

HPLC assessment of carotenoids' stability during food processing

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INTRODUCTION



Among bioactive compounds in food matrix, carotenoids are the most important due to their antioxidant, coloring and provitamin A properties. Unfortunately, they are also among the most sensitive substances to heat, light, oxygen and acidic environment; hence, common food processing techniques induce massive degradations of carotenoids, decreasing both the nutritional value and the appearance of carotenoid-containing foods [1].

Research objective

For assessing the stability of carotenoids during some food processing procedures, the fruits of *Cucurbita maxima Duch*.

(pumpkins) were selected. The main reason of this choice was the diversity of carotenoid structures that are present in these,

this study allowing a complex view in the stability of both carotenes and xanthophylls, involving: neoxanthin, violaxanthin, cucurbitaxanthin A, anteraxanthin, lutein, zeaxanthin, α -cryptoxanthin, β -cryptoxanthin and β -carotene.

MATERIALS & METHODS

Samples from both raw fruits and the obtained products (baked/ boiled/ brined/ fermented pumpkin) were extracted with acetone and ethanol, using added butylhydroxytoluene as antioxidant. Liquid-liquid extraction was then performed to transfer carotenoids in diethyl ether, being followed by saponification (figure 1). High performance liquid chromatography (HPLC) was selected in order to quantify carotenoids, this being the best method for carotenoid analysis available to date [2]. Good HPLC separations during less than 20 minutes were accomplished (figure 2), using a Nucleosil 120-3C₁₈ column, with a gradient involving acetonitrile : water (9 : 1) and ethyl acetate; carotenoids were identified based on their retention times, co-chromatography with standards and their spectra (recorded using a photodiode array detector). Quantification was accomplished based on the external standard method.

RESULTS

A differential degradation of carotenoids was recorded, depending on the processing method. Hence, brining caused severe degradation of violaxanthin, anteraxanthin and neoxanthin; lutein, zeaxanthin and β -carotene were influenced in a smaller degree, while the most stable carotenoid was cucurbitaxanthin A. During the two processing methods explored for assessing the effects of thermal processing involving boiling and baking, it was proved that both xanthophylls and carotenes were affected, zeaxanthin and



Figure 1. Simplified flow diagram for sample processing

violaxanthin being the most sensitive carotenoids. Cucurbitaxanthin A and lutein and were the most stable carotenoids under these circumstances, their degradation being of 21.39% and 25.11%. The decrease with 33.95% of provitamin A during processing is mainly due to the loss of β-carotene. As an effect of heat exposure, β -carotene undergoes both degradation and E- to Z- isomerization, mainly in 9Z- β -carotene, whose concentration increased.



Figure 2. HPLC chromatogram of carotenoids from raw (left) baked (right) mesocarp of *Cucurbita max. Duch* fruits (1neoxanthin, 3 - violaxanthin, 4- luteoxanthin, 7- cucurbitaxanthin A, 7' - anteraxanthin, 9- lutein, 11-zeaxanthin, 12 - α cryptoxanthin, 13- β -cryptoxanthin, 14 - 5, 6-epoxy- β -carotene, 17- β -carotene, 18 - 9Z- β -carotene, 19 -13Z- β –carotene



Figure 3. Carotenoids' degradation during processing

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The 1st International Electronic Conference CSAC 2021 on Chemical Sensors and Analytical Chemistry 01-15 JULY 2021 ONLINE



