Antioxidant activities of young apple polyphenols and its preservative effects on lipids and proteins in grass carp (Ctenopharyngodon idellus) fillets

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ABSTRACT
The aim of this study was to study the antioxidant and antibacterial activities of young apple polyphenols (YAP) and to investigate the application of YAP in grass carp fillets (GCF) stored at 4°C for retarding lipid and protein degradation. The results indicated that YAP had high antioxidant activities in vitro, and the addition of YAP to GCF during low-temperature storage could significantly inhibit the microbial growth; retard the decrease in contents of fatty acids for both unsaturated and saturated ones; and minimize the degradation of salt-soluble myofibrillar protein and sulfhydryl group. The profiles of GCF in the presence and absence of YAP under a scanning electron microscope indicated that YAP could protect GCF from destruction that was resulted from fish deterioration. Therefore, YAP may be developed as natural antioxidants to control lipid and protein degradation and to extend shelf life of freshwater fish fillets.

INTRODUCTION
Grass carp (Ctenopharyngodon idellus), as one of the four major Chinese carps, is one kind of favorite freshwater fish due to its enjoyable taste. It is rich in proteins and lipids, containing essential amino acids and fatty acids (Wu & Mao, 2008). In comparison with red meats, the content of fat in grass carp is lower and the main fatty acids are unsaturated ones that are beneficial to cardiovascular health. In addition, it contains plenty of selenium that has antiaging and anticancer activities. Due to its tender and soft taste and nutritional value, the consumption of grass carp is large, especially in China. For ease of production and processing, the fish is usually required to be stored at a low temperature (4°C or below −18°C). However, during storage some adverse changes may occur to fish. For instance, the lipids in grass carp are highly vulnerable to oxidation because of the relatively high content of polyunsaturated fatty acids, which may cause unpleasant odour, rancid taste and discoloration (Farvin, Grejsen, & Jacobsen, 2012). Besides, proteins may be degraded (denatured) through autoxidation, microbial action, and interaction with the compounds that are resulted from lipid oxidation, such as lipid peroxide. This leads to nutritional (amino acids) and functional (solubility and hydrophobicity) loss (Shi, Cui, Yin, Luo, & Zhou, 2014). To avoid lipid oxidation and protein denaturation, some synthetic or natural antioxidants have been applied in meats.

Butylated hydroxyl toluene (BHT) and butylated hydroxyanisole are two common synthetic preservatives which can delay or prevent food oxidation. However, the synthetic preservatives have been found the potential urinary bladder carcinogenesis at a high dose level (Hirose et al., 1998). Therefore, it is desirable to explore alternatives to synthetic phenolic antioxidants.

In recent years, plant extracts have been studied in preservation of meats. Phenolic extracts of olive have been found to enhance lipid stability in cooked beef and pork due to the antioxidant activity (DeJong & Lanari, 2009). It has been indicated that tea polyphenols dip treatment on silver carp could...
markedly retain its good quality characteristics and extend the shelf life during the iced storage (Fan, Chi, & Zhang, 2008). In addition, tea polyphenols could also be incorporated with chitosan film to make a promising material as a packaging film for extending the shelf life of pork meat patties (Qin et al., 2013). As it is necessary to thin blossom and fruits to increase the yield and improve the apple quality, there are approximately 1.6 million tonnes of thinned young apples being produced every year in China. These unripe fruits are usually discarded in apple groves. In fact, they are a kind of potential agricultural and food resource because the polyphenols content in unripe apples is around 10 times of that in ripe apples (Zheng, Kim, & Chung, 2012). Besides, the discarded young apples rot in the field as time goes on, increasing the soil acidity and thus disturbing the microbial community in grove soil. This, in turn, affects the growth of fruit trees. Therefore, it is valuable and necessary to collect these young apples to develop their application values.

Although the applications of polyphenols in preservation of meats or fishes have been reported, the effect of young apple polyphenols (YAP, as a new and plenty phenolic resource) on changes of grass carp lipids and proteins in terms of composition and content during storage at a low temperature needs to be further shed light on. The objective of this work is to study the preservative activity of YAP on grass carp fillets (GCF) during low-temperature storage through the analysis of composition and contents of fatty acids in the fillets as well as the measurement of biochemical properties of proteins. In addition, the microstructures of fish tissues were observed using a scanning electron microscope (SEM) to demonstrate additional evidence supporting the preservative effect of YAP.

Materials and methods

Preparation of YAP

The thinned young apples (Fuji, Nagafu 2) were collected 30 days after blossoming in Liquan (April, 2015), Shaanxi province, China. The harvest season of ‘Nagafu 2’ apples is in October each year. YAP was extracted and separated from thinned young apples using macroporous resin according to our previously reported method (Sun, Guo, Fu, Li, & Li, 2013). Briefly, 500 g of young apples were ground into 3–4 mm particles and extracted with 5000 mL of 60% ethanol solution at 65°C for 3 h. Then, the extraction solution was filtered and concentrated to remove ethanol in a rotary evaporator at 65°C. The concentrated aqueous solution was then centrifuged at 3500g for 20 min to obtain 3000 mL of supernatant clear extract. After that, the clear extract was loaded onto a glass column (45 × 600 mm) filled with X-5 resin at a flow rate of 1.0 BV/h. Then, 2 BV of distilled water was used to wash the resin column to rinse out impurities such as polysaccharides, proteins et al. After that, 3 BV of 70% ethanol solution was applied to desorb the phenolic compounds at a flow rate of 2.0 BV/h, and the desorption solution was collected and concentrated to remove ethanol, followed by lyophilization to obtain YAP powder. The extracting yield of YAP was 1.47% based on the weight of fresh young apples.

Preparation of GCF

Fresh grass carps (weight 732 ± 46 g; length 340 ± 27 mm) with healthy skins were purchased from a supermarket in Xi’an, China, and transported alive to the laboratory. The grass carps were slaughtered, scaled, gutted, and washed in distilled water, followed by boned and skinned before being sliced into fillets (around 150 × 60 × 10 mm, 100 ± 5 g). Then, the fish fillets were brined with different concentrations of YAP (0.01%, 0.03%, 0.05%, 0.07%, 0.10% (w/v)), 0.05% (w/v) vitamin C, and 0.05% (w/v) potassium sorbate solutions. The fillets brined with distilled water were used as control. The ratio of fillet to solution was 10:1 (g:ml). The fillets were packaged and sealed in ziplock bags and stored in refrigerator at 4°C for 0, 1, 2, 3, 4, 5, 6, and 7 sampling days. Triplicates of eight fillet groups including blank control, potassium sorbate, vitamin C, 0.01%, 0.03%, 0.05%, 0.07%, and 0.10% YAP solutions treatments were conducted.

Determination of phenolic compounds in YAP

The contents of total polyphenols and soluble tannins were determined using spectrophotometry (Del Bubba et al., 2009; Singleton, Orthofer, & Lamuela-Raventos, 1999). The high performance liquid chromatography (HPLC) procedure for determining the contents of individual phenolic compounds in YAP were conducted using a Dionex® HPLC system (P680, California, U.S.A.) equipped with a Dionex® UV-VIS detector (UVD170U, California, U.S.A.) and an Agilent® C18 column (250 × 4.6 mm I.D., 5 μm) (California, U.S.A.). Elution with solvent A (methanol) and solvent B (1% trifluoroacetic acid) in a step gradient way at a flow rate of 1.0 mL/min was carried out as follows: 0–30 min, 90–75% B; 30–80 min, 75–50% B; 80–95 min, 50–90% B. During the run, the detection wavelength was 280 nm, and the injection volume was 20 µL.

Determination of antioxidant activities of YAP

The antioxidant activities of YAP were demonstrated using three radicals-scavenging methods, including 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), superoxide and hydroxyl radicals (Banerjee et al., 2012; Chun, Kim, & Lee, 2003), as well as the anti-lipid peroxidation assay (Wang, Zhao, Sun, & Yang, 2014).

Microbiological analysis

The microbiological analysis was performed according to the method provided previously (Qin et al., 2013) with some modifications. Respective total amount of 15 g of fish sample brined with distilled water, 0.05% vitamin C, 0.05% potassium sorbate, and 0.05% YAP at each sampling day was collected into 100 mL of sterile 0.1% peptone solution (w/v). The fillets were homogenized for 3 min using an IKA® homogenizer (T25 digital ULTRA-TURRAX®, Germany), followed by 10 fold dilutions using sterile 0.1% peptone solution. An aliquot of 0.1 mL of each dilution was plated onto standard plate count agar. The plates were incubated at 37°C for 48 h. All the microbiological counts were expressed as the colony forming units per gram of sample (CFU/g).

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of grass carp was conducted as follows: respective fish sample brined with distilled water, 0.05%...
YAP, and 0.10% YAP at 0, 3, and 7 days was collected and then smashed to surimi using a grinder (JYL-C051, Jujiang Co., Xi’an China). After that, 100 g of respective surimi sample was taken into a beaker containing 200 mL of methanol and 200 mL of chloroform, and then mixed thoroughly. 50 mL of distilled water was added to the mixture after 30 min, followed by centrifuged at 5000g for 10 min. The chloroform phase was retained and then evaporated to remove chloroform and obtain fish oil. 0.2 g of obtained fish oil was withdrawn into a 5 mL centrifuge tube and dissolved with 2.0 mL of n-hexane. Next, 2 mL of 2 M KOH–methanol solution was added to the tube and mixed rapidly. The mixture was then centrifuged at 5000g for 10 min, and the supernatant was collected for GC-MS analysis.

The GC chromatograms of the methyl-estered fish oil were measured using an Agilent® GC system (7890A, California, U.S. A.) coupled with an Agilent® MS system (5975C, California, U. S.A.) and a flame ionization detector (FID detector). A capillary column model for the esterified fatty acids was Rtx®-WAX (30 m × 0.25 mm×0.25 µm) (Shimadzu Co., Kyoto, Japan). The inlet temperature was set at 250°C. The temperature program was as follows: oven initial temperature maintained at 80°C for 2 min at first to 150°C at a heating rate of 5°C/min, then the temperature maintained at 150°C for 2 min before up to a final temperature 200°C at a heating rate of 5°C/min, the temperature 200°C maintained for 28 min. The injection volume was 1.0 µL, and the flowing rate of carrier gas (He) was 0.8 mL/min with the split rate of 100:1. In the mass spectrometer, impact with ionization (EI) mass spectra were recorded at 70 eV energy in full scan mode (35 ~ 500 m/z) to determine the absorbance value at 660 nm. Different concentrations of monopotassium phosphate were used for plot-determining the absorbance value at 660 nm. Different concentrations of monopotassium phosphate were used for plotting the standard curve.

**Table 1**

| Concentration | Antioxidant activity (AE) |
|---------------|---------------------------|
| 0.01%         | 0.78                      |
| 0.05%         | 0.82                      |
| 0.10%         | 0.84                      |

**Results and discussion**

**Antioxidant activities of YAP**

The major chemical compounds in YAP are shown in Table 2, and the individual contents are summarized in Table 1. The content of total polyphenols in YAP was 89.67%, in which soluble tannins accounted for 60.25%, followed by phlorizin (34.05%), epicatechin (11.97%), and chlorogenic acid (10.99%).

The antioxidant properties of YAP are shown in Table 2. The data indicated that YAP had a comparable antioxidant activity to vitamin C and a higher activity than BHT. Plant extracts abundant in polyphenols have been found to have antioxidant activities and the antioxidant potential is largely

**Table 2**

| Concentration | Antioxidant activity (AE) |
|---------------|---------------------------|
| 0.01%         | 0.78                      |
| 0.05%         | 0.82                      |
| 0.10%         | 0.84                      |

**Determination of protein degradation**

The extent of grass carp protein degradation was demonstrated by measuring the content of salt-soluble myofibrillar protein, the content of sulphydryl group, and the ATPase activity.

**Determination of salt-soluble myofibrillar protein**

Salt-soluble myofibrillar protein was extracted according to a previously reported method (Konno, YoungJe, Yoshioka, Shinho, & Seki, 2003) with some modifications. Specifically, 5 g of respective fish sample brined with each concentration of YAP at each sampling day was collected and homogenized with 60 mL of 0.1 M KCl and 0.2 mM Tris-HCl (pH = 7.5) mixture for 40 s, and then centrifuged at 12000 g for 20 min at 4°C. The final supernatant liquid was collected and protein concentration was determined using Bradford method (Kirazov, Venkov, & Kirazov, 1993) with bull serum albumin as standard curve.

**Determination of sulphydryl group**

The content of sulphydryl group was determined using a spectrophotometry (Shi et al., 2014). An aliquot of 1 mL of myofibrillar protein obtained was added to 8 mL of 0.2 M Tris-HCl buffer containing 8 M urea, 2% SDS and 10 mM EDTA. An aliquot of 8 mL of the mixture was mixed with 1 mL of DTNB (0.1%) containing 0.2 M Tris-HCl. The reaction mixture was incubated for 25 min at 40°C, and the absorbance was measured at 412 nm. The molar extinction coefficient of sulphydryl was 13,600 (M⁻¹·cm⁻¹).

**Determination of ATPase activity**

The activity of ATPase was determined using a colorimetric quantitative method (Ko et al., 2016) with some modifications. An aliquot of 1 mL of myofibrillar protein obtained was mixed with 8 mL of 10 mM CaCl₂ in 0.2 M Tris-HCl. 0.5 mL of 20 mM ATP solution was then added to the mixture. The reaction mixture was incubated for 8 min at 25°C before adding 5 mL of 15% trichloroacetic acid to stop the reaction. Next, the mixture was centrifuged at 3500g for 5 min. 2 mL of supernatant was withdrawn to a tube containing 1 mL of 3.5% ammonium molybdate (w/v, in 3 M sulfuric acid). Next, 0.2 mL of 0.25% hydroquinone (w/v, in 15% sodium bisulfite and 0.5% sodium sulfate) was added to the tube and diluted with distilled water to 5 mL. The reaction was mixed thoroughly and stored at room temperature for 1 h, followed by determining the absorbance value at 660 nm. Different concentrations of monopotassium phosphate were used for plotting the standard curve.

**SEM observation**

The microstructures of grass fillets were observed using a SEM (Hitachi Ltd, Tokyo, Japan). Respective fish sample brined with distilled water, 0.01%, 0.05%, and 0.10% YAP at 0, 2, 4, 6 days was collected and cut to a small piece of cuboid (1 cm×1 cm×0.5 cm). The fish sample was then soaked with glutaraldehyde and stored at 4°C for 24 h. Next, the soaked sample was rinsed with phosphate buffered saline (PBS) buffer thoroughly, followed by soaked with tertiary butanol for 2 h. Then, the sample was wiped dry using lens wiping paper carefully before lyophilized to remove water inside the sample. The lyophilized sample was then mounted on carbon adhesive discs attached on aluminum stubs and subsequently coated with a fine layer of gold (15 nm) for better SEM imaging quality. Each dehydrated sample was observed with 1200-fold magnification operated at an accelerating voltage of 20 kV and was photographed using a SEM Hitachi S-570 camera (Hitachi Ltd., Tokyo, Japan).

**Statistical analysis**

The data were presented as the mean ± standard deviations of duplicate measurements and evaluated by one-way analysis of variance (ANOVA) using SPSS 18.0 Statistics (SPSS Inc., Chicago, IL, U.S.A.). The mean values were compared using Dunnett’s t-test at the 95% significant level (P < 0.05)
Table 1. The contents of phenolic compounds in YAP.

| Phenolic compounds | Content (%)       |
|--------------------|------------------|
| Phlorizin          | 34.05 ± 0.25     |
| Epicatechin        | 10.99 ± 1.33     |
| Chlorogenic acid   | 2.22 ± 0.09      |
| Quercetin-3-galactoside | 6.510 ± 1.25 |
| Quercetin-3-rhamnoside | 4.140 ± 0.25 |
| Caffeic acid       | 0.95 ± 0.14      |
| Rutin              | 0.91 ± 0.04      |
| Soluble tannin     | 60.25 ± 2.55     |
| Total polyphenols  | 89.67 ± 2.10     |

Each value is a mean of duplicate analysis ± standard deviation.

Antibacterial activity of YAP

The effect of different additives on total microbial amount in GCF during the storage time is shown in Figure 1(a). All the additives had an antibacterial activity, while YAP showed better effect than vitamin C and potassium sorbate. The bacterial growth velocity in GCF with YAP was significantly slower than that in control. Polyphenols have been shown to interact with microbial proteins and inhibit enzymic activity, affecting microbial metabolism (Tenore, Campiglia, Rotini, & Novell no, 2013). In addition, there are several hydroxyl groups in polyphenols structures, which may chelate metal ions on cellular structures, forming sediment. The chelation can destroy cellular structures, and thus inhibit microbial reproduction (Pastene, Speisky, & Garcia, 2010).

Effect of YAP on composition and contents of fatty acids in GCF

Composition and contents of fatty acids in GCF

It has been reported that the unsaturated fatty acids have many benefits to humans, like hypolipidemic, lowering cholesterol level, and anti-atherosclerosis, etc. Also, it plays an important role in maintaining the integrity of cellular organs, such as mitochondrion and cytomembrane. The unsaturated fatty acids are the necessary nutrients that are essential to growth, development, and pregnancy for humans (Dhakal, Jung, Chae, Shannon, & Lee, 2014). In this study, the composition and contents of fatty acids in GCF were measured by use of GC-MS. In the fresh GCF, 15 types of fatty acids were detected totally, in which there were 5 types of saturated fatty acids. They were myristic acid, pentadecanoic acid, palmitic acid, daturic acid, and octadecanoic acid, accounting for 29.72% of total fatty acids (Table 3). The content of palmitic acid was determined as 24.05%, the highest amount in the saturated fatty acids (Table 3). There were 10 types of unsaturated fatty acids in GCF, including hexadecenoic acid, hexadecadienoic acid, heptadecatrienoic acid, oleic acid, linoleic acid, linolenic acid, eicosadienoic acid, mead acid, eicosatetraenoic acid, and docosahexaenoic acid. The total content of unsaturated fatty acids was 69.49%, much higher than that of saturated fatty acids (Table 3). Oleic acid, as one monounsaturated fatty acid, and linoleic acid, as one polyunsaturated fatty acid were shown to be two unsaturated fatty acids with the highest amount in GCF (32.35% and 26.18%, respectively).

Effect of YAP on composition and contents of fatty acids in GCF

Figure 2 describes the GC chromatograms of fatty acids in GCF which were pretreated with 0.05% and 0.10% YAP.
stored at 4°C for 3 and 7 days. Obviously from the chromatograms, the peak height and area in Figure 2(b,c) were smaller than that in Figure 2(d,e), especially for palmitic acid, oleic acid, and linoleic acid, indicating that higher concentration of YAP showed better preservation effects. After storage at 4°C, all the contents of individual fatty acids in GCF decreased with the storage time (3 and 7 days) (Table 3), as lipid oxidation occurred during the storage process (Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2008). YAP was shown to have strong antioxidant activities in vitro (Table 2), especially have a lipid peroxidation clearance activity; therefore, YAP may develop lipid-preservative ability through scavenging lipid free radicals that were formed through lipid oxidation in GCF during storage. Besides, the growth of aerobic bacteria also consumes lipid components for energy metabolism, which accelerates the degradation and oxidation of fatty acids (Yamauchi et al., 1990). As shown in Figure 1(a), YPA could inhibit the growth and reproduction of bacteria. By this way, the fish fillets could be well preserved in terms of avoiding the degradation of lipid caused by microbial metabolism. Specifically, the contents of three main fatty acids, palmitic acid, oleic acid, and linoleic acid, decreased by 47.90%, 60.06%, and 44.16% after 7 days storage (Table 3), respectively, compared to the fresh GCF, while for the GCF pretreated with 0.10% YAP, the contents declined only by 16.47%, 20.28%, and 17.49%, respectively (Table 3). Although 0.05% YAP could also delay the velocity of degradation of fatty acids in GCF, the extent of this retarding effect was weaker than 0.10% YAP. Generally, the amounts of total saturated fatty acids and total unsaturated ones both decreased gradually during the storage time, but the unsaturated fatty acids were more vulnerable, as the content of total unsaturated fatty acids decreased more after 7 days storage (Table 3). This is consistent with the fact that unsaturated fatty acids are more easily oxidized (Wood, Mnyusiwalla, Chen, & Johnston, 2000), especially for the polyunsaturated fatty acids, such as eicosatetraenoic acid and docosahexaenoic acid. Although the amounts of such polyunsaturated fatty acids in GCF is not high enough (Table 3), the oxidation of them in the initiation step generates free alkyl radicals which may promote the propagation of the free radical chains in the oxidation process of other fatty acids (Schulz & Kunau, 1987). After the addition of YAP in GCF, the decreasing extent of total saturated and unsaturated fatty acids during storage process both reduced (Table 3). Notably, there is no significant (*P* < 0.05) difference between fatty acids contents in GCF in the presence of 0.10% YAP after 3 days and that in the fresh GCF in the beginning (Table 3). Due to the existence of polyhydroxyl groups, YAP can donate hydrogen proton that can combine with the free radicals produced in the initiation step of lipid oxidation in GCF, which may temper or put on hold the free chain reaction (Schulz, 1991). Therefore, YAP can inhibit or retard the fatty acids oxidation. Conclusively, the mechanism in which YAP could retard the decrease of fatty acids in GCF during the storage time may include the antioxidant activity by scavenging the free radicals produced in the lipid oxidation, and the antibacterial activity by inhibiting the growth of some aerobic bacteria that may consume the lipid components in GCF.

**Effect of YAP on degradation of protein in GCF**

**Effect of YAP on content of salt-soluble myofibrillar protein**

Protein is an important component part of fish muscles. According to the differences in protein forms, solubility, and distribution, the proteins in fish muscles can be divided into three types: water-soluble myogen, salt-soluble myofibrillar protein, and insoluble myostomin (Hoke, Mcgeary, &
| Number | Fatty acids     | Molecular formula | Retention time (min) | Control   | 0.05% YAP | 0.10% YAP | 0.05% YAP | 0.10% YAP | 0.05% YAP | 0.10% YAP |
|--------|----------------|-------------------|----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1      | Myristic acid  | C_{14}H_{28}O_{2} | 24.54                | 1.68 ± 0.12a | 0.36 ± 0.01b | 0.03 ± 0.00c | 1.65 ± 0.14a | 1.07 ± 0.05b | 0.75 ± 0.04b | 1.64 ± 0.02a | 1.52 ± 0.54a | 1.19 ± 0.04b |
| 2      | Pentadecanoic acid | C_{15}H_{30}O_{2} | 29.72                | 0.24 ± 0.01a | 0.16 ± 0.00a | 0.07 ± 0.00b | 0.21 ± 0.02a | 0.21 ± 0.01a | 0.07 ± 0.00b | 0.22 ± 0.01a | 0.22 ± 0.01a | 0.19 ± 0.04b |
| 3      | Palmitic acid  | C_{16}H_{32}O_{2} | 30.56                | 1.65 ± 0.10a | 1.07 ± 0.05b | 0.75 ± 0.04b | 1.64 ± 0.02a | 1.52 ± 0.54a | 1.19 ± 0.04b |
| 4      | Daturic acid   | C_{16}H_{34}O_{2} | 31.62                | 0.22 ± 0.01a | 0.16 ± 0.00a | 0.07 ± 0.00b | 0.21 ± 0.02a | 0.21 ± 0.01a | 0.07 ± 0.00b | 0.22 ± 0.01a | 0.22 ± 0.01a | 0.19 ± 0.04b |
| 5      | Octadecanoic acid | C_{18}H_{36}O_{2} | 33.92                | 1.65 ± 0.10a | 1.07 ± 0.05b | 0.75 ± 0.04b | 1.64 ± 0.02a | 1.52 ± 0.54a | 1.19 ± 0.04b |
| 6      | Hexadecenoic acid | C_{16}H_{30}O_{2} | 34.67                | 3.53 ± 0.09a | 2.16 ± 0.02b | 1.89 ± 0.00c | 3.62 ± 0.07a | 2.25 ± 0.59b | 1.94 ± 0.04c | 3.64 ± 0.01a | 3.64 ± 0.01a | 2.66 ± 0.24b |
| 7      | Hexadecadienoic acid | C_{18}H_{34}O_{2} | 35.07                | 4.36 ± 0.01a | 2.45 ± 0.04b | 1.88 ± 0.01c | 4.33 ± 0.01a | 3.57 ± 0.69a | 2.07 ± 0.14b | 4.25 ± 0.01a | 4.06 ± 0.01a | 3.21 ± 0.04b |
| 8      | Heptadecatrienoic acid | C_{17}H_{28}O_{2} | 36.97                | 0.12 ± 0.01a | 0.06 ± 0.00b | 0.04 ± 0.00c | 0.06 ± 0.01a | 0.09 ± 0.02b | 0.05 ± 0.01c | 0.13 ± 0.01a | 0.11 ± 0.00a | 0.10 ± 0.03b |
| 9      | Oleic acid     | C_{18}H_{34}O_{2} | 37.25                | 0.22 ± 0.01b | 0.16 ± 0.00a | 0.07 ± 0.00b | 0.21 ± 0.02a | 0.21 ± 0.01a | 0.07 ± 0.00b | 0.22 ± 0.01a | 0.22 ± 0.01a | 0.19 ± 0.04b |
| 10     | Linoleic acid  | C_{18}H_{34}O_{2} | 37.64                | 1.65 ± 0.10a | 1.07 ± 0.05b | 0.75 ± 0.04b | 1.64 ± 0.02a | 1.52 ± 0.54a | 1.19 ± 0.04b |
| 11     | Linolenic acid | C_{18}H_{36}O_{2} | 39.49                | 4.36 ± 0.01a | 2.45 ± 0.04b | 1.88 ± 0.01c | 4.33 ± 0.01a | 3.57 ± 0.69a | 2.07 ± 0.14b | 4.25 ± 0.01a | 4.06 ± 0.01a | 3.21 ± 0.04b |
| 12     | Eicosatrienoic acid | C_{20}H_{36}O_{2} | 40.89                | 0.12 ± 0.01a | 0.06 ± 0.00b | 0.04 ± 0.00c | 0.06 ± 0.01a | 0.09 ± 0.02b | 0.05 ± 0.01c | 0.13 ± 0.01a | 0.11 ± 0.00a | 0.10 ± 0.03b |
| 13     | Mead acid      | C_{20}H_{34}O_{2} | 44.89                | 1.65 ± 0.10a | 1.07 ± 0.05b | 0.75 ± 0.04b | 1.64 ± 0.02a | 1.52 ± 0.54a | 1.19 ± 0.04b |
| 14     | Eicosatetraenoic acid | C_{22}H_{36}O_{2} | 47.24                | 4.36 ± 0.01a | 2.45 ± 0.04b | 1.88 ± 0.01c | 4.33 ± 0.01a | 3.57 ± 0.69a | 2.07 ± 0.14b | 4.25 ± 0.01a | 4.06 ± 0.01a | 3.21 ± 0.04b |
| 15     | Docosahexaenoic acid | C_{22}H_{36}O_{2} | 49.21                | 0.22 ± 0.01b | 0.16 ± 0.00a | 0.07 ± 0.00b | 0.21 ± 0.02a | 0.21 ± 0.01a | 0.07 ± 0.00b | 0.22 ± 0.01a | 0.22 ± 0.01a | 0.19 ± 0.04b |

Each value is a mean of duplicate analysis ± standard deviation. Different letters (a–c) for each fatty acid in the respective group (control, 0.05% YAP and 0.10% YAP) represent significantly different mean values ($P < 0.05$). Different letters (a–c) for each fatty acid in the three groups (control, 0.05% YAP and 0.10% YAP) at Day 7 represent significantly different mean values ($P < 0.05$). The content is the relative percent (%) in total fatty acids of respective fresh fish fillets at Day 0.
All the three types of proteins play an important role in fish processing, storage, flavor, and taste. Myofibrillar protein is the main component of muscular proteins, accounting for 55–70% in total protein content in fish muscles. Myofibrillar protein has a good solubility in neutral saline solution with the ironic strength of above 0.5. Based on this property, it can be extracted out and studied. The effect of different YAP additions on the content of salt-soluble myofibrillar protein in GCF during the storage time is shown in Figure 1(b). Obviously, 0.10% YAP could delay the decreasing process of myofibrillar protein content to a large extent. There are a few factors affecting the dissolution quantity of myofibrillar protein. Firstly, it is closely related to the denaturation of protein. For example, during storage at a low temperature, actomyosin may form insoluble aggregation of supramolecule, which eventually results in the decrease in solubility of myofibrillar protein (Yongsawatdigul & Park, 2003). The formation of disulfide bonds caused by oxidation of sulfhydryl groups during storage may lower the dissolution amount as well (Sompongse, Itoh, & Obatake, 1996). Besides, myofibrillar protein may be hydrolyzed by endogenous protease or microbial metabolism (Matsukura, Okitani, Nishimuro, & Kato, 1981). Polyphenols have been shown to have properties of protease inhibition (Goncalves, Mateus, Pianet, Laguerre, & de Freitas, 2011) and antibacterial (Rasooly, Do, & Friedman, 2010). Therefore, YAP may also develop the inhibitory potential against the endogenous protease in fish. Along with the antibacterial activity shown in Figure 1(a), the retarded decreasing velocity of myofibrillar protein dissolution quantity, therefore, may be attributed to the antioxidant, antibacterial, and inhibition of protease properties of YAP.

Figure 2. GC chromatogram of individual fatty acids in GCF in the absence (a) (Day 0), and presence of 0.05% YAP (b and c) and 0.10% YAP (d and e) (Day 3 for b and d, Day 7 for c and e). Three main fatty acids, including palmitic acid, oleic acid, and linoleic acid are labeled according to respective retention time. Other fatty acids and corresponding retention time are listed in Table 2.
Effect of YAP on ATPase activity

Myofibrillar protein is one conjugated protein that is composed of myosin, actin, troponin, and tropomyosin, in which myosin is the main component with the highest content (~54%) (Tikunov, Mancini, & Levine, 1996). The head part of myosin has the activity of ATPase that can decompose ATP, producing ADP and inorganic phosphate. The energy released is used in muscle contraction. It should be noted that during storage, myosin is easily affected by internal and external factors, especially lipid oxidation, which may cause protein degradation or denaturation. The extent of this adverse change can be demonstrated by the decrease in the activity of ATPase. Figure 1(c) showed the changes in the activity of ATPase of GCF in the absence and presence of different concentrations of YAP. During the storage time, the ATPase activity in the fillets without YAP decreased significantly. Especially, the activity decreased dramatically from Day 2 to Day 4, indicating that denaturation occurred to proteins (mainly myosin) to a large extent. For the fillets pretreated with YAP, the decrease in ATPase activity along with the storage time was significantly \( P < 0.05 \) retarded from Day 0 to Day 4, and this retarding effect showed a concentration-dependent relationship. As the fact that lipid oxidation is one important factor affecting the ATPase activity (Ahuja, Borchman, & Dean, 1999; Thanonkaew, Benjakul, Visessanguan, & Decker, 2006), and that YAP was shown to retard the fish lipid oxidation process (Table 3), the protective effect on ATPase activity by YAP may also be attributed to its antioxidation property.

Effect of YAP on content of sulfhydryl group

Figure 1(d) shows the content of sulfhydryl group in GCF in the absence and presence of different YAP additions during the storage time. Clearly, compared to the control group (GCF without YAP), 0.10% YAP could significantly \( P < 0.05 \) retard the decreasing process of sulfhydryl content. Commonly, during storage the sulfhydryl inside the protein structures is easily oxidized to the disulfide bonds, which leads to structure destruction of protein and corresponding decrease in sulfhydryl content (Shi et al., 2014). YAP could inhibit the oxidation of sulfhydryl due to its strong antioxidant activity, and this effect increased with YAP concentration increasing.

SEM profiles of GCF

The SEM profiles of GCF with and without YAP during storage process are shown in Figure 3. In the beginning
(Figure 3(a)), the organizational structure of muscle tissues arranged tightly; the surface was smooth; and the sarcomere was plump. After 2 days storage at 4°C, the surface of GCF without YAP became rough, and the texture became loose. The protein deterioration leads to the loss of cross-link between proteins and water molecules, and thus the muscular tissues may be destroyed. It is obvious in Figure 3(d) that there came into being some clear holes on the surface of fillets, indicating that it was spoiled without the protection of preservatives. A similar changing trend was observed for GCF pretreated with 0.01% YAP. After 6 days storage, there were still some holes symbolizing fillet deterioration. This means YAP with low concentration cannot access to the expected fresh-keeping effect. While 0.05% and 0.10% YAP could develop favorite preservation effect, which can be demonstrated by the fact that after storage at 4°C for 6 days, the fillets with both the two concentrations of YAP still had compact muscular structure and smooth surface, and no holes were observed (Figure 3 (l,p)). Therefore, the SEM profiles of GCF indicated that YAP are effective in delaying the softening of fish tissues and in inhibiting the degradation of muscle fibers, and thus have a strong preservation activity. In addition, YAP with higher concentration showed better preservation effect.

In this study, polyphenols from young apples, as a new kind of mixed phenolic compounds, was shown a preservation effect on GCF in terms of inhibiting the degradation of proteins and lipids. Therefore, YAP may have a potential of extending the shelf life of freshwater fish. On the other hand, as polyphenols with bitter and astrigent tastes (Lesscheave & Nobel, 2005), the taste of fillet in the presence of YAP should be further measured through sensory test to ensure that the introduction of YAP into fish fillets does not result in unpleasant taste. Besides, the hydroxyl groups of polyphenols have been reported to interact (bind) with the amino and sulfhydryl groups of protein molecules through hydrogen bonding and electrostatic interactions (Siebert, Troukhanova, & Lynn, 1996). This may change the microstructure of cross-link between protein and water molecules, inducing the change in textural properties of fish fillets. Therefore, further work is also to be done to analyze the effect of YAP on the textural characters by use of texture profile analysis.

Conclusion

YAP, with soluble tannins, phlorizin, chlorogenic acid, epicatechin, and quercetin glycosides as the main phenolic compounds, was shown to effectively scavenge oxyradicals in vitro and inhibit bacterial growth in GCF. The addition of YAP to GCF could significantly retard the degradation of fatty acids and proteins, protecting fish tissues from destructure and deterioration. The preservative effect of YAP on GCF may arise from its antioxidant and antibacterial properties. YAP, as a new potential phenolic extracts, may be used in preservation of freshwater fishes stored at a low temperature.

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