Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Research International 38 (2005) 847–853
FOOD RESEARCH INTERNATIONAL
www.elsevier.com/locate/foodres

Analysis of a complex mixture of carotenes from oil palm (*Elaeis guineensis*) fruit extract

A. Mortensen *

Colour Research, Development & Application, Chr. Hansen A/S, Bøge Allé 10-12, DK-2970 Hørsholm, Denmark

Received 1 June 2004; accepted 21 January 2005

Abstract

A carotene extract from the fruits of the oil palm (*Elaeis guineensis*) was analysed by HPLC employing a C_{30} column for better separation efficiency. A multitude of *cis*-isomers of α-, β- and γ-carotene were separated. Detailed assignment was possible by subjecting pure standards of α-, β- and γ-carotene to isomerisation and comparing spectral data and order of elution to literature data. α- and β-carotene were found to be the most abundant carotenoids comprising 12.3% and 17.9%, respectively, of a (roughly) 30% oil suspension of oil palm carotenes in vegetable oil. A large proportion (about 40%) of α- and β-carotene was in the form of *cis*-isomers. The γ-carotene content was found to be 0.38% and other carotenes like phytoene, phytofluene, ζ-carotene, lycopene and possibly β-zeacarotene were found as well but were not quantified.

Keywords: Oil palm fruit; *Elaeis guineensis*; α-Carotene; β-Carotene; γ-Carotene; HPLC

1. Introduction

Oil palms (*Elaeis guineensis*) are grown extensively in Southeast Asia and Equatorial Africa because the fruits are rich in vegetable oil and because the oil palm produces more oil per area than any other plant (Poku, 2002). The kernel as well as the mesocarp contain oil. The oil obtained from the yellow, fleshy mesocarp is called crude palm oil and is rich in carotenes (Poku, 2002). A number of carotenes have been found in palm oil: phytoene, phytofluene, ζ-carotene, neurosporene, α-zeacarotene, β-zeacarotene, lycopene, δ-carotene, α-carotene, β-carotene and γ-carotene (Tay & Choo, 2000; Tan, Grady, & Gawienowski, 1986; Yap, Choo, Ooi, Ong, & Goh, 1991) of which α- and β-carotene comprise more than 90% (Tay & Choo, 2000; Yap et al., 1991). The other carotenes are present in small amounts and are precursors in the biosynthesis of α- and β-carotene.

The crude palm oil is usually processed to yield either a red or bleached cooking oil or detergents. During this processing, the carotenes are often destroyed, but they may also be isolated and suspended in vegetable oil at a concentration of up to 30%. This product can be used as a food colour or in health products such as dietary supplements.

Before the oil is pressed from the fruits, the fruits are subjected to heat treatment employing pressurised steam or boiling (Poku, 2002). Heating causes *cis*/*trans*-isomerisation of the carotenes (Doering, Sotiriou-Leventis, & Roth, 1995). Thus, a complex mixture of highly apolar carotenes and their *cis*-isomers is obtained making separation difficult. A decade ago a new stationary phase (called C_{30}) was developed (Sander, Sharpless, Craft, & Wise, 1994). This new column has since then gained wide acceptance for analysis of carotenoids and has proven useful for the simultaneous separation of 11 different carotenoids used as food colourants (Breithaupt, 2004). Furthermore, this column not only provides excellent separation of closely related carotenes like α- and

* Fax: +45 45 74 89 11.
E-mail address: alan.mortensen@dk.chr-hansen.com.

0963-9969/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2005.01.009

β-carotene, but also a better separation of their *cis*-isomers than was previously possible using a conventional C_{18} column (Sander et al., 1994).

2. Materials and methods

Oil palm carotenes were suspended in vegetable oil at a concentration of 30%. α- and γ-carotene were obtained from CaroteNature and β-carotene (30% oil suspension) from BASF. Methyl *tert*-butyl ether (MTBE) and methanol were from Merck (HPLC grade). HPLC analysis was performed on a Merck-Hitachi system employing a photodiode-array detector for detection. The column (thermostatted at 10 °C) used was from YMC (C_{30}, 3 μm, 250 × 4.6 mm) and a linear gradient going from 0% to 100% B in 75 min (A: MTBE:methanol:water (15:81:4) and B: MTBE:methanol (10:1)) at a flow rate of 1.1 ml/min was employed. Samples for HPLC analysis were dissolved in MTBE and filtered prior to injection. α-, β- and γ-carotene were isomerised by dissolving the carotene in MTBE, adding a small crystal of iodine and leaving the sample under fluorescent light for 1 h. Response curves of α-, β- and γ-carotene as a function of concentration were constructed – the concentration of the standard solutions was determined by using published absorption coefficients ($E^{1\%}_{1\,cm}$): α-carotene (2710 in hexane), β-carotene (2500 in cyclohexane) and γ-carotene (2780 in hexane) (Britton, 1995; CaroteNature, private communication).

3. Results and discussion

3.1. Assignments

In Fig. 1 is shown the chromatogram of oil palm carotenes in the region where α- and β-carotene elute. As expected, based on the close structural similarity between α- and β-carotene, α- and β-carotene elute close together. A large number of isomers are formed in relatively high concentration (Fig. 1) due to the heat treatment the carotenes have undergone (vide supra). In assigning the peaks, standards of *trans*-α- and *trans*-β-carotene were used to assign the largest and second-largest peaks to *trans*-β- and *trans*-α-carotene, respectively. Because standards of *cis*-α- and *cis*-β-carotenes are not readily available (except for 9- and 13-*cis*-β-carotene), assignments of the *cis*-isomers were done using the following procedure. Firstly, isomerised samples of *trans*-α- and *trans*-β-carotene were used to clarify which peaks in Fig. 1 belonged to α-carotene and which belonged to β-carotene. Secondly, relative abundance and spectral data of the individual isomers were used (see below) to assign (most of) the mono-*cis*-isomers and, thirdly, assignments in the literature were used to corroborate

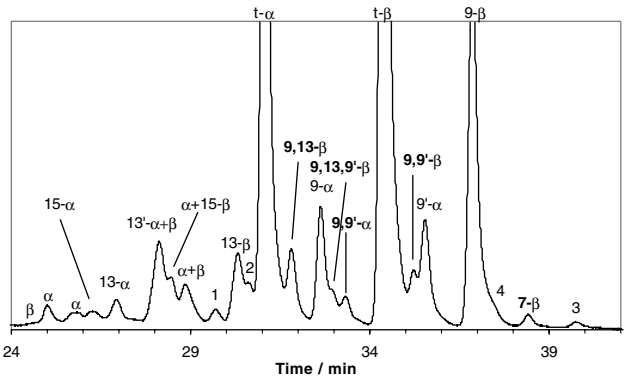

Fig. 1. Chromatogram at 456 nm of oil palm carotenes. Peaks are labelled as follows: number indicates position of *cis*-bond and greek letter indicates carotene, e.g., 15-α means 15-*cis*-α-carotene. Assignments in bold are tentative. See text for details.

the assignments and assign a few di-*cis*-isomers. In assigning mono-*cis*-isomers, the following guidelines (Britton, 1995) are useful: (1) the absorption maximum is blue-shifted a few nm compared to the *trans*-isomer (di- and poly-*cis*-isomers show a larger shift) and (2) a new band around 340 nm (the *cis*-band) is observed, the intensity of which increases the closer the *cis*-bond is to the centre of the molecule (the *cis*-band may be completely absent in di- and poly-*cis*-isomers). Furthermore, 9-*cis*- and 13-*cis*-β-carotene are the most stable mono-*cis*-isomers (based on calculations, not actual measurements) followed by 7-*cis*, 15-*cis* and 11-*cis* (Doering et al., 1995) which is reflected in the relative abundance of these isomers: 9-*cis* and 13-*cis* are often observed in carotenoid-containing samples, 15-*cis* is sometimes observed, whereas 7-*cis* and 11-*cis* are rarely/never observed.

Based on this, it is possible to assign (most of) the mono-*cis*-isomers of α- and β-carotene without using standards. However, based on spectral data alone it is not possible to distinguish between 13-*cis*- and 13′-*cis*- or 9-*cis*- and 9′-*cis*-α-carotene. It has been shown that 13-*cis*-α-carotene elutes before 13′-*cis*-α-carotene and 9-*cis* elutes before 9′-*cis* (Emenhiser, Englert, Sander, Ludwig, & Schwartz, 1996) under conditions similar to those used here. Therefore, it is possible to unambiguously assign 15-, 13-, 13′-, 9- and 9′-*cis*-α-carotene and 15-, 13- and 9-*cis*-β-carotene (Fig. 1). However, this only accounts for around half the number of peaks. Comparison with the literature assignments allows a tentative assignment of some of the remaining peaks. Thus, 9,13-di-*cis*-β-carotene has been shown to elute between 13-*cis*- and *trans*-β-carotene (Breitenbach, Braun, Steiger, & Sandmann, 2001; Lacker, Strohschein, & Albert, 1999). The peak at 31.8 min is a good candidate for 9, 13-di-*cis*-β-carotene due to its relative intensity and because the intensity of the *cis*-band of this compound matches that of 9,13-di-*cis*-β-carotene (*cis*-band more intense than in 9-*cis*-β-carotene but less intense than in 13-*cis*-β-carotene) (Lacker et al., 1999). Other di- and