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Colour and carotenoid changes of pasteurised orange juice during storage

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 $\begin{array}{l} Chemical \ compounds \ studied \ in \ this \ article: \\ \beta-Cryptoxanthin \ (PubChem \ CID: 5281235) \\ Lutein \ (PubChem \ CID: 5281243) \\ Zeaxanthin \ (PubChem \ CID: 5280899) \\ \alpha-Carotene \ (PubChem \ CID: 5280489) \\ \beta-Carotene \ (PubChem \ CID: 5280489) \\ \zeta-Carotene \ (PubChem \ CID: 5280788) \\ Mutatoxanthin \ (PubChem \ CID: 5280735) \\ Antheraxanthin \ (PubChem \ CID: 5281223) \\ \end{array}$

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ABSTRACT

The correlation of carotenoid changes with colour degradation of pasteurised single strength orange juice was investigated at 20, 28, 35 and 42 °C for a total of 32 weeks of storage. Changes in colour were assessed using the CIELAB system and were kinetically described by a zero-order model. L^* , a^* , b^* , ΔE^* , C^*_{ab} and h_{ab} were significantly changed during storage (p < 0.05). Activation energies for all colour parameters were 64–73 kJ mol⁻¹. Several carotenoids showed important changes and appeared to have different susceptibilities to storage. A decrease of β -cryptoxanthin was observed at higher temperatures, whereas antheraxanthin started to decrease at lower temperatures. Depending on the time and temperature, changes in carotenoids could be due to isomerisation reactions, which may lead to a perceptible colour change. Although the contribution of carotenoids was recognised to some extent, other reactions seem of major importance for colour degradation of orange juice during storage.

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1. Introduction

Orange juice is one of the most popular beverages in the world, with its attractive colour, refreshing taste and high nutritional value; it remains the most widely consumed fruit juice (Neves, Trombin, Lopes, Kalaki, & Milan, 2011). In fresh juice, the bright orange colour is determined by the composition and concentration of its naturally occurring pigments, carotenoids (Meléndez-Martínez, Vicario, & Heredia, 2009).

Colour is an important characteristic of food. The deliverance of a good impression through colour will determine consumers' acceptability and their purchase decision. Also, colour plays an important role as a quality indicator. According to Van Boekel (2008), different chemical and biochemical reactions which occur in a food product can be detected visually by its colour.

During processing and storage, colour of orange juice can be changed depending on the conditions that favour degradation reactions. Since colour degradation is quite complex, there could be more than one mechanism responsible for the colour changes. On the one hand, fading of the desired colour of the natural carotenoid pigments (Kidmose, Edelenbos, Nørbæk, & Christensen, 2002; Rodriguez-Amaya, 2001), and on the other hand, development of pigmented substances due to enzymatic and/or non-enzymatic reactions (Roig, Bello, Rivera, & Kennedy, 1999).

In recent years, several studies have been conducted to reveal the relationship between fresh orange juice colour, carotenoid structure and composition (Meléndez-Martínez, Britton, Vicario, & Heredia, 2007, 2008; Meléndez-Martínez, Escudero-Gilete, Vicario, & Heredia, 2010). Colour can be quantified and characterised through an objective colour measurement such as CIELAB





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(Francis, 1995). This colour system was found to be useful for estimating the presence of bioactive components including carotenoids (Sant'Anna, Gurak, Ferreira, & Tessaro, 2013). A strong correlation between CIELAB colour values and the individual or total carotenoid content of orange juice was reported by Meléndez-Martínez, Vicario, and Heredia (2003). Furthermore, this system has also proved to be effective for tracking colour changes during processing and storage (Wrolstad, Durst, & Lee, 2005).

It is known that each carotenoid has its own distinctive colour properties. The carotenoid structure, the length of the chromophore, the arrangement of conjugated double bonds in the end ring and the geometrical (*cis/trans*) isomers of carotenoids influence its perceived colour (Meléndez-Martínez et al., 2010). Most carotenoids absorb between 400 and 500 nm, which is the blue-green region of the visible spectrum. Thus, carotenoids exhibit yellowish to reddish colours, in which a wide range of orange juice colours can be recognised.

Due to their highly unsaturated structure, carotenoids are prone to various degradation reactions, which affect not only their colour but also their biological activity. The presence of oxygen, especially in combination with light and heat, can lead to oxidative degradation, forming an epoxide and free radicals. Subsequent reactions yield low-molecular-weight compounds and in some cases volatile compounds (Rodriguez-Amaya, 2001). Both isomerisation and oxidation reactions lead to a decrease in yellowness and further oxidation reactions result in carotenoid bleaching or colour loss (Kidmose et al., 2002; Sant'Anna et al., 2013). The stability of carotenoids is influenced by several factors, such as the processing intensity, storage time and temperature, the availability of oxygen and light, and the type of carotenoid involved (Rodriguez-Amaya & Kimura, 2004). The impact of different processing and storage conditions on orange juice carotenoids has been investigated by several authors (Cortés, Torregrosa, Esteve, & Frígola, 2006; Lee & Coates, 2003; Plaza et al., 2011; Vervoort et al., 2011). Nevertheless, to the best of our knowledge, no study has yet been conducted on the changes of carotenoids during storage both at ambient and at elevated storage temperatures: specifically, on the impact of these changes on colour changes. With the purpose of gaining insight into the colour stability of pasteurised orange juice during storage, two specific objectives were formulated. First, kinetic modelling of the colour changes during storage at different storage temperatures was aimed at. The second goal was to study the carotenoid changes at different storage times and temperatures and their correlation with colour changes.

2. Materials and methods

2.1. Sample preparation and storage

Single strength orange juice (11.2 °Brix, pH 3.7, titratable acidity 0.8%) was prepared by reconstituting frozen Brazilian orange concentrate (65 °Brix) with water (1:5, w/w). The juice was immediately subjected to pasteurisation (92 °C, 30 s) followed by filling at 85 °C into monolayer PET bottles (500 mL), cap twisting and inversion of the bottles to disinfect the bottle tops. The bottles were then cooled to ambient temperature by submerging them into a circulating and chlorinated water tank. After processing, the juices were stored at 20 and 28 °C for 32 weeks, at 35 °C for 12 weeks and at 42 °C for 8 weeks, in dark incubators (IPP500; Memmert, Schwabach, Germany).

At each sampling time (Fig. 1), three bottles were taken from storage, mixed and divided uniformly into smaller tubes (\pm 30 mL). These tubes were immediately frozen in liquid nitrogen and stored at -80 °C until use. At the time of analysis, tubes were thawed in a circulating water bath at 25 °C.

2.2. Colour measurement

The colour of orange juice was analysed using a Hunterlab ColorQuest colorimeter (Hunterlab, Reston, VA). The instrument $(45^{\circ}/$ 0° geometry, Illuminant D65, 10° observer) was calibrated with a black and white ceramic tile (*X* = 78.66, *Y* = 83.31, *Z* = 88.40) before the measurement. Subsequently, samples were placed in a glass cell and covered with a white plate. Colour measurements were carried out in triplicate with five readings for each sample. The recorded *XYZ* tristimulus values were then converted to CIE *L**, *a** and *b** colour values. The *L** values represent lightness, ranging from 0 (black) to 100 (white). The *a** values indicate greenness (negative) to redness (positive) and the *b** values quantify blueness (negative) to yellowness (positive). In CIELAB, three other parameters were determined by the following equations:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \tag{1}$$

$$C_{ab}^{*} = \sqrt{a^{*2} + b^{*2}}$$
(2)

$$h_{ab} = \arctan b^{*2}/a^{*2} \tag{3}$$

The total colour difference (ΔE^*) between a stored sample and the pasteurised juice before storage was calculated as the Euclidean distance between two points in a three-dimensional space. ($\Delta L^* = L^* - L_0^*$; $\Delta a^* = a^* - a_0^*$; $\Delta b^* = b^* - b_0^*$; subscript "0" indicates initial colour at week 0). The classification of ΔE^* was based on the one reported by Cserhalmi, Sass-Kiss, Tóth-Markus, and Lechner (2006): 0–0.5 (not noticeable), 0.5–1.5 (slightly noticeable), 1.5– 3.0 (noticeable), 3.0–6.0 (well visible) and >6.0 (great). The chroma (C_{ab}^*), or saturation index, characterises the quantitative attribute of colourfulness and is proportional to its intensity. Hue (h_{ab}) is a qualitative indicator of the chromatic nature of the colour and it is expressed in degrees (0° or 360° for red, 90°, 180° and 270° for yellow, green and blue, respectively) (CIE, 1978).

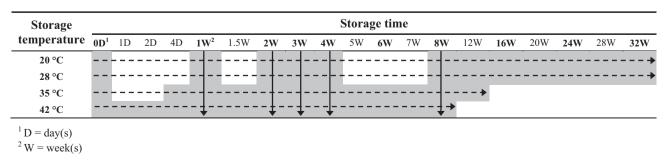


Fig. 1. Overview of all sampling times at each storage temperature. The samples used for colour measurements are indicated by a grey background, whilst the samples for carotenoid analysis are marked by bold fonts. The dashed arrows represent the length of storage at a specific temperature and the solid arrows indicate the investigation of the effect of temperature with respect to storage time.

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