



Heat and light colour stability of beverages coloured with a natural carotene emulsion: Effect of synthetic versus natural water soluble antioxidants



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ABSTRACT

Natural carotenoid emulsions are commonly used to colour beverages. However, carotenoids are frequently prone to oxidation, which results in undesirable colour loss. This study investigates the effect of the direct addition to the beverage of water soluble antioxidants on the colour stability in both direct light (sun test) and heat conditions. A synthetic antioxidant, ascorbic acid, is compared to a natural one, a rosemary extract rich in rosmarinic acid. The addition of ascorbic acid results in improved stability to light but has negative effect for heat stability. The addition of rosemary affects both heat and light stability favourably, even though it is not as efficient to prevent carotenoid degradation in the sun test. It can be concluded that the rosemary extract is a good natural alternative to synthetic ascorbic acid. Its combination with ascorbic acid could be very efficient to improve both light and heat stability of beverages coloured with natural carotenoids.

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1. Introduction

Recent consumer concerns about the safety of synthetic colourants have led to an increased use of natural colourants in food, confectionery and beverages. However, natural alternatives are generally less stable to environmental factors such as heat, light and pH. As colour is an essential marker for food quality, research is increasingly being carried out in order to improve the stability of natural colours (Ghidouche, Rey, Michel, & Galaffu, 2013; Jespersen, Stromdahl, Olsen, & Skibsted, 2005).

Carotenoids are pigments responsible for the yellow, orange and red colours found in many fruits and vegetables, as well as algae and bacteria. Many algae and fungi are used to produce commercial sources of carotenoids. Algal carotenoids are obtained from *Dunaliella salina*, a green micro-alga found in sea salt fields. Carotenoids are isoprenoid compounds, with a C40 carbon skeleton from which all the different types are derived (Britton, 1995). Their distinctive colour is provided by conjugated double-bond system which constitutes the light absorbing chromophores. In general the higher the number of double bonds the higher the wavelength of maximum absorption (Melendez-Martinez, Britton, Vicario, & Heredia, 2006). Algal carotenoids are mainly composed of 9-cis- β -carotene (Leach, Oliveira, & Morais, 1998). After

extraction from the algae, carotenoids are standardised in concentration in vegetable oil.

Due to their lipophilic nature, carotenoids have to be emulsified in water in order to colour beverages. The pigment is dispersed in an oil phase which is then homogenised at high shear to form an oil in water emulsion. The emulsion is stabilised by different emulsifiers adsorbed at the oil–water interface to achieve long-term stability (Delgado-Vargas, Jimenez, Paredes-Lopez, & Francis, 2000; Mao, Dubot, Xiao, & McClements, 2013; Qian, Decker, Xiao, & McClements, 2012a).

Carotenoids are well known for their anti-oxidant properties. They are prone to degradation from several agents. They can undergo auto-oxidation, and react with singlet oxygen as well as metal ions to form radicals. Such reactions can lead to further degradation of the carotenoids and the formation of short chains molecules, epoxides and apocarotenoids, resulting in undesirable colour loss. These different oxidation mechanisms were comprehensively reviewed by Boon et al. (2010).

To counteract degradation reactions it is common to incorporate antioxidants such as tocopherols into the oil phase of the emulsion. In general, it results in a prolonged product shelf-life, although it fails to match that of synthetic colourants (Boon, McClements, Weiss, & Decker, 2009; Mercadante, Capitani, Decker, & Castro, 2010). Rodrigues et al. (2013) studied the degradation of isolates (90% pure) of lycopene and β -carotene in both low moisture and aqueous model

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systems. They found that both carotenoid degradation followed first order kinetics in the presence and absence of light. In their work no antioxidants were added to the model systems. Sampaio et al. (2013) have investigated the degradation of carotenoids in palm oil. They found that the high concentration of commonly occurring natural antioxidant tocopherols helped improve the stability of carotenoids. Qian, Decker, Xiao, and McClements (2012b) investigated the addition of oil and water soluble antioxidants on the stability of β -carotene emulsion. They found that the rate of degradation decreased upon addition of water soluble ascorbic acid and oil soluble vitamin E acetate and coenzyme Q10. However their utilisation in combination was less effective than when used individually. Kiokias and Oreopoulou (2006) mention the reduction of carotenoid radical formation upon addition of tocopherols.

The present research studies the potential increase in colour stability of a mixed-carotene (algal origin) emulsion in a lemonade-type beverage. It is hypothesised that the addition of water-soluble antioxidants directly to the beverage will result in improved colour stability. Two antioxidants were chosen for this study. Synthetic ascorbic acid is compared to a natural rosemary extract for both heat and light stability.

Ascorbic acid is known for being a free radical scavenging antioxidant (Boon, McClements, Weiss, & Decker, 2010; Mercadante et al., 2010). Amongst potential natural sources of antioxidants, rosemary has perhaps received the most attention from researchers. The antioxidant activity of rosemary extract is mainly attributed to carnosic acid, carnosol and rosmarinic acid. The natural rosemary extract used in this study contains water soluble rosmarinic acid. This is one of the most potent antioxidants amongst simple phenolic acids (Erkan, Ayranci, & Ayranci, 2008; Frankel, Huang, Aeschbach, & Prior, 1996; Ibarra et al., 2010; Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005).

2. Materials and methods

2.1. Materials

The carotenoid emulsion (Naturex, France) contains 1% natural mixed-carotene extracted from *D. salina*. It has a typical colour strength E_1^1 of 21–25 at 448 nm in acetone. The emulsion contains between 0.1 and 0.3% of alpha-tocopherol and ascorbyl palmitate included in the oil phase. Acid ascorbic powder (purity >99%) was obtained from North China Pharmaceutical Co. (China). The water soluble rosemary liquid extract (StabilEnhance® WSR, Naturex, France) contains 4 to 4.5% rosmarinic acid.

2.2. Beverage preparation

The natural mixed-carotene colour emulsion was added at 0.1% to a non-sparkling ready to drink (RTD) beverage (7°brix, pH 3.5). The RTD base contains the following ingredients (Table 1).

The different antioxidants were added at 0.005, 0.015 and 0.05% in the beverages.

2.3. Stability test

The stability of the beverage was investigated in both light and heat conditions. The light stability was tested using an accelerated ageing

Sun Test (Heraeus suntest CPC Xenon exposure system) equipped with xenon light to simulate natural daylight. The heat stability was tested at 40 °C in a laboratory oven (Picksten oven, UK). The samples were removed at regular intervals for colour measurements. For the light stability in the sun test, samples were taken every hour for the first 10 h and then every 3 h. For the heat stability study, samples were measured every 3 days. The lemonade-type beverages with added antioxidants (ascorbic acid or rosemary extract) were compared to a control containing no added antioxidants.

2.4. Colour fading measurement

The carotene degradation was measured by recording colour changes of the beverages at different time intervals as described above. The different bottles were opened at each measurement. The cylindrical colour coordinates (L^* , C^* , h) were measured on a Konica Minolta spectrophotometer (cm-3600d, Japan). However, it is more convenient to compare a single value, therefore the total colour difference ΔE_{00} (Eq. (1)) is given. The equation is based on the lightness difference ΔL , the saturation difference ΔC and the hue difference ΔH . The equation includes corrections using weighing coefficients (S_L , S_C , S_H) and constants called parametric coefficients (k_L , k_C , k_H)

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + \left(R_T \left(\frac{\Delta C'}{k_C S_C}\right) \left(\frac{\Delta H'}{k_H S_H}\right)\right)}. \quad (1)$$

2.5. Degradation kinetics of the colour values

In general, colour variation exhibits a zero or first order time dependence relationship (Saxena, Maity, Raju, & Bawa, 2012; Pathare, Opara, & Al-Said, 2013). From these basic kinetic equations, it is possible to obtain a reaction rate constant K . The kinetic equation is usually applied to the three colour parameters L^* , a^* and b^* of the Lab colour system, which represent the colour of a product in a uniform colour space. The newer L^* , C^* and h system (introduced above) was developed to correct the limitation of the Lab system which was shown not to match the perception of colour by the human eye.

However, by interest of comparison with other studies the Lab system was used in this paper to investigate the degradation kinetics. The parameter L^* describes the lightness (similar to L^* in the LCH system), a^* and b^* are the chromaticity coordinates. The parameter a^* is the value on the red to green axis. The parameter b^* represents the yellow to blue axis. In this study the parameter b^* (yellow) shows the most variation.

The kinetic reaction was only fitted to parameter b^* as the fit to the other two parameters did not show satisfactory results. In addition, parameter b^* is the one showing the most variation and thus represents the most interest. As a result, only the modelling of the b^* parameter is presented. Zero and first order kinetic models (Eqs. (2) and (3)) were fitted to each set of data of b^* and the most suitable fits are introduced. The goodness of fit was evaluated with the correlation coefficient R^2 which is a measure of the amount of variation around the mean (Kumar, Sarkar, Sharma, & Jha, 2012).

$$C = C_0 \pm k_0 t \quad (2)$$

$$\ln C = \ln C_0 \pm k_1 t \quad (3)$$

where C is the measured value, C_0 is the initial value of the corresponding response, t is the time and k_0 and k_1 are the reaction rate constant for zero and first order, respectively.

Table 1
Ingredient list of the non-sparkling ready-to drink base.

Saccharose	43%
Potassium sorbate	0.09%
Sodium benzoate	0.07%
Citric acid	0.87%
Distilled water	55.97%

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