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ORIGINAL PAPER

The inhibitory effect of chlorogenic acid on lipid oxidation of grass carp (*Ctenopharyngodon idellus*) during chilled storage

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Abstract

Grass carp (*Ctenopharyngodon idellus*) is a kind of freshwater fish which is rich in polyunsaturated fatty acids and easily exposed to lipid oxidation during refrigeration. The effect of chlorogenic acid (CGA) on lipid oxidation, protein oxidation, enzymatic activities, and color stability of grass carp muscle during chilled storage was investigated. The lipid oxidation was inhibited by CGA, as evidenced by lower thiobarbituric acid values, peroxide values, carbonyl valence, less free fatty acid content, and higher amount of unsaturated fatty acid compared to the control group. CGA also had a positive effect on the whiteness value and the stability of protein oxidation of fish samples. In addition, the inhibitory study of CGA on endogenous lipase and lipoxygenase activities of fish muscle can help to partly illustrate the mechanism that retains its freshness effect. The results indicate that CGA is a novel natural additive which can be used to inhibit lipid and protein oxidation and be applied in the storage of aquatic products or some similar fields.

Keywords Chlorogenic acid · Grass carp · Chilled storage · Lipid oxidation

Introduction

Grass carp (*Ctenopharyngodon idellus*) is a species of freshwater fish with the highest annual yield in the world. It is preferred by most consumers due to its delicious taste, rich nutrition, and moderate price. Chilled storage is an efficient and popular preservation method during fish transportation and processing. Usually, the activity of some endogenous enzymes and the growth of spoilage microorganisms in fish muscle tissue are effectively inhibited at low temperatures (Babakhani et al. 2015). However, grass carp is rich in protein and polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are easily exposed to oxidation. Oxidative changes (lipid and protein oxidation) are key factors affecting the deterioration in quality of freshwater fish. Oxidizing reactions in fish tissue lead to rancid flavors, discoloration, toxic components, loss of nutrients, and shelf life reduction (Chauhan et al. 2018). Meanwhile, other oxidative products such as free radicals can destroy the cells and macromolecules of the organism (Cerutti 1985). Therefore, in order to maximize utilization of grass carp resources, it is very important to develop efficient approaches to reduce lipid and protein oxidation. The addition of antioxidants is a valid method to prevent lipid and protein oxidation. The possible carcinogenesis and mutagenicity of synthetic antioxidants, such as tert-butyl hydroquinone (TBHQ) and butylated hydroxyl toluene (BHT), have been reported, which have recently made natural antioxidants become popular. Polyphenols are one of the representative natural antioxidants applied widely in the inhibition of protein and lipid oxidation due to their high antioxidant capacity (Farvin and Surendraraj, 2019, Turguta, et al., 2017, Sun, et al., 2019). Chitosan films with grape (*Vitis vinifera*) seed extract, which contain high amounts of catechin, epicatechin, and phenolic acid, evidently increased the shelf-life of refrigerated salmon (*Oncorhynchus*) (Alves et al. 2018). Icing with rosemary (*Rosmaricus officinalis*) extract improved the sensory score and decreased biogenic amine content of sardines

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(*Sardinella aurita*) (Özyurt et al. 2012). Icing medium containing red alga (*Gracilaria verrucosa*) extract, which is rich in quercetin-7-methyl ether, delayed the development of chemical parameters of Indian mackerel (*Rastrelliger kanagurta*) deterioration (Abimannan et al. 2018). Tea polyphenols are natural polyphenols extracted from green tea (*Camellia sinensis*) leaves and were shown to be effective quality-preservation material for aquatic products (Nie et al. 2018; Ju et al. 2018; Feng et al. 2017).

Chlorogenic acid (CGA) is a kind of polyphenol which is widely present in plants. It is the active ingredient of herbs such as honeysuckle (*Lonicera japonica Thunb*) and eucommia (*Eucommia ulmoides Oliver*). CGA has various biological activities such as antibacterial, anti-inflammation, and maintaining blood sugar balance *etc.* (Feng et al. 2016; Wu et al. 2016). It also shows good antioxidant properties on scavenging 1,1-diphenyl-2-picrylhydrazyl radical, superoxide anion radical, and hydroxyl radical (Xiang and Ning 2008) and is regarded as an anti-staling agent in food preservation. The addition of CGA inhibited the oxidation of tung oil and oil-in-water emulsions (Sasakia et al. 2010). CGA extracted from Eucommia ulmoides leaves showed significant effect on apple and pear anticorrosive (He and Yang 2015). Chlorogenic acid-gelatin conjugates delayed protein decomposition and lipid oxidation during the storage of sword prawn (Fua et al. 2017).

There are some reports about food preservation that used CGA as one kind of film material, but to our knowledge, the application of CGA on freshwater fish preservation has not yet been studied. Furthermore, most studies focused on assessing the general freshness rather than the inhibition of lipid oxidation during food storage. Therefore, the aim of this work was to explore the quality changes of grass carp with regard to lipid oxidative stability using CGA as a natural inhibitor during chilled storage. At the same time, protein oxidation and color stabilities which are very closely related to lipid oxidation were also measured. Moreover, the mechanism for inhibiting lipid oxidation of CGA for fish muscle was explained by determining endogenous lipase and lipoxygenase activities. Thus, this study provides a comprehensive investigation of the inhibition of lipid oxidation of CGA during fish refrigeration.

Materials and Methods

Chemicals and Reagents

Chlorogenic acid was purchased from Melonepharma (Dalian, China). Disodium ethylenediaminetetraacetate dehydrate (EDTA-Na2), 4-nitrophenyl butyrate (4-NPB), and 35-component fatty acid methyl esters mixture were obtained from Sigma–Aldrich Chemical Co (St. Louis, MO, USA). Boron trifluoride-methanol solution and 5, 5′-dithiobis 2-nitrobenzoic acid (DTNB) were provided by Yuanye Biological Technology Co. Ltd. (Shanghai, China). Linoleic acid was purchased from Aladdin (Shanghai, China). Methanol (HPLC) was obtained from Fisher Scientific (USA). All other reagents and chemicals used were of analytical grade.

Preparation of Fish Samples

Fresh grass carp with an average weight of 3.5 ± 0.5 kg ($n = 20$ in total and four parallel pieces of fish in every test) was purchased from a local fish market (Wuhan, Hubei Province, China). The fish was decapitated, gutted, deboned by hand, and peeled, then cut into pieces (6 cm × 2 cm × 1 cm) after the red meat was removed due to its uneven distribution. The fish muscles were respectively dipped into 0.05% (lower), 0.1% (medium), and 0.3% (higher) (W/V) CGA aquatic solution (Ju et al., 2018) for 5 min, drained and air-dried for 10 min, then put into small packages individually. Fish muscles treated with distilled water were used as the control group (CONT). All treated samples were stored at 4 ± 1 °C and taken for further analysis at days 0, 4, 8, 12, and 16.

Lipid Extraction

The crude lipid was extracted following a previous method (Folch et al. 1957). The fish sample (50.00 g) was grinded and mixed with 150 mL of chloroform-methanol solution (2:1, v/v). The mixture was shaken for 10 min then another 100 mL chloroform-methanol solution (2:1, v/v) was added and filtered after standing for 1 h. The filtrate was mixed with salt solution (containing 0.73% NaCl and 0.05% $CaCl_2$ (w/v)), chloroform, methanol, and water in proportions 8:4:3 by volume. The mixture was centrifuged at 4000×*g* for 10 min. The chloroform phase was poured into a flask and evaporated at 40 °C using an evaporator (Yarong RE-3000, Shanghai, China). The residual solvent was removed by flushing nitrogen.

Determination of Lipid Oxidative Stability

Conjugated Diene

The lipid sample (0.0100–0.0500 g) was dissolved in 5 ml isooctane, and the absorbance of the sample was measured with UV-VIS at 234 nm (Srinivasan et al. 1996). The concentration of conjugated diene was calculated using the molar extinction coefficient of 25,200 $M^{-1}cm^{-1}$ and the results were expressed as mol of conjugated diene compounds per kilogram of the lipid sample.

Peroxide Value

The peroxide value was determined using a ferric thiocyanate method (Shantha and Decker 1994). The crude lipid sample

(0.1000 g) was mixed in a glass tube with 9.8 ml chloroform-methanol solution (7:3, v/v), then 50 μl of 3.94 M ammonium thiocyanate solution and 50 μl ferrous chloride (prepared with barium chloride solution and ferrous sulfate solution) solution were added and mixed for 2–4 s. After 5 min incubation, the absorbance of the sample was measured with UV-VIS at 500 nm. Results were expressed as milliequivalents (meq) of peroxide per kilogram of lipid.

Thiobarbituric Acid Reactive Substances

2-thiobarbituric acid was determined using a former method (Mi et al. 2016) with slight modification. Minced fish muscle (5.00 g) was homogenized (IKA Ultra-Turrax T18 Basic, Staufen, Germany) with 25 mL 7.5% (w/v) trichloroacetic acid (containing 0.1% EDTA) for 1 min at 3000 rpm, then centrifuged (Fitchell SF-TDL-5A , Shanghai, China) at a speed of 4000×*g* for 10 min. The supernatant (5 mL) was mixed with thiobarbituric acid (20 mM, 5 mL) solution; the mixture was heated in boiling water for 30 min and cooled with flowing water. The absorbance was measured with ultraviolet-visible spectrophotometer (UV-VIS) at 532 nm (UNIC UV-2600 UV-Vis, Shanghai, China). The standard curve was prepared by using 1, 1, 3, 3-tetraethoxypropane. The results were expressed as milligrams of malondialdehyde (MDA) per kilogram of fish sample.

Free Fatty Acid

The content of free fatty acid was determined by titration. The minced fish sample (5.00 g) was homogenized with 15 ml ether-ethanol solution (2:1, v/v) at 3000 rpm for 1–2 min, then centrifuged at a speed of 4000×*g* for 5 min and the supernatant collected. The operation was repeated once as described above. Ten milliliters of supernatant was drawn off into a Erlenmeyer flask, using 1% phenolphthalein as an indicator; the mixture was titrated with potassium hydroxide solution (0.05M) (Zhao et al. 2009). The free fatty acid content was calculated in 100 g fish muscle sample.

Carbonyl Value

Measurement of carbonyl value (COV) was based on a former method previously defined (Han et al. 2014) with little modification. Briefly, the crude lipid sample (0.0300–0.0500 g) was dissolved in 10 ml *N*-butanol (purified using reflux heating). One milliliter of this solution was mixed with the same volume of 0.05%, 2, 4-dinitrophenylhydrazine, then heated in 60 °C water bath for 20 min. The solution was cooled down to room temperature then 8 mL 2.5% potassium hydroxide-*n*-butanol was slowly added and centrifuged at speed of 3000×*g* for 5 min. The supernatant was collected and measured with UV-VIS at 420 nm. The carbonyl value was expressed as milliequivalents of carbonyl compounds per kilogram of lipid.

Fatty Acid Composition Analysis

Fatty acid methyl esters (FAMEs) were prepared using KOH in methanol. The determination of the fatty acid composition was performed using Agilent 7890A gas chromatography/mass spectrometer (GC-MS) system (Agilent Technologies, CA, USA). The separation was achieved using a column Thermo TG-5MS (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness; Waltham, MA, USA). After holding at 80 °C for 1 min, the temperature was gradually raised to 200 °C at the speed of 10 °C/min, to 225 °C with 5 °C/min, and to 250 °C with 2 °C/min, maintained for 5 min, with the injector at 290 °C. Using nitrogen as the carrier gas, the flow rate was set at 1.20 mL/min and the sample volume injected was 1 μL. The standard FAMEs were run under the same conditions. FAMEs in samples were identified by comparing with the retention times of standard FAMEs, and fatty acid compositions were expressed as the area percentage of total FAMEs.

Protein Oxidation Products

The fish meat sample (1.00 g) was homogenized with 20 ml buffer solution (containing 0.60 M NaCl, 0.05 M NaH_2PO_4-Na_2HPO_4, pH = 7.2) at 10,000 rpm for 30 s. The mixture was centrifuged for 10 min at a speed of 10,000×*g* (Xiong et al. 2016). The protein content in supernatant was determined by the Lowry method.

Protein Carbonyls

Two milliliters of 10 mM 2, 4-dinitrophenylhydrazine (dissolved in 0.5 M phosphoric acid) was mixed with 2 mL protein solution and incubated for 10 min at room temperature. Then, 1 mL 6.0 M sodium hydroxide solution was added, to react at room temperature for another 10 min. Absorbance of the solution was measured at 450 nm (Mesquita et al. 2014). Content of the protein carbonyls was calculated using the molar extinction coefficient of 22,308 M^{-1} cm^{-1}.

Sulfhydryl Content

The mixture of 4.5 mL 20 mmol/l Tris–hydrochloric acid buffer (containing 12% sodium dodecyl sulfate, 10 mM EDTA and 8 M urea, pH = 6.8), 0.5ml Ellman solution (0.1% 2-nitrobenzoic acid dissolved in Tris–hydrochloric acid buffer, pH = 6.8) and 0.5 mL protein solution was placed in 40 °C for 25 min.The absorbance of the sample solution was measured at 412 nm. The content of sulfhydryl was calculated using the molar extinction coefficient of 13,600 M^{-1} L cm^{-1}.

Color Measurement

The color was determined with colorimeter (Shang Guang WSC-S, Shanghai, China). The color was measured in three different places on both sides of each steak. L* (brightness), a* (redness -greenness), b* (yellowness -blueness) values were recorded. The whiteness (W) value was calculated using the following formula (Park 1995):

$$W = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Enzymatic Activities Assay

Crude Extraction of Endogenous Enzymes

The extraction of lipase was carried out following a former method (Smichi et al. 2013). The fish samples were homogenized with ten times volume of 25 mM Tris–HCl buffer (containing 150 mM NaCl, 2 mM benzamidine, pH = 8.0) for 3 × 10 s, then stirred with a magnetic bar (Thermo, Massachusetts, USA) at 4 °C for 45 min. The mixture was centrifuged for 20 min at 4 °C at 10,000×*g*. The supernatant was collected for lipase activity measurement.

The assay of lipoxygenase (LOX) activity was measured according to a former method (Gata et al. 1996). The sample was added to 50 mM phosphate buffer (containing 1 mM dithiothreitol, 1 mM EDTA, pH = 7.4) with the proportion of 5 mL per 1 g fish. The mixture was homogenized at 12,000 rpm for 4 × 10 s, then stirred with a magnetic bar at 4 °C for 30 min. The suspension was centrifuged at 4 °C for 20 min at 10,000×*g*.

Enzyme Activity

The lipase activity was measured using the 4-NPB method (Kuepethkaew et al. 2017). The substrate solution was prepared by dissolving 15 mg 4-NPB into 5 mL isopropanol. The reaction was carried out by mixing into a tube 200 μL diluted lipase with 2.4 mL 100 mM Tris–HCl buffer (containing 0.5 % Triton X-100, 25 mM NaCl, pH = 8.0) and 200 μL substrate solution at 37 °C for 10 min. The absorbance was measured with UV-VIS at 410 nm, and one unit of enzyme activity was defined as the enzyme liberated per μmol of *p*-nitrophenol in 1 min.

Substrate solution was prepared by dissolving 140 mg linoleic acid in 10 mL distilled water, containing 180 μL Tween 20, the pH of the solution adjusted to 9.0 with 2M NaOH, and it was diluted with distilled water to a final volume of 50 mL. Subsequently, 200 μL crude enzyme was mixed with a citric acid buffer (2.4 mL, 50 mM pH = 5.5) in a tube. After incubation at room temperature for 5 min, 200 μL substrate solution was added to initialize the reaction. The absorbance at 234 nm after 1 min reaction was recorded. One unit of LOX was defined as an increase in absorbance at 234 nm of 0.001 per min under assay condition.

Statistical Analysis

The collected data were analyzed by two-way analysis of variance (ANOVA). Duncan's multiple-range tests were used to evaluate the differences between different groups. Statistical significance between mean values was calculated at a significance level of $P < 0.05$. All treatments were carried out in triplicate ($n = 3$) and the results are expressed as means ± standard. Analyses were carried out using IBM SPSS Statistics 19 software.

Results and Discussion

Effect of Chlorogenic Acid on Lipid Oxidative Stability during Grass-Carp Refrigeration

Changes of Conjugated Diene

The conjugated diene value is one parameter which is used to measure the primary stage of lipid oxidation process. Conjugated compounds represent degradation products of polyunsaturated fatty acids, such as linoleic acid and linolenic acid, which are important polyunsaturated fatty acids that exist in grass carp muscles. Figure 1 illustrates that the conjugated diene value increased continuously in all groups during 0–12 days of cold storage. It decreased in the control group after 12 days. The possible reasons for this phenomenon might be that hydroperoxide decomposed into secondary oxidation products such as aldehydes and ketones or formed into a polymer (Johnson and Kummerow 1957). The conjugated diene value

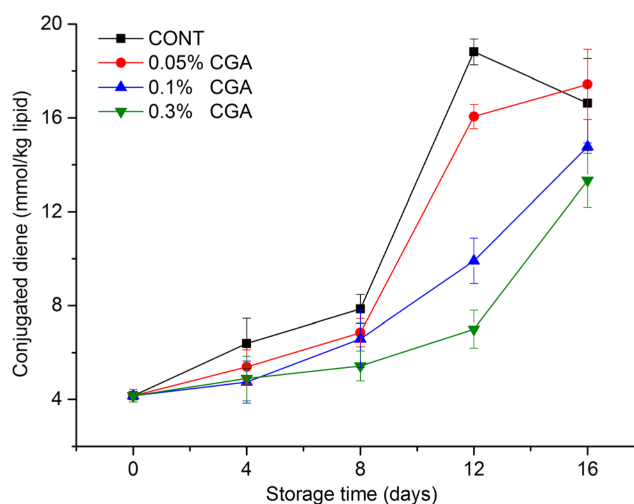

Fig. 1 Changes in TBARS value of grass carp treated with chlorogenic acid during chilled storage

in 0.3% CGA treated samples was significantly lower ($P < 0.05$) than that in the control group. Although there was no significant ($P > 0.05$) effect of CGA on inhibiting the conjugated value before 8 days, it remained significant ($P < 0.05$) during 8–16 days of cold storage. A similar tendency occurred in the conjugated diene value found during refrigerated storage of red drum fillets (Mi et al. 2016).

Changes of Peroxide Value

As an important index to evaluate the generation of lipid hydroperoxide, peroxide value (PV) is the main product in the primary phase of lipid oxidation. The changes of PV in all groups during refrigerated storage are shown in Fig. 2. The PV value was gradually increased during the12 days of storage in all groups, indicating that the lipid oxidation always exists even in low temperatures. The formation rate of hydroperoxide was faster than the decomposition rate during the early stage of storage, so hydrogen peroxide gradually accumulated. Concentration of hydroperoxide in the control group was significantly ($P < 0.05$) higher than that in the 0.1% or 0.3% CGA groups during 12 days. After 12 days of cold storage, the PV in the control and 0.05% CGA groups sharply declined, as the hydroperoxide might have decomposed into secondary oxidation products in the later stage of lipid oxidation (Chauhan et al. 2018). However, PV in the grass carp muscle treated with 0.1% and 0.3% CGA kept rising; thus, higher concentration of CGA may be delayed due to hydroperoxide decomposition. These results indicate that higher concentration of CGA showed better inhibitory effect on lipid oxidation. Similar results were found in minced Atlantic mackerel (*Scomberscombrus*) when water extract of *Polysiphonia fucoides* was added to inhibit the degradation of peroxide during refrigeration (Babakhani et al. 2016).

Changes of Thiobarbituric Acid Reactive Substances

The thiobarbituric acid reactive substances (TBARS) value is a key index to evaluate the lipid oxidation. The primary oxidation product of polyunsaturated fatty acids is hydroperoxide, and TBARS reflect the content of malonaldehyde (MDA), which is the secondary lipid oxidation product generated from the degradation of lipid hydroperoxide (Aubourg 1993). The TBARS values gradually increased in all groups during chilled storage (Fig. 3); the amount of MDA was significantly higher than the fresh fish after 16 days in all the samples ($P < 0.05$), which suggests that hydroperoxide was continually broken down to secondary products. Groups treated with CGA had lower TBARS values than the control group, and the content of MDA was decreased with the rise of chlorogenic acid concentration. This observation indicated that CGA could reduce the degradation of lipid hydroperoxide during chilled storage. During 16 days storage, although the TBARS values of the 0.05% CGA group showed no significant difference compared with the control group ($P > 0.05$), the 0.3% CGA group illustrated significant decrease in the TBARS values ($P < 0.05$). The content of MDA was significantly ($P < 0.05$) reduced from the 0.3% CGA group and the 0.1% CGA group after 8 days. That means CGA could slow down the lipid oxidation or peroxidation during grass carp storage. A similar finding was reported that pepper (*Zanthoxyoum Bungeanum maxim*) leaf extract which is rich in chlorogenic acid and quercetin could reduce TBARS values and inhibit the lipid peroxidation of salted silver carp during processing (Li et al. 2015).

The content of MDA in the control group was 0.62 ± 0.03 mg MDA/kg fish after 4 days, but for the 0.3% CGA treated group, it was decrease to 0.50 ± 0.01 mg MDA/kg fish after 12 days, which was even lower than the content in the control group after four days. Thus, it was demonstrated that higher concentrations of CGA

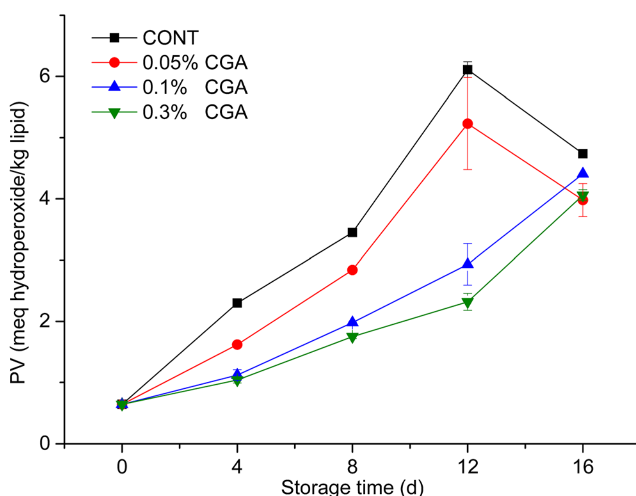

Fig. 2 Changes in PV of grass carp treated with chlorogenic acid during chilled storage

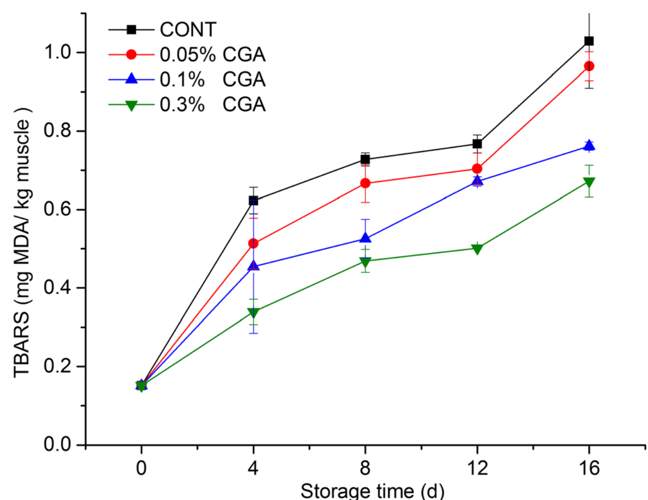

Fig. 3 Changes in FFA content of grass carp treated with chlorogenic acid during chilled storage

treatment could effectively limit lipid oxidation and extend the shelf life of grass carp during cold storage.

Changes of Free Fatty Acid

Free fatty acid is mainly obtained from the hydrolysis of glycerol fatty acid ester; in addition, the decomposition of unstable hydroperoxide can also produce some short-chain fatty acids (Qiu et al. 2014). Thus, free fatty acid (FFA) was selected as one key index to evaluate the degree of lipid oxidation in this study. The content of FFA was very low in fresh grass carp muscle and increased in all groups during storage (Fig. 4), indicating that grass carp fish fillet was continuously hydrolyzed. The FFA content of the control group was significantly ($P < 0.05$) higher than that of the 0.1% CGA and 0.3% CGA groups. Basically, the content of FFA increased from low concentration of CGA treatment to higher concentration during storage. A sharp increase in FFA content was observed in the control and 0.05% CGA groups during 4–8 days, but the same tendency to increase for the 0.1% CGA and 0.3% CGA groups occurred during 8–12 days. Results indicate that high concentration of CGA could affect the production of FFA. Similar results were reported in recent work (Miranda et al. 2018). In such study, the FFA content of lean fish progressively increased and the high concentration batch of *Bifurcaria bifurcata* alga extract treatment significantly retarded FFA formation during chilled storage. Previous studies showed that microbial activity also contributed to the increase of FFA content and was mostly responsible for lipid hydrolysis after 9 days of storage (Campos et al. 2012; Miranda et al. 2018). This indicates that CGA has effects on inhibiting microbial activities in grass carp fish muscle.

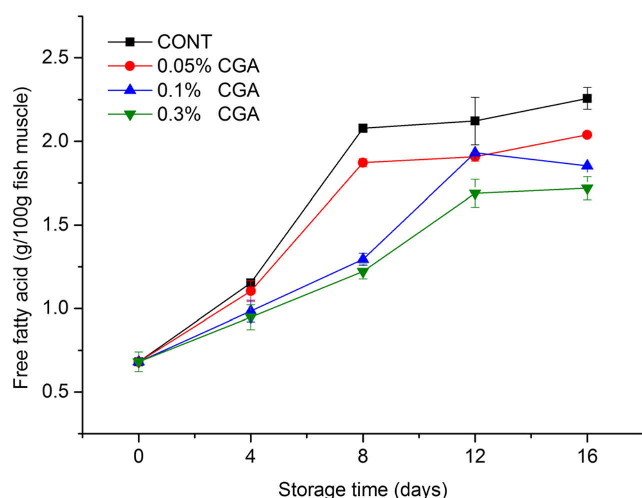

Fig. 4 Changes in COV of grass carp treated with chlorogenic acid during chilled storage

Changes of Carbonyl Value

The COV reflects the total amount of carbonyl compounds such as aldehyde and ketone, which are the crucial secondary products of lipid oxidation. Therefore, the COV is usually regarded as one of the important indexes for lipid oxidation. Results of COV measurement about the grass carp storage are shown in Fig. 5. It is clear to see that COV went up during 0–8 days in all groups, and it reached the peak value in the control group on day 8. The rise in COV due to the constant production of free fatty acids provided enough peroxide which can decompose carbonyl compounds (Li et al. 2016). The COV declined rapidly in the control group after the 8th day, but it increased in the treated groups during 8–12 days of storage. The COV peaked in the treated groups on the 12th day. Polymerization, degradation, or other reaction of carbonyl compounds in the late period of lipid oxidation led to the reduction in COV (Li and Huan 2017). The highest contents of carbonyl compound in 0.1% CGA (50.53 meq/kg lipid) and 0.3% CGA groups (36.65 meq/kg lipid) were significantly ($P < 0.05$) lower than that of the control group (69.76 meq/kg lipid). The results reveal that CGA treatment could evidently decrease the formation of carbonyl compounds to some extent.

Changes in Fatty Acid Profiles

Usually, fatty acid profiles are closely related to lipid oxidation, and changes in the content of different fatty acids in grass carp muscles might reflect the extent of lipid oxidation. Therefore, fatty acid profiles of fish muscle samples were assessed in this study. The changes of fatty acid composition in total lipid extracted from the CONT and 0.3% CGA groups are listed in Table 1. Fresh grass carp meat contained a high proportion of polyunsaturated fatty acid (PUFA), especially linoleic acid (C18:2n-6), eicosatetraenoic acid (C20:4n-6),

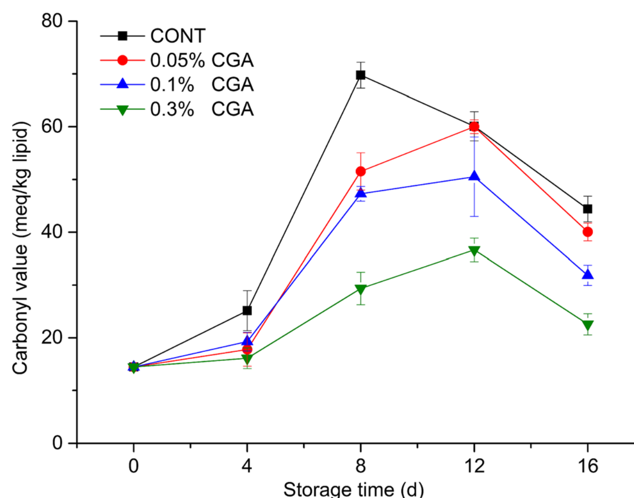

Fig. 5 Changes in conjugated diene value of grass carp treated with chlorogenic acid during chilled storage

Table 1 Fatty acid composition (%) of total lipids from grass carp meat during chilled storage

Fatty acid	Day 0	Day 4		Day 8		Day 12		Day 16	
		CONT	0.3% CGA	CONT	0.3% CGA	CONT	0.3% CGA	CONT	0.3% CGA
C12:0	0.03 ± 0.00cB	0.04 ± 0.00b	0.03 ± 0.00B	0.02 ± 0.00c	0.03 ± 0.00B	0.04 ± 0.00bc	0.05 ± 0.00A	0.07 ± 0.00a	0.05 ± 0.01A
C14:0	0.35 ± 0.01dB	0.43 ± 0.01d	0.31 ± 0.01C	0.40 ± 0.16c	0.36 ± 0.11C	0.95 ± 0.05a	0.83 ± 0.03A	0.69 ± 0.02b	0.86 ± 0.00A
C15:0	0.08 ± 0.00 aAB	0.07 ± 0.00a	0.07 ± 0.01B	0.08 ± 0.02a	0.06 ± 0.01C	0.10 ± 0.01a	0.10 ± 0.00A	0.12 ± 0.00a	0.10 ± 0.00A
C16:0	12.71 ± 0.53cC	12.70 ± 0.71c	11.58 ± 0.33D	14.87 ± 0.89b	13.63 ± 0.57C	17.30 ± 0.58a	15.77 ± 0.05B	17.77 ± 1.07a	16.86 ± 0.05A
C17:0	0.10 ± 0.00cB	0.07 ± 0.01d	0.08 ± 0.01C	0.07 ± 0.02d	0.06 ± 0.00D	0.13 ± 0.01b	0.13 ± 0.00A	0.18 ± 0.00a	0.13 ± 0.00A
C18:0	4.65 ± 0.55bB	4.10 ± 0.43b	4.56 ± 0.05B	4.22 ± 0.22b	3.50 ± 0.63C	4.67 ± 0.27b	4.75 ± 0.02B	6.21 ± 0.82a	5.08 ± 0.06A
C20:0	0.18 ± 0.01aA	0.10 ± 0.01b	0.11 ± 0.02C	0.13 ± 0.03ab	0.08 ± 0.01D	0.17 ± 0.00a	0.17 ± 0.01A	0.15 ± 0.03ab	0.15 ± 0.01B
C22.0	0.14 ± 0.00bC	0.16 ± 0.00a	0.17 ± 0.07A	0.05 ± 0.01c	0.04 ± 0.00D	0.13 ± 0.00b	0.18 ± 0.00A	0.17 ± 0.01a	0.15 ± 0.00B
C16:1n-7	2.38 ± 0.47abA	2.87 ± 0.26a	1.93 ± 0.03B	2.17 ± 0.48b	2.65 ± 0.08A	2.64 ± 0.43ab	2.36 ± 0.01AB	1.45 ± 0.03c	2.32 ± 0.07AB
C18:1n-9	22.60 ± 0.72aA	21.13 ± 1.07a	17.29 ± 0.33C	21.18 ± 2.06a	21.87 ± 0.30AB	20.84 ± 0.49b	19.08 ± 1.32BC	13.71 ± 1.93c	18.53 ± 0.06C
C20:1n-9	0.71 ± 0.05abB	0.43 ± 0.04c	0.54 ± 0.08C	0.60 ± 0.22b	0.51 ± 0.21C	0.92 ± 0.04a	0.94 ± 0.06A	0.70 ± 0.08b	0.83 ± 0.03B
C22:1n-9	5.31 ± 0.16eE	7.46 ± 0.24b	8.56 ± 0.19B	6.61 ± 0.22c	7.22 ± 0.67D	5.83 ± 0.23d	9.65 ± 0.05A	9.66 ± 0.19a	7.69 ± 0.18C
C24:1n-9	0.22 ± 0.05bB	0.23 ± 0.01a	0.36 ± 0.01A	0.08 ± 0.01d	0.09 ± 0.02D	0.12 ± 0.01c	0.18 ± 0.01BC	0.21 ± 0.01ab	0.16 ± 0.01C
C18:2n-6	12.85 ± 0.88aA	13.05 ± 1.01a[a]	9.77 ± 0.79B	14.12 ± 1.42a	13.98 ± 0.33A	11.45 ± 0.34b	10.71 ± 0.96B	9.73 ± 0.89c	10.58 ± 0.25B[a]
C18:3n-3	1.12 ± 0.38bB	0.89 ± 0.31c	1.04 ± 0.15B[a]	1.27 ± 0.29c	1.20 ± 0.29B	3.55 ± 0.05a[a]	3.05 ± 0.18A	2.80 ± 0.15b	3.17 ± 0.06A[a]
C18:3n-6	0.27 ± 0.00aA	0.22 ± 0.01b	0.20 ± 0.01B	0.21 ± 0.07b	0.20 ± 0.06B	0.14 ± 0.01c[a]	0.11 ± 0.02C	0.08 ± 0.01d	0.12 ± 0.00C[a]
C20:2	0.63 ± 0.00aA	0.60 ± 0.01a	0.64 ± 0.06A	0.51 ± 0.06b	0.46 ± 0.00C	0.48 ± 0.06b	0.50 ± 0.04B	0.45 ± 0.05b	0.49 ± 0.03BC
C20:3n-3	0.12 ± 0.00bD	0.09 ± 0.00c	0.20 ± 0.01C[a]	0.11 ± 0.01b	0.09 ± 0.01E	0.39 ± 0.01a	0.41 ± 0.00A	0.38 ± 0.01a	0.38 ± 0.02B
C20:3n-6	2.33 ± 0.20aB	2.41 ± 0.12a	2.74 ± 0.19A[a]	2.09 ± 0.23a	1.86 ± 0.32C	1.16 ± 0.63b	1.23 ± 0.05D	1.41 ± 0.06b[a]	1.25 ± 0.07D
C20:4n-6	17.78 ± 1.23aA	18.69 ± 1.63a	19.90 ± 1.25A	18.39 ± 1.46a	18.36 ± 0.64A	16.97 ± 1.34a	17.50 ± 1.12A	19.08 ± 0.37a[a]	18.04 ± 0.2A
C20:5n-3	1.42 ± 0.02abB	1.36 ± 0.01ab	2.11 ± 0.21A[a]	1.53 ± 0.42ab	1.42 ± 0.64B	1.15 ± 0.09b	1.18 ± 0.11B	1.62 ± 0.23a[a]	1.33 ± 0.00B
C22:2	0.21 ± 0.01bD	0.29 ± 0.00a	0.35 ± 0.02A	0.08 ± 0.00c	0.08 ± 0.01E	0.16 ± 0.01c	0.28 ± 0.01B	0.29 ± 0.00a	0.22 ± 0.00C
C22:6n-3	13.80 ± 0.42aB	12.60 ± 0.88a	17.43 ± 0.70A[a]	11.21 ± 1.29a	12.26 ± 1.10C	10.73 ± 0.86a	11.85 ± 1.48C	13.09 ± 2.68a	11.50 ± 0.30C
ΣSFA	18.24 ± 0.01dB	17.68 ± 0.63d	16.93 ± 0.73C	19.84 ± 0.88c[a]	17.76 ± 0.06BC	23.48 ± 0.82b[a]	21.98 ± 0.12B	25.35 ± 1.59a[a]	23.38 ± 0.00A
ΣMUFA	31.22 ± 1.89aA	32.12 ± 0.14a[a]	28.69 ± 0.91A	30.63 ± 3.59a	32.34 ± 4.97A	30.34 ± 0.80a	32.20 ± 1.46A	25.73 ± 2.26b	29.53 ± 0.32A[a]
ΣPUFA	50.54 ± 1.90aB	50.20 ± 1.88a	54.39 ± 2.21A	49.52 ± 4.32a	49.90 ± 5.00B	46.17 ± 0.02a	45.82 ± 1.58C	48.93 ± 2.21a	47.09 ± 0.32C
Σn-6	33.22 ± 0.74bB	34.37 ± 0.68a	32.61 ± 1.06B	34.81 ± 0.89a	34.40 ± 1.81A	29.71 ± 0.62c	29.56 ± 0.20C	30.30 ± 1.2c	29.99 ± 0.52C
Σn-3	16.47 ± 1.13aB	15.94 ± 0.45a	20.79 ± 1.21A[a]	14.12 ± 3.46a	14.97 ± 1.27C	15.82 ± 0.70a	15.48 ± 1.41AB	17.89 ± 3.06a	16.39 ± 0.22AB

Different small letters in the same row represent significant differences ($P < 0.05$) between means in the CONT group

Different capital letters in the same row represent significant differences ($P < 0.05$) between means in 0.3% CGA group

[a] Difference ($P < 0.05$) between the CONT group and 0.3% CGA group at the same day

and DHA (C22:6n-3). Palmitic acid (C16:0) and oleic acid (C18:1n-9) were dominant in saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA), respectively.

The concentration of total SFA increased during storage, as well as the percentages of C16:0 and stearic acid (C18:0). The content of total SFA was significantly ($P < 0.05$) lower in fish muscle treated with 0.3% CGA after 8 days of preservation. The content of C16:0 in the control group was also higher than in the CGA-treated group during 16 days of cold storage. These results indicate that CGA was efficient in retarding the SFA formation. A decrease was observed in the concentration of SFA during 0–4 days, and a similar phenomenon was found in a previous report, that total SFA content decreased slightly during 4-day cold storage of mussel (*Mytilus edulis*) and meat (Zhou et al. 2019).

It is well known that cis-MUFA has an obvious effect on the decrease of cholesterol. The amount of total MUFA declined during 12–16 days of storage in the CONT; however, there was no change ($P > 0.05$) in the 0.3% CGA group during the entire cold storage period. The proportion of dodecanoic acid (C22:1n-9) increased in both groups and was higher in the CGA-treated samples. The results demonstrate that CGA had an effect on maintaining the content of total MUFA and to some extent prevented MUFA from oxidative reactions.

PUFA is expected to be the easiest oxidized component, and the content of PUFA decreases due to lipolysis, autoxidation, and catalysis reactions that occur during the preservation process. PUFA in the grass carp muscle is primarily comprised of long-chain n-3 PUFA and n-6 PUFA, which are essential for human health. In this study, the content of total PUFA in grass carp muscle treated with 0.3% CGA increased during 0–4 days; no obvious change was found in the control group. However, the amount of PUFA in the 0.3% CGA group decreased significantly ($P < 0.05$) during 4–16 days of preservation, but that in the control group remained unchanged ($P > 0.05$). The percentage of total PUFA indicated no significant ($P > 0.05$) difference between the two groups throughout the storage. Putri Widyanti reported that C18:2n-6 and linolenic acid (C18:3n-3) can transform into long-chain PUFAs through the enzymatic effect, which may explain the increase in some kinds of fatty acids (Harlina et al. 2019). The changes in proportions of C18:2n-6, C18:3n-3, EPA, and DHA demonstrated that the inhibitory effect on PUFA oxidation of CGA was significant ($P < 0.05$) during 0–4 days.

The proportion of n-6 PUFAs/n-3 PUFAs in fresh fish was 2.02. Proportions in fish treated with and without CGA were 1.83 and 1.69 on the 16th day. This indicates that n-6 PUFAs degraded faster than n-3 PUFAs during the period of chilled storage.

Effect of Chlorogenic Acid on Protein Oxidation

There is a relationship between lipid oxidation and protein oxidation during the food preservation system. Primary and secondary products of lipid oxidation may accelerate protein oxidation, and protein oxidation can also mediate lipid oxidation (Choe et al. 2017). Therefore, the effect of CGA on protein oxidation is a supplementary explanation to the assessment of CGA as a potential inhibitor of lipid oxidation.

Carbonyl content is one of the most frequently used indicators for evaluating protein oxidation. Some amino acids, such as arginine, histidine, lysine, and proline, generate carbonyl compounds through oxidative degradation. Moreover, the production of carbonyl compounds has bad effects on the quality of meat (Babakhani et al. 2015). Figure 6 a shows the changes in protein carbonyls in the groups with different concentration of CGA treatment. After 16 days of storage, the carbonyl content increased significantly ($P < 0.05$) in all groups, and the carbonyls of all the CGA-treated groups were

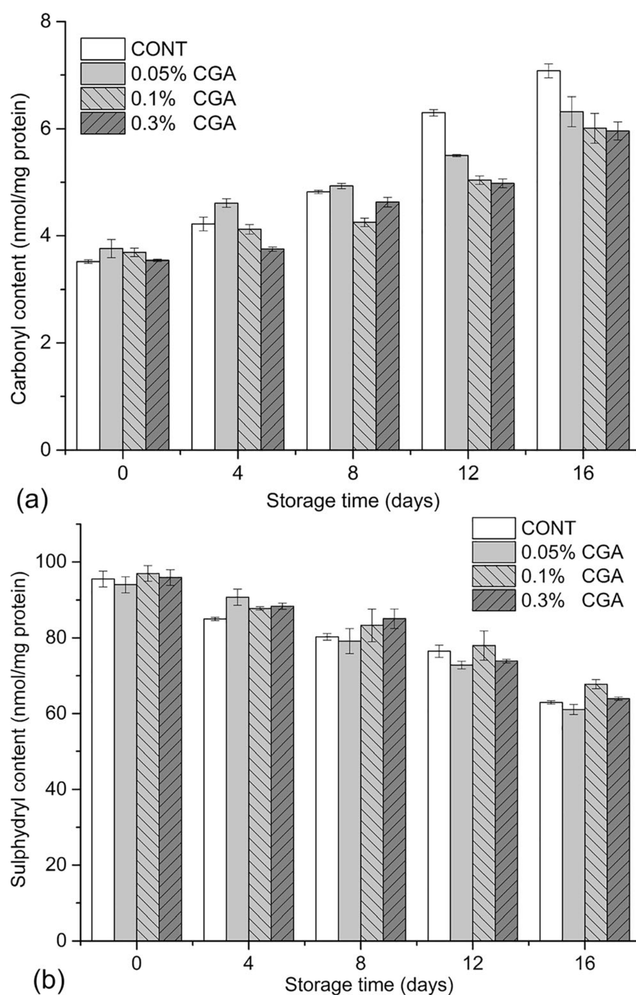

Fig. 6 Changes in protein oxidation of grass carp treated with chlorogenic acid during chilled storage. **a** Carbonyl content. **b** Sulfhydryl content

significantly ($P < 0.05$) lower than the control group. The results show that protein oxidation occurred in grass carp muscle precisely during cold storage, and CGA was efficient in retarding amino acid decomposition and protein oxidation. However, during 16 days of the storage period, the 0.3% CGA group did not show a better inhibitory effect on the generation of carbonyl compounds than the 0.1% CGA group, which did not coincide with the results of lipid oxidation.

Sulfhydryl content is used to evaluate cysteine oxidation, which induces the formation of intermolecular disulfide bridges. The loss of sulfhydryl content has been used as an important index of protein oxidation in aquatic products. Sulfhydryl group content declined sharply for all samples in 16 days storage, as shown in Fig. 6b, indicating that the sulfhydryl group oxidized during storage. The reason might be that sulfhydryl converted into disulfide bonds and other oxidation products preceding protein oxidation (Dean et al. 1997). The greatest decrease in sulfhydryl was observed in the control group (from 95.48 to 62.59 nmol/mg protein); total sulfhydryl content in the 0.1% and 0.3% CGA-treated samples was significantly higher than the control group ($P < 0.05$). No difference was found between the 0.3% and 0.1% CGA groups during 0–8 days, the sulfhydryl content was even higher in the 0.1% CGA-treated samples after 8 days of storage. This suggests that the ability to inhibit protein oxidation cannot be improved with the increase of CGA concentration. These results correspond with those in carbonyl content.

The previous dipping treatment of CGA may form a coating on the grass carp surface, while the antioxidant activity and the antimicrobial property of polyphenol provide the fish with good protection from deterioration during chilled storage. There are other studies that reached a similar conclusion (Miranda et al. 2018; Wu et al. 2016).

Effect of Chlorogenic Acid on Color Stability

Color is one of the important criteria for consumers to evaluate meat quality and influence consumption desire. The effect of CGA on color changes of fish muscle are shown in Table 2. At the beginning of storage (day 0), no difference ($P > 0.05$) was found between the control group and the CGA-treated groups in whiteness (W) value. After 16 days storage, the W value significantly ($P < 0.05$) increased in all groups, because of the reduction of water retention and adhesion of moisture on the surface of fish muscle. At the same time, the L* value of fish samples rose, which was affected by the degree of protein oxidation. Compared with fresh fish muscle, a* value ($P < 0.05$) significantly decreased in all groups after 16 days, while

Table 2 Measurement of color stability in grass carp during chilled storage

Parameters	Storage time (days)	Sample			
		CONT	0.05% CGA	0.1% CGA	0.3% CGA
L	0	50.39 ± 0.43aB	50.82 ± 0.73aC	50.53 ± 0.32aC	50.19 ± 0.74aC
	4	50.93 ± 1.06bB	51.35 ± 0.66bBC	52.23 ± 0.57aB	52.85 ± 0.41aAB
	8	51.76 ± 0.85cA	52.77 ± 1.18abA	53.63 ± 0.60aA	52.05 ± 0.82bcB
	12	52.30 ± 0.59bA	52.28 ± 0.53bA	53.67 ± 0.70aA	52.60 ± 0.51bB
	16	52.01 ± 0.41bA	52.14 ± 0.55bAB	52.40 ± 0.58bB	53.54 ± 0.99aA
a*	0	0.08 ± 0.23aA	0.51 ± 0.37aA	0.67 ± 0.23aA	0.24 ± 0.09aA
	4	0.15 ± 0.16aA	− 1.16 ± 0.14bB	− 1.10 ± 0.24bC	− 1.23 ± 0.11bB
	8	− 0.56 ± 0.28abB	0.08 ± 0.31aA	− 0.58 ± 0.24abB	− 1.12 ± 0.04bB
	12	− 1.20 ± 0.21aC	− 1.39 ± 0.22aB	− 1.77 ± 0.32bD	− 1.02 ± 0.14aB
	16	− 1.55 ± 0.43bC	− 1.22 ± 0.25aB	− 1.03 ± 0.25aC	− 1.16 ± 0.16aB
b*	0	− 0.77 ± 0.29aB	− 0.69 ± 0.16aAB	− 0.63 ± 0.05aA	− 0.94 ± 0.29aB
	4	0.56 ± 0.26aA	− 0.38 ± 0.21bA	− 0.53 ± 0.17bA	− 0.84 ± 0.20bAB
	8	0.28 ± 0.45aA	− 0.22 ± 0.14aA	− 0.08 ± 0.26aA	− 0.01 ± 0.34aA
	12	− 1.18 ± 0.25aBC	− 0.95 ± 0.06aAB	− 0.93 ± 0.57aA	− 0.79 ± 0.21aB
	16	− 1.86 ± 0.36bC	− 1.78 ± 0.41bB	− 0.81 ± 0.34aA	− 1.18 ± 0.29bB
W	0	50.38 ± 0.70aB	50.80 ± 0.54aC	50.52 ± 0.68aC	50.16 ± 0.69aC
	4	50.92 ± 0.89bB	51.23 ± 0.65bBC	52.20 ± 0.53aB	52.82 ± 0.49aAB
	8	51.74 ± 0.74aA	52.75 ± 0.94aA	53.62 ± 0.78aA	52.03 ± 0.57aB
	12	52.26 ± 0.79bA	52.24 ± 0.86bA	53.61 ± 0.76aA	52.56 ± 0.67bB
	16	51.95 ± 0.62bA	52.10 ± 0.74bAB	52.36 ± 0.43bB	53.40 ± 0.57aA

Different small letters within the same row represent significant differences ($P < 0.05$) between groups. Different capital letters within the same column represent significant differences ($P < 0.05$) between days

b* value showed no difference ($P > 0.05$) in CGA-treated groups. The results indicate that the color of fish muscle turned from reddish to greenish during cold storage; this phenomenon might be due to the oxidation of myoglobin pigment in grass carp muscle (Zakaria and Sarbon 2018). The W values in the higher CGA concentration-treated groups were significantly ($P < 0.05$) increased than those in the control group at the end of storage. Chlorogenic acid improved the color of grass carp muscle, probably because polyphenols distorted or broke protein covalent bonds, and to a certain extent, the structure of protein got denatured and agglutinated (Feng et al. 2016). Similar changes in luminosity and red color were found in chilled minced pork coated with chitosan essential oil films, which had good oxygen barrier capacity (Bonilla et al. 2014). The whiteness of hake (*Gadus*) also increased after 7 days of conventional storage (0.1 MPa, 5 °C) (Otero et al. 2017).

Effect of Chlorogenic Acid on Enzyme Activity

Lipase and LOX are two kinds of active endogenous enzymes which are involved in lipid oxidation during fish preservation. The actions of lipase and LOX are closely associated with biochemical reactions in lipid oxidation, such as the hydrolysis of triglyceride and the formation of hydroperoxide (Fidalgo et al. 2015). In order to obtain a more comprehensive understanding of the inhibition of CGA to lipid oxidation, the changes in endogenous lipase and LOX activities of fish samples were measured as illustrated in Fig. 7.

From Fig. 7a, it is shown that the lipase activities of both groups increased ($P < 0.05$) during 0–4 days and declined during 4–16 days on the whole. A similar tendency was also reported in a former study (Zhou et al. 2019). The lipase activity of the 0.3% CGA-treated group is significantly ($P < 0.05$) lower than the control during 8 days of storage. There were no differences in lipase activities between CGA treated and control groups from days 12 to days 16 of storage. The results indicate that CGA can induce lipase activity in fish muscle to go down which maybe one of the main reasons of its inhibitory effect on lipid oxidation. The changes in LOX activities of fish muscle during storage are depicted in Fig. 7b. The LOX activity increased significantly ($P < 0.05$) during 0–4 days in the control group; however, it showed no difference ($P > 0.05$) in the 0.3% CGA-treated samples. The activity of LOX in the 0.3% CGA-treated group was significantly ($P < 0.05$) lower than that in the control group during 0–12 days of cold storage. Results demonstrate that CGA can be an effective inhibitor of lipid oxidation during grass carp refrigeration by decreasing LOX activity. With the effect of the oxidation progress, the lipid and fatty acids available might be limited for LOX, which explains why lipid oxidation products are not formed to a high level at the end of storage (Sae-leaw and Benjakul 2014). The trend of endogenous enzyme activities is confirmed with the results of parameters of lipid oxidation such as FFA and PV during storage.

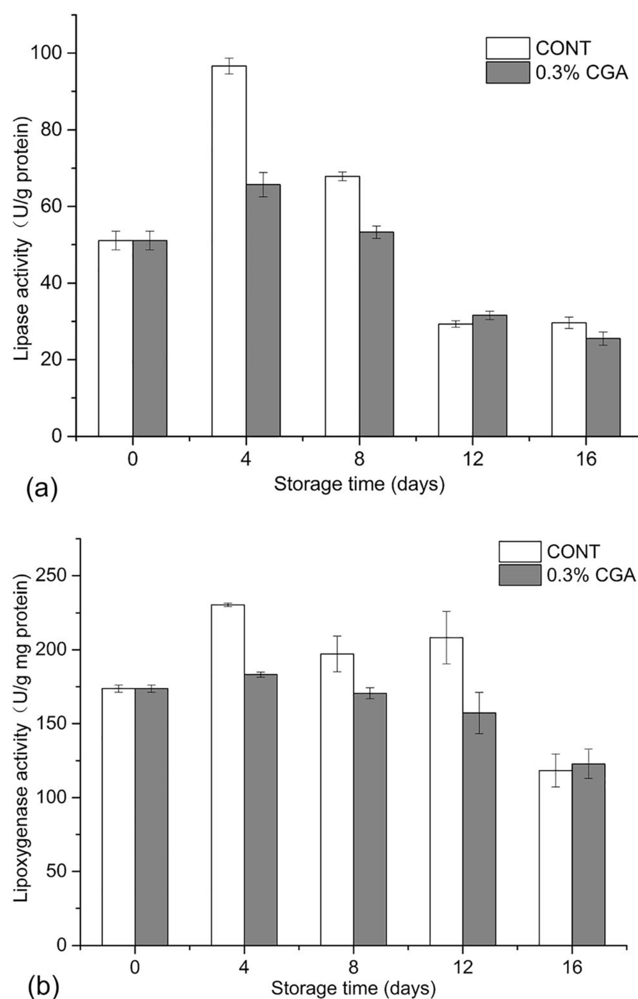

Fig. 7 Changes in **a** lipase and **b** lipoxygenase activities of grass carp supplement with chlorogenic acid during chilled storage

Conclusion

The present study investigated the inhibiting effect of CGA with different concentrations (0, 0.05%, 0.1%, and 0.3%) on lipid and protein oxidation in grass carp during chilled storage. The results showed that CGA has good efficiency in retarding further deterioration of grass carp and extended its shelf life during the storage period. According to the TBARS value, PV, COV, FFA content and CD content, the inhibition effect of CGA increased as the concentration rose; thus, 0.3% CGA provided the best fresh-keeping effect according to the formation of primary and secondary oxidative products. CGA treatment also prevented the increase of SFA and the decrease of MUFAs in grass carp muscle, but the degradation of PUFA

was less affected. The degree of protein oxidation in fish samples treated with 0.1% and 0.3% CGA was less than the control group. The significant enzyme activity decrease of lipase and LOX in CGA-treated groups provided useful information to our understanding of the mechanism inhibiting lipid oxidation of CGA during the storage period. Therefore, CGA as a novel natural additive could be used to inhibit lipid and protein oxidation and has good prospects to its application on aquatic products.

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