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Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay

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ABSTRACT

The purpose of this study was to assess the antioxidant activity of carotenes and xanthophylls measured by various methods, compared to α-tocopherol, BHA and BHT. Four assays were selected to achieve a wide range of technical principles. Besides αTEAC, which uses $ABTS^{\cdot+}$ radical cation, ferric reducing activity (measured by using FRAP assay), and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) scavenging assay were used. In addition, a luminol-chemiluminescence based peroxyl radical scavenging capacity (LPSC) assay, was used. Most of the compounds showed significant differences in their activity of scavenging radicals depending on the assay used. Of the 22 compounds tested, only a few such as lutein, zeaxanthin and capsanthin gave comparable results in the various assays. Surprisingly, in contrast to α-tocopherol, BHA and BHT, carotenoids did not show any $DPPH^{\cdot}$ scavenging activity. To standardise the relative contribution of the assays used, weighted means of the values obtained in αTEAC, FRAP, DPPH and LPSC assay were calculated. This strategy was used to assess the antioxidant capacity of several juices and oil samples. The highest lipophilic antioxidant capacity in all assays was observed for sea buckthorn berry juice, followed by tomato juice, carrot juice and orange juice. Within the oil samples, the order of antioxidant capacity depended on the assay used.

1. Introduction

Carotenoids, a class of natural fat-soluble compounds mainly *de novo* synthesised by plants (Rodriguez-Amaya, Kimura, Godoy, & Amaya-Farfan, 2008), are one class of major food micronutrients in human diet (Maiani et al., 2009). In plants, they have potential antioxidant properties due to their chemical structure (Stahl & Sies, 2003). In the human organism, carotenoids are part of the antioxidant defense system, too. The quantitative most important carotenoids in human diet are β-carotene, lycopene, lutein, β-cryptoxanthin, zeaxanthin, and astaxanthin (Riccioni, 2009). Numerous observational studies have supported the hypothesis that antioxidants like carotenoids and vitamin E or metabolites of these nutrients are associated with cardiovascular diseases (CVD) (Lichtenstein, 2009). Carotenoids could be used as an inexpensive mean of prevention, and as a possibly treatment, even though human intervention trials showed controversial results, with some positive findings, many null findings, and some suggestion of harm in certain high-risk populations (Riccioni, 2009). Recent smaller interventional studies with carefully chosen populations, such as those under high levels of oxidative stress, have yielded largely positive results (Lichtenstein, 2009).

Carotenoids play a role in protecting plants against photooxidative processes. They are efficient antioxidants, e.g. in scavenging singlet molecular oxygen (Di Mascio, Kaiser, & Sies, 1989) and peroxyl radicals (Stahl & Sies, 2003). Certain convenient methods were developed for a quick, simple and reliable quantification of the antioxidant capacity. In general, the methods to determine the total antioxidant capacity were divided into two major groups: assays based on the single electron transfer (SET) reaction, displayed through a change in colour as the oxidant is reduced, and assays based on a hydrogen atom transfer (HAT) (Huang, Ou, & Prior, 2005), which measure the activity of the antioxidant to scavenge peroxyl radicals, such as the total radical trapping antioxidant parameter (TRAP) assay, the oxygen radical absorbance capacity (ORAC) assay and the luminol-chemiluminescence based peroxyl radical scavenging capacity (LPSC) assay (Alho & Leinonen, 1999; Huang et al., 2005; Ou, Hampsch-Woodill, & Prior, 2001). The ferric reducing antioxidant power (FRAP), the α-tocopherol/Trolox equivalent antioxidant capacity (αTEAC/TEAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays include electron transfer reaction (Benzie & Strain, 1996; Brand-Williams, Cuvelier, & Berset, 1995; Huang et al., 2005; Re et al., 1999).

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Table 1
Absorptivity values at specific wavelength maxima and used solvents, and solvents used for stock solutions of analysed carotenoids and α-tocopherol (Franke, Murphy, Lacey, & Custer, 2007; Rodriguez-Amaya, 2001).

Carotenoid	Solvent	Wavelength (nm)	Absorptivity value ($E_{1\%,1\ cm}$)	Solvent used for stock solution
Antheraxanthin	Ethanol	444	2350	Methanol
Astaxanthin	*n*-Hexane	478	2100	Acetone
α-Carotene	*n*-Hexane	445	2710	T + CH (1 + 4, v/v)
β-Carotene	*n*-Hexane	450	2590	T + CH (1 + 4, v/v)
Canthaxanthin	Petroleum ether	466	2200	T + CH (1 + 4, v/v)
Capsanthin	Benzene	487	2070	Methanol
β-Cryptoxanthin	Petroleum ether	449	2400	T + CH (1 + 4, v/v)
Echinenone	Petroleum ether	458	2160	Ethanol
Lutein	Ethanol	445	2550	Ethanol
Lycopene	Petroleum ether	470	3450	T + CH (1 + 4, v/v)
Neoxanthin	Ethanol	442	2380	Methanol
Neurosporene	*n*-Hexane	440	2920	Methanol
Phytoene	Petroleum ether	286	915	T + CH (1 + 4, v/v)
Phytofluene	*n*-Hexane	347	1580	T + CH (1 + 4, v/v)
Rubixanthin	Petroleum ether	460	2750	T + CH (1 + 4, v/v)
Violaxanthin	Ethanol	440	2550	Methanol
Zeaxanthin	Ethanol	450	2480	Ethanol
Bixin	Petroleum ether	456	4200	Ethanol
Crocetine	Petroleum ether	422	4320	Ethanol
DL-α-Tocopherol	Ethanol	292	75.8	Ethanol

T + CH: toluene + cyclohexane.

The purpose of this study was to compare the antioxidant activity values obtained by different methods (FRAP, αTEAC, DPPH, LPSC) of a variety of carotenes and xanthophylls, known as antioxidants, and found in human diet. The second aim was to standardise the reporting on antioxidant activity for each single compound by calculating a meanful standardised value based on the results observed in all the antioxidant capacity assays used. In addition, the proposed approach was tested on several juices (tomato, carrot, sea buckthorn berry, and orange) and oil samples (sunflower, olive, walnut, and fish).

2. Material and methods

2.1. Materials

2.1.1. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), $K_2S_2O_8$, 2,4,6-tripyridyltriazine (TPTZ), and 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT) were obtained from Sigma–Aldrich (Taufkirchen, Germany). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Acros Organics (Schwerte, Germany). Manganese dioxide was obtained from Merck KGaA (Darmstadt, Germany). Luminol and 2-*tert*-butyl-4-hydroxyanisol (BHA) were purchased from Fluka (Buchs, Switzerland). DL-α-tocopherol (α-T), β-tocopherol (β-T), γ-tocopherol (γ-T) and δ-tocopherol (δ-T) were purchased from Calbiochem (Darmstadt, Germany) with purities of 97–100% shown by GC. α-Tocotrienol (α-T3), β-tocotrienol (β-T3), γ-tocotrienol (γ-T3) and δ-tocotrienol (δ-T3) were obtained from Davos Life Sciences (Singapore). Rubixanthin was purchased from Apin Chemicals Ltd. (Abingdon, UK). Bixin and crocetine were obtained from Extrasynthèse (Genay, France). All other carotenoids used were obtained from CaroteNature (Lupsingen, Switzerland) with a purity of 94–98% by HPLC. All solvents used were of HPLC grade. HPLC grade water (18 MΩ) was prepared using a Millipore Milli-Q purification system (Millipore GmbH, Schwalbach, Germany). Buffer salts and all other chemicals were of analytical grade.

2.1.2. Food samples

Tomato and carrot juice, as well as sunflower, olive, and walnut oil were bought in a local supermarket. Sea buckthorn berry juice was obtained in a local health food store. The fish oil was from Croda (Nettetal, Germany).

2.2. Sample preparation

Stock solutions of each carotenoid (ca. 100 μg/ml) and α-T (ca. 1 mg/ml) were prepared in the specific solvent of each compound (see Table 1) and stored at −30 ± 2 °C until analysis. Exact concentrations of the stock solutions were determined spectrophotometrically using the absorptivity at the specific wavelength of each compound (see Table 1), except of the two synthetic antioxidants BHA and BHT, in which initial weight was used.

Lipophilic extracts of the various juices were prepared using *n*-hexane (Rösch, Bergmann, Knorr, & Kroh, 2003). To assess the antioxidant capacity of the oils used, 1 g of the samples was diluted with *n*-hexane to a final volume of 25 ml and centrifuged (5 min, 16,900*g*).

2.3. Evaluation of the antioxidant capacity

All experiments were done under subdued light. Before analysis, a defined volume of each carotenoid stock solution was evaporated to dry under a stream of nitrogen at 30 ± 1 °C and the residue was resolved in *n*-hexane (for FRAP and αTEAC), ethanol/*n*-hexane 1 + 1 (v/v, for DPPH assay), or MTBE/DMSO 1 + 19 (v/v, for LPSC assay), respectively. All carotenoid samples were analysed in triplicate at four different concentrations (1, 5, 10, 20 μM). The extract solutions of juices and oil samples were analysed in triplicate on different days, too.

One assay used to assess the antioxidant capacity was the ferric reducing antioxidant power (FRAP) assay, in order to determine the ferric reducing activity of carotenoids and food samples. The procedure was based on the work recently published by our research group (Müller, Theile, & Böhm, 2010). In a reaction tube, 100 μl of carotenoid or extract solution, standard (ca. 4.5–114 μmol α-T/l), or blank (*n*-hexane) and 600 μl of FRAP reagent, consisting of ferric chloride and TPTZ in acetate buffer (pH 3.6), were shaken on a thermoshaker (25 ± 1 °C, 1400 rpm). After 6 min of shaking, the mixtures were transferred into half microcuvettes (1.5 ml, PS), and centrifuged for 30 s at 1000*g* to separate the layers. Finally, the absorbances of the lower layer of samples,