Improving Extraction of Lutein from Egg Yolk Using an Ultrasound-Assisted Solvent Method

XIAOHUA YUE, ZHIMIN XU, WITORN PRINYAWIWATKUL, AND JOAN M. KING

ABSTRACT: Extraction yields of lutein from egg yolk using both hexane solvent (SOL) and ultrasound-assisted solvent (UA-SOL) extraction methods at different levels of saponification were compared. The yields obtained with SOL and UA-SOL were significantly different. The broad range of extraction yield in the SOL method at different levels of saponification was from 6.3 to 63.8 μg/g. Compared to the SOL method, the extraction yield using UA-SOL was significantly higher at each corresponding saponification level. The yield of the UA-SOL method without alkaline solution was the highest (89.9 μg/g) of all. The yield of the UA-SOL decreased as the level of saponification increased. The results indicated that alkaline significantly affected stability of lutein in egg yolk and resulted in underestimation of its concentration. Compared with the traditional saponification solvent extraction method, the UA-SOL extraction method was more effective in extracting lutein from the sample matrix, and presumably by avoiding degradation reactions.

Keywords: lutein, extraction, ultrasound, antioxidant, egg yolk, saponification

Introduction

Lutein is one of the important carotenoids; it has a yellow-orange color. It is mostly found in fruits, vegetables, grains, and eggs (Huck and others 2000; Johnson 2004). It also occurs in animal metabolism systems and is stored in various tissues including blood (Surai and others 2000). Being different from β-carotene and lycopene, which are carried predominantly by low-density lipoprotein, lutein is mainly transported by high-density lipoprotein of plasma because of its relatively higher polarity (Johnson 2000). Lutein plays an important role in preventing cataracts and age-related macular degeneration (Johnson 2004). Lutein acts as a blue-light filter and protects the underlying tissues from phototoxic damage. Recently, lutein and other carotenoids in human plasma were reported to have antioxidative function such as the scavenging of free radicals and singlet oxygen and thus reducing the risk of certain cancers (Slattery and others 1988; Riso and Porrini 1997; Handelman 2001; Schunemann and others 2002). Evidences from human studies have indicated that dietary intake of lutein can increase the lutein level in plasma and the eye’s retina (van het Hof and others 1999; Bernstein 2002).

Egg yolk is one of the major lutein sources in our foods (Johnson 2004). Lutein in egg yolk is highly bioavailable, compared with other sources. It was reported that egg yolk intake significantly increased plasma lutein (Handelman and others 1999). Like other carotenoids, the hydroxyl groups and multiple double bonds of lutein (Figure 1), however, make it susceptible to some chemicals and to harsh conditions, such as saponification (Oliver and others 1998). A solvent method following saponification has been widely used in the extraction and quantification of carotenoids (Britton 1985; Riso and Porrini 1997; Handelman and others 1999; Larsen and Christensen 2005). The purpose of saponification is to hydrolyze the ester linkages of glycerides, phospholipids, and sterols, and to disrupt the matrix for the release of lutein. This is required for a complicated sample matrix such as egg yolk because it contains much fat and protein. The saponification could also hydrolyze the lutein esters in the sample to yield free form of lutein. However, a major concern is that saponification could result in degradation of the lutein (Oliver and others 1998; Larsen and Christensen 2005).

Ultrasound-assisted solvent (UA-SOL) extraction has been used in determining lutein and other compounds in animal and plant tissues as well as food packaging materials (Cooper and others 1998; Song and others 2000; Rostagno and others 2003; Li and others 2004). Those studies have demonstrated that ultrasound can increase the extraction yield of targeted compounds. Most likely the high frequency of ultrasound breaks down the sample micelle or matrix to facilitate access of solvent to the hydrophobic compounds contained within. Unlike saponification, which breaks the cell matrix through alkaline conditions, there is no chemical involvement in the UA-SOL, and therefore no chemical degradation of targeted compounds. Furthermore, the accompanying turbulence would agitate the extraction solvent, thus increasing contact between solvent and the targeted compounds, which is the result of greatly improving extraction efficiency. In this study, the extraction yields of lutein from egg yolk using both hexane solvent (SOL) and UA-SOL extraction methods at different levels of saponification were compared. The use of ultrasound-assisted extraction may greatly improve the extraction yield of lutein from foods and other biological samples with high fat content.

Materials and Methods

Chemicals

Hexane, methanol, and acetone were purchased from Fisher Scientific Inc. (Fair Lawn, N.J., U.S.A.). Lutein and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Hen eggs were obtained from a local market and stored at 4 °C prior to analysis.

Sample preparation and saponification

Four egg yolks were combined and homogenized before use. One tenth gram (exact to 0.001 g) of the egg yolk was placed in a 25-mL
test tube. One mL of ascorbic acid aqueous solution (0.1 g/mL) was added to the tube, which was then vortexed for 30 sec. Then the solution in each tube was mixed with 0, 0.2, 0.4, or 0.6 mL of alkaline (saponification) solution (10% w/v NaOH in water) for producing different levels of saponification. Distilled water (0.6, 0.4, 0.2, and 0 mL) was added into the corresponding tubes with 0, 0.2, 0.4, and 0.6 mL of alkaline solution, respectively. All tubes were capped and vortexed for 30 sec, then incubated in a 60°C shaking water bath for 30 min.

Hexane solvent (SOL) extraction

After the incubation, 3 mL of hexane was added to each cooled tube that was then vortexed. The tube was centrifuged at 1000 × g for 20 min and the separated hexane layer was transferred to a clean 25-mL test tube. Then the residual sample was re-extracted with another 3 mL hexane, vortexed for 1 min and centrifuged. The hexane layer was removed and combined with the previous. Hexane was evaporated at 30°C under vacuum using a CentriVap Mobile system (Labconco, Kansas City, Mo., U.S.A.), and 1 mL methanol was added to dissolve the extract.

Ultrasound-assisted solvent (UA-SOL) extraction

During the first hexane extraction, an ultrasound probe (60 Sonic Dismembrator; Fisher Scientific Inc., Fair Lawn, N.J., U.S.A.) was inserted into the sample solution. The sample solution was sonicated at 10 Watts RMS (root means squared value) for 10 min and centrifuged at 1000 × g for 20 min. The hexane layer was removed, and the residual sample was extracted with another 3 mL hexane without sonication. The extracted hexane layers were combined, evaporated, and redissolved in 1 mL methanol.

HPLC analysis of lutein

Lutein from the extracted egg yolk was analyzed using a HPLC system. The HPLC system included a Waters 510 pump, a 715 Ultra WISP injector, photo diode array detector (Milford, Mass., U.S.A.), and a 25 cm × 4.6 mm diameter 5-μm C18 Discovery column (Supelco, Bellefonte, Pa., U.S.A.). The mobile phase was methanol:acetone (90:10) with a flow rate at 0.8 mL/min. The HPLC was controlled by Waters Millennium chromatography software and the lutein peak was monitored at 450 nm (Li and others 2002). Lutein concentration was calculated using a calibration curve prepared with the pure lutein standard.

Statistics

Each treatment was quadruplicated. Results are presented as means with standard deviation. Significant differences in means were computed using the t-test (p < 0.05).

Results and Discussion

A representative chromatogram of lutein from the egg yolk sample prepared by UA-SOL method is shown in Figure 2. The extraction yields of lutein from the egg yolk using both SOL and UA-SOL extraction methods at different levels of saponification are shown in Figure 3. Extraction yield was significantly affected by the level of saponification for both SOL and UA-SOL extraction methods. The order of the extraction yield (from high to low) obtained by the SOL method was at 0.2, 0, 0.6, and 0.4 mL added alkaline solution. For the SOL method, the extraction yield without saponification (0 mL of alkaline solution) was 24.9 μg/g, which was approximately 2.5 times lower than at 0.2 mL of alkaline solution. It suggested that lutein in egg yolk is mostly blocked within the yolk matrix that is very rich in fat and protein. Solvent alone is not able to completely break the matrix to liberate the lutein. Saponification is an important step in lutein extraction for samples containing much lipid and protein because the sample matrix could prevent the penetration of extraction solvent. During saponification, the chemical linkages in the sample matrix are hydrolyzed and broken down to liberate lutein, which can then be extracted readily by the solvent. However, the extraction yield of lutein was significantly decreased when the level of saponification was increased. At 0.4 mL and 0.6 mL of the alkaline solution, the yields were about 10 times lower than at 0.2 mL alkaline solution. The saponification may have caused degradation of the lutein compound.

Lutein contents in hen egg yolk have been reported in a range of 150 to 435 μg/yolk by using saponification with 100 μL of 30% potassium hydroxide in 50 μL of yolk (Handelman and others 1999). The lutein content was approximately 7 to 21 μg/g of yolk if the average weight of yolk was assumed to be about 20 g. Those results were much lower than that (63.8 μg/g) observed at 0.2 mL of alkaline solution in the SOL method in this study. This could be due to the level of saponification that was high enough to cause the degradation of lutein. Also, because lutein is very sensitive in an alkali condition, a small inconsistency in adding alkali solution or a slight difference in saponification conditions could result in a much larger difference in

Figure 1 — Molecular structure of lutein

Figure 2 — A representative chromatogram of lutein in hen egg yolk sample prepared by the ultrasound-assisted solvent (UA-SOL) method without saponification. HPLC conditions: column, 25 cm × 4.6 mm diameter 5-μm C18; mobile phase, methanol:acetone 90:10; flow rate, 0.8 mL/min; detector wavelength, 450 nm.

Figure 3 — Extraction yields of lutein from hen egg yolk using the solvent (SOL) and ultrasound-assisted solvent (UA-SOL) extraction methods at different levels of saponification. Significant difference (p < 0.05) between two extraction yields is expressed by different letters.
the apparent lutein level. The extraction of lutein from egg yolks of different wild birds without saponification was carried out by Surai and others (2001). They reported 14.2, 14.9, and 45.7 μg/g of lutein in gull, moorhen, and coot egg yolks. Use of solvent alone without saponification has commonly been employed in extracting lutein and its esters from plants and vegetables (Li and others 2002; Tsao and others 2004; Seo and others 2005). Higher yields of lutein could be achieved by solvent extraction without saponification because of a lower level of fat in plants and vegetables.

In Figure 3, the extraction yield of lutein when using UA-SOL extraction method was significantly higher than that using the SOL method at each level of saponification. The yield when using the UA-SOL method without saponification was the highest among all yields. It was about 30 μg/g higher than was the highest yield from the SOL method. It suggests that the ultrasound sonication with only solvent is superior over any methods with saponification in extracting lutein. The sonication could significantly break down the egg yolk matrix to release lutein. Although saponification with 0.2 mL alkaline solution increased the extraction yield in the SOL extraction method, this increase was not observed with the UA-SOL method. Unlike the SOL method, there was no significant difference in the extraction yield between 0 and 0.2 mL alkaline solution in the UA-SOL method. It may be that the supposed increase of lutein yield at saponification with 0.2 mL alkaline solution was equally annihilated by the serious degradation under alkaline solution, which was accelerated by powerful ultrasound agitating at the same time. Sonication increases the chance of reaction of alkali with lutein or other oxidation reactions. Higher yields of lutein were obtained by the UA-SOL than SOL method when 0.4 and 0.6 mL of alkaline solutions were used for extraction. The lutein extraction yield decreased with increased saponification levels in the UA-SOL extraction method but not as pronounced as with the SOL method. Therefore, it suggests that the ultrasound method greatly assisted the recovery of lutein from the egg yolk samples by breaking down the sample matrix in a physical rather than chemical manner. Ultrasound may also distribute the extraction solvent more uniformly in each extraction, and thus makes the extraction more efficient. The advantage of using ultrasound-assisted extraction has been reported in sample preparation for analyzing organics in soil, animal and plant tissues, and food packaging materials (Cooper and others 1998; Song and others 2000; Rostagno and others 2003; Li and others 2004). This study demonstrated that ultrasound could increase the extraction yield of targeted lutein compound as well.

Conclusions

This study demonstrated that the drawbacks of using alkali saponification during the extraction of lutein from hen egg yolk could be overcome by using UA-SOL extraction. Because saponification is replaced by the UA-SOL extraction, the degradation of lutein can be avoided; therefore, the measured level of lutein will be much closer to the actual value. UA-SOL extraction could replace the traditional extraction methods for lutein analysis, especially in biological samples with higher fat and protein contents, such as egg yolk.

References


