E. Muntean, et all. Journal of Agroalimentary Processes and Technologies, Volume XII, No. 1 (2006), 89-94
Full Paper - Food Control Section

HPLC ASSESSMENT OF CAROTENOIDS STABILITY IN CAKES COLORED WITH A NATURAL EXTRACT

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Abstract

A new possible direction of use for a plant extract obtained from the epicarp of Cucurbita pepo L. var. Giromontina fruits (zucchini) is described: that of coloring agent for cakes. The stability of carotenoids during baking and storage was assessed using reverse - phase high performance liquid chromatography. On a Nucleosil 120 - 5C_{18} column, a good separation of all carotenoids was achieved using a gradient involving the following mobile phases: A - acetonitrile: water = 9: 1 and B - ethyl acetate (both with 0.5% EPA), the total separation time being less than 20 minutes.
Keywords: *HPLC, chromatography, food colorants, carotenoids, food analysis, cake*

Introduction

The initial perception of food products is visual and for this reason color is one of the main characteristics which determines the buying decision, being usually closed - related with food quality. Color is a meaningful factor in a rapid quality assessment of food products; consumers became negative when the color is unexpected, interpreting this as an indicator of a possible degradation or of a food fraud.Consequently, it is a widespread practice in industry to enhance or even to change the color of foods, adding natural or synthetic colorants (Douglas, 2002; Otterstatter, 1998).

Many foods are colored naturally through the ingredients used or the preparation and cooking processes used but added naturally-derived colors have been used for several centuries. Among natural colorants, carotenoids are often preferred when producers need yellow, orange or red colors, since beside their coloring properties; they are also biologically active compounds. Natural extracts have been used as

food colorants for centuries - especially annatto, saffron, tomato and paprika. Nowadays, nature - identical carotenoids are produced on a large scale, but natural extracts are still used.

Among other food products, cakes are often the subject of coloring, as the color of eggs' yolk 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 matrix: the extract obtained from the epicarp of *Cucurbita pepo L.var.giromontina* fruits. Up to the present, high performance liquid chromatography proved to be the best available technique in the analysis of carotenoids (Huck, 2000; Khachik, 1992; Muntean, 2006; Olivera, 2000) and for this reason, we used it in this research.

Experimental

Carotenoid extract utilized for coloring cakes was obtained from the epicarp of *Cucurbita pepo L. var. giromontina* (Muntean, 2006).

Cakes were obtained using the ingredients presented in table 1.The eggs' albumen was separated by yolks, and then the albumens were mixed with sugar and rum. The obtained foam was divided in two equal parts using a balance.

Table 1. The recipes used in cake preparation

Ingredients	U.M.	Colored samples	Reference samples
Eggs	Pcs.	3	3
Wheat flour type 000 (Bãneasa)	g	100	100
Sugar	g	125	125
"Floriol" sunflower oil	ml	50	75
Cucurbita extract	ml	25	-
Vanilla sugar	g	10	10
Rum	ml	15	15

An emulsion was prepared from egg yolks and oil, this being then also divided in equal masses: one was utilized as it was, while the other was mixed with *Cucurbita* extract. Dough preparation was achieved by mixing the albumen foam with the emulsion, followed by the addition of wheat flour and baking powder. After homogenization, the dough was poured in buttered trays, being baked for 30 minutes at

300^0C; finally, the cake was cooled, then discharged from trays and packed in polyethylene bags.

Reaching to the above mentioned added volume of extract was possible after several trials, in which were used different volumes of extract; an acceptable cake coloration can be obtained using volumes of 20 – 35 ml extract (reported to the amounts in the recipe). During experiments, ingredients were divided in two equal batches: one for a reference sample, other for colored samples, for a proper evaluation of the influence of added colorant. After cooling, samples were collected from both core and crust of each cake; cakes were then packed in polyethylene bags, being stored for a week in a room with a mean temperature of 20^0C, avoiding the direct exposure to sun light. After this period, cake samples were collected again. The same procedure was followed for both reference and colored samples.

Carotenoid analysis was performed as described in a previous work (Muntean, 2006).

Results and discussions

Determination of the total carotenoids' concentration in the reference samples lead to the value of 0.51 μg/ g core (0.72 μg/ g dry weight).

Table 2. Mean values of carotenoid concentrations in cake samples ([μg/ g dry weight])

Peak index	Carotenoids	Reference cake samples		Colored samples		
		Initial	After 7 days	Dough	Cake	After 7 days
1	Neoxanthin	0	0	0.95	0,76	0,37
2	Violaxanthin	0	0	0.83	0,34	0,21
3	Lactucaxanthin	traces	traces	1.47	0,89	0,57
4	Lutein	0,48	0,45	11.39	6,51	3,99
z	Zeaxanthin	0.17	0.13	0.25	0,18	0.17
5	α-cryptoxanthin	traces	traces	traces	traces	traces
6	β-cryptoxanthin	traces	traces	traces	traces	traces
8	β-carotene	traces	traces	1.92	0,45	0

The HPLC pattern of reference samples is that presented in the chromatogram from figure 1, revealing lutein as major carotenoid, this

being accompanied by smaller amounts of lactucaxanthin, besides traces of α-cryptoxanthin, β-cryptoxanthin and β-carotene (table 2).The main origin of all carotenoids is in the egg's yolk; however, the concentration of these carotenoids is too small, so that the cake color is pale – yellow.

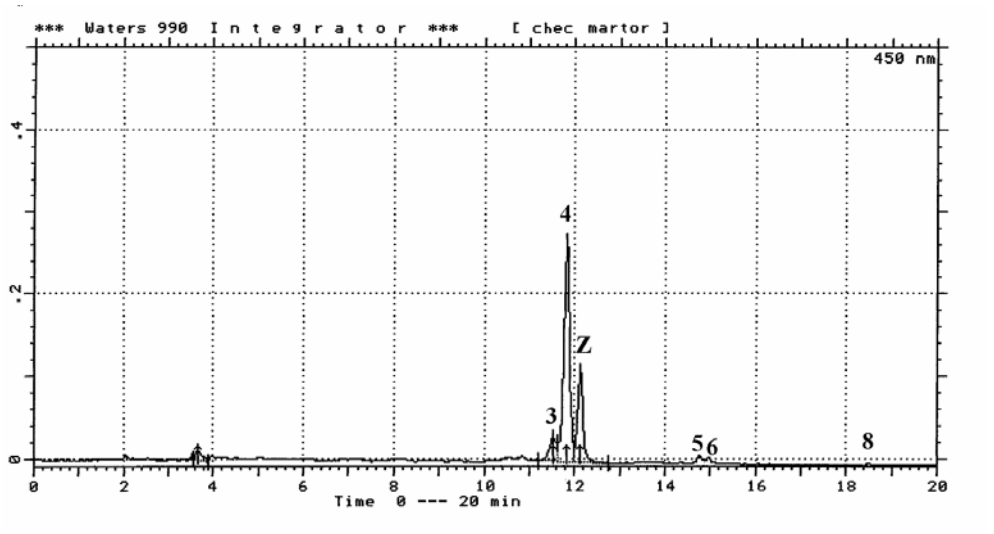

Fig. 1. HPLC chromatogram of the carotenoids extracted from the reference cake's core samples (peaks' identity in table 1)

The addition of extract increases the carotenoid concentration up to the value of 6.92 μg/ g (9.38 μg/ g dry weight) and changed the color in strong yellow with an orange shade, the concentrations of individual carotenoids being listed in table 2.

Figure 2 presents the chromatogram of cake with added carotenoids, showing an increased level of lutein and three new carotenoids: neoxanthin, violaxanthin and 15, 15' Z - β - carotene, all originating from the extract.

Cooking has not the same effects in the whole cakes mass; the crust's temperature is almost equal with that in the oven, while in the core the temperature is lower. For this reason, after cooking the crust contains no more carotenoids, but only degradation products of these. HPLC analysis revealed several peaks, most of them at low retention times

corresponding to unidentified polar carotenoid degradation products, with spectra having absorbtion maxima in the domain 330 – 360 nm (figure 3).

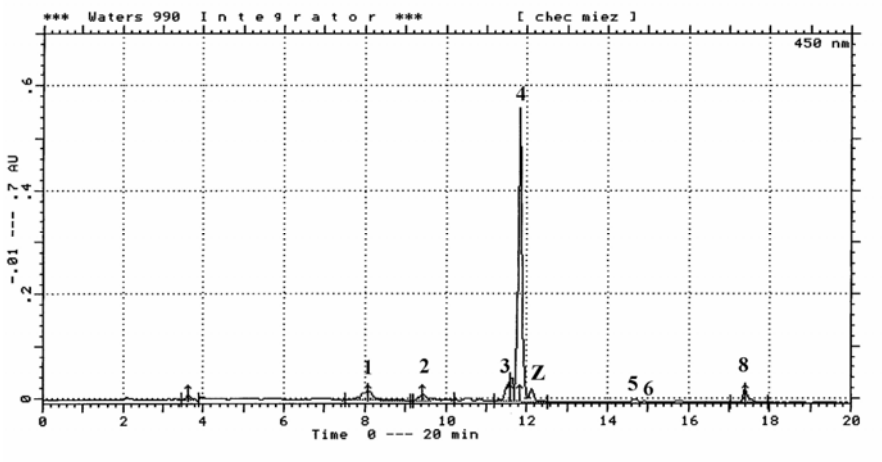

Fig. 2. HPLC chromatogram of the carotenoids extracted from the colored cake's core samples (peaks' identity in table 1).

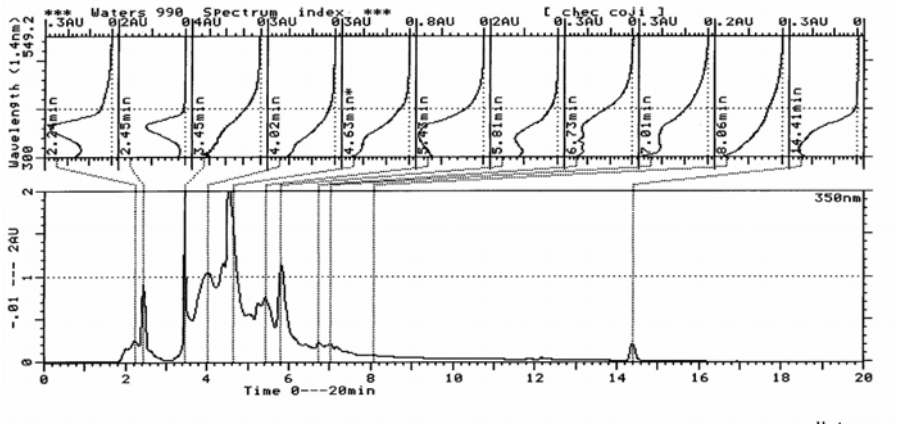

Fig. 3. HPLC chromatogram (spectrum index plot) of the carotenoids extracted from the cake's crust samples

Studying the data presented in table 1 in order to compare the carotenoids' stability, one can observe that degradation is a general characteristic of all carotenoids, being more important during baking. After a seven days storage at room temperature, the carotenoids' concentration in the sample cakes decreased from 0.51 μg/ g core (0.72

μg/ g dry weight) to 0.37 μg/ g core (0.46 μg/ g dry weight). For samples with added extract, the concentration decreased from 6.92 μg/ g (9.38 μg/ g dry weight) to 4.82 μg/ g cake (6.11 μg/ g dry weight), during the same period. However, in the last case the concentration of carotenoids is high enough, so that the color of cake is still a good one in the colored product.

Conclusions

The appearance of cakes 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 can be avoided. The color stability is high enough to maintain a commercial aspect during a normal shelf life.

Reference

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