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Stability Of Carotenoid Extracts Of Cucurbita maxima Towards Enzymatic Cooxidation And Aroma Compound Generation

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***Abstract*— Ambercup squash (Latin name *Cucurbita maxima*), is an excellent source of carotenoids, particularly of β-carotene. These compounds are also potential aroma precursors, among which β-ionone, 5,6-epoxy-β-ionone, dihydroactinidiolide and β-cyclocitral constitute essential aroma notes. They can be produced by β-carotene degradation through an attack by enzyme-generated free radicals and a cleavage at the C9-10 bond. The aim of this study was to investigate the stability of carotenoid extracts from ambercup squash towards enzymatic cooxidation as well as the generation of volatile compounds during the cooxidation process. The stability of β-carotene samples against oxidation was evaluated through β-carotene degradation of the samples. The production of aroma compounds during oxidation was quantified by gas chromatography. Results showed that ambercup squash β-carotene is more stable against a free radical stress than its synthetic pure counterpart. However, when compared with results obtained with pure β-carotene, the quantity of generated aroma during oxidation of ambercup squash is small in comparison with that of synthetic β-carotene. Further studies are conducted to improve the aroma production yield of ambercup squash and the cleavage at the C9-C10 bond.**

Keywords-Cucurbita maxima; carotenoid; stability; oxidation; cleavage compounds

I. INTRODUCTION

Carotenoids are a large group of compounds widely distributed in the plant, microbial and animal reigns. They have an isoprenoid structure which confers them specific properties. In particular, their conjugated double-bond system plays a role in their free radical scavenging antioxidant and anticarcinogenic properties [1]. The majority of epidemiological studies showed clearly the positive impact of carotenoid-rich diets to reduce risk of several cancers [2, 3]. However, some contrasting results have been published including the two well-known intervention studies which concluded to an increase lung cancer incidence for smokers taking supplemental β-carotene. Several hypotheses were proposed to explain these contradictory results [2]. Among them, the presence of other antioxidant compounds or of oxidation products in the carotenoid extracts has been mentioned. As a result, the antioxidant properties of carotenoid extracts depend on the composition and thus, on the source of the extract. When the oxidation and cleavage of carotenoids occur before ingestion (during maturation or extraction), the presence of volatile compounds can also modify the sensory perception of plants as carotenoid derived aroma compounds are a very important source of sensory properties for plant products [4]. The main flavoring compounds resulting from the cleavage of β-carotene are shown in Fig. 1. These compounds are present in numerous β-carotene containing plants but their amount is very different depending on the species, showing that the reactivity of carotenoids in the various plant environments is very different. In particular, some cleavage products can be further oxidized, resulting in another set of compounds with various sensory properties.

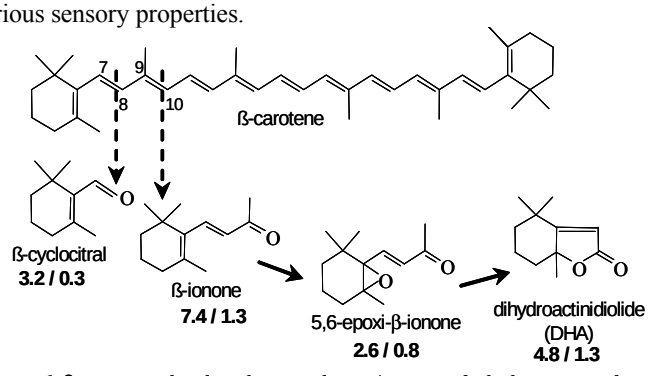

Figure 1. β-Carotene and its degradation products. X/Y: Ratios of volatile generation from pure carotene/ambercup squash carotene

Cucurbita maxima, a *Cucurbitaceae*, called ambercup squash, is of great interest as it is a valuable natural source of carotenoids, including β-carotene which is well known for its antioxidant properties. In this paper, the reactivity of ambercup squash is investigated through the degradation of carotene and the apparition of cyclic volatile compounds.

II. MATERIALS AND METHODS

A. Materials

Synthetic β-carotene (purity > 97%) was obtained from Fluka (Sigma-Aldrich, St. Quentin Fallavier, France). Chloroform, Tween 80, enzyme xanthine oxidase (XO grade III from buttermilk) were purchased from Sigma (Sigma-Aldrich). Ambercup squash is purchased in a local store and stored at 4°C until analyzed.

B. Methods

1) Dispersion of synthetic β-carotene

Dispersion of β-carotene was performed as described previously [5]. 500 mg of β-carotene was first dispersed in 6 ml of Tween 80. The mixture was homogenized for 3 min at room temperature with an IKA Ultra-Turax T25 System (IMLAB, Lille, France) set to 24,000 rpm before solubilization in 500 ml of chloroform under moderate magnetic stirring. Chloroform was then removed from the emulsion by rotary evaporation under reduced pressure according to Ben-Aziz et al. (1971). The residue was dissolved in EDTA 0.25%, filtered and diluted in phosphate buffer.

2) Extraction of β-carotene from ambercup squash

500 g of ambercup squash were milled with 1 l of hexane in a kitchen blender. The hexane phase containing β-carotene was then collected and evaporated by rotary evaporation under reduced pressure. The residue was dissolved in EDTA 0.25%, filtered and diluted in phosphate buffer.

3) Cooxidation of β-carotene in aqueous media

Reactions were carried out as previously described [5] at 37°C in an enzyme reactor under stirring at 250 rpm. The aqueous phase was composed of phosphate buffer (50 mM, pH 8), acetaldehyde (48 mM) and enzyme XO (27 x 10-3 IU/ml). The total volume was 100 ml. The initial concentration of β-carotene was evaluated at 450 nm using a spectrophotometer (V-570 UV/VIS/NIR, JASCO Corporation, Tokyo, Japan) according to the Beer-Lambert Law with = 2592. The kinetics of degradation was followed by determining the concentration of β-carotene remained in the solution using spectrophotometry.

4) Analyses

To extract aroma from the aqueous phase, 4-ml samples were extracted with diethyl ether (1:1) after the addition of methyl isoeugenol (10 mg/l). The organic phase was then concentrated in a Cuderna column at 44.5°C to a final volume of 1 ml and then analyzed in HP6890GC Series gas chromatograph (CPG) equipped with a FID (flows: H2 30 ml/min, air 300 ml/min) and a capillary HP-Innowax HP 1909 1N-113 column. The temperature of the oven was constantly increased from 75°C to 250°C at 1.5°C/min. The flow of the vector gas was set to 4.0 ml/min. The injection of 1 µl was done in a 10:1 ratio split mode.

III. RESULTS AND DISCUSSION

A. Degradation of carotenoids of ambercup squash and comparison with the degradation of pure β-carotene

Carotenoids exhibit antioxidant properties which can turn prooxidant in highly oxidative environments. In our study, a radical generating enzyme was used (Bovine xanthine oxidase (XO) and acetaldehyde as the substrate) to establish highly oxidizing conditions. This model has been previously developed by Bosser and Belin [5]. They identified several radical species in the medium such as the superoxide anion, hydroxyl, alkyl, and peroxyl radicals. In their system, pure β-carotene was bleached and degraded rapidly. In our work, very rapid degradation was also observed with synthetic β-carotene (Fig. 2) in the first three hours, in which about 20% of carotene are degraded, and slower thereafter (60% are degraded after 22 hours). These results are compared to the degradation of carotene from the carotene extracts of ambercup squash. This extract contains apolar compounds from the ambercup squash such as β-carotene, α-carotene and other compounds. With this preparation containing a similar initial concentration of β-carotene, the degradation was more rapid in the first 6 hours of reaction, then the degradation was negligible and the carotene concentration after 24 hours was at about 58% of the initial value (Fig. 2).

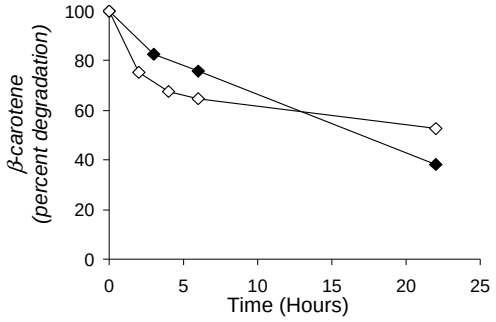

Figure 2. Kinetics of β -carotene degradation. (◆) synthetic β -carotene; (◇) β -carotene from ambercup squash extract.

Thus, for both substrates (pure carotene or carotene of ambercup squash extract), the degradation began rapidly in the first hours and then slowed down. These results are conform to those obtained previously by Bosser and Belin [5] and can be explained by a decrease in the generation of free radicals after some hours. However, for ambercup squash extracts, the degradation was more rapid in the beginning and was almost stopped, suggesting that the mixture could scavenge free radicals without degradation of carotenes. These results are similar to those obtained with the gâc fruit (Latin name *Momordica cochinchinensis*) [6], which may reflect the specific composition or supramolecular structure of the carotene extracts from vegetable. This difference in composition or structure could result in a difference in cleavage products. To investigate this point, we analyzed the apparition of β-carotene cleavage products from degradation of synthetic carotene and carotene of ambercup squash extract.

B. *Generation of volatile carotene degradation products*

The main cyclic volatile compounds resulting from the degradation of β-carotene were analyzed after a 24-h cooxidation. Several compounds can be generated resulting from a cleavage at different sites. The cleavage at the C6-7 bond results in β-cyclocitral, at the C7-8, dihydroactinidiolide and at the C9-10, β-ionone which can be further oxidized to the 5,6-epoxide or to the lactone [7, 8]. Ratios of generation from synthetic and ambercup squash's carotene are given in Fig. 1. Important differences between these two sources are observed. Indeed, whereas for synthetic carotene, the main cleavage site is C9-10 [8], only small quantity of these products is encountered for the ambercup squash's carotene extract (quantity of β-cyclocitral < 5,6-epoxide < β-carotene = DHA) (Fig. 1). Even more than 50% of carotene of ambercup squash were degraded, the yield of generated aroma is low compared to that of pure β-carotene. This suggests that the accessibility of the cleavage bonds to radicals is different for ambercup squash and other compounds were generated rather than the four studied. Several hypotheses can explain this difference. In one hand, the composition of radical species developed in the presence of ambercup squash can be different due to radical scavenging effects resulting in the low concentration the four studied aroma. Another possibility is that the supramolecular organization of carotene is different in ambercup squash, protecting both the C9-10 and the C7-8 bonds. As carotenes are very hydrophobic, they tend to aggregate and several organizations can be encountered [9, 10].

IV. Conclusion

The results presented here show that ambercup squash β-carotene is more stable against a free radical stress than its synthetic pure counterpart. This could be due to the presence of other radical scavenging species or of synergistic scavenging effects but also to a different supramolecular aggregation of carotene. In relation to this, the favored cleavage sites of β-carotene seemed very different in ambercup squash. These results confirm the potential interest of ambercup squash as an antioxidant or radical scavenging source but they also bring new insights into the carotenoid derived aroma compound generation. Both fields of antioxidant properties of ambercup squash and volatile compounds of this fruit are currently under investigation.

Acknowledgment

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