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Epimerisation of lutein to 3′-epilutein in processed foods

József Deli,[a,*] Péter Molnár,[a] Erzsébet Ősz,[a] Gyula Tóth[a] and Ferenc Zsila[b]

[a]Department of Biochemistry and Medical Chemistry, University of Pécs, Medical School, PO Box 99, H-7601 Pécs, Hungary
[b]Department of Molecular Pharmacology, Institute of Chemistry, Chemical Research Center, PO Box 17H-1525 Budapest, Hungary

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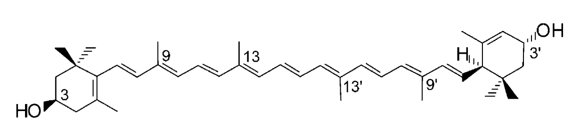
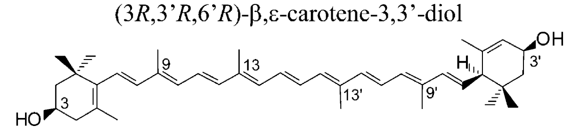
Abstract—Lutein can convert to 3′-epilutein and anhydrolutein I in acidic medium as well as during different cooking methods. To our knowledge, this is the first demonstration of the epimerisation and dehydration of lutein in native samples. During different cooking processes the lutein content of the processed vegetables and fruits dramatically decreases, thus the bioavailability of processed plant food may also decrease. The occurrence of 3′-epilutein in processed food may be important regarding the application of synthetic lutein as food additive.

Dietary carotenoid antioxidants from fruits and vegetables have long been known to play an important role in human health. Their importance is receiving increased attention, and they have now been implicated in contributing to prevention of cancer. Dark-green leafy vegetables, such as spinach, collard greens and sorrel, have relatively high carotenoid (especially lutein[1]) content.

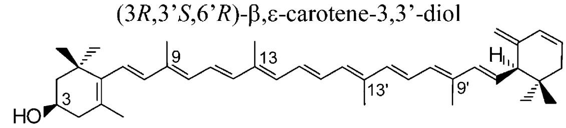
Lutein [(3*R*,3′*R*,6′*R*)-β,ε-carotene-3,3′-diol] is the main xanthophyll found in the major light-harvesting pigment–protein complex of higher plants. It is involved in energy transfer mechanisms during photosynthesis. In fatty acid ester form, lutein is widely distributed in fruits, flowers and yellow autumn leaves as well.[2] Lutein is of particular interest because it has high antioxidant activity and it has been directly implicated in cancer prevention.[3] The carotenoids lutein and zeaxanthin [(3*R*,3′*R*)-β,β-carotene-3,3′-diol] are described as having a positive influence on the prevention and treatment of age-related macular degeneration (AMD). The yellow colour of pigmented macula is attributed to its zeaxanthin and lutein content. Concerning the biological role of these carotenoids two main proposals are considered. On the one hand the xanthophylls act as optical filters, selectively removing partially the blue part of the visible spectrum. On the other hand as antioxidants they inhibit free radical damage and quench singlet oxygen. Both suggestions have merit, and at this point it is unknown whether either, or both, of these mechanisms operate.[4] Whatever the function of the xanthophylls in the macula is, their stereochemistry seems to be important. Nevertheless, the chemistry of the xanthophylls is intrinsically interesting, and may give insight into their function.



Lutein
(3*R*,3’*R*,6’*R*)-β,ε-carotene-3,3’-diol



3’-Epilutein
(3*R*,3’*S*,6’*R*)-β,ε-carotene-3,3’-diol



Anhydrolutein I
(3*R*,6’*R*)-3-hydroxy-3,4-didehydro-β,γ-carotene

Figure 1. Chemical structure of carotenoids.

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* Corresponding author. Fax: +36-72-536-225; e-mail: jozsef.deli@aok.pte.hu

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The 3′-epimer of lutein, 3′-epilutein [(3*R*,3′*S*,6′*R*)-β,ε-carotene-3,3′-diol], was detected as a metabolite of lutein in the human body.[5,6] In contrast with lutein, from plants, 3′-epilutein was only isolated from the petals of *Caltha palustris*[7] and it was detected in anthers of flowers of several roses and in those of peonies.[8]

Determination of the biological activity of different carotenoid stereoisomers requires careful qualitative and quantitative analysis of the functional foods during cooking processes. There is a perception that carotenoids are destroyed by the heat processes involved in cooking vegetables. In fact, carotenoid loss is minimal with moderate cooking, and in many cases, carotenoids become more bioavailable after cooking, probably because heat processing liberates them from cell matrices.[9]

Here we show that in certain conditions, lutein converts to 3′-epilutein, 3′-stereoisomer of lutein. To our knowledge, this is the first demonstration of the epimerisation of lutein in native samples.

Carotenoid composition analyses of vegetable extracts and identification of the carotenoids were performed by high-performance liquid chromatography.[10] Lutein, the newly formed 3′-epilutein and anhydrolutein I (a

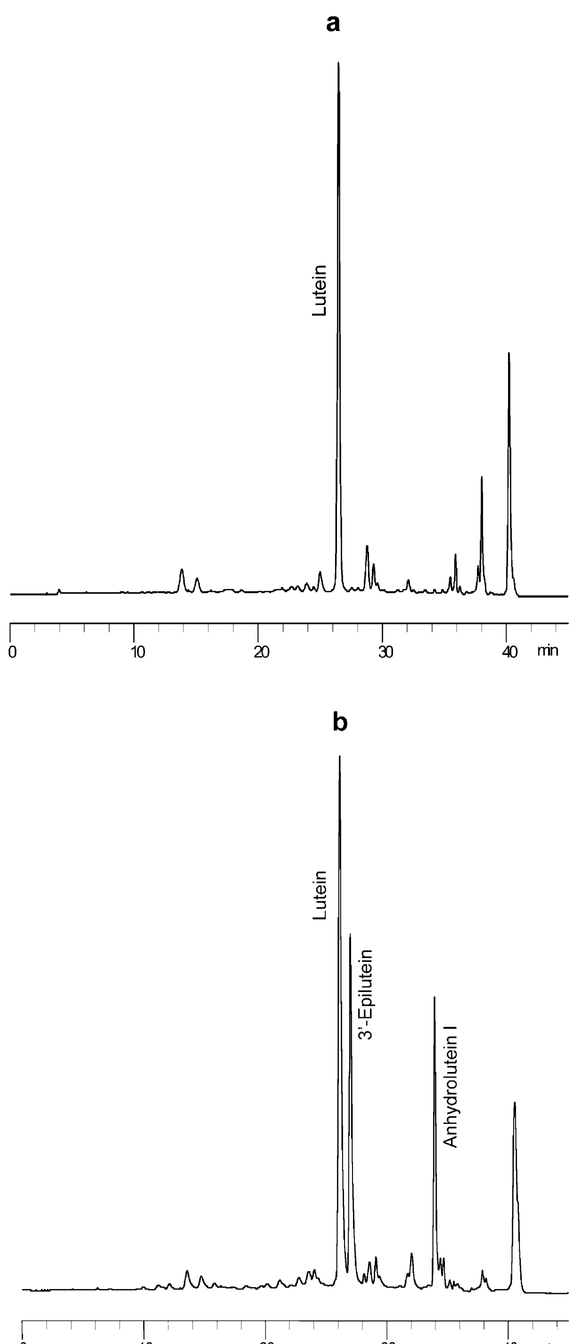

Figure 2. HPLC separation of carotenoids in the extract of raw (**a**) and steamed (**b**) sorrel (*Rumex rugisus Camp.*).

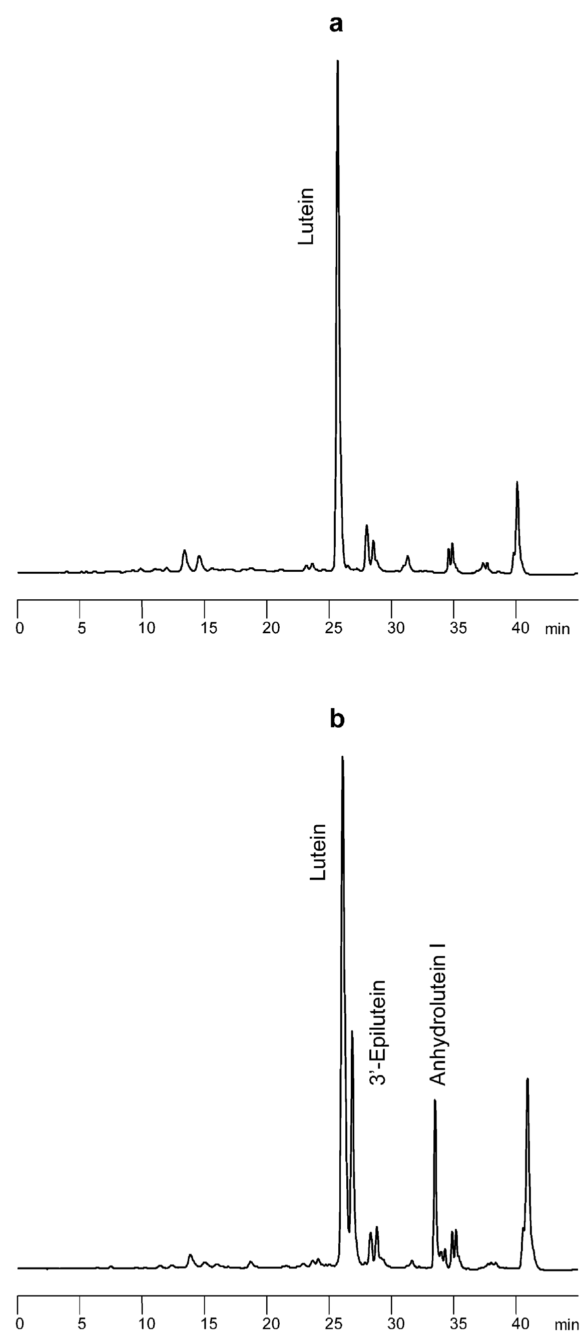

Figure 3. HPLC separation of carotenoids in the extract of fresh (**a**) and bottled (**b**) blackberries (*Rubus tornless*).

dehydration product of lutein: (3*R*,6′*R*)-3-hydroxy-3′,4′-didehydro-β,γ-carotene) (Fig. 1) could be separated from each vegetable sample, isolated by column chromatography and characterised by NMR and CD spectroscopy as well as mass spectrometry. The UV–vis, CD and MS data as well as the proton chemical shift[11] and proton–proton coupling constant values determined from the NMR spectra of isolated compounds were identical to the corresponding data from both our previous works[12,13] and literature.[7,14]

The main carotenoids of fresh spinach (*Spinacia oleracea* L.) were proved to be lutein, β-carotene and violaxanthin. In addition, the presence of neoxanthin and various (*Z*)-isomers, such as (9′*Z*)-neoxanthin, (9*Z*)-violaxanthin, (13*Z*)-violaxanthin, (9*Z*)- and/or (9′*Z*)-lutein, (13*Z*)- and/or (13′*Z*)-lutein as minor components was observed. During different cooking methods on spinach (e.g., steaming, microwaving, stewing and boiling) we could observe only modest changes in the concentrations of the major carotenoids. Some differences were found for minor carotenoids, particularly loss of 5,6-epoxy-carotenoids and increases of (*Z*)-isomers.

In contrast with spinach, sorrel (*Rumex rugisus Camp.*) contains oxalic acid. The main carotenoids isolated from fresh sorrel were also lutein and β-carotene, and according to the acid content, some furanoids (neochromes, mutatoxanthins) could be detected. During different cooking methods, surprisingly, the formation of 3′-epilutein as well as of anhydrolutein I, was observed. According to the HPLC measurements (Fig. 2) the 3′-epilutein and anhydrolutein I content of the stewed sorrel sample proved to be 9% and 8%, while in the case of steamed sorrel 9% and 11%, respectively. Applying other cooking methods a 7–12% of 3′-epilutein and 3–7% anhydrolutein I content was found.

The formation of *3′-epilutein* in acidic conditions is a known reaction in laboratory,[14] but it has not been described yet during the food processing. Since sorrel contains oxalic acid in larger amount, the epimerisation reaction may take place during cooking when heat processing liberates the carotenoids and acid from the cells.

This recognition led us to investigate other vegetables and fruits, which contain floral acids or which are stored in acidic conditions. The epimerisation and dehydration of lutein were also observed in cooked, baked and pickled green paprika and in the case of the extract of pickled dill. Similarly, the formation of 3′-epilutein and anhydrolutein I was detected in some bottled fruits (plum, blackberries), which do not contain these carotenoids in fresh state (Fig. 3, Table 1).

Our results demonstrate that a part of the lutein content of native samples can convert to 3′-epilutein and anhydrolutein I in acidic medium. The proposed mechanism is shown in Figure 4.

The lutein content of processed vegetables and fruits dramatically decreases thus the bioavailability of processed plant foods may also decrease. However, studies

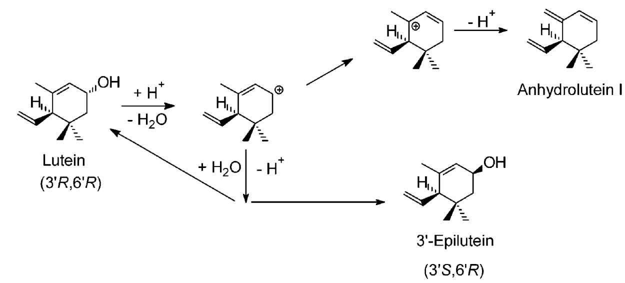

Figure 4. Proposed mechanism for the formation of 3′-epilutein and anhydrolutein I from lutein.

Table 1. The ratio of lutein, 3′-epilutein and anhydrolutein I in different raw and processed vegetables and fruits

Plants	Lutein (%)	3′-Epilutein (%)	Anhydrolutein I (%)
Raw sorrel	36.0	—	—
Steamed sorrel	12.7	9.2	11.3
Stewed sorrel	16.4	8.8	8.0
Boiled sorrel	28.6	9.0	6.5
Microwaved sorrel	24.0	6.4	5.0
Raw paprika	53.5	—	—
Baked paprika	47.5	3.8	5.1
Pickled paprika	49.2	12.5	2.2
Pickled dill	28.7	13.8	3.0
Raw plum	34.2	—	—
Bottled plum	29.3	7.0	4.7
Raw blackberries	58.6	—	—
Bottled blackberries	37.4	17.4	7.9

on the biological activity of 3′-epilutein have not been published yet. This study suggests to investigate the dietary intake and the biological role of 3′-epilutein. On the other hand, the occurrence of 3′-epilutein in processed food may be important concerning the application of synthetic lutein as food additive. According to our results an epimeric mixture at C(3′) synthesised in a more efficient way, compared to lutein may also have applications in food chemistry.

Acknowledgements

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11. (a) ^{1}H NMR (400 MHz, $CDCl_3$) data of the characteristic end group of 3′-epilutein: δ 0.84 (s, CH_3(16′)), 0.93 (s, CH_3(17′)), 1.38 (dd, H-2′α), 1.62 (dd, H-2′β), 1.63 (s, CH_3(18′)), 2.15 (d, H-6′), 4.22 (m, H-3′), 5.47 (bs, H-4′); (b) ^{1}H NMR (400 MHz, $CDCl_3$) data of the characteristic end group of anhydrolutein I: δ 0.88 (s, CH_3(16′)), 0.90 (s, CH_3(17′)), 1.94 (m, H-2′α), 2.01 (m, H-2′β), 2.65 (d, H-6′), 4.80 (s, H-18′b), 4.87 (s, H-18′a), 5.71 (m, H-3′), 6.18 (m, H-4′).
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