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NEWSLETTER

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EUROPEAN NETWORK TO ADVANCE CAROTENOID RESEARCH AND APPLICATIONS IN AGRO-FOOD AND HEALTH

CAROTENOIDS AS ANTIOXIDANTS: POSSIBLE HEALTH IMPLICATIONS

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Carotenoids: Introduction

Carotenoids are fat-soluble colouring compounds widely spread in nature, having various biological functions in plant and also in animal organisms. This is mainly due to their chemical structure. Carotenoids belong to the natural poly-unsaturated isoprenoids. Often consisting of eight isoprene units, they form a basic structure of 40 carbon atoms. Thus, many carotenoids belong to the tetraterpenes. At the end of the carbon chain various functional groups can be located, resulting in the enormous variety of more than 750 carotenoids known today. According to their structure, they are distinguished in oxygen-free carotenes and oxygen-containing xanthophylls. Around 60 of them present in food have an effect on human nutrition. In human plasma the following carotenoids are found (**Figure 1**):

- lycopene (0.61–1.38 µmol/L)
- α-carotene (0.03–0.22 µmol/L)
- β-carotene (0.13–0.53 µmol/L)
- lutein (0.14–0.34 µmol/L)
- zeaxanthin (0.03–0.05 µmol/L)
- β-cryptoxanthin (0.15–0.37 µmol/L)

Other carotenoids and their metabolites like phytoene and phytofluene are contained in lower concentrations in human plasma[1].

Antioxidant activity/capacity of carotenoids

Various methods have been developed for quantification of antioxidant activity or antioxidant capacity. Most of these test systems were firstly described for hydrophilic antioxidants, being the major part of antioxidants in foods as well as in physiological systems. However, lipophilic antioxidants are also important in specific foods (e.g. plant oils) being rich in lipids. They are relevant as well *in vivo* (e.g. in membranes of cells). Thus, several test systems were optimized/developed for lipophilic antioxidants[2]. All tests determine antioxidant activity/capacity as a sum parameter without looking for individual compounds (e.g. carotenoids or tocopherols). The methods are divided into two major groups: assays based on the single electron transfer (SET) reaction and assays based on a hydrogen atom transfer (HAT). A third possible mechanism include radical addition to antioxidants (formation of adducts).

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CAROTENOIDS AS ANTIOXIDANTS: **POSSIBLE HEALTH IMPLICATIONS**

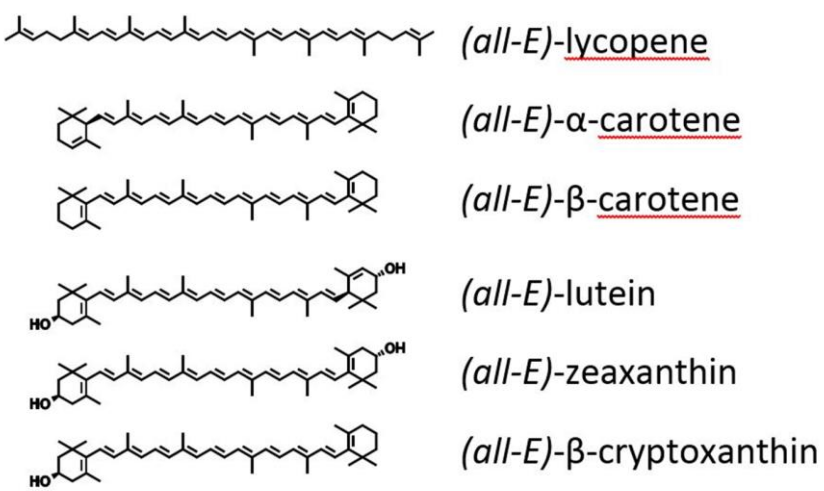

Figure 1. Main carotenoids in human plasma.

The ferric reducing antioxidant power (FRAP), the α-tocopherol/Trolox equivalent antioxidant capacity (αTEAC/TEAC) and the 2,2,-diphenyl-1-picrylhydrazyl (DPPH) assays belong to the SET-based assays. The oxygen radical trapping antioxidant capacity (ORAC) assay and the luminol-chemiluminescence based peroxyl radical scavenging capacity (LPSC) assay are two examples of HAT-based tests[3].

Carotenoids undergo “bleaching”, that means they lose their colour, when exposed to radicals or oxidizing species. This process involves interruption of the conjugated double bond system either by cleavage or by addition to one of the double bonds[4].

Carotenoids as antioxidants

Due to their lipophilic properties, carotenoids develop their antioxidant activity mainly in cell membranes and lipoproteins. In many epidemiological, clinical and intervention studies, data regarding the carotenoids β-carotene, lycopene, lutein and zeaxanthin have been collected which confirm that a nutrition high in carotenoids from fruit and vegetables can decrease the risk of chronic diseases like atherosclerosis, coronary heart diseases, diabetes type 2 or asthma. Various *in vitro* test systems for determining the antioxidant capacity showed that carotenoids (concentration: 10 µM) have a distinct antioxidant activity against a number of radical species. The importance of the antioxidant effects *in vivo*, however, is controversially discussed: interactions of various antioxidants during resorption and metabolism can cause changes in the antioxidant *in vivo* mechanisms. Under certain circumstances, carotenoids are also able to develop pro-oxidant effects. When taken up in physiologically relevant doses and in the status of high oxygen partial pressure, e.g. in the lung, or at strong oxidative stress, carotenoids showed pro-oxidant properties[1].

As different methods to determine the antioxidant activity can yield widely diverging results, it has recently been recommended to use more than one assay to determine the antioxidant potential of food extracts or single compounds[5-7]. Our studies at Friedrich Schiller University Jena used various assays based on different mechanisms[2]. Lycopene as well as hydroxyl carotenoids were the most effective carotenoids in reducing ferric ions (FRAP assay). Within the αTEAC assay, lycopene and α-carotene as well as β-carotene were more efficient quenchers of $ABTS^{\cdot+}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation) than most of the xanthophylls. Only the xanthophylls rubixanthin and β-cryptoxanthin showed comparable activities to the carotenes. In contrast, the keto carotenoids demonstrated the highest activity in scavenging peroxyl radicals. None of the carotenoids showed any activity to scavenge DPPH[3].

CAROTENOIDS AS ANTIOXIDANTS: **POSSIBLE HEALTH IMPLICATIONS**

A ranking of all compounds investigated is possible within each assay[8]. However, for summarising the results of one compound within all assays a standardisation is recommended. Thus, the use of a weighted average of the results obtained in all assays based on the reference compound was proposed by Tabart et al. in 2009[9]. However, a simple mathematical mean is not adequate, because one of the methods (LPSC) gave much higher values. This would give this assay undue preponderance in the mean. In contrast, carotenoids did not show any activity to scavenge DPPH radicals. This would lead to an underestimation of the antioxidant potential of carotenoids when calculating a simple mean. Therefore, a compensing antioxidant activity as weighted mean was calculated, including the results determined by using the FRAP, αTEAC, DPPH and LPSC assays. A weighted factor was calculated by dividing the antioxidant activity of a compound determined using a given method by the average activity determined for the whole set of compounds in the same method[3]. As the carotenoids did not scavenge DPPH radicals, weighted averages excluding the DPPH results were also calculated. The xanthophylls β-cryptoxanthin and rubixanthin as well as the carotene lycopene showed the highest antioxidant potentials, with a weighted average of 1.8-1.9 (**Figure 2**)[3].

Metabolites of carotenoids as antioxidants

Isomerisation and oxidation are the main reactions proposed for the metabolism of carotenoids[10]. Thus, *in vivo* besides the native carotenoids there will be various isomers and oxidation products. HPLC methods using C_{30} columns allowed to better separate different isomers (**Figure 3)**.

Regarding oxidation products of carotenoids, recently apo-lycopenoids with aldehyde ending functions, i.e. apo-lycopenals, have been detected in heated tomato products and also in plasma of volunteers after consumption of tomato food products[11].

Investigating β-carotene isomers [(all-E)-, (9Z)-, (13Z)-, (15Z)-β-carotene] as well as β-carotene metabolites (β-apo-8'carotenal, β-apo-8'-carotenoic acid ethyl ester, 6'-methyl-β-apo-6'carotene-6'-one) by using FRAP, αTEAC and LPSC assays led to interesting results. While the different isomers did not react in FRAP assay and showed higher αTEAC values than the metabolites, the metabolites had higher antioxidant potential in LPSC assay. Weighted averages showed for the isomers.

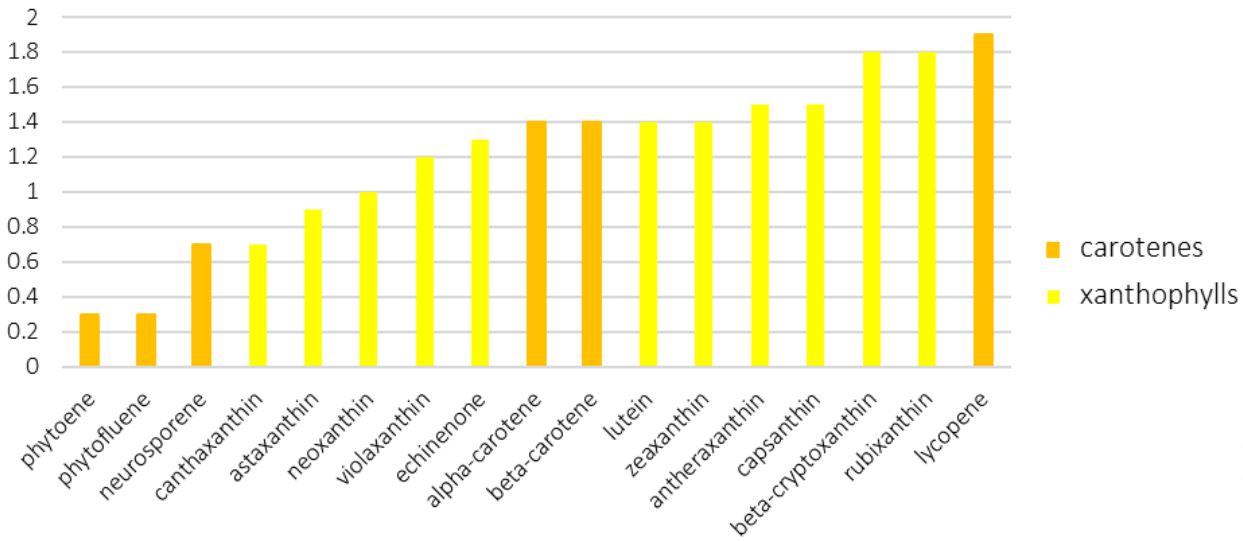

Figure 2. Standardised antioxidant activities of common carotenoids: weighted averages including FRAP, αTEAC and LPSC results (modified from Müller *et al. (*2011)[3]).

CAROTENOIDS AS ANTIOXIDANTS: **POSSIBLE HEALTH IMPLICATIONS**

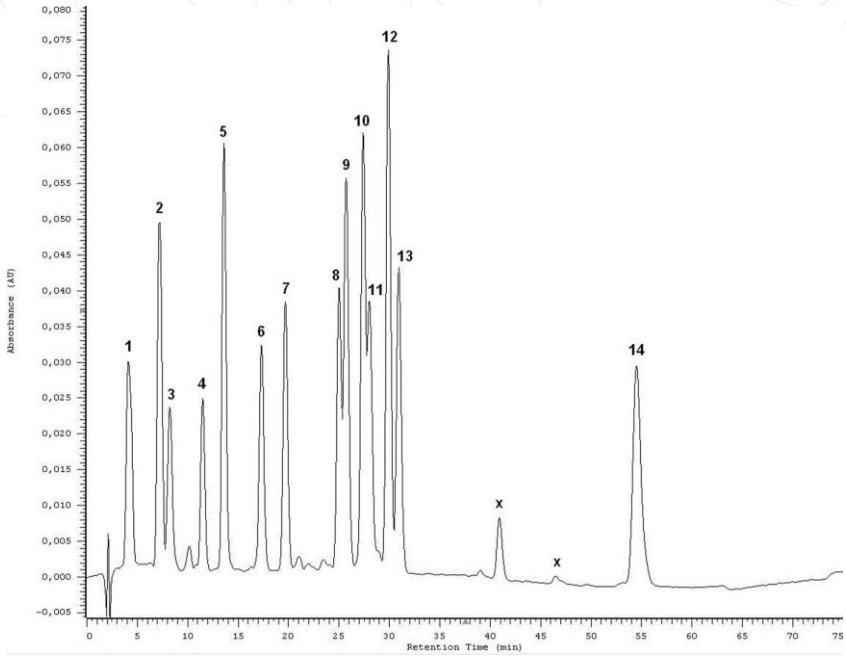

Figure 3. HPLC chromatogram (DAD 450 nm) of 14 carotenoids, C30 column, 250 x 4.6 mm, 5 µm (Trentec, Gerlingen Germany), column temperature: 17 °C, mobile phase: methyl tert.-butyl ether (solvent A) and methanol (solvent B) using a gradient procedure as follows: 1) Initial conditions 10% solvent A and 90% solvent B, 2) a 45-min linear gradient to 55% solvent A, 3) 55% solvent A and 45% solvent B for 15 min, 4) a 10-min linear gradient to 10% solvent A, flow rate 1.3 mL/min. **1** = (all-E)-violaxanthin, **2** = (all-E)-lutein, **3** = (all-E)-zeaxanthin, **4** = (all-E)-canthaxanthin, **5** = (all-E)-β-apo-8'-carotenal (internal standard), **6** = (all-E)-β-cryptoxanthin, **7** = (all-E)-echinenone (internal standard), **8** = (15Z)-β-carotene, **9** = (13Z)-β-carotene, **10** = (all-E)-α-carotene, **11** = (all-E)-rubixanthin, **12** = (all-E)-β-carotene, **13** = (9Z)-β-carotene, **x** = lycopene (Z)-isomers, **14** = (all-E)-lycopene.

comparable antioxidant activity to that of α-tocopherol. The activity of the breakdown products of β-carotene was twice as high[4]. Lycopene (Z)-isomers [(5Z)-, (9Z)-, (13Z)- (7Z,9Z,7'Z,9'Z)] showed comparable reducing activities (αTEAC assay) to (all-E)-lycopene and had higher peroxyl radical scavenging activity (LPSC) than (all-E)-lycopene[12]. In contrast, none of the lycopene metabolites (various apo-lycopenoids) tested was similar or more active when using FRAP and αTEAC assays. Within the LPSC assay, only the long-chain metabolites apo-6'-lycopenal and apo-8'-lycopenal were as efficient as (all-E)-lycopene[13].

Natural antioxidants exist in combination and are consumed together in foods. Thus, investigation of interactions between antioxidants is important to get a more realistic picture. Antioxidants do not always react in addition of their activities. Possible interactions are synergism or antagonism. Recently, lipophilic antioxidants were mixed in 1:1, 2:1 and 1:2 M ratios and analysed on their antioxidant capacity by using αTEAC and PCL (photochemiluminescence) assays[14]. Besides α-tocopherol, the carotenoids lycopene, β-carotene and lutein were investigated. When using the αTEAC assay, all combinations of two carotenoids each showed lower experimental antioxidant capacity (EAC) than theoretical antioxidant capacity (TAC: calculated by addition of single values), resulting in ratios of EAC/TAC less than 1 (**Figure 4**). Thus, antagonistic effects were observed between the carotenoids. In contrast, mixtures of lycopene and β-carotene as well as mixtures of β-carotene and lutein showed synergistic effects (higher experimental values than theoretical values, ratios EAC/TAC higher than 1 in PCL assay. Only the mixtures of lycopene and lutein resulted in antagonistic effects[14]. These results show that not only single compounds need

CAROTENOIDS AS ANTIOXIDANTS: **POSSIBLE HEALTH IMPLICATIONS**

to be investigated. The food matrix with various hydrophilic and lipophilic antioxidants might have completely different biological effects. Thus, although sum parameters like antioxidant capacity are not very precise and only *in vitro* data, they will give an idea of the processes in whole food matrices and within the human organism.

Preventive aspects due to the antioxidant properties of carotenoids

Carotenoids play a role in protecting plants against photo oxidative processes. Although they are part of the antioxidant defense system in the human organism[3], their preventive role solely as antioxidants is still under controversial debate. A recent review[15] critically discussed the antioxidant role of the carotenoid lycopene in the prevention of cardiovascular diseases. As the oxidation of human low density lipoproteins is a fundamental mechanism in the initiation of atherosclerosis, a beneficial role of lycopene as antioxidant in the prevention of cardiovascular diseases is suggested. However, the data are still scarce and controversial. While chemical and *in vitro* cell studies showed preventive properties of lycopene, there is only a small number of intervention trials supporting these potential benefits of lycopene[15]. As already discussed for the interactions of carotenoids as antioxidants, foods with their complex matrix often do not have special effects only due to the presence of one ingredient. Thus, future studies have to clarify the beneficial role of carotenoids as antioxidants in combination with other food ingredients. The population used for intervention trials is another important aspect to be taken into account[15].

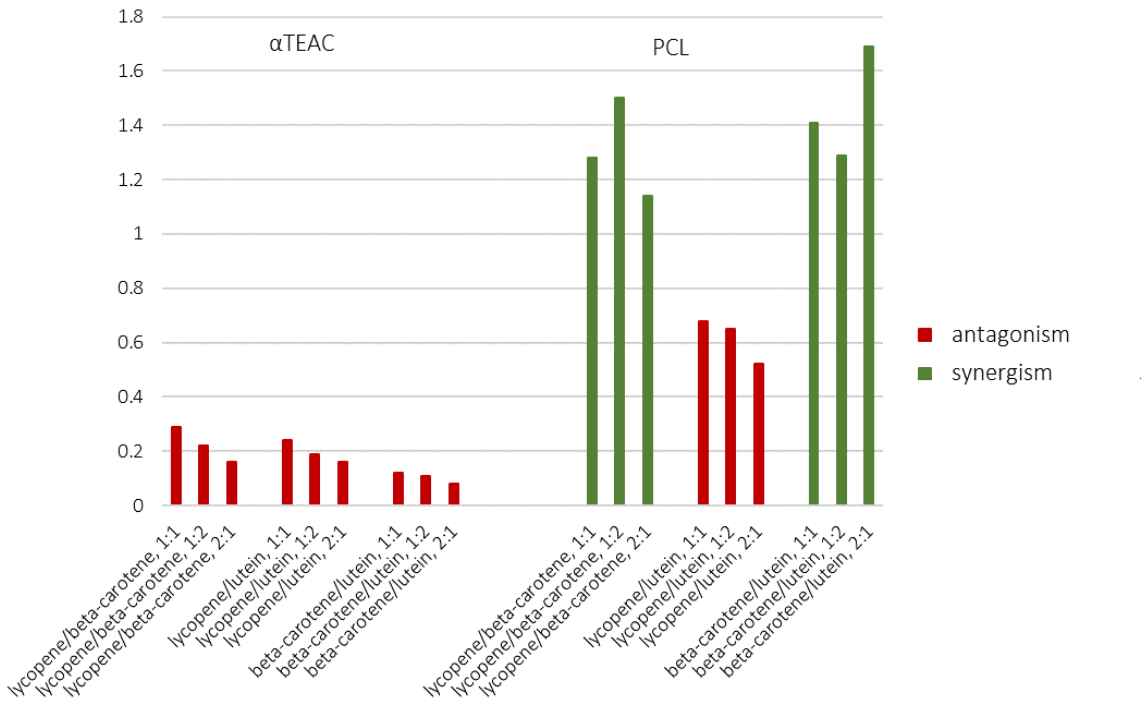

Figure 4. Results of synergism and antagonism or additive effects (antioxidant capacity) of the different combinations (1:1, 1:2, 2:1 M ratios) of two carotenoids each by using αTEAC and PCL assays, all results show the ratios EAC/TAC, EAC: experimental antioxidant capacity, TAC: theoretical antioxidant capacity, all ratios are significant different from 1.0 ($p < 0.05$) (modified from Karmowski *et al.* (2015)[14]).

CAROTENOIDS AS ANTIOXIDANTS: POSSIBLE HEALTH IMPLICATIONS

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