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HPLC ASSESSMENT OF CAROTENOIDS' STABILITY DURING LACTIC ACID FERMENTATION OF ZUCCHINI

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Abstract

The effects of lactic acid fermentation on carotenoids from fruits of Cucurbita pepo L. var.giromontina are assessed using high performance liquid chromatography. As a consequence of exposure to an acidic pH, a differential degradation of carotenoids occurred. The most unstable carotenoid proved to be violaxanthin, but high percentages of degradation were recorded also for neoxanthin, lactucaxanthin, lutein and β-carotene – which proved to be the most stable carotenoid. As provitamin A activity of the studied matrix is mainly due to β-carotene, the degradation of this carotenoid was followed by a similar loss of provitamin A activity, which decreased from 53.32 μg RE/ g dry weight to 43.40 μg RE/ g dry weight.
Keywords*: lactic fermentation, zucchini, Cucurbita pepo L. var.giromontina, HPLC, preservation.*

Introduction

Lactic acid fermentations include those fermentations in which the fermentable sugars are converted to lactic acid by microorganisms such as *Lactobacillus spp., Leuconostoc spp., Pediocccus spp., Streptococcus spp., Bifidobacterium spp.* etc. (Demain, 2000; McFeeters, 2004). Such bacteria perform an essential role in the preservation and production of foods ranging from fermented fresh vegetables such as cabbage, cucumber, to fermented cereals, yogurt, sour dough bread, fermented milk, fish and meat products (Kilcast, 2000). These bacteria grow readily on most food substrates and lower the pH rapidly to a point where other competing organisms are no longer able to grow; thus, the food becomes resistant to microbial spoilage and to development of toxins (Kalantzopoulos, 1997). Lactic acid fermentation is responsible for processing and preserving vast amounts of food, insuring also its safety; vegetable foods are preserved around the world by lactic acid fermentation. In addition, this type of fermentation provides the consumer with a wide variety of flavors, aromas and textures which enrich the human diet.

The oldest known product of lactic fermentation was sauerkraut (Pederson, 1969), but most vegetables can be fermented in this way: cucumbers, cauliflower, green tomatoes, green beans, Brussels sprouts, mixed vegetables, radishes, carrots and nearly all vegetables - even some fruits such as olives, papaya and mango are acid fermented in the presence of salt around the world (Steinkraus, 1983). So far cucumber, cabbage and olives are the only vegetables that are fermented in large volumes for human consumption (Gardner, 2001; Montet, 2006; Nout, 1992), these processes being most economically relevant.

Lactic acid fermentation of vegetables, applied as a preservation method, is further investigated because of the growing amount of raw materials processed in this way in the food industry. The main reasons for this interest are the agricultural, nutritional, physiological and hygienic aspect of the fermentation process; also, it is a potential process for making new products from other vegetables.

Lactic fermentation has many benefits: it is feasible in both large and small scale, it is inexpensive, it does not require additives, it requires little or no heat and confers organoleptic characteristics to the foodstuff according to consumers' habits and requirements (Steinkraus 1983 and 2004).

The biochemical processes involving major nutrients in lactic acid fermentation are well documented; less documented is the behavior of minor nutrients. Among them, carotenoids are important food constituents and deserve a better approach; maybe the lack of information in this particular area is due to the well-known instability of carotenoids to heat, acidic pH, light and oxygen.

For assessing the stability of carotenoids during brining process, the fruits of *Cucurbita pepo L.var.giromontina* ("zucchini") were selected. The main reason of this choice was the high carotenoid content in this plant matrix, especially in the fruit's epicarp (Muntean, 2006). More than that, zucchini have not been investigated yet as fermented product, though they are frequently included in mixed vegetable pickles.

Zucchini is an annual herbaceous plant, a popular summer squash with a thin edible skin and soft edible seeds. It has a cylindrical shape and a slightly curved, smaller stem end; the skin is light or dark green in color and sometimes it has faint yellow stripes. Zucchini are used when still unripe and since the younger ones are preferred, they are often sold still bearing the corolla; they have a mild, delicate flavor.

Dietetically, zucchini are highly recommended; boiled and seasoned with oil and lemon juice they are easily digestible and therefore good for convalescents or people on strict diets. Zucchini are thought to have a considerable diuretic action and to be effective against constipation because of the mucilage they contain. They are very good after being dipped in batter or in egg and bread crumbs and fried, stuffed, stewed separately or with other vegetables such as eggplants, tomatoes, potatoes, peppers, etc.

According to the USDA National Nutrient Database for Standard Reference, the chemical composition of raw zucchini is as follows: water - 92.73%, protein - 2.71%, total lipids - 0.40%, ash -1.05%, carbohydrates - 3.11%, total dietary fiber -1.1%. The concentration of microelements expressed in mg/ 100 g, are: calcium - 21, iron - 0.79, magnesium – 33, phosphorus - 93, potassium - 459, sodium - 3, zinc - 0.83, copper - 0.097, manganese - 0.196, selenium - 0.3. The concentration of vitamins, in mg/ 100 g, are as follows: ascorbic acid - 34.1, thiamin - 0.042, riboflavin - 0.036, niacin - 0.705, pantothenic acid - 0.367, vitamin B-6 - 0.142, folate - 0.02, folate - 0.02, vitamin A – 490 IU. Lipids, in g/ 100 g, are: total saturated fatty acids - 0.083, 12:0 - 0.003, 16:0 - 0.071, 18: - 0.009, total monounsaturated fatty acids - 0.031, total polyunsaturated fatty acids - 0.169. Amino acids are also known, in g/ 100 g: tryptophan - 0.024, threonine - 0.066, isoleucine - 0.098, leucine - 0.159, lysine - 0.151, methionine - 0.039, cystine - 0.029, phenylalanine - 0.096, tyrosine - 0.073, valine - 0.123, arginine - 0.115, histidine - 0.059, alanine - 0.142, aspartic acid - 0.332, glutamic acid - 0.291, glycine - 0.103, proline - 0.085, serine - 0.111. From these data, it is obvious an informational gap in carotenoid composition of zucchini.

In this paper, carotenoid stability during lactic acid fermentation of zucchini has been described. Some practical consequences linked to the loss of provitamin A activity are also involved. High performance liquid chromatography was selected in order to quantify carotenoids, this being the best method for carotenoid analysis available to date.

Experimental

Plant material: Immature fruits of zucchini (*Cucurbita pepo L. var. giromontina*) were used for lactic acid fermentation; immediately after harvesting, fruits were washed, peeled and sliced in pieces 10 cm length and 1 cm width.

Fermentation: The zucchini slices of epicarp and mesocarp were blanched in hot water (1 min at 70°C), then were introduced in 400 ml glass jars (epicarp slices at the bottom, mesocarp pieces above). Brine solutions of three different salt concentrations (2, 4 and 6%) were prepared by dissolving NaCl in distilled water, and 200 ml of the prepared brine solution were added to each jar. Pickled zucchini obtained with 2% brine solution were found to be the most acceptable organoleptically, so that fermentation experiments were re-started using this concentration, using a bigger number of jars, in order to have three replicates for each measurement. All the jars were stored at room temperature (22 ± 2°C), in the dark (for avoiding the degradation caused by light), for 28 days. Samples were collected after 7, 14, 21 and 28 days of storage; three jars were opened each time and after the removal of brine, the pieces of mesocarp were cut in smaller parts, being then mixed. Average samples of ~10 g were selected for each analysis; from these, the water content and the concentration of carotenoids were established.

Analysis of carotenoids: zucchini pieces were homogenized in a blender and the obtained mash was used for carotenoid extraction. Carotenoids were analyzed by high performance liquid chromatography, using the procedure described in a previous work (Muntean, 2003). Identification of carotenoids was made on the basis of visible spectral characteristics, retention times, HPLC co-chromatography with standards, relative elution order compared to authentic standards and literature data; quantification was achieved using the internal standard method, with echinenone as internal standard. The provitamin concentrations were expressed in retinol equivalents (RE), according to the requirements of FAO/WHO. The reported results were calculated as a mean of three replications.

Results and Discussion

The total carotenoid content from the raw zucchini was 0.15 µg/ g (1.82 µg/ g dry weight) for mesocarp and 431.17 µg/ g (3612.68 µg/ g dry weight) for epicarp. The HPLC chromatogram presented in figure 1 reveals the carotenoid pattern of the epicarp, which is dominated by lutein; small amounts of neoxanthin, violaxanthin, lactucaxanthin and β-carotene are present, together with traces of α-cryptoxanthin, β-cryptoxanthin, α-carotene, and 15, 15'Z-β, β-carotene. The mesocarp has a similar pattern.

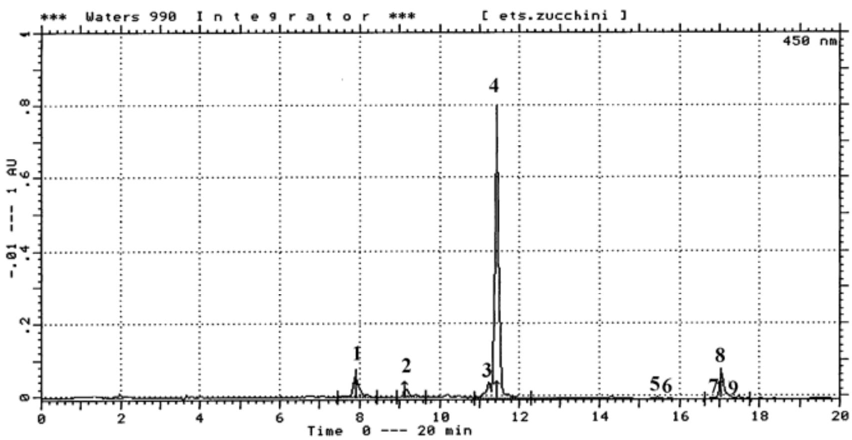

Figure 1. HPLC chromatogram of the saponified total extract obtained from the raw fruits mesocarp of *Cucurbita pepo L. var.giromontina.*
The identities of peaks are: 1 - neoxanthin, 2 - violaxanthin, 3 – lactucaxanthin, 4 - lutein, 5 - α-cryptoxanthin, 6- β-cryptoxanthin, 7 - α-carotene, 8 - β-carotene, 9 – 15, 15'Z-β, β-carotene.

The brine's pH recorded in the first day was 5.57. There was a gradual decrease of pH due to the accumulation of organic acids, mainly lactic acid: 4.81 after 7 days, 4.76 after 14 days, 4.64 after 21 days and finally 4.10 after 28 days. As a consequence of the acidic environment but also of the initial processing, the carotenoids were degraded. The results recorded during experiments are summarized in table 1; these results reveal the changes in carotenoid composition in the epicarp, which is richer in carotenoids; the small carotenoid content recorded in the mesocarp made difficult the quantification for individual carotenoids.

Table 1. The carotenoids' concentrations recorded during experiments in zucchini epicarp expressed in μg/ g dry weight.

Peak index	Carotenoid	Start	After 7 days	After 14 days	After 21 days	After 28 days
1	Neoxanthin	319.85	102.35	79.96	67.16	60.77
2	Violaxanthin	120.26	24.15	13.23	8.42	traces
3	Lactucaxanthin	172.65	77.69	65.61	51.79	47.34
4	Lutein	2605.97	1303.98	1041.38	1013.27	995.20
5	α-cryptoxanthin	traces	traces	traces	traces	traces
6	β–cryptoxanthin	traces	traces	traces	traces	traces
7	α-carotene	traces	0	0	0	0
8	β - carotene	310.37	294.85	279.33	263.81	252.28
9	15Z - β-carotene	19.15	23.81	20.13	18.01	16.20
[μg R.E/ g dry weight]		53.32	51.13	48.23	45.47	43.40

For a better image of the comparative carotenoids stability, the percentages of degradation were calculated for the major carotenoids, being plotted vs. time in figure 2. This figure emphasize two types of behavior: violaxanthin, neoxanthin, lactucaxanthin and lutein were strongly affected by brining (especially during the initial processing stages), while storage had a smaller influence on their degradation. β-carotene was degraded mainly due to storage.

From table 1 and from figure 2 one can remark that the most important degradations were recorded as a result of initial processing stages (peeling, slicing, blanching at 70°C, first stage of fermentation); after the first week, a massive degradation occurred (between 49 and 79%), while in the other 3 weeks the total degradation was much smaller (between 11.85 and 23%). The most unstable carotenoid proved to be neoxanthin, which was almost completely destroyed during 28 days; neoxanthin, lactucaxanthin and lutein were influenced in a lesser extent, while the most stable carotenoid was β-carotene which during 28 days was degraded only in 13.72%.

A distinct case is that of 15Z-β-carotene: its concentration increases in the analyzed samples, touching a maximum after one weeks, then decreases. This can be explained admitting that initially it results as an izomerisation product of β-carotene, but finally suffers degradations too, due to the acidic environment.

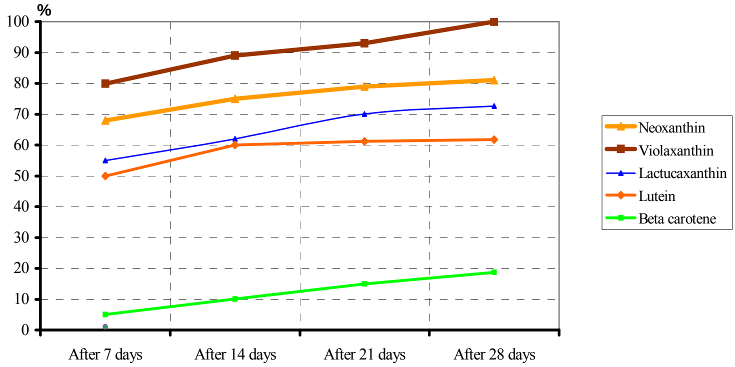

Figure 2. Carotenoids' degradation during experiments [%]

Traces of α-cryptoxanthin and β-cryptoxanthin are present in all stages (under the quantification limit); their constant presence at trace levels can indicate that they are short life intermediates in the oxidative degradation of carotenes to xanthophylls.

From a nutritional point of view, lactic acid fermentation caused only relatively small changes in provitamin A value: from 53.32 μg RE/ g dry weight to 43.40 μg RE/ g dry weight. This aspect can be explained by higher stability of β-carotene, the main provitamin A carotenoid present in this matrix, during fermentation.

Conclusions

The presented data allows a comparative insight in carotenoid behavior during lactic acid fermentation. A proof that oxidative damage occurs is the constant presence α-cryptoxanthin and β-cryptoxanthin (both being oxidation products of carotenes). Izomerization was demonstrated for the case of β-carotene, which was converted in 15 Z - β-carotene. The poor stability of epoxycarotenoids reported in other circumstances was confirmed by the poor stability of violaxanthin and that of neoxanthin. Despite the overall decrease in carotenoid content, zucchini pickles remains still a valuable source of lutein. The results of this study also show that zucchini can be successfully processed using lactic acid fermentation; the final product can be used as both a condiment or as a side-dish. Sensory evaluation rated the pickled zucchini acceptable based on texture, taste, flavor and aftertaste. A new product such as zucchini pickles may serve as an additional source of pickle with beneficial probiotic properties.

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