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Evaluation of carotenoid and capsaicinoid contents in powder of red chili peppers during one year of storage

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ABSTRACT

The color and the pungency of red chili pepper powder, depending on the carotenoid and capsaicinoid contents, are important properties for this food ingredient. In this study the patterns of these two classes of compounds have been analyzed in samples of red chili powder during one year of storage at room temperature (20–24 °C) and at low temperature (−18 °C), in order to investigate the eventual chemical changes occurring during storage, and to find a possible correlation between the behavior of the two groups of molecules.
During storage at room temperature, both carotenoid and capsaicinoid amounts were found to decrease progressively following a linear kinetics. After 12 months free carotenoids decreased to 20% of the initial value, and total capsaicinoids to 75%. All classes of carotenoids were found to be highly correlated with total capsaicinoids, thus showing that the molecules as the object of the study were subjected to similar kinetics. During storage at low temperature carotenoids decrease with lower rate (free carotenoids reached 66% of the initial value after 12 months) and capsaicinoids were almost unaltered.

1. Introduction

Chili peppers are consumed worldwide with an always increasing demand, in the fresh form and in the form of paste, powder and oleoresin. Their popularity derives from a combination of different factors such as color, taste and pungency (Wahyuni, Ballester, Sudarmonowati, Bino, & Bovy, 2013).

During storage of the powdered red berries, a progressive loss of color intensity is observed, causing the product to change from brilliant red to dull brown; the loss of color can be associated with a change in quality. Color deterioration of red-pepper powder during processing and storage is a serious problem; in fact, recently, quality and marketability of chili peppers products have depended not only on color content but also on the expected color stability during storage. The oxidative degradation of carotenoids seems to be the main reason for color loss (Kim, Park, & Hwang, 2004).

Pungency, an important tasty characteristic of red chili peppers, has also been found to decrease during storage (Schweiggert, Schieber, & Carle, 2006; Topuz & Ozdemir, 2004). Factors such as high temperature, humidity, water activity, exposure to light and contact with oxygen have been found to play an important role in causing deterioration (Rhim & Hong, 2011).

The interest in carotenoids from a nutritional standpoint has recently greatly increased, because of their important health benefits (Arab & Steck, 2000; Britton, Liaaen-Jensen, & Pfander, 2009; Caris-Veyrat, 2008; Hernandez-Ortega et al., 2012; Troconis-Torres et al., 2012) and in some cases, provitamin A activity.

Carotenoids are based on a C_{40} tetraterpenoid skeleton. These compounds are divided into two groups: hydrocarbons (commonly known as carotenes) and oxygenated compounds (generally named as xanthophylls). Carotenoids can be present in nature as free carotenoids or in a more stable form esterified with fatty acids, in the case of the oxygenated compounds. Moreover, in carotenogenic fruits, the esterification greatly increases during the fruit ripening process (Hornero-Mendez & Minguez-Mosquera, 2000). Carotenoid esters are major components in fruits and their thermal and photochemical stabilities, as well as the antioxidant capacity are important attributes. Carotenoid ester stability and bioavailability are related both to the carotenoid moiety itself and to the type of esterified fatty acids (Perez-Galvez & Minguez-Mosquera, 2005). The knowledge of a specific carotenoid class profile could be used to guarantee the genuineness of a product. There are a limited number of reports considering the carotenoid ester composition in *Capsicum* varieties (Breithaupt & Schwack, 2000; Giuffrida et al., 2013;

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Goda, Sakamoto, Nakanishi, Maitani, & Yamada, 1995; Minguez-Mosquera & Hornero-Mendez, 1994; Schweiggert, Kammerer, Carle, & Schieber, 2005; Schweiggert, Kurz, Schieber, & Carle, 2007). Compared to free xanthophylls, the studies pointing out the stability of xanthophyll esters are relatively scarce and the approaches used are very different (Fu et al., 2010; Perez-Galvez & Minguez-Mosquera, 2002). Moreover, reports on the pigment composition during frozen fruit storage are very limited (Biacs & Wissgott, 1997; Cano & Mario, 1992; Dias, Filomena, Camoes, & Oliveira, 2014; Lee & Coates, 2002; Sheehan, O'Connor, Sheehy, Buckley, & FitzGerald, 1998). Considering the important nutritional value of fruits and vegetables, the phytochemical response with potential health properties to freezing and storage becomes very relevant. In general, domestic kitchens have −20 °C freezers and the use of longer-term storage at freezing temperature of fresh products is increasing, both in private homes and in supermarkets. Pungency is determined by the presence of a group of molecules belonging to the family of capsaicinoids, capable of interacting with vanilloid receptors occurring on the tongue. Capsaicin and dihydrocapsaicin are the most predominant compounds responsible for the hot taste. Some minor compounds, such nordihydrocapsaicin and homocapsaicin have been found to lead to a less intense response (Wahyuni et al., 2013).

Previous studies on capsaicinoid stability report a progressive decrease of capsaicin and dihydrocapsaicin after cell disruption occurring when peppers are minced (Kirschbaum-Titze, Hiepler, Mueller-Seitz, & Petz, 2002). This phenomenon was found to be possibly linked to oxidative conversion; in particular, peroxidase (Contreras-Padilla & Yahia, 1998) and lipoxygenase (Orak & Demirci, 2005) activities were demonstrated to be involved. However, also non-enzymatic degradation was found to be responsible for capsaicinoid loss during storage (Schweiggert et al., 2006).

Reports on the pigment composition during fruit storage under low temperature are very limited (Biacs & Wissgott, 1997; Cano & Mario, 1992; Dias et al., 2014; Lee & Coates, 2002; Sheehan et al., 1998), and a parallel analysis of capsaicinoid composition has never been investigated in order to find a possible correlation between the two classes of compounds. This work shows, for the first time, a study on the long term stability (12 months) of various classes of carotenoids and capsaicinoids (Fig. 1) at room temperature, to monitor slight changes eventually occurring under conditions reproducing those of market storage, and at low temperature to investigate whether it could represent an improved storage condition. The aim was also to provide new data on the long term stability of capsaicinoids and different carotenoid classes present in a carotenoid rich matrix, and to provide useful information in order to predict the expected color stability during storage in carotenogenic fruits.

2. Materials and methods

2.1. Chemicals

All the reagents and solvents used were of analytical or HPLC grade and were purchased from Sigma-Aldrich (Milan, Italy). Carotenoid standards, namely, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, luteindi-palmitate, and physalein, were purchased from Extrasynthese (Genay, France). Capsaicin and dihydrocapsaicin were purchased from Sigma-Aldrich (Milan, Italy).

2.2. Samples

The powder of chili peppers was obtained from red berries belonging to *Capsicum annuum* sp. cultivated in the region of Emilia-Romagna, in Northern Italy, by Azienda Agraria Sperimentale Stuard (Parma, Italy). Berries of similar size were picked up at harvest time and subjected to a drying process at 60 °C for 48 h. Then, fruits were minced by a household grinder to obtain a fine powder. Aliquots of the powder were stored for 12 months in the dark, at two different temperature conditions: some aliquots were stored at room temperature (RT, between 20 and 24 °C), and others at −18 °C. Each aliquot was analyzed for its carotenoid and capsaicinoid contents at different intervals: at initial conditions, and respectively after 6, 9, and 12 months of storage.

2.3. Carotenoid extraction and analysis

The carotenoid pigments were extracted according to the recommended procedures (Minguez-Mosquera & Hornero-Mendez, 1993; Rodriguez-Amaya, 2001): 2 g of each sample was mixed with 1 g of sodium bicarbonate, homogenized and extracted three times to color exhaustion with 15 mL of acetone. The extracts were combined and the acetone was evaporated (under vacuum at 35 °C) until a 10 mL final volume was obtained. The concentrate was transferred into a separatory funnel with 20 mL of diethyl ether, shaken and left to settle. An amount of NaCl solution (10%) sufficient enough to separate the phases and to transfer the pigments to the ether was added. This solution was treated several times with anhydrous Na_2SO_4 solution (2%) to remove all the water. The ether phase was evaporated to dryness at 30 °C. The dry residue was then dissolved in a mixture of methanol/methyl-tert-butyl ether (MTBE) (1:1, v/v), filtered under membrane filtration (0.45 μm), and analyzed by HPLC. Samples were stored at −20 °C until they were analyzed.

The analyses were performed by HPLC-DAD on a Shimadzu Prominence® LC-20A (Shimadzu, Milan, Italy), consisting of a CBM-20A controller, two LC-30AD dual-plunger parallel-flow pumps, a DGU-20 A_5 degasser and an SPD-M20A photo diode array detector (8 μL detector flow cell volume). The data were processed with the software Labsolution ver. 5.10.153 (Shimadzu).

Separations were performed following a previously reported methodology on a YMC C_{30} column (250 × 4.6 mm–5 μm); the mobile phases consisted of methanol/MTBE/water (83:15:2, v/v/v; eluent A) and methanol/MTBE/water (8:90:2, v/v/v; eluent B), using a gradient program as follows: from 0 min to 20 min 0% B; at 120 min 70% B; and at 125 min 100% B. The flow rate was 1.0 mL/min and the injection volume was 20 μL. The UV–Vis spectra were acquired in the range of 250–600 nm, while the chromatograms were extracted at 450 nm (sampling frequency: 15,625 Hz; time constant: 0.64 s) (Giuffrida, Dugo, Dugo, Torre, & Mondello, 2011; Giuffrida et al., 2013). Analyses were performed in triplicate.

2.4. Capsaicinoid extraction and analysis

The capsaicinoids were extracted from the chili powder following the procedure reported in a previous work (Giuffrida et al., 2013): 0.5 g was added to 20 mL of acetone and stirred for 30 min at the temperature of 50 °C. After centrifugation, the extraction procedure was repeated and the extracts were evaporated to dryness, resuspended in 2.5 mL of water: acetonitrile 55:45 v/v, and filtrated through 0.2 μm nylon filter (Econofilter, Agilent Technologies).

Qualitative and quantitative analyses of the capsaicinoid profile were carried out by HPLC-UV–DAD (Agilent 1200, Agilent Technologies Palo Alto, CA, USA) equipped with a reversed phase column (Poroshell Agilent 120 SB-C18 (3.0 × 50 mm, 2.7 μm)) thermostated at 30 °C. The amount of sample injected was 2 μL. Separation of the compounds was performed employing an isocratic mixture of water:acetonitrile 55:45 v/v. Wavelength set for detection was 280 nm. The complete separation of the peaks of interest was obtained in about 3 min (Giuffrida et al., 2013). Two aliquots were analyzed in duplicate (n = 4).

2.5. Method validation

Validation of the whole analytical methods was performed according to Eurachem guidelines in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy and recovery.