The nutritional value for human retina of lipids in *Gou Qi Zi*

Lei Zhou,[1] PhD, Josephine Ngai,[2] MPh, Ivan Leung,[2] PhD, Mark O. M. Tso,[3] DSc, Kwok-Wai Lam,[1] PhD
[1] *Singapore Eye Research Institute, The National University of Singapore, Singapore.*
[2] *Department of Ophthalmology, The Chinese University of Hong Kong, Shatin, Hong Kong, China.*
[3] *The Wilmer Ophthalmological Institute, Johns Hopkins University, Baltimore, MD, USA.*

Correspondence and reprint requests:
Kwok-Wai Lam, 2111 Lexington Ridge Drive, Lexington, MA 02421, USA.

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Abstract

Aims: To investigate whether *Gou Qi Zi* contains sufficient carotenoids and fatty acids to supply the special lipid requirements of the human retina.
Materials and methods: Lipids were extracted from *Gou Qi Zi* by ethanol-hexane, then separated on a silica column and eluted by dioxane-hexane. The eluant was monitored by a photodiode array detector followed by a mass spectrometry detector.
Results: Before hydrolysis, only one pigmented lipid was detected in the chromatogram. The molecular size was identical to that of dipalmityl zeaxanthin. The major non-pigmented lipids have the molecular size of triglycerides and diglycerides. After hydrolysis, a marked increase in oleic, linoleic acid, and long chain fatty acids with 22 and 24 carbons was observed.
Conclusion: The lipid content in *Gou Qi Zi* matches the specific requirements of the human retina for long chain polyunsaturated fatty acids and zeaxanthin.

Key words: *Carotenoid, Gou Qi Zi, Linoleic, Linolenic, Palmitic, Retina, Triglycerides, Zeaxanthin*

Introduction

Gou Qi Zi (*Lycium barbarum*) is commonly used as an additive in Chinese cooking, primarily because of its flavour but also for health benefits. The most widely accepted health benefit is improvement in visual acuity.[1-4] However, the molecular mechanism of its beneficial effect is uncertain. In response to the current emphasis in Hong Kong to modernise traditional Chinese medicine, we investigated the lipids in *Gou Qi Zi* to ascertain whether they match some of the unique lipid requirements of the human retina.

The dark red color of *Gou Qi Zi* initiated our efforts to identify the type of carotenoids in *Gou Qi Zi*.[5] We observed a high content of zeaxanthin in *Gou Qi Zi*, matching the special carotenoid requirements of the human retina.[6-9] During our study, we noticed a large amount of non-pigmented lipids co-eluted with zeaxanthin. This report describes the characterization of fatty acids in the non-pigmented lipids. The fatty acids in *Gou Qi Zi* also match the unusual requirement of the human retina for long chain polyunsaturated fatty acids.

Materials and methods

Extraction

Lipids from *Gou Qi Zi* were extracted using ethanol-hexane as previously described.[5] The hexane extract was evaporated with nitrogen. The residue was dissolved in 1 ml of dioxane-hexane (16:84, v/v) and stored in the dark at room temperature.

Hydrolysis by potassium hydroxide

After evaporating the hexane in the extract, the residue was redissolved in 0.2 ml dioxane-hexane (16:84) and mixed with 0.2 ml of 12N potassium hydroxide (KOH) in methanol,

and incubated at 50°C for a specified time. At the end of incubation, the sample was mixed with 0.2 ml of 12N hydrochloride, then extracted with 0.8 ml of hexane. The hexane extract was evaporated using nitrogen and redissolved in 50 µl of dioxane/hexane (16:84), then injected into a silica column. The column was eluted by the same solvent at 1 ml/min.

Chromatography on a silica column

The column and solvent selection was established in our previous study to separate varieties of carotenoids.[10] A specified amount of the specimen was injected into a silica column (1.9 x 300 mm) and eluted by dioxane/hexane (16:84, v/v) at 1 ml/minute. The eluant was monitored by a photodiode array detector followed by a Micromass Platforom LCZ4000. The source blocks for positive or negative atmospheric pressure chemical ionization (±APCI) heater temperature were set to 140°C and 300°C, respectively. The corona and cone voltages were 3.0 and 70 volts, respectively throughout the experiments. The nitrogen gas flow rate was maintained at 500 l/hour. All mass spectra were recorded under a full scan operation for positive and negative ions, with a scan range from molecular size (m/z) 150 to 1500.

The lipid extract was examined first according to the light absorption chromatogram. The pigmented lipid was demonstrated in the 450 nm chromatogram (**Figure 1a**, red line) and the non-pigmented lipids in the 210 nm chromatogram (**Figure 1a**, black line). The ion sizes present within a specified retention time were examined by mass spectrometer in the -APCI or +APCI ionization mode. The

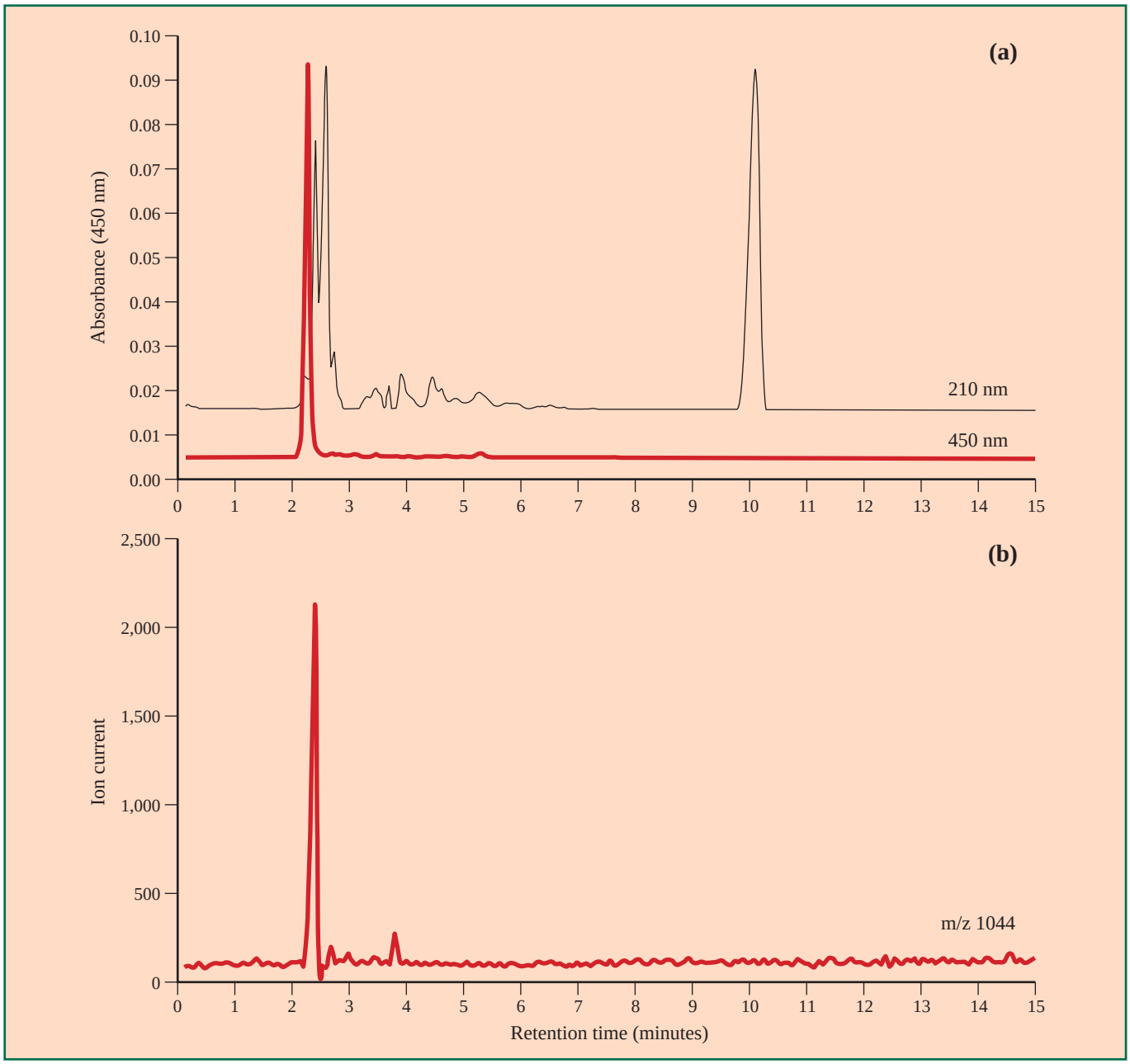

Figure 1. Chromatogram of the lipids extracted from *Gou Qi Zi*. (a) Light absorption chromatogram — red line 450 nm, black line 210 nm; (b) chromatogram of a selected ion size, m/z 1044.

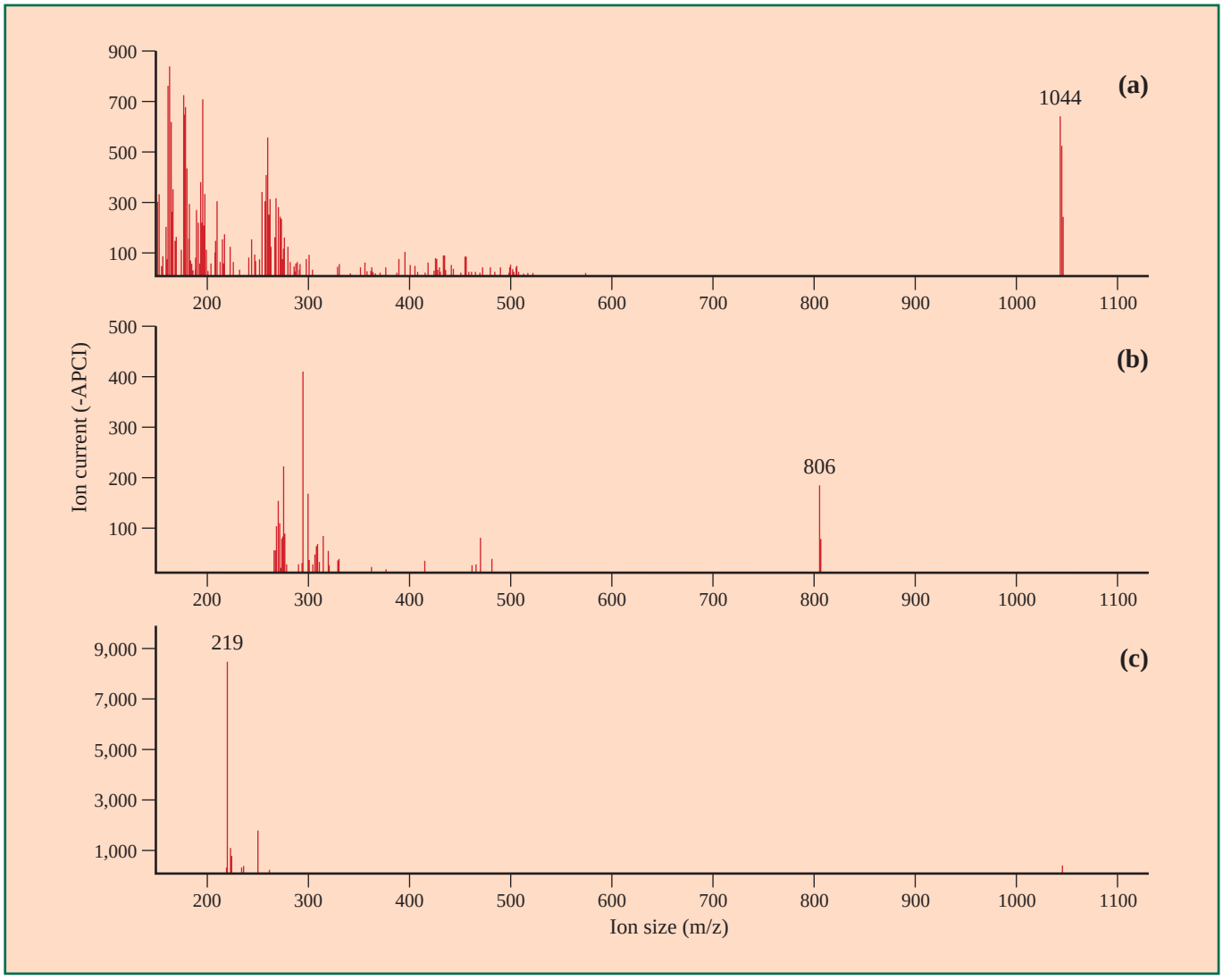

Figure 2. Negative atmospheric pressure chemical ionization (-APCI) mass spectrum at different retention times in the chromatogram shown in Figure 1a. (a) 2 to 3 minutes; (b) 5 to 5.5 minutes; (c) 10 to 10.5 minutes.

mass spectrometer allows identification of the sizes of the molecular ions eluted at a specified retention time (**Figures 2** and **3**). The chromatogram was re-examined for each selected ion size to determine the retention time of each ion size in the chromatogram (**Figure 4**). These steps allowed us to identify the molecular size of non-pigmented lipid eluted closely with dipalmityl zeaxanthin. Mass spectrometer detects the fatty acids that are difficult to detect by light absorption. The total mass chromatogram (**Figure 5**) was used to visualize the location of the major group of ions eluted from the chromatogram. The fatty acids under the major mass were visualized in the mass spectrum shown in **Figure 6**.

Results and observations

Light absorption chromatograms of lipids in *Gou Qi Zi*

Carotenoids have strong absorption at 450 nm. Only one major peak was observed at 2.4 minutes in the 450 nm chromatogram (**Figure 1a**, red line). A very small peak was noticeable at 5.3 minutes. Most lipids have no color and have weak absorption at low wavelength. The complexity of non-pigmented lipids is noticed in the 210 nm chromatogram (**Figure 1a**, black line). The ion size of dipalmityl zeaxanthin is 1044. The selected ion chromatogram for m/z 1044 (**Figure 1b**) showed a sharp peak at 2.4 minutes, coinciding with the pigmented carotenoid peak in the 450 nm chromatogram shown in **Figure 1a**. The selected ion chromatograms for the low molecular weight ions (<500) did not produce distinct peaks. The negative results are not presented.

-APCI mass spectrum at the retention time of 2 to 3 minutes

The -APCI mass spectrum for the major carotenoid peak (2-3 minutes) showed the presence of m/z1044, the ion size of dipalmityl zeaxanthin (**Figure 2a**). In addition, heterogeneous groups of different ion sizes were observed below m/z 500. The heterogeneous appearance must have resulted from the presence of heterogeneous non-pigmented lipids indicated in the 210 nm chromatogram (**Figure 1a**, black

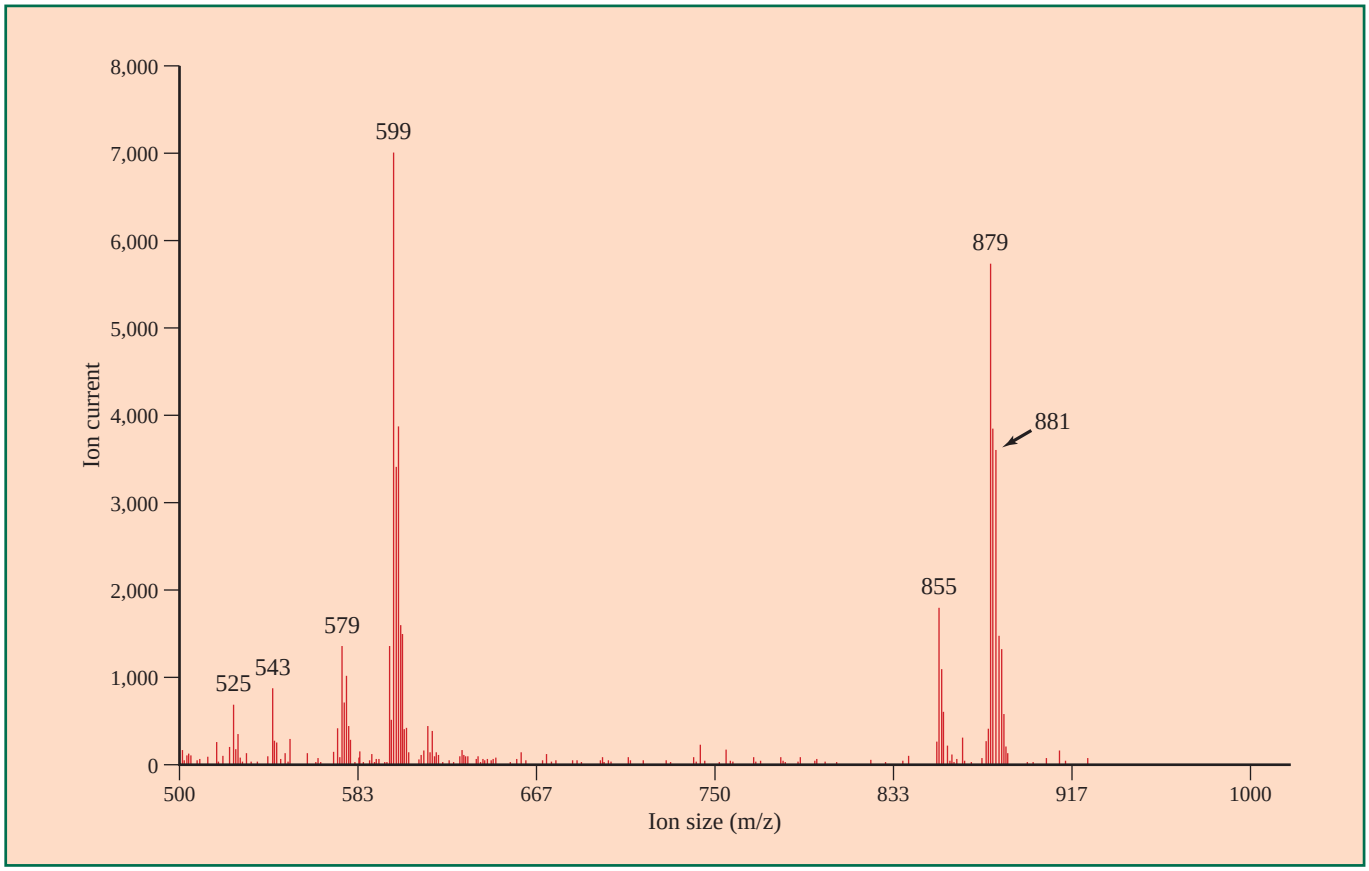

Figure 3. Positive atmospheric pressure chemical ionization mass spectrum at the retention time between 2 to 3 minutes for the chromatogram shown in Figure 1a.

line). The very small peak at the retention time of 5.3 minutes in the 450 nm chromatogram (**Figure 1a**, red line) contained the ion size of monopalmityl zeaxanthin (m/z 806, **Figure 2b**). The peak observed at 10.2 minutes in the 210 nm chromatogram (**Figure 1a**, black line) had an ion size of m/z 219 (**Figure 2c**). The identity of this ion is not known.

+APCI mass spectrum at the retention time of 2 to 3 minutes

Carotenoids produce very little signal in the +APCI spectrum. Therefore, we chose -APCI to identify the molecular size of the carotenoids in *Gou Qi Zi* as shown in **Figures 1** and **2**. The +APCI mass spectrum (**Figure 3**) obtained at the retention time of dipalmityl zeaxanthin (**Figure 1**, 2-3 minutes) showed strong signals for ion sizes of 579, 599, 855, 879, and 881, which match the ion sizes of diglycerides and triglycerides. The signals of these ions detected by +APCI were very strong. The selected ion chromatograms (**Figure 4**) for these ion sizes detected by +APCI had a much higher magnitude than that of dipalmityl zeaxanthin detected by -APCI, yet they did not coincide exactly with the retention time of dipalmityl zeaxanthin (m/z 1044, **Figure 4**, red line).

Total -APCI mass chromatogram

When the detector was set for total -APCI mass mode, a major peak was noticed at 3.5 minutes and a small peak at 10.2 minutes (**Figure 5**). The small peak at 10.2 minutes coincided with one of the major peaks in the 210 nm chromatogram (**Figure 1**). It had an -APCI ion size of m/z 219 (**Figure 2c**). The major mass detected at 3.5 minutes showed the occurrence of free fatty acids (**Figure 6a**). The major fatty acids observed were palmitic acid (m/z 255) and stearic acid (m/z 283). A small amount of linoleic acid (m/z 279) was noticeable. In addition, a trace amount of long chain fatty acids containing 22 and 24 carbons were barely noticeable (**Figure 6a**). These were free fatty acids existing in the lipid extract before hydrolysis.

As described above, large amounts of diglycerides and triglycerides were eluted closely with dipalmityl zeaxanthin between 2 to 2.6 minutes (**Figures 1a, 3**, and **4**), clearly separated from the free fatty acids. Free fatty acids were eluted together at 3.5 minutes (**Figures 5** and **6a**). We removed the free fatty acid by repeated chromatography. The resulting lipids contained dipalmityl zeaxanthin, diglycerides, and triglycerides as shown in **Figures 3** and **4**. Hydrolysis of this lipid preparation released high amounts of polyunsaturated fatty acids (**Figure 6b**). The major fatty acids observed after hydrolysis were palmitic acid (m/z 255), stearic acid (m/z 283), oleic acid (m/z 281), and linoleic acid (m/z 279). Before hydrolysis only a trace amount of long chain fatty acids with more than 20 carbons were observed (**Figure 6a**). After hydrolysis, a small amount of behenic (C_{22}, m/z 339), lignoceric (C_{24}, m/z 367), and cerotic (C_{26}, m/z 395) acids were clearly observed (**Figure 6b** and

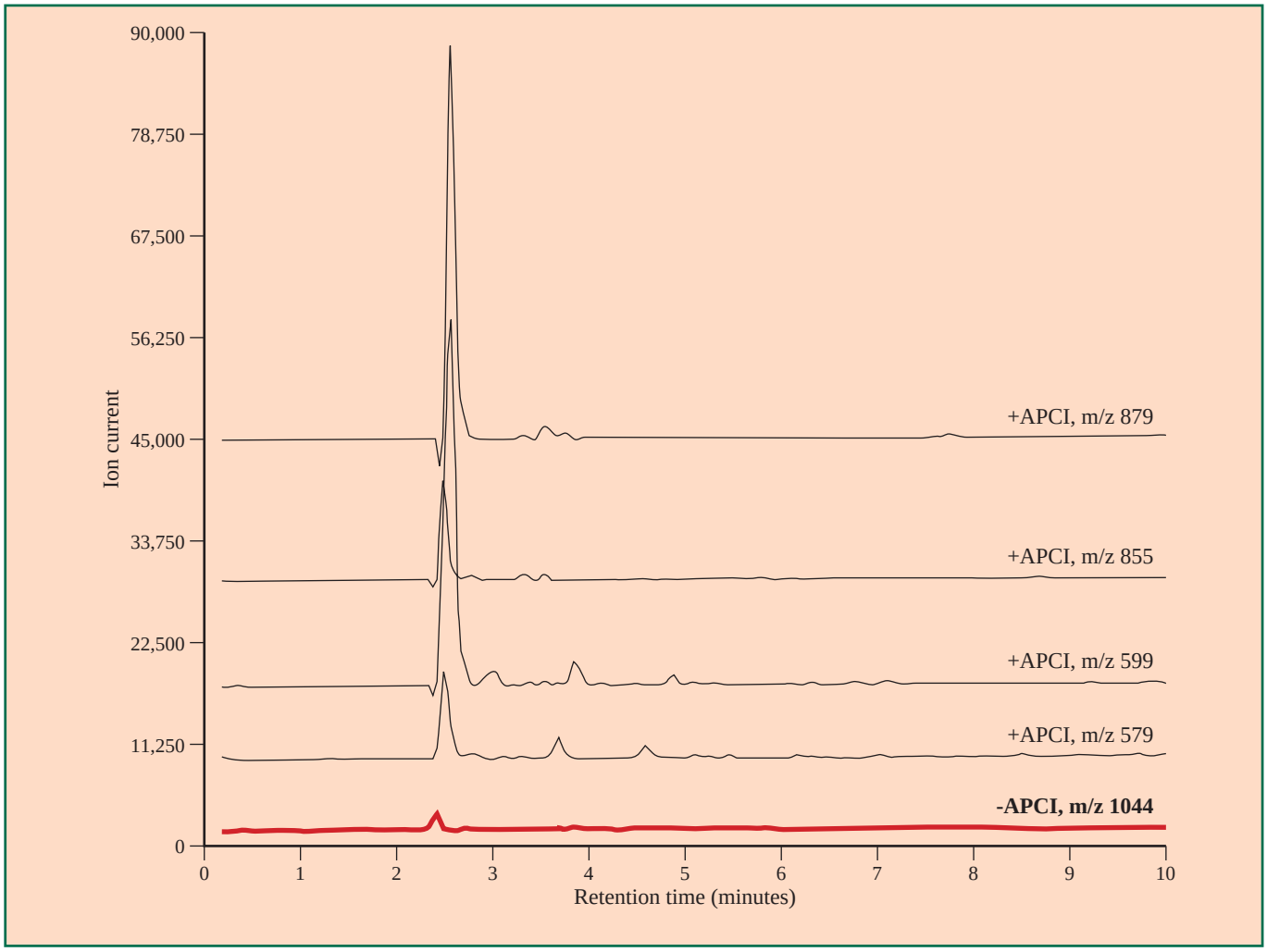

Figure 4. Selection ion chromatograms. The positive atmospheric pressure chemical ionization (+APCI) chromatogram was graphed based on the ion current of four different ion sizes observed in Figure 3. The selected ion m/z 1044 detected by negative atmospheric pressure chemical ionization (-APCI) was included to indicate the retention time of dipalmityl zeaxanthin.

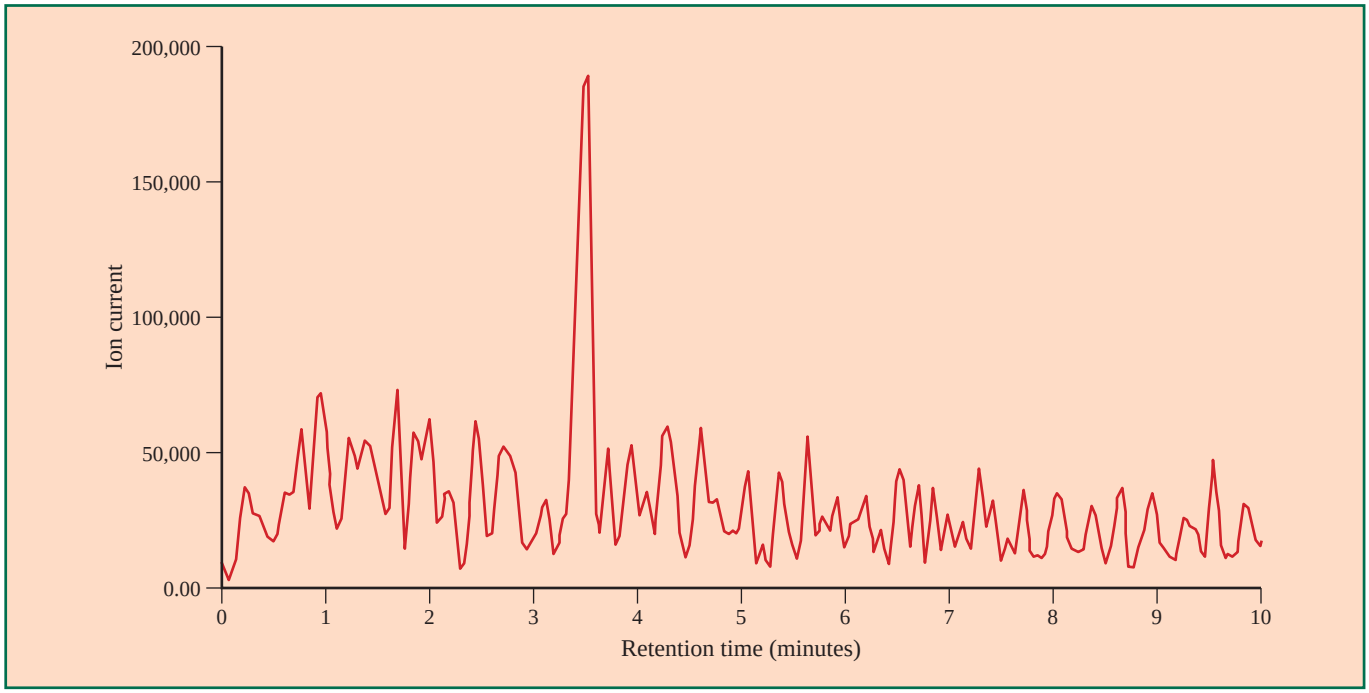

Figure 5. Re-graphing of Figure 1b using the negative atmospheric pressure chemical ionization total mass mode.

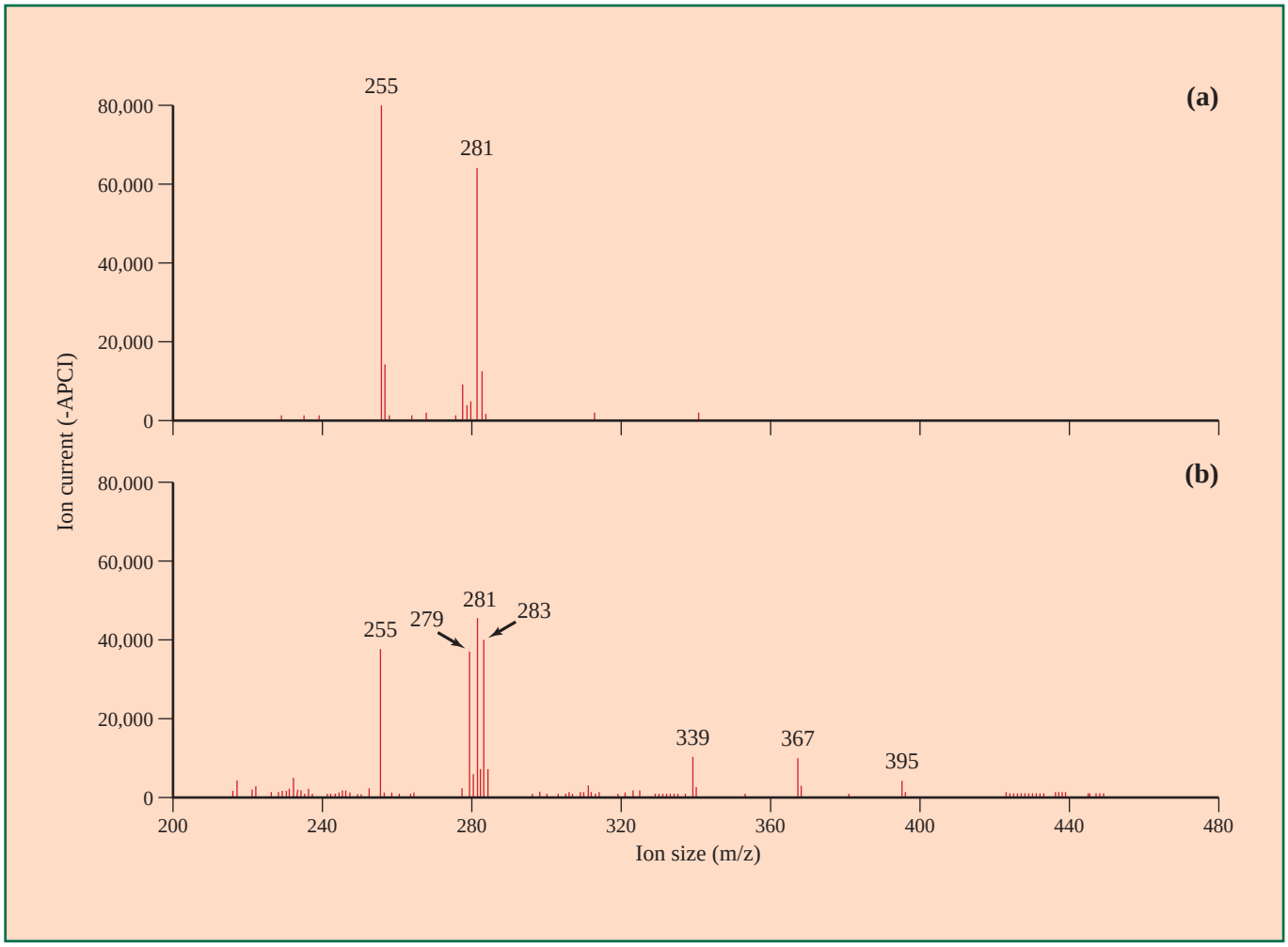

Figure 6. Negative atmospheric pressure chemical ionization (-APCI) mass spectrum at 3.2 to 3.8 minutes of the chromatogram shown in Figure 5. (a) Before hydrolysis; (b) after hydrolysis.

Table 1. Composition of fatty acids after hydrolysis.

Fatty acid	Number of carbons	Number of double bonds	% of total
Hexadecanoic	16	0	20.2
Octadecanoic	18	0	20.8
Octadecenoic:1	18	1	24.7
Octadecenoic:2	18	2	16.5
Octadecenoic:3	18	3	1.2
Dodecahexanoic	20	0	1.5
Docosahexanoic	22	0	5.1
Docosahexenoic	22	8	2.6
Tetracosahexanoic	24	0	4.5
Tetracosahexenoic	24	6	0.5

Table 1). A small amount of 7 or 8 double bonds of the long chain fatty acid were also noticeable (**Table 1**). During hydrolysis free fatty acids (palmitic, oleic, and linoleic acid) increased to a maximal level at 20 minutes and decreased thereafter (**Figure 7**). Dipalmityl zeaxanthin was reduced rapidly upon hydrolysis. Monopalmityl zeaxanthin increased with hydrolysis time up to 10 minutes and decreased thereafter. Free zeaxanthin approached the maximal level at 20 minutes and remained constant thereafter (**Figure 8**).

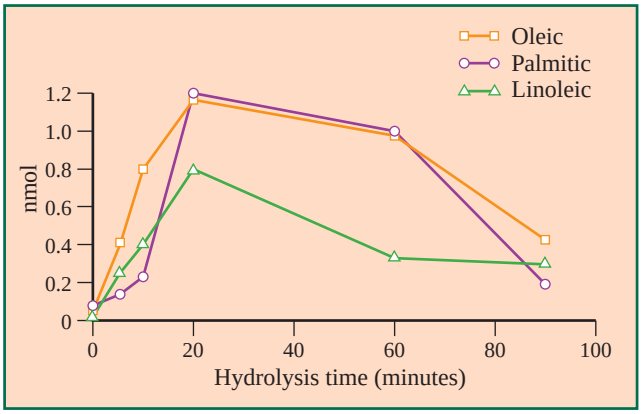

Figure 7. Amounts of fatty acids released at different times during hydrolysis.

Discussion

The human retina has a very special feature in carotenoid and lipid content. While more than 10 different carotenoids are present in normal blood,[10] only zeaxanthin is taken up into the macular.[6-9] Most fatty acids in human tissues are palmitic and stearic acids containing 16 or 18 carbons, respectively. A fair amount of fatty acid in the retina is docosahexenoic acid containing 22 carbons and 6 double bonds.[11]

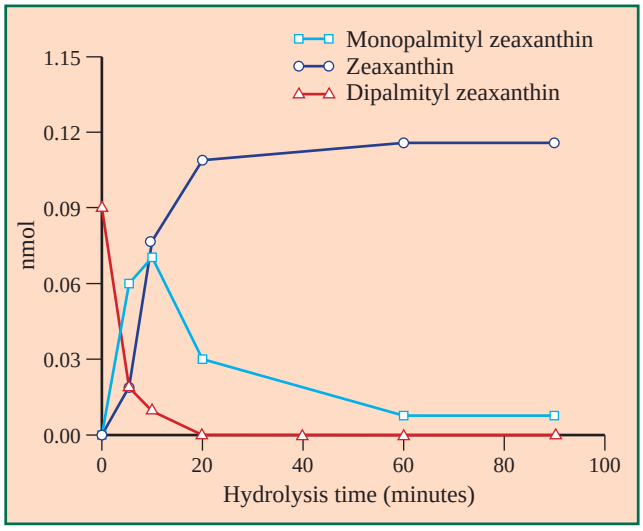

Figure 8. Changing concentration of dipalmityl zeaxanthin, monopalmityl zeaxanthin, and free zeaxanthin at different times during hydrolysis.

The carotenoid and fatty acids content in *Gou Qi Zi* have similar selectivity to carotenoid and fatty acids as that of the human retina. More than 500 varieties of carotenoids have been identified in plants and animals.[12-15] Only zeaxanthin is found in *Gou Qi Zi*. The 450 nm chromatogram was used to visualize varieties of carotenoids in a lipid extract of a specimen. There was only one major peak, dipalmityl zeaxanthin, and a very small peak, monopalmityl zeaxanthin, observed in the lipid extract from *Gou Qi Zi*. The chromatograms of other fruits and vegetables contain many peaks in the 450 nm chromatogram because of the heterogeneous types of carotenoids present in each specimen. Zeaxanthin accounts for a very small percentage of carotenoids in commonly consumed fruits and vegetables.[12-14]

When we investigated the chemical structure of carotenoid in *Gou Qi Zi*, we noticed many non-pigmented lipids co-eluted with dipalmityl zeaxanthin. Their molecular sizes match that of triglycerides and diglycerides. The glycerides in *Gou Qi Zi* consisted of large amounts of essential polyunsaturated fatty acids such as oleic and linoleic acid and a small amount of linolenic acids. In addition, some long chain fatty acids with 22 and 24 carbons were clearly detectable after hydrolysis (**Figures 6b** and **Table 1**). Small amounts of long chain fatty acids with 7 or 8 double bonds were also noticeable (**Table 1**).

Fatty acids are the major building blocks of biomembranes. The large amounts of long chain polyunsaturated fatty acids are essential to maintain the intact structure of the consecutively folded membrane of the outer segments of the rods. Loss of these fatty acids results in degeneration of the retina.[16] Polyunsaturated fatty acids are essential dietary components. Human tissues cannot synthesize linoleic acids. The retina can carry out elongation and dehydrogenation of linoleic acid to form docosahexenoic acids.[16] The high amounts of oleic, linoleic, and fatty acids with 22 carbons match the special requirement of the human retina for long chain fatty acids in addition to the specific requirement for zeaxanthin we reported before.[5]

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