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Thermal degradation kinetics of xanthophylls from blood orange in model and real food systems

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ABSTRACT

Thermal degradation kinetics of the major blood orange xanthophylls (*cis*-violaxanthin, lutein, β-cryptoxanthin, zeaxanthin and *cis*-antheraxanthin) were investigated at 45, 60, 75, and 90 °C in real juice and three model systems formulated to evaluate the impact of xanthophyll form (esterified or free) and pH (acid or neutral). Xanthophylls were monitored by HPLC-DAD and kinetic parameters were identified by non-linear regression. A second order model best fitted the degradation curves of xanthophylls. All degradation rates were the lowest in real juice. Esterified forms were more stable than were the free forms. In all acidic media, β-cryptoxanthin exhibited the lowest degradation rates followed by lutein and zeaxanthin. In comparison, the epoxy carotenoids *cis*-violaxanthin and *cis*-antheraxanthin degraded around 3-fold faster in their esterified form. In their free form, *cis*-antheraxanthin degraded 30-fold faster while *cis*-violaxanthin instantaneously disappeared because of the isomerisation of its 5,6-epoxy groups into 5,8-epoxy. By contrast, in neutral medium, free epoxy-xanthophylls were about 2-fold more stable than were the free hydroxy xanthophylls lutein, zeaxanthin and β-cryptoxanthin. Kinetic behaviours of xanthophylls were closely dependent on their chemical structures.

1. Introduction

Citrus fruits are well known for containing several phytochemicals, such as carotenoids, vitamin C and polyphenols (Ladaniya, 2008; Plaza et al., 2011). The nutritional value of citrus fruits, owing particularly to their carotenoid content, is now well established in the prevention of and/or protection against several human diseases (Mishima et al., 2003). Citrus carotenoids are classified by structure into two major classes: carotenes and xanthophylls. The carotenes (e.g. β-carotene, lycopene) are non-polar hydrocarbons. The more polar xanthophylls (lutein, zeaxanthin, β-cryptoxanthin and astaxanthin) contain either a hydroxyl or keto function in the end-group (Aizawa & Inakuma, 2007; Basu, Vecchio, Flider, & Orthoefer, 2001; Rodríguez-Bernaldo de Quirós & Costa, 2004).

Citrus fruits are largely produced and consumed, fresh or processed. Among citrus fruits, orange represents the more complex carotenoid profile with a large diversity of xanthophylls. The major ones are *cis*-violaxanthin, β-cryptoxanthin, lutein, zeaxanthin and *cis*-antheraxanthin (Dhuique-Mayer, Caris-Veyrat, Ollitrault, Curk, & Amiot, 2005; Fanciullino et al., 2006). Furthermore, in juice matrices, xanthophylls can be found, either in their free form, or partially or totally esterified with fatty acids (Giuffrida, Dugo, Salvo, Saitta, & Dugo, 2010; Melendez-Martinez, Vicario, & Heredia, 2009; Pérez-Galvez & Minguez-Mosquera, 2005; Schlatterer & Breithaupt, 2005).

Many researchers have studied carotenoid stability in food or model systems. These molecules are known to be relatively stable in their natural environment, but become sensitive to light, temperature and chemical exposure during processing (Minguez-Mosquera & Hornero-Mendez, 1994; Schieber & Carle, 2005). Moreover, the stability of xanthophylls was shown to be increasingly affected by the degree of their esterification and the nature of the fatty acids (Biacs, Daood, Pavisa, & Hajdu, 1989; Fu et al., 2010; Subagio, 1999). β-Cryptoxanthin esters or lutein esters, in solvents, were more stable to heat treatment than were the free forms (Fu et al., 2010; Subagio, 1999). As well, the results of Biacs et al. (1989) demonstrated that the esters of capsanthin and capsorubin were more stable to oxidation than were the free capsanthin and capsorubin in linoleic acid. The study of Mertz, Brat, Caris-Veyrat, and Gunata (2010) found that zeaxanthin esters of tamarillo fruit nectar heated at 80, 90, and 95 °C, were less heat-sensitive than were the free forms of zeaxanthin and β-cryptoxanthin (Mertz et al., 2010).

Among parameters affecting the carotenoid stability, pH medium can influence the degradation of xanthophylls. Several studies have shown that organic acids, released during the processing of fruit juices, cause rearrangements of 5,6-epoxide groups of violaxanthin

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to 5,8-epoxide groups. These isomerizations lead to carotenoid profile modifications (Dhuique-Mayer, Tbatou, et al., 2007; Mercadante & Rodriguez-Amaya, 1998). Indeed, Mercadante and Rodriguez-Amaya (1998) noticed that violaxanthin, the major carotenoid pigment in mango, was not found in processed juice. This observation was also done on citrus juices and the disappearance of violaxanthin in heated or processed juice and the appearance of auroxanthin were also observed in different citrus studies (Dhuique-Mayer, Tbatou, et al., 2007; Melendez-Martinez et al., 2007, 2010). Regarding the kinetic aspect of xanthophyll degradations, few studies are available compared with that on β-carotene. Some authors have conducted their kinetic study on a real food matrix. Hidalgo and Brandolini (2008) studied the thermal degradations of lutein, zeaxanthin and β-cryptoxanthin in cereals in a temperature range of −20–38 °C and showed that the degradation rate values and activation energies were the highest for lutein, and higher for zeaxanthin than for β-cryptoxanthin (Hidalgo & Brandolini, 2008). Dhuique-Mayer, Tbatou, et al. (2007) also found that β-cryptoxanthin was the most stable among xanthophylls of citrus juice from 75 to 100 °C but did not compare degradation rates between them. In contrast, Aparicio-Ruiz, Minguez-Mosquera, and Gandul-Rojas (2011) showed that, in a range temperature of 60–120 °C, β-cryptoxanthin was more labile than was lutein in virgin olive oil. Their resulting activation energies for β-cryptoxanthin were very different and show that kinetic data on xanthophyll degradation are very matrix-dependent. (Aparicio-Ruiz et al., 2011; Dhuique-Mayer, Tbatou, et al., 2007). This fact reveals a need to overcome the matrix effect in order to improve understanding of the xanthophyll degradation and prediction of their losses during food processing. Thus, to study their degradation, some authors used model systems such as commercial standards or carotenoid extracts from natural products dissolved in various solvents, such as zeaxanthin in methanol or lutein in benzene (Milanowska & Gruszecki, 2005; Rios, Borsarelli, & Mercadante, 2005; Sanchez, Carmona, Ordoudi, Tsimidou, & Alonso, 2008; Subagio, 1999; Zepka & Mercadante, 2009). However, the problem was that transposition to a real food matrix was difficult. In addition, in this case, xanthophylls were in their free form while they were mainly esterified naturally. Some authors performed studies in systems similar to a food matrix, i.e. natural carotenoid extract in an aqueous phase (Rios, Fernández-García, Mínguez-Mosquera, & Pérez-Gálvez, 2008; Sanchez & Carmona, 2008). Only Zepka and Mercadante (2009) conceived a juice-like model containing xanthophylls (β-cryptoxanthin) based on cashew apple carotenoid extract solubilized in a water/ethanol mixture. These approaches seemed to be a good compromise to conduct a kinetic study, simplifying the medium composition while enabling comparison with the initial matrix. The lack of degradation kinetic data of xanthophylls, in both real and model matrices, justified our study. In addition, to our knowledge, there is no available data on the kinetic parameters of *cis*-violaxanthin, the major xanthophyll in orange juices. The aim of this study was to obtain kinetic data of the degradation reaction of each major xanthophyll from blood orange, in both their natural matrix and in three different aqueous model systems, isolated in their esterified or free form. The effects of the pH and esterification were also tested. This approach could provide original kinetic data on chemical structure of the xanthophylls and environmental conditions relating to their thermal stability.

2. Materials and methods

2.1. Blood orange juice

Sanguinelli blood oranges (*Citrus sinensis* L. Osbeck) were harvested at optimal maturity, which is defined by calculating the ratio, acid/soluble solids, and the colour of oranges, in the agricultural field Benyoub (Bejaia, Algeria). Oranges were cut in half and pressed, using a domestic juicer (Moulinex Masterchef 470, France). The juice was filtered with cheesecloth to remove the pulp. The freshly pressed juice was placed in amber glass bottles, and stored under nitrogen at −20 °C prior to analysis or heat treatment.

2.2. Preparation of model systems

2.2.1. General

Fig. 1 illustrates the whole procedure followed in our work. Juice-like systems were formulated with carotenoid extracts of blood orange juices, in their ester form (model 1), or in their free form in acidified or non-acidified solvent (models 2 and 3, respectively).

2.2.2. Preparation of carotenoid extract

The first carotenoid extraction step was performed according to Dhuique-Mayer et al. (2005). 80 g of orange juice were extracted with a mixture of ethanol/hexane 4/3 v/v (0.1% BHT), then with ethanol (0.1% BHT) and finally with hexane. Carotenoid extract for model 1 did not undergo saponification (native form) and was directly formulated. Carotenoid extracts for models 2 and 3, were saponified to obtain the free forms. The saponification was performed according to Dhuique-Mayer et al. (2005) and the free carotenoids were extracted with dichloromethane and hexane (Dhuique-Mayer et al., 2005).

2.2.3. Formulation of model systems

The three extracts were formulated in an aqueous solvent in order to reproduce a juice-like system, according to Zepka, Borsarelli, Azevedo, da Silva, and Mercadante (2009) with a slight modification because of the carotenoid profile differences between cashew apple and blood orange. In our study, xanthophylls were solubilized and emulsified in 40 ml of ethanol/pH 3.5 citrate buffer mixture at a ratio 25/75 (v/v) instead of ethanol/acidified water mixture (16/86) in Zepka and Mercadante (2009). Addition of a few drops of hexane was necessary to stabilize the emulsion of ester forms in the solvent mixture mentioned above. Emulsion stability was controlled at room and at all heat treatment temperatures. To assess the pH effect, the non-acidified model system was prepared by substitution of citrate buffer by distilled water. This neutral model did not reflect the real juice but it was studied in order to evaluate the impact of acidity on xanthophyll degradation kinetics. Since the extract came from 80 ml of orange juice and the final system represented 40 ml, a concentration factor (CF = 2) was applied in all model systems. Then, after formulation, the model systems were submitted to heat treatment.

2.3. Heat treatments

Kinetics of thermal degradation of xanthophylls were conducted at four temperatures 45, 60, 75 and 90 °C. For each temperature, 2 ml of blood orange juice or model juices were heated in sealed Pyrex tubes. The tubes were immersed in an oil bath with a temperature control (AM 3001K, Fisher-Bioblock Scientific, France). A digital temperature probe (EKT 3001, Heidolph, Germany) fitted to a reference tube was used to measure the medium temperature during the thermal experiments. Five sampling points were selected, from 5 to 240 min, according to the temperature; three replicates were done for each temperature. The time for juice to reach the temperature set up was below 4 min, and the cooling time was about 1 min. Then, the thermal transient could be negligible and the treatment could be considered isothermal. Each tube was stored under nitrogen and kept frozen (−20 °C) until analysed.