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Short communication

Ultrasound assisted extraction in quantifying lutein from chicken liver using high-performance liquid chromatography☆

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Abstract

Four sample preparation methods, (1) solvent (SOL), (2) saponification and solvent (SP), (3) ultrasound assisted solvent (UA), and (4) saponification and ultrasound assisted solvent (SP-UA), were used for quantifying lutein in chicken liver samples by HPLC. The lutein concentrations obtained by using SOL, UA, SP, and SP-UA were significantly different with values from 10.4 μg/g (UA) to undetected (SOL). Efficiency of the four different methods for extracting lutein from high to low were the UA, SP, SP-UA, and SOL method. The measured value of lutein in the liver sample using the UA method was approximately two and three times higher than that obtained from the SP and SP-UA method, respectively. The methods with saponification significantly affected the stabilities of lutein in liver samples. The lutein concentration measured with the solvent only method was either much lower than any of the other extraction methods or undetectable. This indicated that little lutein in those samples was in a form that could be extracted directly by solvent. Compared with the saponification method, the ultrasound assisted solvent method could effectively extract lutein from sample matrix and thus avoid chemical degradation reactions, which would be especially important for complex biological tissue such as liver.

Keywords: Lutein; Ultrasound; Saponification; HPLC; Carotenoid; Extraction; Chicken liver

1. Introduction

Lutein is a non-provitamin A carotenoid and yellowish pigment. The chemical structure is depicted in Fig. 1. Lutein is mostly found in fruits, vegetables, grains, and eggs [1]. Lutein also occurs in animal metabolism systems and is stored in tissues and blood [2]. Lutein is predominately transported by high-density lipoprotein of plasma because of its relatively high polarity [3]. One major health function of lutein is to prevent age-related macular degeneration (AMD) and cataracts [4]. Recently, lutein was reported to have the capability of reducing the risk of certain cancers, such as colon cancer [5]. This may be due to its antioxidant function, which is effective as a scavenger of free radicals that could cause cell mutation [3].

Lutein possesses two hydroxyl groups and multiple double bonds, which make lutein susceptible to some chemicals and harsh conditions. Traditional solvent only methods in sample preparation are generally used in preparing plant samples for lutein analysis by HPLC [6–9]. However, for animal cell samples, the solvent only method may have lower extraction yield of intracellular lutein because the organic solvent is not able to break down cell membrane to release those compounds inside. A solvent method following saponification has been widely used in extraction of the compounds within animal cells for HPLC analysis. The purpose of saponification is to hydrolyze the ester linkages of glycerides, phospholipids, and sterols, destroy pigments, and disrupt the cell membrane matrix to release intracellular compounds. However, a major concern is that saponification could result in degradation of the compounds of interest.

Ultrasound assisted extraction has been used in analyzing trace organic compounds in soil, animal, and plant tissues, and food packaging materials [10–18]. Those studies demonstrated that ultrasound could increase the extraction yield of targeted compounds in sample preparation. It is possible that the frequency of ultrasound could break down the sample micelle or

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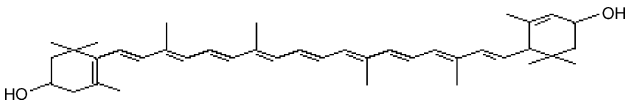

Fig. 1. Molecular structure of lutein.

matrix to facilitate access of solvent to the hydrophobic compounds contained within. Unlike saponification, which breaks the cell matrix through alkaline conditions, there would be no chemical involvement in the ultrasound assisted extraction, which could prevent possible chemical degradation of targeted compounds. Furthermore, the ultrasound power would agitate the extraction solvent, thus increasing the contact between solvent and targeted compounds, which could greatly improve the extraction efficiency.

In this study, the HPLC measured levels of lutein in chicken liver using solvent only, saponification, ultrasound assisted, and saponification with ultrasound assisted sample preparation methods were compared. The use of ultrasound assisted extraction may greatly improve the approach to the extraction of lutein from biological samples and increase sensitivity and reliability of HPLC analysis.

2. Experimental

2.1. Chemicals

Hexane and methanol were purchased from Fisher Scientific Inc. (Fair Lawn, NJ). Lutein was purchased from Sigma Chemical Co. (St. Louis, MO). Homogenized chicken liver samples were obtained from the Department of Animal Science at Louisiana State University Agricultural Center and stored at −20 °C prior to analysis.

2.2. Solvent only sample preparation method (SOL)

Three millilitres of hexane were added to 0.50 g of chicken liver in a 25-mL test tube. The tube was purged with nitrogen and capped, then was incubated at 70 °C for 30 min and vortexed every 5 min. The tube was centrifuged at 1000 × *g* for 10 min and the hexane layer was transferred to a clean test tube. Then the residual liver sample was re-extracted with another 3 mL hexane, vortexed for 5 min and centrifuged. The hexane layer was transferred to combine with the previous one. Hexane was evaporated at 30 °C under vacuum using a CentriVap Mobile system (Labconco, Kansas City, MO) and 1 mL of methanol was added to dissolve the extract for HPLC analysis.

2.3. Saponification and solvent extraction method (SP)

The saponification solution included 10 g NaOH, 1 g butylated hydroxytoluene (BHT), 50 mL ethanol, and 50 mL water. A 0.8 mL water and 0.2 mL saponification solution were added to 0.50 g chicken liver in a 25-mL test tube, flushed with nitrogen and sealed. Then the tube was incubated at 70 °C for 20 min and vortexed every 5 min. After saponification, the sample was extracted twice with 3 mL hexane each as described in the solvent only sample preparation method. One millilitre of methanol was added to dissolve the dried extract for HPLC analysis.

2.4. Ultrasound assisted solvent sample preparation method (UA)

Three millilitres hexane was mixed with 0.50 g chicken liver in a 25-mL test tube and incubated in a 10 °C water bath controlled using a refrigerated recirculator. An ultrasound probe (60 Sonic Dismembrator, Fisher Scientific Inc., Fair Lawn, NJ) was inserted into the sample solution. The sample solution was sonicated at 10 W Root Means Squared value (RMS) for 10 min. After being centrifuged, the hexane layer was removed to a clean tube and the residual sample was extracted with another 3 mL hexane. The extracted hexane layers were combined and evaporated. The dried extract was re-dissolved in 1 mL methanol for HPLC analysis.

2.5. Saponification and ultrasound assisted solvent sample preparation method (SP-UA)

Saponification was initiated by combining 0.8 mL water and 0.2 mL of the saponification solution with 0.5 g chicken liver sample. The saponification procedure was as described in Section 2.3. Then 3 mL hexane was added to the sample. The solution was sonicated using the condition described in Section 2.4. The hexane layer was removed after being centrifuged. The residual sample was extracted with another 3 mL hexane. The extracted hexane layers were combined, evaporated, and re-dissolved in 1 mL methanol for HPLC analysis.

2.6. Degradation of lutein during saponification

One hundred microlitres of lutein solution (100 μg/mL of hexane) was added to each 25-mL test tube. After the hexane was evaporated, different mixture of water to saponification solution (1–0 mL, 0.8–0.2 mL, and 0.5–0.5 mL) was added to each tube. Then the tubes were incubated at 70 °C for 20 min and vortexed every 5 min. After saponification, the sample solution was extracted twice with 3 mL hexane each as described in Section 2.2. One millilitre of methanol was added to dissolve the dried extract for HPLC analysis after hexane was evaporated at 30 °C under vacuum using the CentriVap Mobile system.

2.7. HPLC analysis

Lutein in the extracted chicken liver sample was analyzed using a HPLC system, that included a Waters 510 pumps, a 715 Ultra WISP injector, diode array detector (Milford, MA), and a 25 cm × 4.6 mm diameter 5-μm C18 Discovery column (Supelco, Bellefonte, PA). The mobile phase was methanol:acetone (10:90) and flow rate at 0.8 mL/min. The HPLC was operated at room temperature and controlled by Waters Millennium chromatography software and the lutein peak was monitored at 450 nm [8].

Table 1
Concentration of lutein in three chicken liver samples prepared by (a) solvent only (SOL), (b) saponification and solvent (SP), (c) ultrasound assisted solvent (UA), (d) saponification and ultrasound assisted solvent (SP-UA) methods and analyzed by HPLC[a]

Sample	Concentration of lutein (μg/g)			
	SOL	SP	UA	SP-UA
1	ND a	2.9 ± 0.6 b	6.0 ± 0.2 c	1.9 ± 0.3 d
2	2.1 ± 0.5 a	4.5 ± 0.8 b	10.4 ± 0.3 c	2.9 ± 0.5 a
3	ND a	2.5 ± 0.7 b	5.5 ± 0.1 c	0.7 ± 0.2 d

ND: not detected.
[a] Significant difference between two extraction methods in each sample is expressed by different letter.

2.8. Statistics analysis

Each extraction method was replicated three times. Results are presented as means with standard error. Significant differences in means were computed using the *t*-test with a *P*-value of 0.05.

3. Results and discussion

Table 1 lists the lutein concentrations in three different chicken liver samples using four sample preparation methods. The measured value of lutein in the liver sample using the UA method was two and three times higher than that obtained from the SP and SP-UA method, respectively. The order of sample preparation method for obtaining lutein concentration from high to low was UA, SP, SP-UA, and SOL. The solvent only method produced measured values of lutein that were either much lower than any of the other extraction methods or undetectable. This suggested that lutein in chicken liver tissue is mostly present within the liver cell and is not liberated by using solvent alone, which may not break the cell membrane completely. Compared with biological tissue samples, most plants and vegetables contain a higher level of lutein with simpler structure and permeable cell wall, which may not cause much difficulty in the lutein extraction [6–9]. The results from SP method in Table 1 indicate that during saponification, the chemical linkages in the sample matrix are hydrolyzed and broken down to liberate lutein, which could be extracted readily by the extraction solvent. However, compared with the UA method, the lower concentration of lutein obtained from the SP method suggests that alkali solution may cause chemical degradation of lutein. In the experiment of lutein degradation during saponification, the percentages of retained lutein after adding 0.2 and 0.5 mL of the alkali solution were 60 and 45% of the lutein without adding alkali solution, respectively. Thus, the methods with saponification significantly affected the stabilities of lutein in liver samples, even though saponification could disrupt sample matrix to release lutein during extraction.

The concentrations of lutein using the UA method were the highest among the four methods. This suggests that the ultrasound method greatly assisted the recovery of lutein from the liver samples by breaking down the sample matrix and cell structure in a physical rather than chemical manner. Ultrasound may cause greater cell disruption and stronger agitation, and thus distribute the extraction solvent more uniformly in each extraction and keep the extraction efficiency more consistent. The results of UA method demonstrated that ultrasound could increase the extraction yield of targeted compounds in sample preparation.

Although both the UA and SP methods yielded higher levels of lutein, the level of lutein decreased significantly when the two methods were combined in the extraction. From Table 1, the concentrations of lutein using the SP-UA method were much lower than when using the SP method alone. This suggests that the alkali condition could cause more serious degradation of lutein when combined with UA method, possibly because it increases the chance of reaction of alkali with lutein or other oxidation reactions.

4. Conclusions

This study demonstrates that the disadvantages of using solvent or saponification during the extraction of lutein from chicken liver could be overcome by using ultrasound assisted solvent extraction in sample preparation for lutein analysis. Because saponification is replaced by ultrasound assisted solvent extraction, the degradation of lutein is avoided; therefore, the measured level of lutein will be much closer to the actual value. Ultrasound assisted solvent extraction could replace the traditional extraction methods, especially in biological samples with lower lutein levels, such as liver tissue.

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