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Food Chemistry 67 (1999) 173–176

Food Chemistry

www.elsevier.com/locate/foodchem

# The content of zeaxanthin in *Gou Qi Zi*, a potential health benefit to improve visual acuity

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Received 2 December 1998; received in revised form 28 February 1999; accepted 28 February 1999

**Abstract**

*Gou Qi Zi* was obtained from 6 different sources. The specimens were homogenised in water-ethanol followed by extraction with hexane. Total carotenoid content in the hexane extract was estimated by the absorbance at 450 nm. Chromatography on a silica column eluted by dioxane-hexane showed only 1 carotenoid peak which was converted to zeaxanthin upon hydrolysis. The zeaxanthin concentrations should be useful as an objective parameter for grading of *Gou Qi Zi*. 

*Keywords:* Zeaxanthin; *Gou Qi Zi*; Health; Activity

## 1. Introduction

Since the isolation of β-carotene from carrot in 1831, over 500 varieties of carotenoids have been reported in the literature (Pfander, 1992). In spite of the large number of dietary carotenoids absorbed into the blood circulation (Barua & Furr, 1992), only zeaxanthin is present in human macular, while lutein occurs outside of the macular (Bone et al., 1997; Bone, Landrum, Hime, & Cains, 1993; Handelman, Dratz, Reay, van Kuijk, 1988). The specific location of zeaxanthin-lutein in human retina has drawn speculation on the possible health benefit of these carotenoids against age-dependent macular degeneration (Bone, Landrum, Fernandes & Tarsis, 1988; Hammond, Wooten & Snodderly, 1997; Malinow, Feeney-Burns, Peterson, Klein & Neuringer, 1980). Lutein is abundant in commonly consumed vegetables and fruits. However, lutein occurs mainly outside of the macular. The major carotenoid in the macular is zeaxanthin which exists only in a trace amount in common diet.

*Gou Qi Zi*, Lycium barabarum, is a small red berry commonly used in home cooking in China because of its flavor as well as general health benefits. It is also used in traditional Chinese herbal medicine for the improvement on visual acuity. The dark red colour of this berry indicates the presence of carotenoids. The total carotenoid concentration in *Gou Qui Zi* reported in Chinese literature is (40–90 μg/g) (Chai et al., 1986; Sing, 1991; Xie, 1956). Chromatographic data of lipid extract from each fruit or vegetable usually show the occurrence of a heterogeneous mixture of carotenoids (Mangels, Holden, Beecher, Forman & Lanza, 1993; Ong & Tee, 1992). The composition of different types of carotenoid in *Gou Qi Zi* has not been reported.

The recent finding on the specific location of zeaxanthin in the macular and the traditional usage of *Gou Qi Zi* for the improvement of visual acuity led us to the present investigation on the types of carotenoids present, and the concentration of zeaxanthin in *Gou Qi Zi*.

## 2. Materials and methods

β-Carotene was purchased from Sigma Chemical Company. Lycopene, lutein, zeaxanthin were purchased from Indofine Chemical Company, Belle Mead, NJ.

### 2.1. Extraction

*Gou Qi Zi* was obtained from 6 different sources. Specimens 1 and 2 were freshly harvested dried berries

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0308-8146/99/$ - see front matter 
PII: S0308-8146(99)00119-3

sent to us by wholesale dealers from Inner Mogolia and Ling-Xia respectively. The other 4 specimens were purchased from open market subjectively graded by the distributors.

Four grams of each specimen were homogenised in 5 ml of water in a Waring Blender for 3 min. The final volume of the homogenate was diluted to 10 ml with water, mixed with 10 ml of ethanol to denature the proteins, and then shaken vigorously with 40 ml of hexane for 3 min by vortex. After separation of the aqueous and hexane layers by centrifugation for 10 min at 20,000×*g*, the aqueous layer was extracted for 2 more times with 40 ml of hexane. The hexane extracts were combined and evaporated to dryness under nitrogen. The residue was dissolved in 1 ml of dioxane:hexane (16:84, v/v) and stored in the dark at room temperature.

### 2.2. Chromatography on a silica column

Carotenoids in the extract were analysed by chromatography on a silica column (1.9×300 mm) eluted by dioxane:hexane (16:84,v/v) at 2 ml/min. The absorbance of the eluant was recorded (Figs. 1 and 2) by a photo diode array detector (Waters Associates, Medford, MA). This is a very simple procedure to separate carotenes and xanthophylls. It was used also to demonstrate the conversion of the major carotenoid in *Gou Qi Zi* to free zeaxanthin after hydrolysis (Fig. 3).

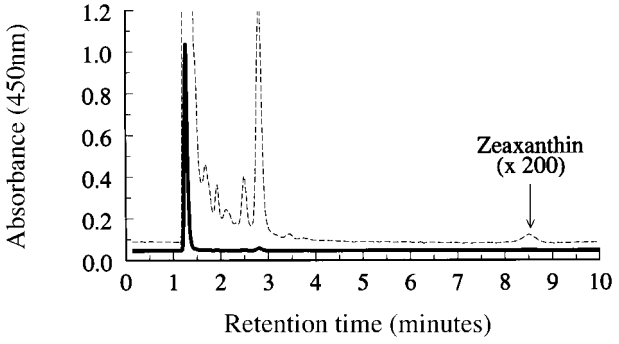


Fig. 1. Chromatogram of carotenoid extracted from *Gou Qi Zi*.

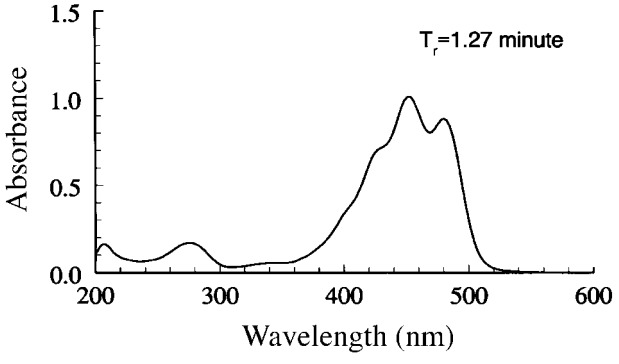


Fig. 2. Light absorption spectrum of the carotenoid ester eluted at 1.3 min.

### 2.3. Hydrolysis by KOH

After evaporating the hexane extract, the residue was re-dissolved in 0.2 ml of dioxane-hexane (16:84) and mixed with 0.2 ml of 6 N KOH (KOH was dissolved in methanol), and incubated at 50°C for 3 h. At the end of incubation the sample was mixed with 0.2 ml of 6 N HCl, then extracted with 0.8 ml of hexane. Extraction by hexane was repeated twice. The hexane extract was evaporated to dryness under nitrogen and re-dissolved in 50 μl of dioxane:hexane (16:84), then injected to the silica column. The column was eluted by the same solvent at 2 ml/min. The control sample was mixed with KOH and HCl, and extracted by hexane in the same manner, except that the incubation step at 50°C was omitted.

## 3. Results

### 3.1. Types of carotenoid present in Gou Qi Zi

Carotene and xanthophill have a large difference in their affinity on a silica column. These two classes of carotenoids are easily resolved by eluting the column with 16:84 dioxane:hexane. The chromatogram of the lipids extracted from *Gou Qi Zi* showed the occurrence of only 1 carotenoid at the retention time of 1.3 min (Fig. 1, solid line). The amount of the minor carotenoids was so small that they are visible only after magnifying the chromatogram 100 fold (Fig. 1, broken line).

The retention time of the major carotenoid in *Gou Qi Zi* is very similar to that of β-carotene, and lycopene. The effect of KOH distinguished the carotenoid of *Gou Qi Zi* from the other carotenes. The chromatograms of carotenes were not changed by KOH. After incubation of the carotenoid from *Gou Qi Zi* with KOH, the retention time was changed to that of zeaxanthin (8.6 min) (see Fig. 3). The retention time of lutein was 8.1 min.

### 3.2. Total carotenoid and zeaxanthin in Gou Qi Zi

Absorbance at 450 nm detect all yellowish compounds in the hexane extract. The hexane extract from the fresh specimens obtained from wholesale dealers had a higher color than the specimens purchased from different stores in the market place (Table 1).

The zeaxanthin concentration determined after hydrolysis accounts for 17% (Table 1, column 1 divided by column 2) of the carotenoid estimated by absorbance of the crude extract measured at 450 nm. Zeaxanthin concentration in the fresh specimen is also higher in the fresh specimens obtained from the wholesale dealers than those purchased from the open market. The zeaxanthin concentration is also related to the subjective

Table 1
Zeaxanthin concentration in *Gou Qi Zi*[a]

| Sample | Total carotenoid | Zeaxanthin | |
|---|---|---|---|
| | μg/g | μg/g | % of total |
| (1) Inner Mogolia[c] | 176 ± 88 | 43.3 ± 8.3 | 24.6 |
| (2) Ning Xia[c] | 164 ± 51 | 35.6 ± 7.4 | 21.7 |
| (3) Grade A (shop 1)[b] | 194 ± 69 | 27.7 ± 7.3 | 14.2 |
| (4) Grade A (shop 2)[b] | 106 ± 9 | 12.4 ± 1.2 | 11.6 |
| (5) Grade B (shop 2)[b] | 70 ± 18 | 12.0 ± 3.2 | 17.1 |
| (6) Grade B (shop 1)[b] | 92 ± 24 | 11.5 ± 2.3 | 12.5 |
| Average ± SD | 134 ± 51 | 23.7 ± 13.8 | 17.0 ± 5.2 |

[a] The mean values were calculated from 5 analyses of each specimen.
[b] Samples 3–6 were purchased from different stores.
[c] Samples 1 and 2 were products from Inner Mogolia and Ning Xia respectively obtained from a wholesale dealer.

grading of *Gou Qi Zi*. Grade A had higher zeaxanthin content than grade B.

## 4. Discussion

Although *Gou Qi Zi* has been widely used in China for centuries with an expected health benefit to the eye, the mechanism of its effect is not known. A common problem in understanding the function of herbal medicine is the absence of a suitable bioassay procedure to identify the therapeutically active agents. One way to deal with this intrinsic difficulty is to identify the major organic components of the herb under investigation. An important biochemical feature of *Gou Qi Zi* is the high carotenoid content.

The present chromatographic data indicate that the majority of carotenoid in *Gou Qi Zi* is different from the commonly known carotenes and xanthophylls. The chemical structure of an ester of zeaxanthin was indicated from the appearance of zeaxanthin in the chromatogram after hydrolysis in KOH. Acylation of the hydroxyl group of zeaxanthin in *Gou Qi Zi* must have greatly reduced the affinity of zeaxanthin to the silica column resulting in the low retention time of the major carotenoid in the crude extract of *Gou Qi Zi*. We have analysed the extract from spinach, carrot, turnip, onion, garlic, tomato, eggplant, chilli pepper, red bell pepper, green pepper, banana, papaya, mango, persimmon, the skin of pear, lychee, red plum. All of them contain a group of carotenoids different from that of *Gou Qi Zi*. Only red pepper has a trace amount of the carotenoid of *Gou Qi Zi*.

Total carotenoid values described in Chinese medical reports varies from 40 to 88 μg carotenoid per g of *Gou Qi Zi* (Chai et al., 1986; Sing, 1991; Xi, 1956). The method of their analysis was not described in their reports. Chromatographic separation of carotenoids in *Gou Qi Zi* has not been reported. In this study total carotenoid concentration was calculated from the 450 nm absorption of the crude extract, using the extinction coefficient of zeaxanthin (16). The total carotenoid content in *Gou Qi Zi* purchased from the stores had concentrations comparable to the published value. Both total carotenoids and zeaxanthin concentrations correlated to the arbitrary commercial grading of the 6 specimens. The zeaxanthin concentration in *Gou Qi Zi* is a good scientific criterion for grading.

We have tested the stability of zeaxanthin in alkali hydrolysis. There was negligible loss of zeaxanthin after incubation of pure zeaxanthin with KOH. However, the amount of zeaxanthin recovered after hydrolysis of the crude extract accounts for only about 17% of total carotenoid estimated by absorbance at 450 nm. After hydrolysis, only zeaxanthin is observed in the chromatogram. There was a negligible amount of other carotenoids was observed in the chromatogram. Therefore, the high total carotenoid values in the crude extract is over-estimated due to an unknown coloured substance present in the crude extract.

The most interesting finding is the selective accumulation of zeaxanthin ester in *Gou Qi Zi*. The present data show a unique natural supply of a stable form of zeaxanthin for human health benefit. The hydroxyl group of zeaxanthin could be acylated or glycosylated. The detailed chemical structure remained to be investigated. Huang (1993) speculated that dipalmityl zeaxanthin is present in *Gou Qi Zi*. Experimental evidence was not presented. We were able to use mass spectrometry to document the hydrolyzed product as zeaxanthin. However, the carotenoid ester in *Gou Qi Zi* is not valatile and cannot be detected by mass spectrometry.

The occurrence of lutein and zeaxanthin in the retina (Landrum, Bone & Kiibum, 1997) has led some investigators to speculate on the beneficial effects of dietary

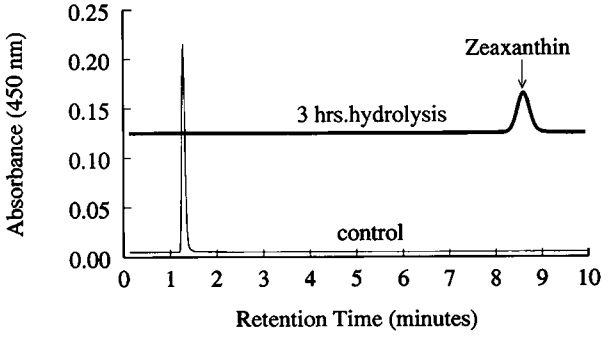


Fig. 3. Hydrolysis products of the carotenoid from *Gou Qi Zi* by KOH: a specimen of the zeaxanthin ester recovered from chromatography on a silica column as shown in Fig. 1 was mixed with an equal volume of 6 N KOH and incubated at 50°C for 3 h (upper tracing). The result was compared with a control sample kept at room temperature for 3 h (lower tracing).

supplementation with these carotenoids. While Lutein is abundant in vegetables, fruits, and in normal human serum, the amount of zeaxanthin is very small (Mangels et al., 1993; Khachik, Beecher, Goli & Lusby, 1993). Thus, if dietary supplementation of zeaxanthin has a beneficial effect to the health of the retina, the present data indicate that *Gou Qi Zi* is an excellent dietary source for zeaxanthin. The hydroxyl groups in a compound can be damaged by oxidative degradation. Acylation or glycolsylation of zeaxanthin should prevent oxidation of the hydroxyl groups. The natural occurrence of the ester form of zeaxanthin accounts for the stability of the medicinal effect of *Gou Qi Zi* in the dried berries. The occurrence of the remarkably high amounts of zeaxanthin ester in *Gou Qi Zi* is a fascinating natural design to provide one stable source of zeaxanthin in one type of berry for a health benefit to human.

## Acknowledgements

The authors are grateful for the technical assistance by Josephine Ngai, Ivan Leung, and Dana Wong. This study is supported in part by the Earmarked Grants from the Research Council of Hong Kong (CUHK 40595M). The authors are grateful to the advice from Dr. Chun Tao Che concerning the hydrolysis procedure.

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