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Impact of blanching, freezing and frozen storage on the carotenoid profile of carrot slices (*Daucus carota* L. cv. Nutri Red)

Diana Behsnilian*, Esther Mayer-Miebach

Department of Food and Bioprocess Engineering, Max Rubner-Institut (MRI), Federal Research Institute of Nutrition and Food, Haid-und-Neu-Str. 9, 76131 Karlsruhe, Germany

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ABSTRACT

The Nutri Red carrot (*Daucus carota* L. cv. Nutri Red), a red carrot variety which together with β-carotene contains large amounts of lycopene, could represent an interesting alternative to tomatoes as a source of lycopene in the European diet. However, carotenoid retention during processing of this carrot variety has not yet been sufficiently addressed. The interest here is focused on the evaluation of the potential carotenoid degradation and/or isomerisation resulting from blanching, freezing and during the subsequent frozen storage of Nutri Red carrots. Carrots were sliced, blanched, air blast frozen and stored at −50 °C, −30 °C, −18 °C and −15 °C for up to two years. β-carotene, α-carotene, and lutein remained stable along processing and long-term frozen storage. All-*trans*-lycopene was degraded at all storage temperatures. A first-order and a two-first-order reaction models were applied for the description of lycopene degradation. The temperature dependence of the rate constants was adequately modelled by the Arrhenius equation at temperatures above −30 °C. Although substantial losses of all-*trans*-lycopene were detected, the carrot slices still exhibited lycopene contents similar to that of some tomato varieties after one year storage at −18 °C. Hence, both raw and frozen Nutri Red carrots represent an excellent alternative lycopene source.

1. Introduction

Fruit and vegetables are sources of a wide variety of micronutrients and other bioactive compounds such as carotenoids and polyphenols (Watzl & Leitzmann, 2005). Currently, based on the scientific evidence regarding the health-promoting effects of a fruit and vegetable rich diet (Boeing et al., 2012), the health authorities of many countries recommend an intake of at least five daily servings of raw and processed fruit and vegetables as one of the strategies for reducing the risk of some cancer types, heart diseases and many other non-communicable diseases (FAO/WHO, 2005).

Freezing, canning and drying count among the technologies that contribute to widen the choice of high quality processed fruit and vegetables, independent of seasonal conditions or geographic situation. After blanching and freezing the retention of most bioactive compounds contained in fruits and vegetables is generally very good (Puupponen-Pimïa et al., 2003; Rickman, Bruhn, & Barrett, 2007). However, different studies (Kidmose & Martens, 1999; Morais et al., 2002; Fish & Davis, 2003) show a reduction of the total carotenoid content during long term frozen storage of plant material, having both the blanching and the freezing methods applied a significant impact on the nutritional quality of frozen plant material. Furthermore a number of parameters affect carotenoid retention along their long-term frozen storage, including the plant matrix itself (e.g. cultivar), post-harvest storage conditions (e.g. temperature), the processing conditions (e.g. shape and size of the material, blanching step) and the storage conditions (e.g. temperature, packaging) (Sharma & Le Maguer, 1996; Lisiewska & Kmiecik, 2000; Morais et al., 2002; Fish & Davis, 2003; Cremona, Sandei, Taddei, & Leoni, 2004; Bouzari, Holstege, & Barrett, 2014).

In the European diet carrots are the main dietary source for α- and β-carotene, while tomatoes are the main source for lycopene (O'Neill et al., 2001; Maiani et al., 2009). The Nutri Red carrot, a carrot variety which together with β-carotene contains large amounts of lycopene, could represent an interesting alternative source of lycopene. However, little is known concerning carotenoid retention during processing of this carrot variety.

Our interest here is focused on the evaluation of the potential carotenoid degradation and/or isomerisation resulting from

* Corresponding author.
E-mail address: diana.behsnilian@mri.bund.de (D. Behsnilian).

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blanching and freezing Nutri Red carrots. Furthermore, the carotenoid profile was evaluated throughout frozen storage for up to two years at four different temperatures in the range from −50 °C to −15 °C.

2. Materials and methods

2.1. Carrots

At the time of this study only the seeds of the lycopene containing carrots (*Daucus carota* L. cv. Nutri Red) were commercially available, not the carrots. Therefore, the material used for the study was grown from May to August in a soil bed greenhouse at the Max Rubner-Institute in Karlsruhe, Germany. No special light or temperature conditions were used for the growing. Immediately after harvest (80 days after sowing) the full-sized ripe carrots were stored at 1 °C and 97% relative humidity, until further processing within one week.

2.2. Processing and storage

Carrots were washed, sliced (3–4 mm thickness, 15–25 mm diameter) and blanched (3 kg in 80 L water at 95 °C for 1.5 min). Under the time and temperature conditions applied for blanching peroxidase is inactivated, while β-carotene is not degraded (Mayer-Miebach & Spieß, 2003; Leemens et al., 2009). The blanched carrots were frozen in a batch fluidised bed freezer developed at the Max Rubner-Institut (Karlsruhe, Germany), with a 0.13 m^2 bed surface, operating with an air temperature of −30 °C and air velocity of 6 ms^{-1}. The temperature evolution of the samples during freezing was measured with a thermocouple (0.5 mm diameter, ± 0.1 °C; Ahlborn GmbH, Holzkirchen, Germany) introduced into the geometrical centre of a carrot slice. The mean freezing rate of the slices (10 batches) was 5 ± 1 °C min^{-1}. The weight of carrot slices was registered before and after each processing step. Working at a temperature of −30 °C the frozen carrots were portioned (250 g) and packed in frozen storage food-grade polyamide/polyethylene bags under vacuum (VacSy, Zepter International). Three samples for each storage time (0, 1, 3, 6, 12 and 24 months) and temperature (−50 °C, −30 °C, −18 °C and −15 °C) were analysed.

2.3. Analytical methods

Raw, blanched, frozen and frozen stored carrot samples were homogenised (Büchi Labortechnik GmbH, Konstanz, Germany) and analysed for moisture and carotenoid content. The frozen samples were brought to a temperature of around 0 °C in the dark before homogenisation. The moisture content of homogenates was determined gravimetrically by drying in an oven at 105 ± 1 °C until constant weight, according to the AOAC method 984.25 (AOAC, 2000). The assay was done in triplicate for each sample.

2.3.1. Carotenoids extraction and analysis

Carotenoids extraction was performed according to Mayer-Miebach and Spieß (2003) with modifications. Carrot homogenates were extracted with acetone, re-dissolved in petroleum ether, washed with water and dried, the total carotenoid content determined by spectral photometric measurement (Lambda 40, Perkin Elmer, Connecticut, USA) (λ 445 nm; $A^{1\%}_{1cm}$ 2500), evaporated to dryness, flushed with nitrogen gas and stored at −86 °C until HPLC-analysis. For HPLC-analysis the extracts were re-dissolved in tetrahydrofuran with 0.01% butylated hydroxytoluene. The HPLC system consisted of a quarternary pump, auto-sampler, column oven, photodiode array detector (Hitachi High-Technologies Corporation, Tokyo, Japan). A C30 reversed phase column (250 mm × 4.6 mm, 5 μm, YMC Europe GmbH, Schermbeck, Germany) was used as the stationary phase together with a linear gradient of methyl-t-butyl-ether in methanol for 90 min as the mobile phase. Calibration standards were used to identify *trans*-isomers and quantify all isomers: all-*trans*-β-carotene (Sigma-Aldrich Chemie GmbH, München, Germany), all-*trans*-lycopene (BASF AG, Ludwigshafen, Germany) and lutein (Roth GmbH, Karlsruhe, Germany). The identities of α-carotene and of all *cis*-isomers were assigned based on retention time and specific ultra violet/visual absorption (Schierle et al., 1997) and mass spectroscopic analysis (binary pump, auto-sampler, column oven, photodiode array detector, mass spectrometer equipped with APCI, Agilent Technologies, California, USA) in the positive ionisation mode with total-ion scanning range 500–600 mz^{-1}, drying gas flow 6 L min^{-1}, nebulizer pressure 300 psi, dry gas temperature 250 °C, vaporizer temperature 400 °C, capillary voltage 2500 V, fragment voltage 70 V and corona current 4 μA. All experiments were carried out under subdued light to prevent photodegradation and isomerisation. All chemicals used were purchased from Merck KGaA, Darmstadt, Germany, except otherwise stated.

2.4. Data analysis

2.4.1. Carotenoid retention

The impact of blanching and freezing on carotenoid retention was evaluated according to equation (1):

$$c_R = \frac{c_t \cdot m_i \cdot X_{S_i}}{c_0 \cdot m_0 \cdot X_{S_0}} \quad (1)$$

Hereby is c_0 the α-carotene, β-carotene, lycopene, or lutein content per kg solids ($mgkg_S^{-1}$), m_0 the weight (kg) and X_{S0} the solid fraction ($kg_S kg^{-1}$) of the raw material; c_i is the α-carotene, β-carotene, lycopene or lutein content ($mgkg_S^{-1}$), m_i the weight (kg) and X_{Si} the solid fraction ($kg_S kg^{-1}$) of the blanched or the frozen material; and c_R is expressed in mg per mg in the raw material ($mgmg^{-1}$).

2.4.2. Kinetic parameters

A first-order reaction model (equation (2)) and a two-first-order reaction model (equation (3)) were applied for the evaluation of the kinetic of lycopene degradation during frozen storage:

$$c = c_0 \exp[-k \cdot t] \quad (2)$$

$$c = c_0[a_1 \exp(-k_1 t) + a_2 \exp(-k_2 t)] \quad (3)$$

Hereby is c the carotenoid content per kg frozen material at time t ($mgkg^{-1}$); c_0 the carotenoid content per kg frozen material at time zero ($mgkg^{-1}$); k, k_1 and k_2 rate constants (d^{-1}); a_1 and a_2 pre-exponential factors for different degradation reactions and t the storage time (d).

The temperature dependence of lycopene degradation was determined by the Arrhenius equation:

$$k = k_0 \exp\left[-\frac{E_a}{R \cdot T}\right] \quad (43)$$

Hereby is k the temperature dependant rate constant for the first-order degradation reaction at temperature T (Kelvin), k_0 the pre-exponential factor, E_a the activation energy ($Jmol^{-1}$) and R the universal gas constant (8.314 J $mol^{-1}K^{-1}$).