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Effect of ultrasonic waves on the stability of all-trans lutein and its degradation kinetics

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Abstract

Ultrasound treatment has been widely applied in the extraction of biologically active compounds including carotenoids. However, there are few reports on their effects on the stability of these compounds. In the present study, the stability of all-trans lutein, one of the carotenoids, was investigated under the action of ultrasound. Results showed that ultrasound induced the isomerization of all-trans lutein to its isomers, namely to 13-cis lutein, 13'-cis lutein, 9-cis lutein and 9'-cis lutein as analyzed by HPLC coupled with DAD and LC-MS; and the percentage of the isomerization increased with increasing both

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ultrasonic frequency and power. The stability of all-trans lutein in dichloromethane was worst among multiple kinds of solvents. Interestingly, the retention rate of all-trans lutein improved as the temperature increased, which runs counter to the Arrhenius law. Under ultrasound irradiation, the degradation mechanism might be different with various temperatures, the degradation of all-trans lutein followed first-order kinetics at 20 °C, while second-order kinetics was followed at 30-50 °C. As the ultrasonic reaction time prolonged, lutein epoxidation nearly occurred. Those results presented here emphasized that UAE techniques should be carefully used in the extraction of all-trans lutein.

Keywords: Ultrasonic wave

Stability All-trans lutein

Degradation

1. Introduction

Carotenoids continue to be a fast-moving topic of considerable interest because of their important functions, including serving as food colourant for humans and preventing heart disease, cancer and other diseases [1].

Carotenoids also have several kinds of important biological activities, such as

radical scavenging, singlet oxygen trapping and other protective activities [2, 3]. Carotenoids form a large isoprenoid family; the non-oxygenated carotenoids are called carotenes (e. g. α - and β -carotene), whereas their oxygenated derivatives are explicitly specified as xanthophylles (e. g. lutein, zeaxanthin and β -cryptoxanthin). Lutein, a xanthophyll of a carotenoid, is anticipated as a therapeutic product to prevent human eye diseases. Findings show that ingestion of lutein containing foods or supplements results in increased macular pigment optical density, and may help to improve visual function in patients suffering from two common eye diseases of ageing, cataract and age-related macular degeneration (AMD) [4, 5], moreover, it has been reported in epidemiological studies that lutein reduces the risk of some chronic diseases such as colon cancer, breast cancer, type 2 diabetes, and heart disease [6].

In recent years, ultrasound waves have been widely used to extract carotenoids from natural sources [7-10]. The ultrasound treatment can enhance extraction, decrease the temperature required and reduce the time of extraction. Ultrasound generates the growth of bubbles inside liquids causing the cavitation phenomenon to occur. This phenomenon results in enhanced mass transfer. Nevertheless, ultrasonic wave was less efficient than conventional techniques in certain cases where oxidation and degradation of active compounds occurred under prolonged sonication [11, 12]. The interaction

between the highly reactive hydroxyl radicals (resulted from sonication) and the active compounds might be responsible for the degradation.

Lutein mainly exist in trans-isomer forms in fruits and vegetables, which are polyene compounds with many conjugate double bonds, and are sensitive to light, oxygen and heat [13], so the structure of all-trans lutein is prone to damage during extraction and fruits and vegetables processing. Although the stability of lutein has always been a subject of intense research [14], to the best of our knowledge, there are no data on the effect of ultrasonic waves on the stability of all-trans lutein and its lutein stereoisomers. Little information is available involving sonochemical studies of the structure and property changes of other carotenoids. Zhao et al. [11] found that all-trans astaxanthin in a model system degraded to unidentified colorless compounds under ultrasound treatment. The degradation increased by increasing both treatment time and ultrasonic power. Sun et al. [9] indicated that the relatively low temperature of ultrasound assisted extraction (UAE) could limit the degradation of all-trans β -carotene during extraction compared to the conventional extraction, low extraction efficiency and degradation during UAE may occur if conditions were not optimized. The literature mentioned above only preliminarily explored the stability of some other carotenoids under special conditions of ultrasound treatment, so far, the study on the stability of all-trans lutein under ultrasound treatment remains unclear.

The objective of this study was to determine the effects of different ultrasound treatments on the stability of all-trans lutein. This study may help us to effectively control the extraction outcome, reduce degradation, and may establish a better understanding of the mechanism of ultrasound-assisted extraction of all-trans lutein.

2. Materials and methods

2.1. Materials

All-trans lutein with high purity 92.3% was obtained from laboratory-made samples. Methanol, acetonitrile (ACN) and methylene chloride of HPLC grade were purchased from Tedia (Fairfield, OH, USA), Ethanol, ethyl acetate, tetrahydrofuran, toluene, methylene chloride and acetone of AR grade were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Nitrogen, with purity of 99.99%, was purchased from Nanjing Wenda Special Gas Co., Ltd. (Nanjing, China).

2.2. Ultrasonic treatment

Ultrasonic treatments were performed on a Kunshan KQ-300GVDV ultrasonic cleaner (Kunshan Ultrasonic Instruments Ltd. Co., China) with frequencies of 45, 80 and 100 kHz and a maximum input power of up to 300 W. Actual power dissipated was calculated by measuring the rise in temperature of a fixed amount of water (3000 mL) in the ultrasonic bath for a

given time [15]. The conical flask with a flat bottom diameter of 8.1 cm mentioned below was just located in the cleaning bath under the ultrasound source to reduce the experimental errors caused by uneven power transfer and the surface of reactants was slightly lower than the level of water in the cleaning bath. Ice bathing was replenished as needed to control the temperature of the reaction solution. A reflux condenser was attached to the conical flask. The conical flask was sealed and purged with nitrogen to ensure an inert atmosphere for the reaction vessel.

20 mg all-trans lutein was placed in a 250 mL conical flask and 20 mL solvent was added. The mixture was submitted to ultrasonic irradiation under nitrogen atmosphere for a certain period of time, and thereafter, the solvent was evaporated. The residue was taken-up in 5 mL acetone; the test solution was filtered through a 0.45 μ m microporous membrane. After that, the samples were stored in a dark room at 4 °C until the analysis of lutein composition. All operations were carried under dark conditions.

2.3. Experiment design

Firstly, the effects of commonly used organic solvents (ethanol, ethyl acetate, tetrahydrofuran, toluene, dichloromethane), ultrasonic frequencies (45, 80, 100 kHz) and ultrasonic powers (120, 180, 240, 300 W) on the stability of lutein were investigated under a ultrasonic irradiation time for 2.5 h where nearby all the lutein would be completely extracted in sample matrix. Then

thermal degradation kinetics of lutein was studied under optimal ultrasonic conditions with minimum all-trans lutein loss, and further tests were also conducted to investigate the products of degradation by prolonged ultrasonic duration up to 192 h.

2.4. HPLC analysis

For HPLC, YMC Carotenoid C30 column (4.6 mm \times 250 mm i.d., 5µm, YMC, Wilmington, NC) and HPLC (Agilent 1200 series, USA) was performed. Elution was carried out using solvent dichloromethane, ACN and methanol (20:30:50, V/V/V) at a flow rate of 1.0 mL/min and the eluents were examined by UV light at a wave-length of 450 nm.

For MS, experiments were carried out on an Agilent 1290 Infinity LC/Agilent Technologies 6460 MS. The positive ion mode (APCI) was used to detect lutein and its isomers, with total ion current (TIC) scanning range 50-1200 m/z, corona current 4 μ A, capillary voltage 4500V and nitrogen as nebulizer gas (purity 99.9% and flow rate 4 L/min) and vaporizer temperature at 350 °C.

Lutein and its isomers were identified through the HPLC retention time, UV-visible absorption maxima, peak ratio III/II and electron ionization and chemical ionization mass spectroscopy (EIMS and CIMS) fragmentation patterns of these compounds combined with their previously published UV-visible spectra [16-18].

The relative concentrations of lutein and its isomers were performed using the calibration curve of all-trans lutein (Sigma-Aldrich).

2.5. All-trans lutein degradation kinetics under ultrasonic waves

Kinetic experimental data were presented in the dimensionless form C/C_0 at different ultrasonic time intervals t, where C is the all-trans lutein concentration and C_0 is the amount of all-trans lutein placed initially in the vials.

The reaction order is evaluated by trial-and-error procedures according to Berrios et al. [19]. If the reaction order is zero, C and t is liner, otherwise, if the degradation kinetics follows first or second-order model, $\ln(C/C_0)$ or 1/C has a linear relationship with t. The kinetic types were expressed by the following equations,

$C = -k_0 t + C_0$	(1)
$\ln(C/C_0) = k_1 t$	(2)
$1/C = k_2 t + 1/C_0$	(3)

Where k_0 , k_1 , k_2 is the kinetic constant.

3. Results and discussion

3.1. Isomer identification

Fig.1a showed the chromatogram of all-trans lutein in dichloromethane. After sonication for 2.5 h at 50 °C, four kinds of lutein cis isomers were

finally formed as shown in Fig.1b with Peak 1, 2, 4 and 5. After sonication for 24 h at 50 °C, three new kinds of lutein cis isomers and epoxides were formed as shown in Fig.1c. The identification of these compounds (Peak 1, 2, 4 and 5) was based on the HPLC retention time, UV-visible absorption maxima, peak ratio III/II and electron ionization and chemical ionization mass spectroscopy (EIMS and CIMS) fragmentation patterns, which made it possible for direct detection and identification of native geometric isomers of lutein (data not shown).

3.2. Effect of solvent type on the stability of all-trans lutein

Five different solvents (ethanol, ethyl acetate, toluene, tetrahydrofuran, and dichloromethane) were screened for investigating the stability of all-trans lutein under ultrasonic treatment (ultrasonic frequency of 45 kHz, ultrasonic power of 240 W, temperature of 30 °C) for 2.5 h, the change of the C/C₀ value of all-trans lutein was shown in Fig.2. Higher C/C₀ value of all-trans lutein was observed under ultrasound in the five organic solvents compared to the identical conditions without ultrasonic treatment. And all-trans lutein showed different extents of degradation, the stability of all-trans lutein in dichloromethane was worst, while a similar degradation was observed for ethanol, ethyl acetate, toluene and tetrahydrofuran solvents. Similar results were observed by Sun et al. [9], where the dichloromethane hardly had any extractability in the ultrasonic condition on the extraction of the all-trans

 β -carotene from citrus peels; the results suggested that the lower extraction yield in dichloromethane under ultrasonic condition might be attributed to the degradation of the all-trans β -carotene extracted. Variation of the solvent affected the stability of all-trans lutein, and also influenced the impact of ultrasound effect. The vapor pressure and kinematic viscosity effects on cavitational and bubble collapse intensity and diffusional rates of solvents into the gaseous cavity might be the crucial factors [20].

The change of the C/C_0 value of lutein cis isomers was shown in Fig.3. 13-cis lutein, 13'-cis lutein and 9-cis lutein in any organic solvents were all produced under ultrasonic treatment, and 13-cis lutein was found to be the major cis isomer. In contrast, C/C_0 of 13-cis lutein in dichloromethane was lower, while the C/C_0 of 9-cis lutein and 13'-cis lutein was higher; it indicated that ultrasonic waves might cause stronger chemical effects in dichloromethane than that in ethanol, and which resulted in a faster rate of degradation of 13-cis lutein. The subsequent degradation of 13-cis lutein might proceed faster than its formation [21, 22].

According to our experiments, dichloromethane caused obvious degradation of all-trans lutein under ultrasound treatment, which could be selected as a control to take further investigation on the stability of all-trans lutein under ultrasonic conditions, and ethanol, generally recognized as a safe (GRAS) solvent, could be considered as an alternative solvent for use in ultrasound

extraction of all-trans lutein. So both dichloromethane and ethanol were selected as reaction solvents.

3.3. Effect of ultrasonic frequency on the stability of all-trans lutein

Fig.4 showed the effect of different ultrasonic frequencies (45, 80 and 100 kHz) on the stability of all-trans lutein under ultrasonic treatment (ultrasonic power of 240 W, temperature of 30 °C) for 2.5 h. It could be seen that C/C_0 of all-trans lutein in both ethanol and dichloromethane under ultrasonic frequency of 80 kHz and 100 kHz was lower than that of 45 kHz, so the concentration of all-trans lutein gradually decreased with increased ultrasonic frequency. The retention rate of all-trans lutein in ethanol under ultrasonic frequency of 45 kHz and 100 kHz was 0.93 and 0.88, respectively, which was consistent with the results of Ye et al. [8]. They found that under ultrasound assistance at the frequency of 20-50 kHz, the structure of carotenoids was not destroyed, the heat effect of 25 kHz was stronger than that of 20 kHz and accelerated the speed at which particles diffuse. The formation of sonochemical effect requires a bubble of a certain size. But as frequency increases, the production of cavitation becomes less likely, there was no sufficient time for the bubble to grow large enough to collapse totally.

The change of the C/C_0 value of lutein cis isomers was shown in Fig.5. 13-cis lutein was the major isomer in both ethanol and dichloromethane under the ultrasonic treatments with different ultrasonic frequencies. 9-cis lutein and

13'-cis lutein had lower C/C₀ values, but there was no significant difference (P<0.05) among all tested ultrasonic frequencies. Only 9'-cis lutein in dichloromethane under ultrasonic treatment with ultrasonic frequency of 100 kHz was produced, which indicated that 9'-cis lutein was less susceptible to formation than the other lutein cis isomers.

3.4. Effect of ultrasonic power on the stability of all-trans lutein

Fig.6 showed the effect of different ultrasonic powers (120, 180, 240 and 300 W) on the stability of all-trans lutein under ultrasonic treatment (ultrasonic frequency of 45 kHz, temperature of 30 °C) for 2.5 h. To estimate the actual input power being used, power dissipation was calculated based on calorimetric study. It was observed that power dissipated in the reaction vessel was 36.6, 50.4, 61.8 and 73.7 W, respectively. Variation in the power dissipated suggested that there was a variation in caviation intensity [15]. The C/C_0 of all-trans lutein decreased gradually with ultrasonic power from 120 to 300 W, it indicated that cavitation bubbles were easily generated by ultrasonic treatment in a short time, and collapsed more violently with increasing ultrasonic power, then the formed cavitation bubbles increased the shear field and improved the rate of degradation. This was similar to that described in the literature. Zhao et al. [11] found that the ultrasound degraded all-trans astaxanthin to unidentified colorless compound (s) as suggested by HPLC analysis and UV/vis measurements, and the degradation likewise increased as

both treatment time and ultrasonic power increased from 100 to 600 W. Qiao et al. [23] investigated the sonochemical effects on seven free phenolic acids under ultrasound treatment in a model system, and found that it is easier to form cavitation bubbles and the bubbles collapse more violently with increasing ultrasonic power output in the range 45-225 W. Meanwhile, higher the ultrasonic power was, the difference of the retention rate of all-trans lutein between ethanol and dichloromethane was larger, and more violent cavitation effect in dichloromethane under sonication produced.

The change of the C/C_0 value of lutein cis isomers was shown in Fig.7. Total retention rate of lutein cis isomers ascent, in the meantime the all-trans lutein decreased as the ultrasonic power increased, it suggested that the occurrence of cis-trans isomerization during ultrasound.

3.5. Degradation reaction order of all-trans lutein under ultrasonic treatment

Fig.8 showed that the effect of different temperatures (20, 30, 40 and 50 °C) on the stability of all-trans lutein under ultrasonic frequency of 45 kHz, power of 240 W, and reaction time of 2.5 h with ethanol or dichloromethane as a solvent. It could be seen that as the temperature of the solution increased, the value of C/C_0 grew rapidly, the reaction rate of all-trans lutein gradually decreased. It suggested that there was more likely transient (vaporous) cavitation occurring. This is confirmed by the decrease in the reaction rate

with increase in temperature. A possible explanation of the temperature effect at 45 kHz would thus be that the vapour which enters the bubble during its formation cushions the collapse of the bubble, the temperature of the hotspot formed by the cavity collapse decreases [24, 25]. As a result, the rate decreases as bulk temperature increases. Contrary to vaporous cavitation, the effective Arrhenius activation energy should not be negative in this case. So lower effect of ultrasound at higher temperatures has been attributed to the cushioning effect of vapour contained inside the bubble during cavitation.

Table 1 summarizes the results of correlation coefficient (R) of zero-order, first-order and second-order kinetic model under ultrasonic treatment at different temperatures. According to the trial-and-error procedure, the kinetics of all-trans lutein degradation under ultrasonic treatment at 20 °C followed a first-order reaction with correlation R_1 above 0.985, while a second-order reaction with R_2 above 0.986 was followed at 30, 40 and 50 °C, respectively. The difference of reaction order indicated that degradation mechanism of all-trans lutein was different under different temperatures. The reason might be that under the ultrasound at 20 °C, the cavitation bubble for sonochemical reaction is the dominant factor on all-trans lutein degradation, but as the temperature increased, thermal effects enhanced, which could play a role combined with cavitation. Several studies about the kinetics of carotenoids under different treatments have been reported in the literature. Sun et al. [26]

found that degradation kinetics of all-trans β -carotene under ultrasound fitted first-order reaction at -5 to 15 °C, and fitted second-order reaction at 25 °C. Henry et al. [27] reported that the oxidative degradation reaction of the carotenoids such as all-trans β -carotene, 9-cis β -carotene, lycopene and lutein in safflower seed oil follows a first-order kinetic model during heat treatment at 75, 85 and 95 °C. Ahmed et al. [28] investigated the degradation kinetics of carotenoids and visual color of papaya puree at selected temperatures (70 to 105 °C), and found that degradation of carotenoids and visual color followed first order reaction kinetics. Dependence of the rate constant followed the Arrhenius relationship.

The corresponding initial degradation rate constant (k) of all-trans lutein were presented in Table 2. In both ethanol and dichloromethane, k was found to decrease markedly and quickly as the increasing temperatures under ultrasonic treatments, it could be shown that in the 20-50 °C temperature range, the degradation rate in ethanol increased by 5.5-fold.

3.6. Newly formed compounds during prolonged ultrasonic treatment

The stability of all-trans lutein was further studied by prolonged ultrasound (ultrasonic frequency of 45 kHz, power of 240 W, temperature of 50 °C, solvent of dichloromethane or ethanol) for 192 h. From Fig.9, it could be seen that the C/C₀ value of all-trans lutein decreased markedly (P<0.05), after 144 h, the retention rate of all-trans lutein was very low. During the ultrasonic

treatment, in addition to 13-cis lutein and 13'-cis lutein, 15-cis lutein, 5,6-epoxy lutein and 5,8-epoxy lutein were newly formed compounds, both 9-cis lutein and 9'-cis lutein were not detected (Fig.1c). The 13'-cis lutein and 13-cis lutein were the major isomers and 15-cis lutein was present at low levels. It meant numerous degradation products of all-trans lutein were formed during exposure to the prolonged experimental conditions, and the trans-to-cis conversion rate was lower in lutein or that cis isomers of lutein are perhaps less stable and more reactive. 13-cis lutein might be more readily formed from all-trans lutein than 9-cis lutein during ultrasound because of the low activation energy [29]; meanwhile 9-cis lutein underwent fast degradation as soon as they were generated from all-trans lutein. The formation of 5, 6-epoxy lutein and 5, 8-epoxy lutein indicated lutein epoxidation occurring under ultrasonic wave combined with thermal effect [30, 31].

4. Conclusion

This study demonstrated that ultrasound had significant effects on the stability of all-trans lutein. And the effects were pronouncedly different with various ultrasonic conditions. The stability of all-trans lutein in dichloromethane was worst. Under various ultrasonic treatments at 20-50 °C for 2.5 h, the all-trans lutein produced the same isomers, namely 13-cis lutein, 13'-cis lutein, 9-cis lutein and 9'-cis lutein, and 9'-cis lutein was ultimately

formed in designated time. With increased ultrasonic frequency and power, the retention rate of all-trans lutein gradually decreased in both ethanol and dichloromethane, meanwhile an identifiable upward change trend of isomers was generally similar. Interestingly, the retention rate of all-trans lutein improved as the temperature increased, which runs counter to the Arrhenius law. Under ultrasound, the degradation mechanism might be different under various temperatures, at 20 °C, the degradation of all-trans lutein followed first-order kinetics, while fitted second order at 30-50 °C. As the ultrasonic reaction time prolonged, lutein epoxidation nearly occurred. All the results emphasize the careful application of both ultrasound-assisted extraction techniques in the extraction of lutein or other unstable biological molecules.

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Accemption



Fig.1. Chromatogram of all-trans lutein in dichloromethane before (a) and

after 2.5 h (b), 24 h (c) of ultrasonic treatment at 50 °C. Peak 1,13-cis lutein; peak 2, 13'-cis lutein; peak 3, all-trans lutein; peak 4, 9-cis lutein; peak 5, 9'-cis lutein; peak 6, 5, 6 - epoxy lutein; peak 7, 5, 8 -epoxy lutein;

poy



Fig.2. Effect of solvent type on the stability of all-trans lutein under ultrasonic treatment (ultrasonic frequency of 45 kHz, ultrasonic power of 240 W, temperature of $30 \,^{\circ}$ C, reaction time of 2.5 h).



Fig.3. Effect of solvent type on the stability of lutein *cis* isomers under ultrasonic treatment (ultrasonic frequency of 45 kHz, ultrasonic power of 240 W, temperature of $30 \,^{\circ}$ C, reaction time of 2.5 h).



Fig.4 Effect of ultrasonic frequency on the stability of all-trans lutein under ultrasonic treatment (ultrasonic power of 240 W, temperature of 30 $^{\circ}$ C, reaction time of 2.5 h).



Fig.5. Effect of ultrasonic frequency on the stability of lutein cis isomers under ultrasonic treatment (ultrasonic power of 240 W, temperature of 30 °C, reaction time of 2.5 h).



Fig.6. Effect of ultrasonic power on the stability of all-trans lutein under ultrasonic treatment (ultrasonic frequency of 45 kHz, temperature of 30 $^{\circ}$ C, reaction time of 2.5 h).



Fig.7. Effect of ultrasonic power on the stability of lutein cis isomers under ultrasonic treatment (ultrasonic frequency of 45 kHz, temperature of 30 °C, reaction time of 2.5 h).



Fig.8. Effect of temperature on the stability of all-trans lutein under ultrasonic treatment (ultrasonic frequency of 45 kHz, power of 240 W, reaction time of 2.5 h).



Fig.9. Newly formed compounds during prolonged ultrasonic treatment (ultrasonic frequency of 45 kHz, power of 240 W, temperature of 50 °C, solvent of dichloromethane (a) or ethanol (b)).

Table 1

C

Correlation coefficients (R) of zero-order, first-order and second-order kinetic model under ultrasonic treatment at different temperatures.

Solvent	Correlation	Temperature (°C)			
	coefficient	20	30	40	50
Ethanol	R ₀	0.959	0.962	0.976	0.949
Dichloromethane		0.979	0.957	0.939	0.937
Ethanol	R ₁	0.985	0.949	0.948	0.936
Dichloromethane		0.998	0.93	0.949	0.942
Ethanol	R ₂	0.970	0.987	0.993	0.998
Dichloromethane		0.965	0.995	0.996	0.986

Table 2

Rate constant (k) of degradation of all-trans lutein under ultrasonic treatment.

To me another (^{0}C)	D eta constant (h^{-1})	Solvent			
Temperature (C)	Kate constant $(\Pi)^{-}$	Ethanol	Dichloromethane		
20	k ₁	0. 011	0.023		
30	\mathbf{k}_2	0.009	0.018		
40	k ₂	0.005	0.010		
50	k ₂	0.002	0.007		