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Degradation kinetics and isomerization of 5-*O*-caffeoylquinic acid under ultrasound: Influence of epigallocatechin gallate and vitamin C

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ABSTRACT

5-*O*-Caffeoylquinic acid (5-CQA), also known as chlorogenic acid, exhibits various biological activities. Hence the interest in its property change during processing and extraction has increased. The present work studied the influence of ultrasound on the stability of 5-CQA at different pH (pH 4.69, 7.09, 7.69 and 9.22) in water and 50% methanol-water system. Different parameters including solvent, ultrasonic power, time, temperature, duty cycle, and liquid height were investigated during the treatment. Results indicate that ultrasound accelerated the degradation of 5-CQA. Based on Weibull model, the degradation kinetics were described. The rate constant (k) of the degradation increased with the increasing pH, demonstrating the alkali sensitivity of 5-CQA. The isomerization of 5-CQA to 3- and 4-*O*-caffeoylquinic acid was found at neutral and alkaline conditions, which was further boosted by ultrasound. The stability of 5-CQA was improved by adding epigallocatechin gallate (EGCG) and vitamin C (VC) respectively.

1. Introduction

Caffeoylquinic acids (CQAs) are a family of natural phenolic compounds, formed by esterification of quinic acid with one or multiple caffeic acids. Based on the number or the position of caffeoyl groups, CQAs are classified into various derivatives. Mono-CQAs are the most abundant, with 5-CQA predominating, as well as 3-CQA and 4-CQA (Park, 2010). 5-CQA is generally known as chlorogenic acid, is one of the main polyphenols in the human diet. These CQAs isomers are found abundantly in Eucommia ulmoides (Wang, Liu, Dai, Zhang & Tang, 2020), Chrysanthemum (Chen et al., 2015), Prunus mume (Shi, Gong, Liu, Wu & Zhang, 2009), coffee beans (Dawidowicz & Typek, 2017), and other plant species. Various biological activities of CQAs have been reported such as antioxidant, anti-inflammatory, neuroprotective, antihypertensive, and antidiabetic effects (Skała, Makowczynska, Wieczfinska, Kowalczyk & Sitarek, 2020; Meng, Cao, Feng, Peng & Hu, 2013). Owing to these functionalities, more and more application of 5-CQA gas been explored in the pharmaceutical and food fields. It may be useful as an antioxidant added in food to prevent diabetes and obesity. Meanwhile, 5-CQA is commonly employed as a marker in the quality control of various natural products (Wianowska & Gil, 2018).

However, 5-CQA is easily degraded during the processing owing to its structural properties (Zhao, Wang, Yang & Tao, 2010). It has also been found that trans-5-CQA will transfer to cis-5-CQA, 4-CQA and 3-CQA during the pressurized extraction. Concurrently, with the increase of pH value, the contents of 4-CQA and 3-CQA increased continuously (Gong et al., 2013). During extraction, 5-CQA was found to isomerize to 3- and 4-CQA, undergoing transesterification and hydrolysis (Wianowska, Typek & Dawidowicz, 2015). Ultrasound assisted technique has been widely used in the extraction of phenolic compounds. Li, Chen, and Yao (Li, Chen & Yao, 2005) established a ultrasonic method to extract chlorogenic acid from fresh leaves of *Eucommia ulmodies* Oliv., which showed a high reproducibility and a short duration. However, the degradation of phenolic compounds induced by ultrasound is also worthy of attention. It has been reported that the stability of polyphenols is affected by high temperature, high pressure

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and hydroxyl radicals caused by ultrasonic cavitation, especially in aqueous solvents (Gong et al., 2016). However, there is no systematic study on the degradation kinetics of CQAs under ultrasound.

The present work therefore studied the effects of different treatment conditions including solvents, time, temperature, ultrasonic power, liquid height and duty cycle on the stability of 5-CQA, as well as the degradation pattern of 5-CQA under ultrasound. Epigallocatechin gallate (EGCG) and vitamin C (VC) as two common antioxidants in food processing were added to protect 5-CQA from degradation and isomerization. The degradation and isomerization of 5-CQA under acidic, neutral and alkaline conditions were explored. In addition, the degradation kinetics of 5-CQA in the presence of either EGCG or VC was also examined, to further reveal the pathway and mechanism in the degradation of CQAs.

2. Materials and methods

2.1. Materials

The standard of 5-CQA (purity ≥ 99.39%), puerarin (purity ≥ 99.71%) and EGCG (purity ≥ 99.78%) were purchased from Chengdu Must Biotechnology Co., Ltd. (Chengdu, China). Ascorbic acid was purchases from Shanghai Aladdin Co., Ltd. (Shanghai, China). Phosphoric acid solution (analytical grade) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Chromatographic grade acetonitrile and methanol were purchased from Scharlau chromatographic reagent company (Barcelona, Spain).

2.2. Ultrasonic treatment

The solution of 5-CQA with a concentration of 0.1 mg/mL was added into the brown glass bottle, and then treated by a probe ultrasonic processor (JY92-IIDN, Ningbo Scientz Biotechnology Co., Ltd. Ningbo, China). During the treatment, the temperature was controlled by a low-temperature thermostatic water bath. The solvent, temperature, time, power, duty cycle and liquid height of the treatment were carried out according to the single factor experimental design. The experiments were conducted in triplicate. After ultrasonic treatment, the samples were detected by HPLC after filtrated through 0.22 μm organic filter membrane.

The effect of solvent (methanol, ethanol, water, 50% (*v/v*) methanol in water, and 50% (*v/v*) ethanol in water), temperature (10, 20, 30, 40, 50, and 60 ℃), treatment time (10, 20, 30, 40, 50, and 60 min), ultrasonic power (0, 135, 270, 405, 54, and 675 W), duty cycle (0, 16.7, 33.3, 50, 66.7, 83.3, and 100%) and liquid height (1, 2, 3, 4, 5, and 6 cm) on the stability of 5-CQA were analyzed. The liquid height was defined as the distance from the bottom of the glass container to the ultrasonic probe. The duty cycle is the ratio of the ultrasonic working time to the time of one cycle.

2.3. Measurement of three mono-CQAs

The content of 5-CQA was measure using an Agilent 1260 series HPLC system equipped with a UV detector and a Phenomenon Luna-C18 column (250 mm × 4.6 mm, 5 μm). The injection volume was 10 μL and the column was maintained at 35 °C. The sample was eluted by acetonitrile (A) and 0.1% phosphoric acid (B) at a flow rate of 0.8 mL/min with the following gradient program (for eluent A): 0–11 min, 10%-18%; 11–16 min, 18%; 16–20 min, 18%-10%. The detection wavelength was 330 nm.

2.4. Degradation kinetics

The solution of 5-CQA (2 mL, 2.8 mmol/L), in 50% (*v/v*) methanol, was mixed with 18 mL buffer of different pH (0.2 M acetic acid buffer, pH 4.6; 0.2 M phosphoric acid buffer, pH 7.05; 0.15 m boric acid buffer, pH 7.96; 0.1 M carbonate buffer, pH 9.22). The mixture was sonicated under 270 W ultrasonic power at 40℃ using a duty cycle of 50%. The liquid height was kept at 3 cm. During the treatment, 100 μL reaction solution was taken out at 0, 10, 30, 60, 90, 120, 150, 180, and 210 min respectively, then mixed with 10 μL puerarin (internal standard, 4.8 mg/mL in 50% (*v/v*) methanol) and 890 μL mobile phase (0.1% phosphoric acid-acetonitrile, 4:1). After mixing, the CQA distribution was measured. The control was treated at the same condition without ultrasound and analyzed in the same way. Three parallels were made for each sample.

Weibull distribution is a probability distribution function, which can be used to fit the degradation kinetics of compounds. The degradation kinetics of 5-CQA in different pH buffer were fitted by Weibull model:

$$C_i/C_0 = \exp[-(kt)^n]$$

where C_i is the concentration of CQA at a certain time; C_0 is the initial concentration; k is the reaction rate constant; n is a shape constant ($n > 1$, the function is an increasing function; $n < 1$, the function is a decreasing function; $n = 1$, the function satisfies the exponential distribution).

According to above equation, the degradation parameters k and n can be calculated by nonlinear least square fitting with origin2019 software. Afterwards, the degradation half-life ($t_{1/2}$) was calculated as follow:

$$t_{1/2} = (0.693)^{1/n}/k$$

2.5. Effect of EGCG and VC

The solution of 5-CQA (2 mL, 2.8 mmol/L) in 50% (*v/v*) methanol, was mixed with 16 mL buffer of different pH as mentioned above. Before sonication, 2 mL antioxidant (EGCG or VC) was added. The final molar concentration ratio of the antioxidant and 5-CQA was 2:1, 1:1 or 0.5:1. Then the same ultrasonic treatment procedures as mentioned above were repeated.

2.6. Statistical analysis

All experiments were conducted in triplicate, leading to achieve the evaluation expressed as mean ± standard deviation (SD). The experimental data was processed by SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Duncan analysis of variance was used for each factor, $p < 0.05$ showed significant difference. The multivariate analysis of the data on the basis of principal component analysis (PCA) was conducted by SIMCA-P 14.1 (Umetrics, Sweden).

3. Results and discussion

3.1. Ultrasonic effect on the stability of 5-CQA in different solvents

For the extraction of phenolic compounds, alcohols (methanol and ethanol), acetone, diethyl ether, and ethyl acetate are commonly used as extraction solvents. Considering the polarity of phenolic acids, mixtures of alcohol–water or acetone–water are more recommended (Stalikas, 2007). The stability of 5-CQA in different solvents is shown in Fig. S1 (in Supplementary data). The results showed that 5-CQA was relatively stable in pure ethanol and 50% ethanol. The degradation rate of 5-CQA was only 1.05% in pure ethanol. In contrast, 5-CQA was degraded obviously in pure methanol, 50% methanol and pure water. Moreover, the degradation rate of 5-CQA in 50% methanol was the highest, which was 20.79%, followed by that in water (14.13%). The results indicated that the stability of 5-CQA under ultrasound was related to the extraction solvent.

The ultrasonic degradation of 5-CQA mainly results from acoustic cavitation, rooting in the formation, growth, and collapse of

microbubbles (Leong, Wu, Kentish & Ashokkumar, 2010). The viscosity, vapor pressure, and surface tension of the solvent are associated with the sonochemical process and affect the ultrasonic cavitation. Frizzo et al. (2016) confirmed that the dissipated ultrasonic power, measured by calorimetric method, was higher in water than that in ethanol and methanol. The decrease of the vapor pressure of the solvent enhances the dissipated ultrasonic power and cavitation intensity. Since water has lower volatility than both ethanol and methanol, the dissipated ultrasonic power in which is much higher than that in ethanol and methanol (Lorimer & Mason, 1987; Lupacchini et al., 2017). Consequently, the different cavitation effect led to the different stability of 5-CQA in different solvents. Since 5-CQA was the most vulnerable in 50% methanol, the following analysis was conducted in 50% methanol system with different ultrasonic conditions.

3.2. Effect of ultrasonic parameters on the stability of 5-CQA

3.2.1. Effect of temperature

When using 50% methanol as the solvent, 5-CQA was degraded obviously after ultrasonic treatment at different temperatures (Fig. 1A). The degradation degree at 50℃ was the highest. Generally, the degradation was more severe at high temperature. After treated by ultrasound in water, the degradation of 5-CQA was observed, but less than that in 50% methanol system (Fig. 1B). As the temperature rose, the degradation of 5-CQA first increased to the maximum at 40℃, and then decreased.

The increase of temperature was certified to abate the upper cavitation threshold, owing to an increase in the liquid vapor pressure or a decrease in surface tension or viscosity. The reduced gas solubility in the liquid at higher temperatures will also hinder the cavitation (Wood, Lee & Bussemaker, 2017). However, the increasing temperature enhanced the transformation of 5-CQA to other isomers (Dawidowicz & Typek, 2010). Comprehensively, the effect of temperature on the ultrasonic degradation efficiency of 5-CQA is not simply increased or decreased with the increase of temperature.

3.2.2. Effect of time

The influence of different ultrasonic time on the stability of 5-CQA is shown in Fig. 1C&D. Ultrasound led to the degradation of 5-CQA in both 50% methanol system and water system. However, the impact of ultrasonic time on 5-CQA content in 50% methanol system had no distinct regularity. At 20 min, the content of 5-CQA was the lowest. When exceeding 20 min, 5-CQA content increased slightly, which may resulted from the transformation from the degradation products (Dawidowicz & Typek, 2015). The change of 5-CQA content with the prolongation of ultrasonic time presented a trend to decrease first and then decrease. The lowest content of 5-CQA showed at 40 min. Compared with the result in 50% methanol system, it can be concluded that 5-CQA was more tolerant to ultrasound in water.

3.2.3. Effect of ultrasonic power

The solution of 5-CQA was treated by ultrasound with different ultrasonic power. The concentration change of 5-CQA is exhibited in Fig. 2A&B. Similar to the above results, the degradation of 5-CQA was

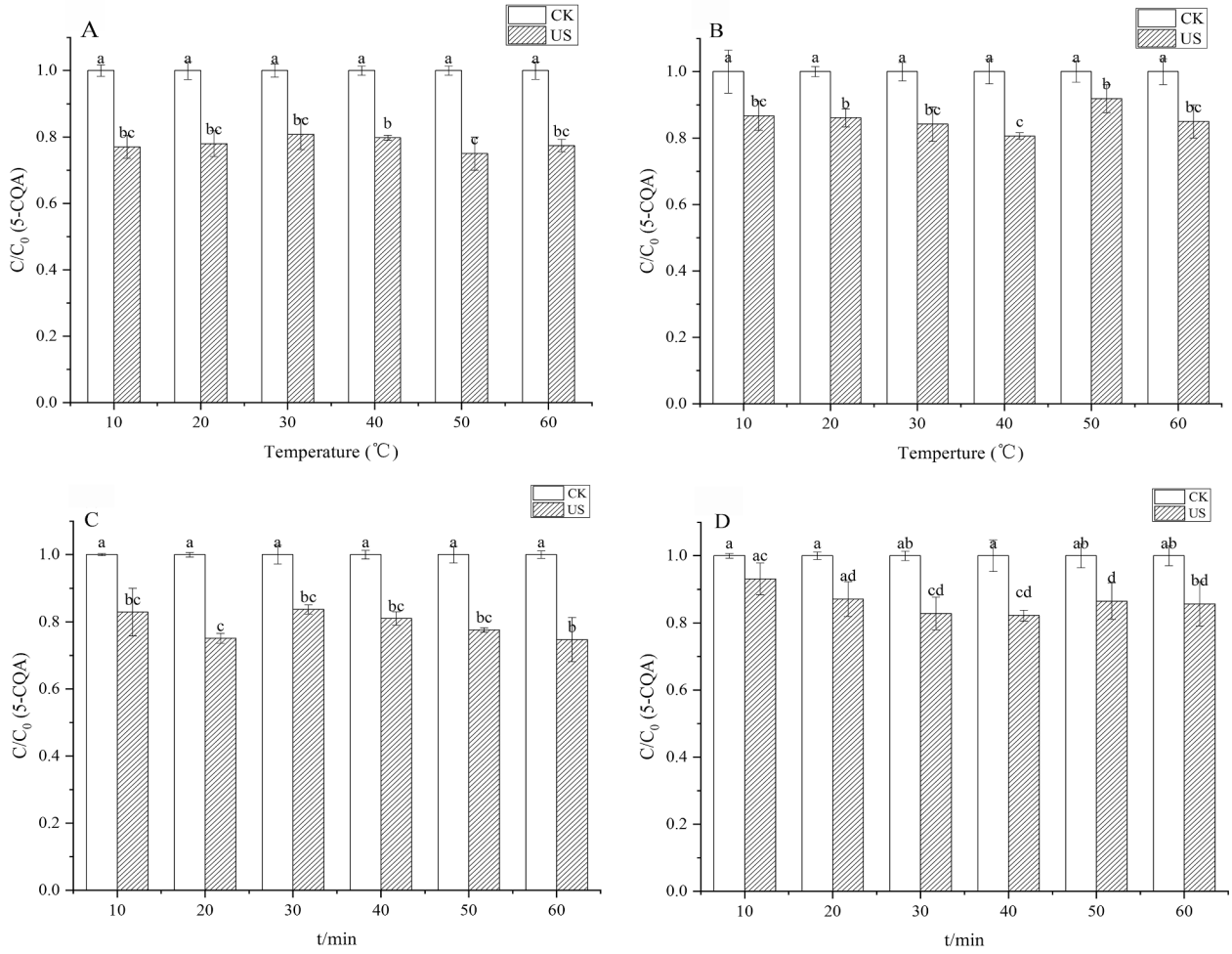

Fig. 1. Effect of the treatment temperature and time on the stability of 5-CQA in 50% methanol (A, C) and water (B, D). CK: control without ultrasound, US: samples treated by ultrasound.

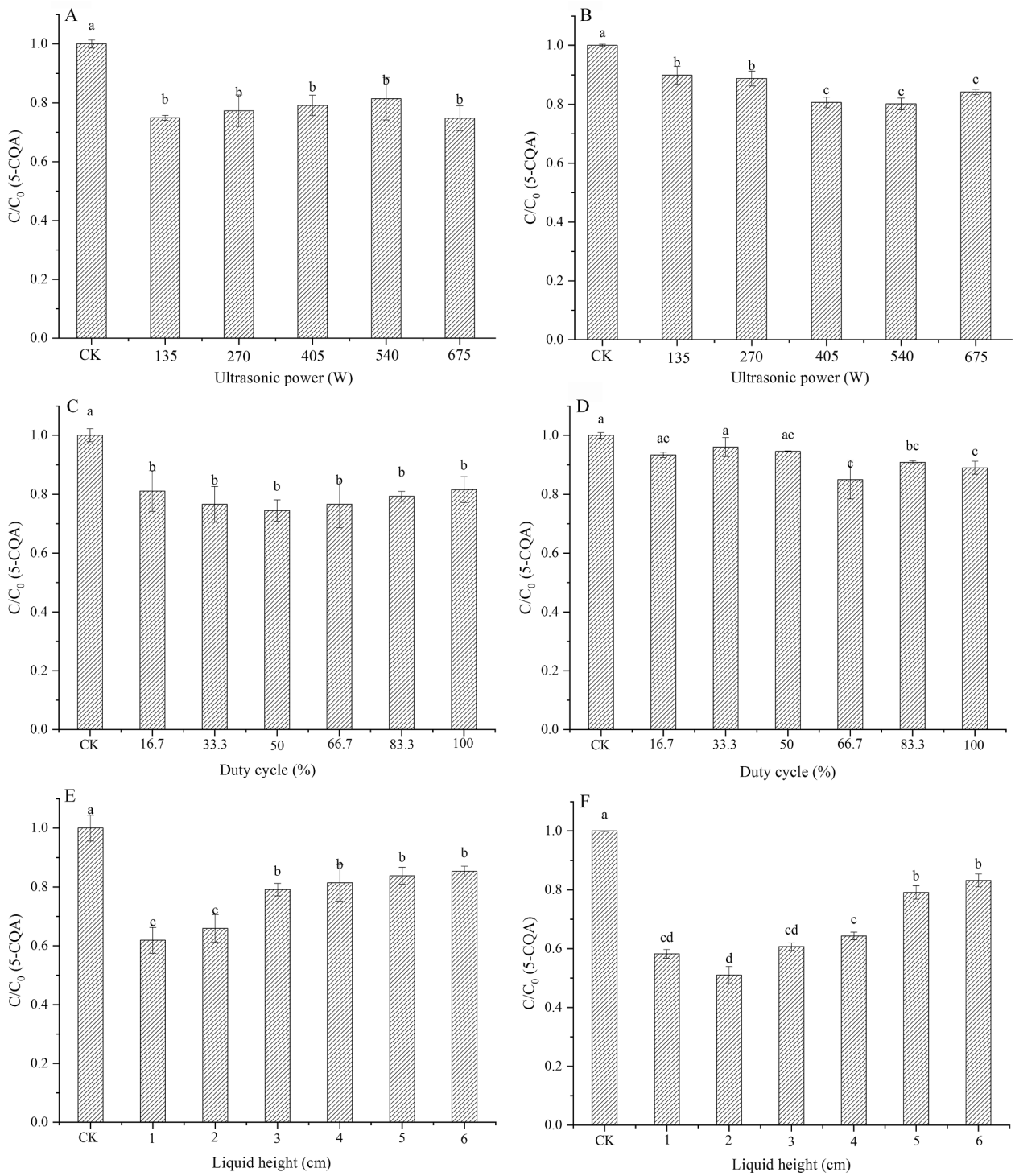

Fig. 2. Effect of ultrasonic power, duty cycle and liquid height on the stability of 5-CQA in 50% methanol (A, C, E) and water (B, D, F).

more severe in 50% methanol system under the same ultrasonic power. In 50% methanol system, the degradation degree of 5-CQA presented no significant change along with the increasing ultrasonic power. At the power of 135 W, 5-CQA content reached the minimum value. In water system, the degradation degree of 5-CQA was decreased as ultrasonic power increased, until reached the minimum at 540 W. The results indicate that higher ultrasonic power promoted the ultrasonic degradation of 5-CQA, but it had a limit. When reaching the limit, the degradation effect by ultrasound weakened. The threshold appeared at a lower power level in 50% methanol.

Generally increasing ultrasonic power leads to the enhancement of cavitation effect. The increasing ultrasonic power was reported to cause the degradation of polyphenols during extraction (Dzah et al., 2020). However, there are upper limit of ultrasonic power to the cavitation threshold. Exorbitant ultrasonic power generates large numbers of microbubbles, impeding the propagation of ultrasonic waves (Pokhrel, Vabbina & Pala, 2016). The abnormally growing bubbles may result in poor cavitation (Wang et al., 2017). Therefore the degradation degree of 5-CQA remained unchanged or even decreased with the boost of power.

3.2.4. Effect of duty cycle

Duty cycle is an important factor in ultrasound assisted degradation. The effect of ultrasonic duty cycle was investigated by varying the ratio of sonication time and stop time (Fig. 2). It can be seen from Fig. 2C that the degradation efficiency was the highest at the duty cycle of 50% in 50% methanol. But no significant difference was found as duty cycle increased from 16.7% to 100%. When using pure water as the solvent, the degradation of 5-CQA is the most significant at the duty cycle of 66.7%. Both pulsed and continuous ultrasound decreased the concentrations of 5-CQA, while pulsed ultrasound was beneficial to avoid unnecessary energy consumption and equipment aging. The effect of duty cycle on ultrasonic effect in different studies was inconsistent. Sun et al. found that the degradation rate of caffeic acid under pulsed ultrasound was higher than under continuous ultrasound (Sun et al., 2013). Luque-García and Luque De Castro declared that duty cycle was not the major factor in the ultrasound assisted extraction (Luque-García & Luque De Castro, 2004).

3.2.5. Effect of liquid height

Fig. 2E&F shows the influence of different liquid height on the ultrasonic stability of 5-CQA in 50% methanol (a) and water system (b). In 50% methanol, the degradation of 5-CQA increased significantly as the liquid height increasing from 1 cm to 3 cm. Further increase of liquid height had no effect on the degradation degree. In water system, the degradation degree was generally decreased with the increasing liquid height. This may have been due to the decreased cavitation intensity on account of the ultrasonic attenuation caused by absorption and scattering (Qiao et al., 2013). The increase of liquid height decreased the axial velocity of ultrasonic wave near the probe, abating the acoustic streaming and local shear forces, which resulted to reduction of the mechanical effect (Wang, Cheng, Ma & Jia, 2020).

Different operating conditions could affect the stability of 5-CQA and the ultrasonic effect. Among the above parameters in 3.2, the liquid height level showed the greatest impact on the ultrasonic degradation of 5-CQA. Treatment time is also an important factor influencing 5-CQA degradation. Duty cycles affected little on the ultrasonic degradation of 5-CQA.

3.3. Degradation and isomerization of 5-CQA at different pH

3.3.1. Degradation kinetics

The degradation curve of 5-CQA fitted by Weibull model in different pH buffer is shown in Fig. 3. The kinetic parameters k, n, R^2 and $t_{1/2}$ of ultrasonic degradation are listed in Table 1.

The rate constant (k) of degradation kinetics was used to indicate the stability of 5-CQA. As shown in Table 1, the rate constant k increased whereas $t_{1/2}$ value decreased significantly with the increasing pH. It reveals that the stability of 5-CQA has a great relationship with pH whether under ultrasonic condition or not. The increasing pH decreased the stability of 5-CQA. Friedman & Jürgens (2000) studied the effect of pH on the stability of some plant phenolic compounds. They found 5-CQA was not stable to high pH, but stable to acid pH. The $t_{1/2}$ values

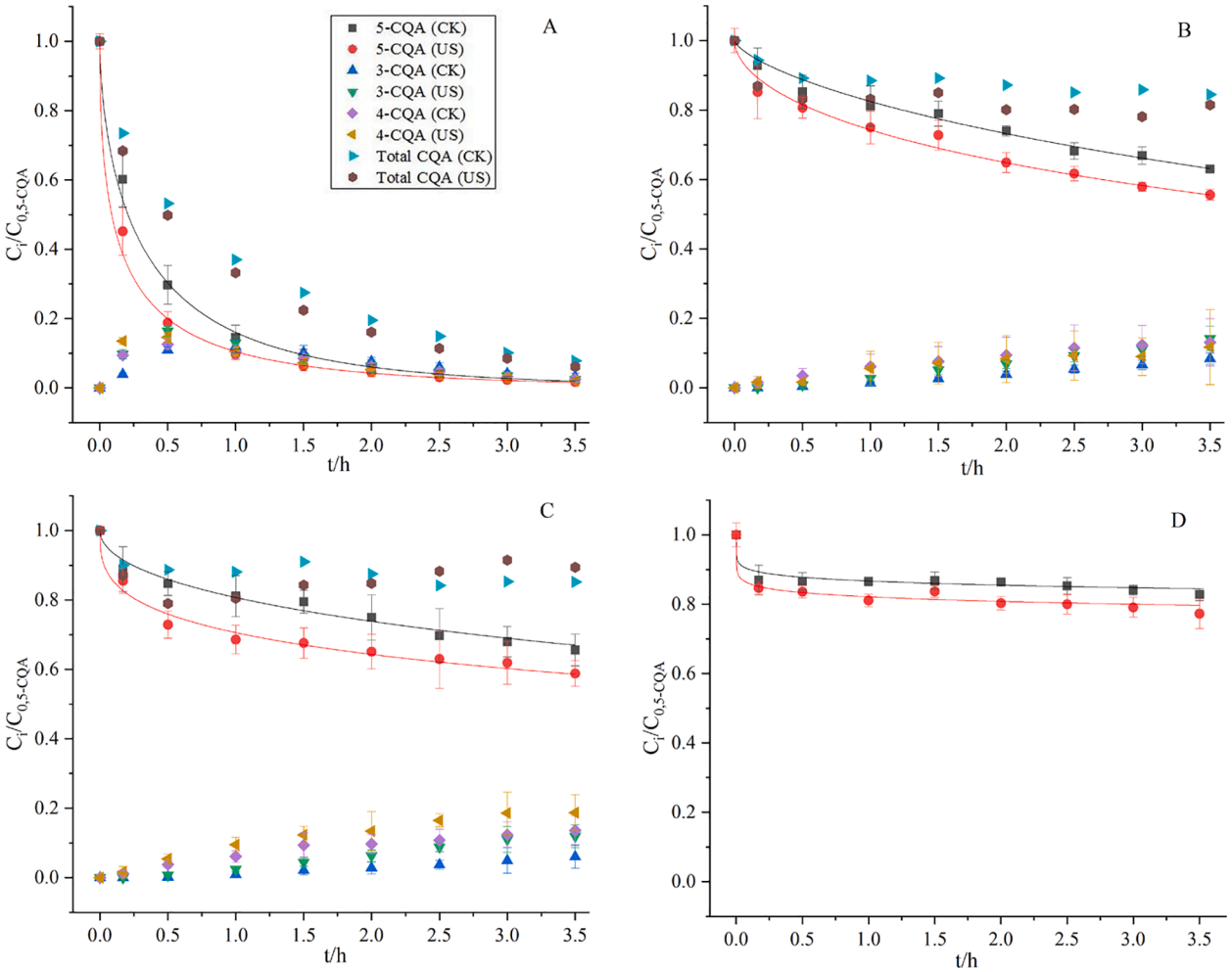

Fig. 3. Ultrasonic effects on degradation and isomerization of 5-CQA at different pH levels (A: pH 9.22; B: pH 7.96; C: pH 7.06; D: pH 4.69; CK: Control without treatment; US: Samples treated by ultrasound).

Table 1
The degradation kinetics parameters of 5-CQA at different pH with and without ultrasound.

pH	Treatment	k	n	$t_{1/2}$/h	R^2
4.96	CK	4.53E-07	0.133	140,853	0.854
	US	5.96E-07	0.113	66,223	0.902
7.06	CK	0.046	0.500	11.45	0.992
	US	0.045	0.341	7.291	0.999
7.96	CK	0.093	0.695	6.325	0.990
	US	0.108	0.544	4.742	0.993
9.22	CK	2.661	0.618	0.208	0.999
	US	5.335	0.485	0.088	0.999

CK: Control without treatment, US: Samples treated with ultrasound.

of sonicated samples at different pH were significantly lower than those of untreated samples, while the rate constants (k) of sonicated samples were larger than those of untreated samples. It can be inferred that the degradation degree of 5-CQA gradually increased with the increase of alkalinity, while ultrasound further accelerated the degradation. Acoustic cavitation induced by ultrasound generates strong physical force and highly active free radicals, which can result in the degradation of compounds (Zhang et al., 2015). With ultrasonic treatment, the pH stability of 5-CQA decreased.

Fig. 3D shows that 5-CQA was relatively stable at pH 4.69, which degraded slightly with the prolong of time. The half-life of 5-CQA at pH 4.96 without ultrasound was 140853 h, which was significantly shortened by ultrasound to 66223 h. Under neutral and alkaline conditions, the stability of 5-CQA gradually weakened with the emergence of the other two isomers (Fig. 3A, B & C). As the treated time increased, the content of 5-CQA decreased. When the time reached 3.5 h, the 5-CQA content decreased about 40% at pH 7.06, and 50% at pH 7.96. Furthermore, the total content of CQA showed a little reduction at pH 7.06 and 7.96. At pH 9.22, the ultrasonic degradation showed the highest rate constant ($k = 5.335$) and the lowest half-life ($t_{1/2} = 0.088$ h), owing to the volatility of CQAs at alkaline condition. Most of 5-CQA was degraded after 3.5 h treatment.

3.3.2. Isomerization

Combined with the data listed in Table S1, it was found that the isomerization happened at neutral and alkaline conditions. At pH 7.06 and 7.96, the content of 3-CQA and 4-CQA gradually increased as the treatment proceeded, reflecting the occurrence of isomerization of 5-CQA. The formation of 4-CQA predominated over that of 3-CQA, indicating that 5-CQA was more likely to transform to 4-CQA. Meanwhile, 3-CQA can't be detected at pH 7.06 and 7.96 until 0.5 h. The reason may be that 5-CQA was transformed to 4-CQA first, and then further transformed to 3-CQA. The isomerization at pH 7.06 was the most remarkable among the four conditions. With ultrasound, the isomerization to 3-CQA and 4-CQA was further enhanced especially at pH 7.06.

At pH 9.22, the initial increase rates of 3-CQA and 4-CQA content were the fastest within 0.5 h. Exceeding 0.5 h, the content of 3-CQA and 4-CQA began to decrease. According to Clifford, Kellard & Birch (1989), 5-CQA in alkaline water solution undergoes isomerization and transformation to 3-CQA and 4-CQA. It has been reported that the concentration of transformation products (3-CQA and 4-CQA) was increased with pH until reach the maximum at the optimal pH, while the optimum pH was lowered with the increasing treatment duration (Dawidowicz & Typek, 2011). Since mono-CQA was unstable at alkaline condition, the prolong of the treatment time further weakened the stability, causing the concentration reduction of both 5-CQA and its isomers. Therefore the content of the total CQA dramatically decreased with the processing. Additionally, 3-CQA is more stable than 4-CQA, so the reduction of 3-CQA was<4-CQA (Li et al., 2015). Ultrasound promoted the formation of 3-CQA and 4-CQA before 1 h, which made their concentration higher than those of control. After 1 h, however, ultrasound resulted in decline of 3-CQA and 4-CQA. The most likely reason is that ultrasound decreased the tolerance of the alkaline environment.

Generally, 5-CQA was slightly degraded at pH 4.69 with no isomerization. The degradation was strengthened with the increase of pH. At pH 7.06 & 7.96, the isomerization and degradation degree of 5-CQA are similar, while at pH 9.22, the degradation of 5-CQA was much stronger than isomerization. Ultrasound accelerated the degradation of 5-CQA at all pH conditions. Ultrasound facilitated the isomerization of 5-CQA to both 3- and 4-CQA, and the facilitating effect was most obvious at pH 7.06.

3.4. Effect of EGCG and VC

3.4.1. Effect of EGCG

Since 5-CQA was relatively stable at pH 4.69 with no isomerization, EGCG was added at pH 7.06, 7.96, and 9.22 to prevent 5-CQA from degradation. Three molar concentration ratios of EGCG and 5-CQA were used, which are 0.5: 1, 1: 1, and 2: 1. The degradation and isomerization of 5-CQA were shown in Fig. 4. The parameters of degradation kinetics were listed in Table S2.

Comparing the degradation of 5-CQA without EGCG, the degradation after adding EGCG was reduced, as the kinetic constant (k) decreased and half-life time ($t_{1/2}$) increased. Reactive oxygen such as superoxide and hydroxyl free radicals are mainly products of ultrasonic cavitation (Sutkar & Gogate, 2009). EGCG is one of the most potent antioxidants. Since the phenol rings in EGCG structure act as electron traps and scavengers of free radicals, leading to protective effect on 5-CQA (Chu, Deng, Man & Qu, 2017). The contrast between the EGCG-added degradation with and without ultrasound indicated that EGCG with appropriate amount could slow down 5-CQA degradation caused by ultrasound. At different pH the appropriate amount was different. At pH 7.06, the protective effect of EGCG on 5-CQA was exhibited at the concentration ratio of 2: 1 (EGCG/5-CQA). At pH 7.96, the highest protective effect was show at the concentration ratio of 1: 1 (EGCG/5-CQA). At pH 9.22, the protective effect of EGCG was increased with its increasing concentration.

3.4.2. Effect of VC

VC added samples were treated with and without ultrasound at different pH (pH 7.06, 7.96 and 9.22). The degradation kinetics of 5-CQA were described by Weibull model. Fig. 5 showed the content changes of 5-CQA, 3-CQA, 4-CQA and total CQA. The parameters of degradation kinetics of 5-CQA, which was described by Weibull model, were calculated and listed in Table S3. The result illustrated that the addition of VC slow down the degradation of 5-CQA.

At pH 7.06, the addition of VC decreased the degradation constant k and prolonged the half-life time $t_{1/2}$, showing the protective effect of VC on 5-CQA. However, with ultrasonic treatment, the degradation of chlorogenic acid was not slowed down by vitamin C. Ordóñez-Santos, Martínez-Girón and Arias-Jaramillo found significant losses of VC after the ultrasound processing (Ordóñez-Santos, Martínez-Girón & Arias-Jaramillo, 2017). The effect of ultrasound on VC may depended on the pH, temperature, ultrasonic parameters and etc. Under this research condition, VC was unstable at pH 7.09 with ultrasound, as a result, no protective effect was of VC on 5-CQA was shown. At pH 7.96, VC impeded the degradation of 5-CQA both with and without ultrasound. When treated without ultrasound, the protective effect of VC increased with the increasing concentration. As mentioned in 3.3, ultrasound accelerated 5-CQA degradation and shortened its half-life time. Comparing the sonicated samples added with and without VC, it can be concluded that the introduction of VC slowed down the degradation of 5-CQA induced by ultrasound. However, the sonicated sample with VC still showed higher degradation constant (k) and shorter half-life time (t) compared with the untreated control sample with VC. This illustrated that VC could slow down the degradation of 5-CQA, and protect 5-CQA from ultrasonic damage partially. At pH 9.22, the protection of VC on 5-CQA improved with the increasing concentration both with and without

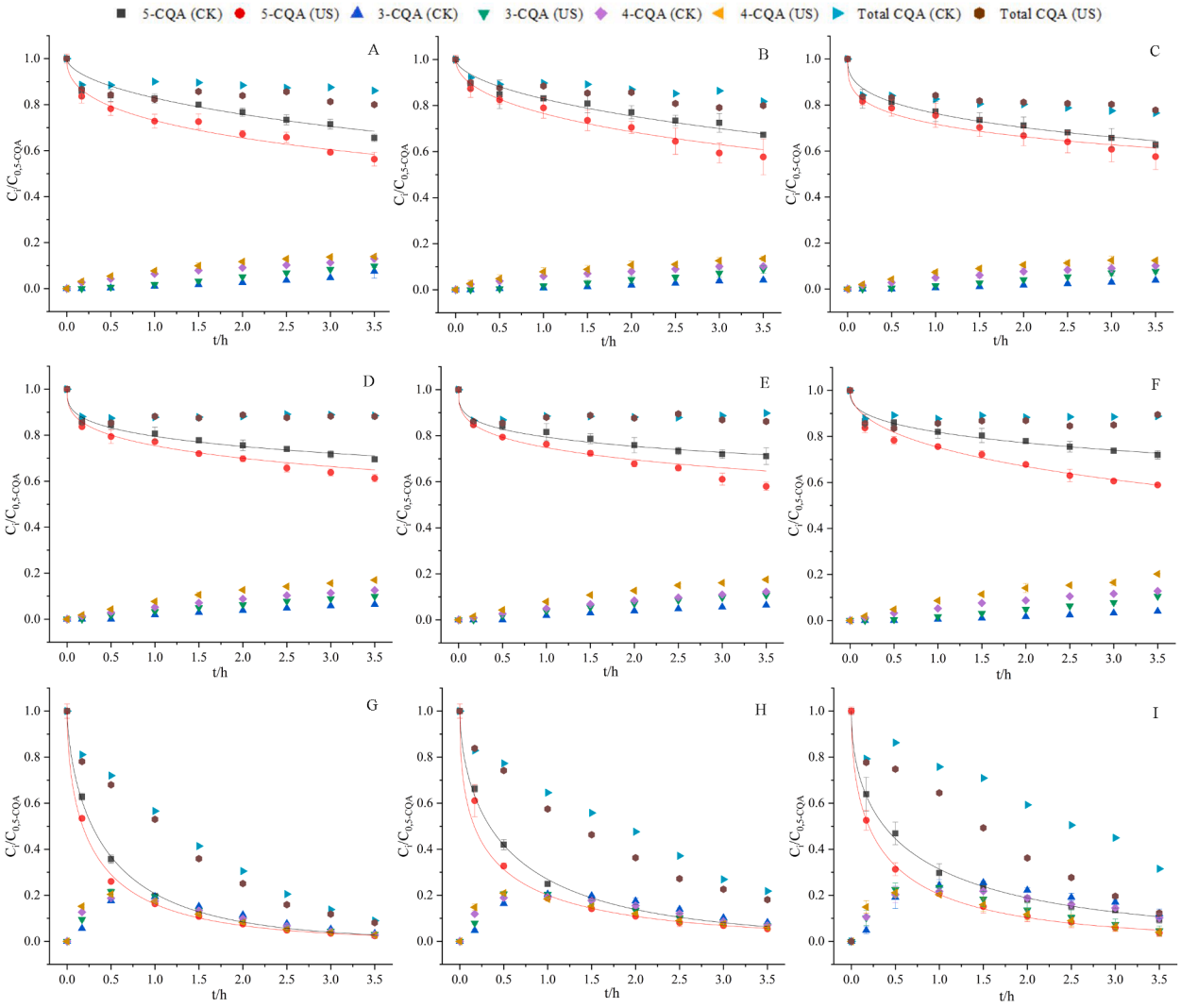

Fig. 4. Effect of different concentrations of EGCG on the stability of 5-CQA with and without ultrasound at pH 7.06 (A-C), pH 7.96 (D-F) and pH 9.22 (G-I). (A, D, G: EGCG:5-CQA = 0.5:1; B, E, H: EGCG:5-CQA = 1:1; C, F, I: EGCG:5-CQA = 2:1; CK: Control without treatment; US: Samples treated by ultrasound).

ultrasound. Some studies have shown that VC can inhibit the isomerization of flavanol and prevent phenolic acids from degradation (Chen et al., 2021; Nardini et al., 2002). The antioxidant potential of VC is useful in reducing free radicals, which helps dimmish 5-CQA degradation caused by the chemical effect of ultrasound (Tariq, 2007).

Generally, both of EGCG and VC could protect 5-CQA from degradation. VC exhibited better protective effect than EGCG on 5-CQA without ultrasound. Instead, EGCG could better weaken the ultrasonic degradation of 5-CQA. In order to better illustrate the protective effect of EGCG on the 5-CQA degradation caused by ultrasound, multivariate statistical analysis was carried out.

3.5. Multivariate statistical analysis

The Kinetic parameters of degradation of 5-CQA at different pH with and without EGCG were obtained as the original material for multivariate statistical analysis by building distinct statistical models. PCA is an unsupervised algorithm, which is generally applied for dimension reduction by finding out principal components (PCs) to maximize the variance and ignoring class labels in a dataset (Song et al., 2021). The PCA model was firstly carried out for exploring major trends on score plot (Fig. S2A). The score plot displayed that all the samples were clustered into two groups (CK and US) clearly, and the first two PCs explained 85.52 cum% of total variance, indicating the good distribution of the samples in space. The DModx plot is proportional to the residual standard deviation (RSD) of the X observation and used for the further identification of the potential outliers and separation of the variables efficiently. Generally, the variable is identified as the true outlier with weighted residual ≥ 2.5 (Song et al., 2020). As shown in Fig. S2B, the result demonstrated that there was no outlier found and the built model was efficient. Afterwards, a partial least squares-discriminant analysis (PLS-DA) was used as supervised classification algorithms. The coefficient plot (Fig. S2C) represented the trends in K value of the samples processed with various pH and EGCG contents in the given pairwise groups during ultrasound. The results indicated that the coefficient levels of some samples mainly including F, A, and D were decreased. In comparison, the levels of I, J, and K were increased. As depicted in Fig. S2D, the permutation test (100 permutations) was used for verification of the statistical model validation. The result demonstrated the model validation with the intercepts of $R^2 = (0.0, 0.0195)$ and $Q^2 = (0.0, -0.602)$ was good.

4. Conclusions

The degradation of 5-CQA at different conditions was studied. Methanol-water mixture and ethanol–water mixture are the commonly

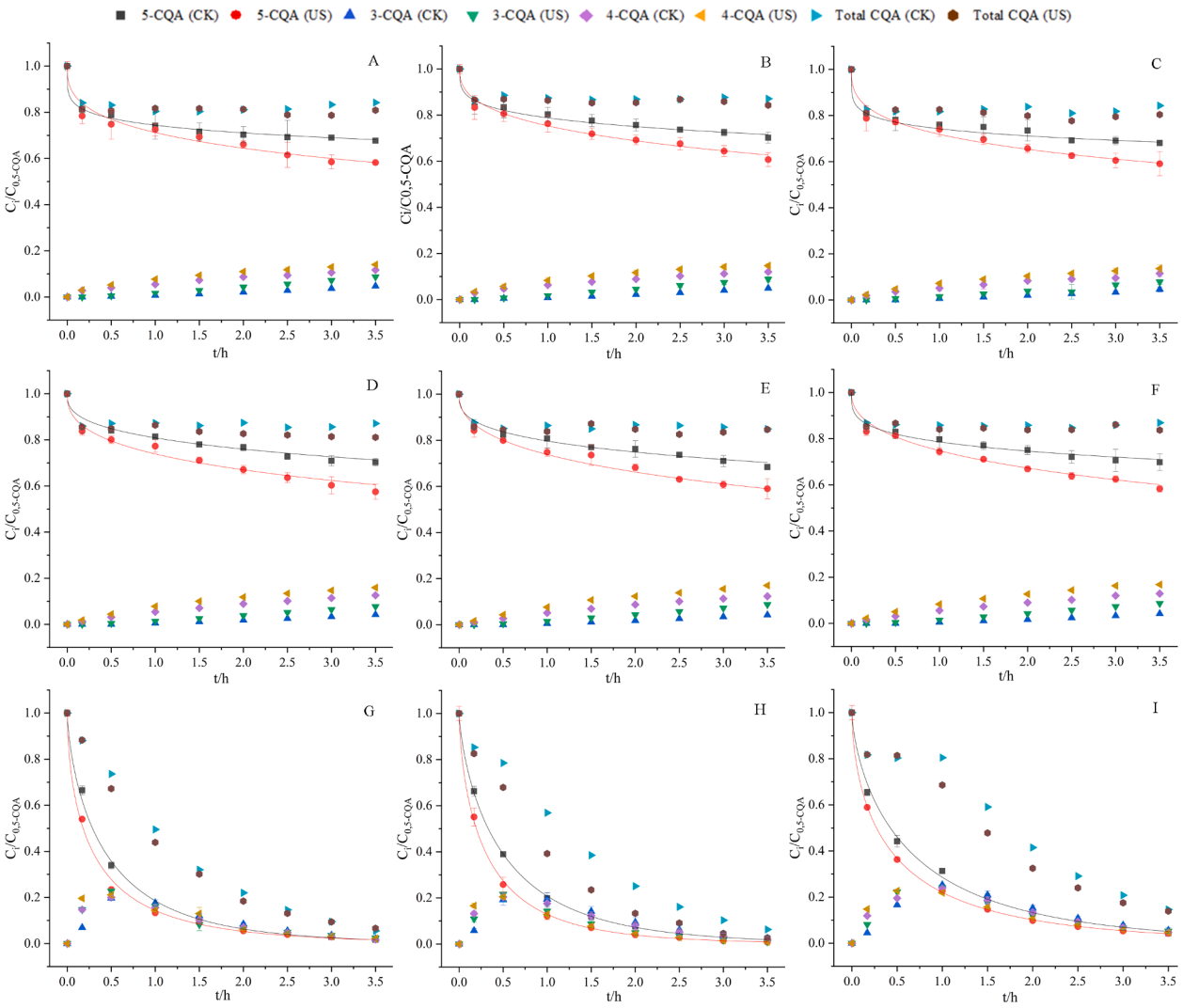

Fig. 5. Effect of different concentrations of VC on the stability of 5-CQA with and without ultrasound at pH 7.06 (A-C), pH 7.96 (D-F) and pH 9.22 (G-I). (A, D, G: VC:5-CQA = 0.5:1; B, E, H: VC:5-CQA = 1:1; C, F, I: VC:5-CQA = 2:1; CK: Control without treatment; US: Samples treated by ultrasound).

used solvent in the extraction of phenolic compounds. In the present study, 5-CQA was found to degrade more obviously in 50% methanol (*v*/*v*). The treatment time, temperature, ultrasonic power, ultrasonic duty cycle and liquid height all made an impact on degradation of 5-CQA, among which liquid height played the most important role. Nevertheless, the action pattern is not a single positive or negative influence. There was an optimum value to reach the maximum degradation rate. Thus, to reduce losses of 5-CQA during processing, we can optimize the operating conditions of ultrasonic treatment.

Based on the degradation kinetics of 5-CQA under different pH conditions, an increasing rate constant (k) and a decreasing half-life time ($t_{1/2}$) were found with the increasing pH, indicating an alkali sensitivity of 5-CQA. Moreover, ultrasound accelerate the degradation of 5-CQA at all pH conditions with an elevated rate constant (k) and a lowered $t_{1/2}$ value. Besides, the difference of degradation speed was enlarged with the increase of pH. The isomerization was found at neutral and alkaline conditions, boosted by ultrasound. Between the two isomer products, 4-CQA accounted for a relatively larger proportion than 3-CQA. EGCG and VC showed protective effect on the degradation of 5-CQA. For the sake of 5-CQA protection, lowering the pH is recommended, which not only improves the stability of 5-CQA, but also reduces the influence of ultrasound. In the food processing involving ultrasound, EGCG and VC can be added as protectant against 5-CQA degradation. Other effective protectants still need to be explored. The results obtained in this study are useful not only for the application of ultrasound technique in the extraction of phenolic compounds but also for food processing involved with 5-CQA.

CRediT authorship contribution statement

Danli Wang: Validation, Writing – original draft, Writing – review & editing. **Jingjing Wang:** Investigation, Methodology, Data curation. **Jiachen Sun:** Investigation. **Shaoping Qiu:** Methodology, Validation. **Bingquan Chu:** Software. **Ruosi Fang:** Visualization. **Ling Li:** Formal analysis. **Jinyan Gong:** Supervision, Funding acquisition, Conceptualization. **Fuping Zheng:** Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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