasta samples were obtained using the same recipe, but with 15 ml sunflower oil instead of the carotenoid extract in sunflower oil. Samples of about 10 g were collected for HPLC analysis from both types of pasta. 50 g colored pasta were boiled in 500 ml water for 10 minutes, then water was discarded and from boiled pasta samples were also collected for HPLC analysis. Amounts of about 50 g pasta were packed in polyethylene bags, being stored for 30 days in a room with a mean temperature of 20^0C, far from direct sun light. After the 30 days, samples were again collected for HPLC analysis of carotenoids.

Carotenoid extraction from samples of about 10 g pasta was accomplished in a blender, using 50 ml acetone, 0.2 g butylated hydroxitoluene (BHT) and 1 g $CaCO_3$. An appropriate amount of echinenone solution (internal standard) was added to each sample. The mixture was filtered under suction with a sintered-glass funnel and the solid material was re-extracted twice with 25 ml acetone. The resulting extract was washed ten times with distilled water, concentrated under reduced pressure in a Buchi rotary evaporator (at 40°C) and dissolved in 25 ml diethyl ether; the extract was saponified with 10 ml solution 30% KOH in methanol at room temperature for 16 hours (overnight). The unsaponifiable fraction was next extracted with diethyl ether and washed repeatedly with distilled water until free of alkali, being finally evaporated to dryness under reduced pressure. The saponified extract was dissolved in 5ml ethyl acetate and an aliquot was used for HPLC analysis.

HPLC analysis 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 80386 computer running a 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, using 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 (obtained using the PDA detector), 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

HPLC analysis of the natural extract (figure 1) revealed only one major carotenoid - 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.

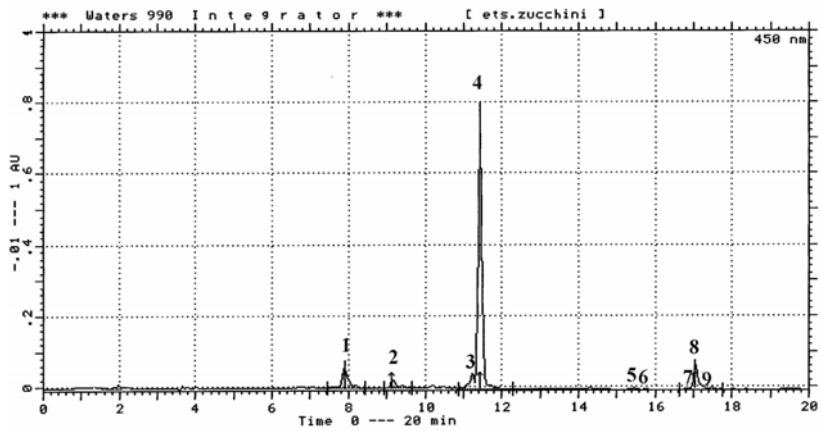

Fig. 1. HPLC chromatogram of the natural extract obtained from the epicarp of *Cucurbita pepo l. var. giromontia* (peak identities are in table 1).

Carotenoids analysis from the reference pasta revealed a concentration of 1.85 μg/g dry weight. The HPLC chromatographic profile of these samples (figure 2) is dominated by lutein, followed by smaller amounts of zeaxanthin and lactucaxanthin; only small amounts of α-cryptoxanthin, β-cryptoxanthin and β-carotene were detected. The origin of all these carotenoids is mainly in the egg's yolk; however, the concentration of these carotenoids is too small, so that the color of reference pasta is an un-commercial pale – yellow. After storage, the HPLC profile of this pasta remains the same, but the concentration of carotenoids decreases at 1.02 μg/ g dry weight.

Table 1. Average concentrations of carotenoids from pasta recorded during experiments ([μg/ g dry weight])

Peak index	Carotenoids	Reference pasta samples		Pasta with natural extract		Boiled pasta
		Initial	After 30 days	Initial	After 30 days	
1	Neoxanthin	0	0	0.32	0	0.25
2	Violaxanthin	0	0	0.41	0.22	0.18
3	Lactucaxanthin	0.08	0.07	0.82	0.43	0.41
4	Lutein	1.23	0.74	10.02	7.03	5.18
Z	Zeaxantin	0.21	0.15	0.19	0.16	0.18
5	α-cryptoxantin	traces	traces	traces	0	0
6	β-cryptoxantin	traces	traces	traces	0	0
7	α-carotene	0	0	0	0	0
8	β-carotene	traces	traces	1.47	0.69	0.38
9	15, 15' Z-β,β-carotene	0	0	0.31	0.47	0.17

Addition of the natural extract had a positive effect, changing the color of pasta in strong yellow with an orange shade, the concentration of the total carotenoids being finally 14.37 μg/ g dry weight; figure 3 presents the chromatogram of pasta with added carotenoids, showing a very high level of lutein and three new carotenoids: neoxanthin, violaxanthin and 15, 15' Z-β-carotene, all originating from the extract.

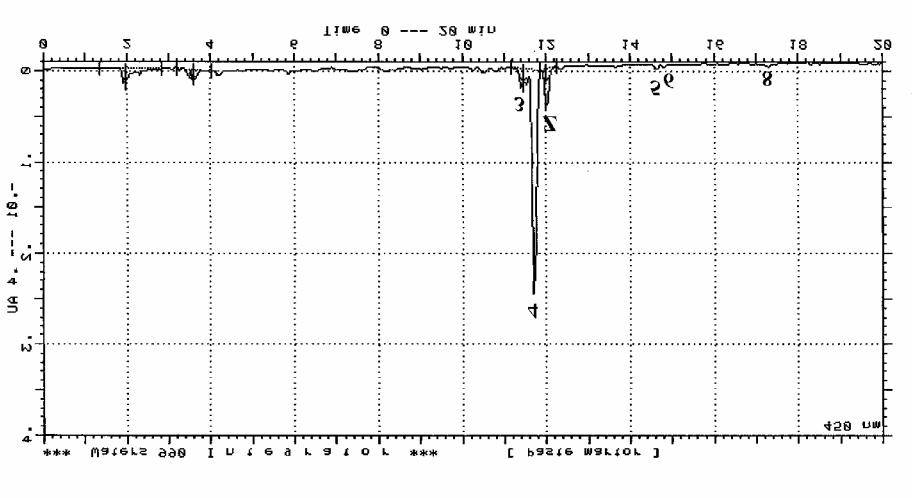

Fig. 2. HPLC chromatogram of carotenoids from reference pasta samples (peak identities are in table 1).

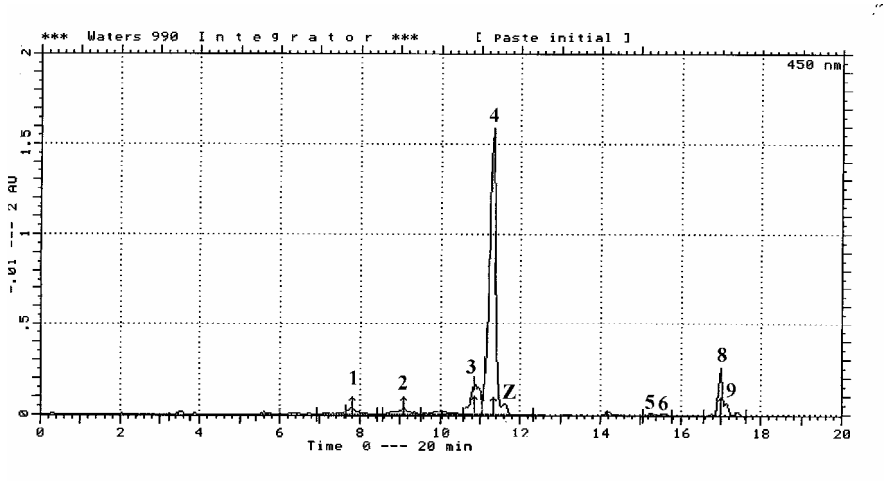

Fig. 3. HPLC chromatogram of carotenoids from pasta colored with the *Cucurbita pepo var. giromontia* extract (peak identities are in table 1).

After a storage of 30 days, the concentration of total carotenoids decreases at 9.76 μg/ g dry weight.

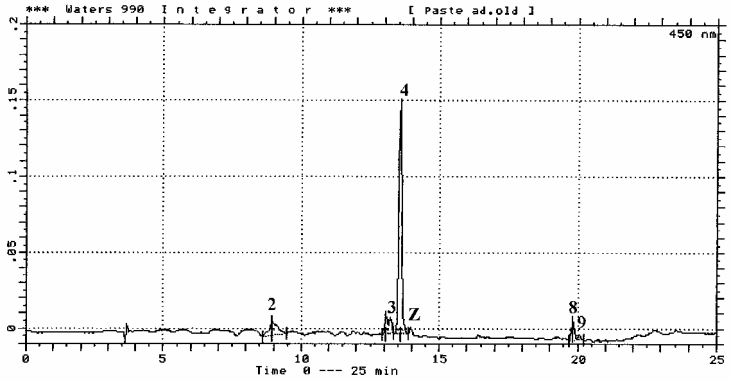

Fig. 4. HPLC chromatogram of carotenoids from pasta stored for 30 days (peak identities are in table 1).

The effect of 10 minutes boiling is a decrease of the total carotenoids concentration at 7.22 μg/ g dry weight. As the remaining water had a yellow shade, this was also analyzed, but only a qualitative HPLC analysis was performed; the similarity between the obtained HPLC profile and the HPLC profile of boiled pasta, together with the turbidity of water samples, lead to the conclusion that the observed color was due to some small dough particles spreaded in water.

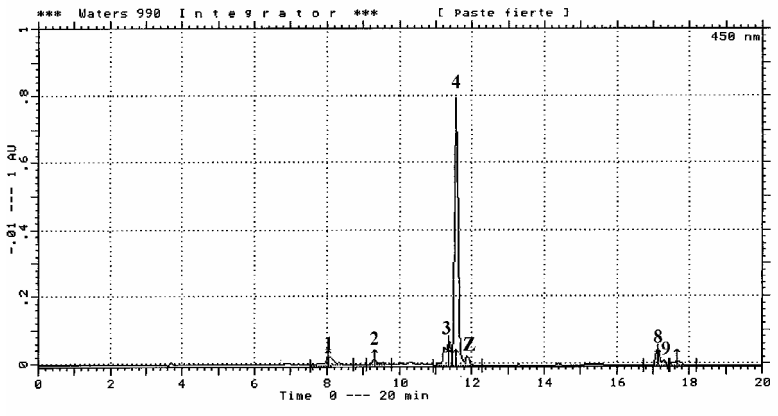

Fig. 5. HPLC chromatogram of carotenoids from boiled colored pasta (peak identities are in table 1).

Conclusions

Carotenoid degradation is a general characteristic of all analyzed carotenoids, excepting 15, 15' Z- β, β-carotene, which is generated during the storage of pasta as an isomerization product of β-carotene.

The appearance of pasta was much improved using the proposed natural extract and we have to emphasize here that this effect was obtained using a natural extract, not a synthetic dye! Thus, the disadvantages related to the health effects when using synthetic dyes, as well as the bad yellow appearance of the boiling water (the result of synthetic dyes' dissolution) can be avoided. The color stability is high enough to maintain a commercial aspect of pasta during a normal shelf life.

Acknowledgement

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