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Grape seed and clove bud extracts as natural antioxidants in silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage: Effect on lipid and protein oxidation

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ABSTRACT

The aim of this study was to investigate the utilization of clove bud extract (CBE) and grape seed extract (GSE) as natural antioxidants for retarding lipid and protein oxidation in silver carp fillets stored at 4 ± 1 °C. The results indicate that CBE exhibited higher total phenolic content, DPPH and Fe^{2+}-chelating activity than GSE. GSE and a 20-times dilution of CBE were found to be effective in retarding lipid and protein oxidation; both treatments resulted in low levels of PV and TBA, and protected against the decrease of L^*, a^*, salt-soluble protein content and total sulfhydryl group. CBE20 more efficiently inhibited lipid oxidation than did GSE. The antioxidant effect of the two extracts on protein oxidation was less pronounced than the effect on lipid oxidation. CBE20 and GSE could be used as natural antioxidants to minimize lipid and protein oxidation and to extend shelf life of fillets.

1. Introduction

With a harvest of 3,713,900 tons in 2011, silver carp (*Hypophthalmichthys molitrix*) is the most abundant freshwater fish in China. It is known to be rich in protein and fat (Li, Sinclair, & Li, 2011). Due to its attractive white colour and high nutritional value, silver carp are often used to produce ready-to-eat and high quality salt fish products, which are well suited for human consumption. In spite of these obvious benefits, one major obstacle in using silver carp is that they are highly susceptible to oxidation because of the relatively high content of polyunsaturated fatty acids. Lipid oxidation leads to unpleasant odour, rancid taste and discolouration (Farvin, Grejsen, & Jacobsen, 2012). Moreover, proteins can be modified by the compounds resulting from lipid oxidation, which leads to nutritional changes in amino acids and a decrease of protein functionality (solubility and hydrophobicity). Many attempts have been made to reduce lipid oxidation and pigment in meats through synthetic or natural antioxidants. Although synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) can markedly delay or prevent the oxidation of substrate, the possible carcinogenic effects of synthetic antioxidants in foods have been found (Lindenschmidt, Tryka, Goad, & Witschi, 1986; Shahidi, 2000). Therefore, there is a general desire to replace synthetic antioxidants with natural ingredients.

In recent years, application of plant extracts as natural antioxidants in meat products have been attempted by different researchers. The effect of kiam wood extract on the postponement of haemoglobin-mediated lipid oxidation of washed Asian sea bass mince was investigated by Maqsood and Benjakul (2013). The efficacy of potato peel ethanol extracts as an antioxidant in minced horse mackerel during chilled storage was observed by Farvin et al. (2012). Mitsumoto, O'Grady, Kerry, and Buckley (2005) showed that tea catechins were stronger natural antioxidants compared with vitamin C in cooked or raw beef and chicken patties during refrigerated storage. Mansour and Khalil (2000) found that addition of freeze-dried extracts from ginger rhizomes and fenugreek seeds to beef patties effectively controlled lipid oxidation and colour changes during chilled storage.

Grape seed is a waste by-product of the food industry and as such presents a cheap source of natural antioxidant due to its phenolic content (Spigno & De Faveri, 2007). The extracts contain bioactive phenolic compounds which have recently been recognized for their efficacy in providing significant antioxidant activity to human foods (Bucić-Kojić, Planinić, Tomas, Jakobek, & Šeruga,

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2009; Gibis & Weiss, 2012; Rosales Soto, Brown, & Ross, 2012). Clove, which is commercially cultivated in the south of China, is an important aromatic spice. Clove bud extracts have likewise been found to possess great antioxidant activity (Verrez-Bagnis, Ladrat, Noëlle, & Fleurence, 2002). Our numerous earlier studies have found that extracts of grape seeds and clove buds are highly efficient in reducing lipid oxidation in minced meat and fish oil. However, the antioxidative effect of grape seed and clove bud extracts in fish fillets had not yet been studied.

The overall objective of this study was to identify whether the addition of grape seed and clove bud extracts can retard both lipid and protein oxidation and extend the shelf life of silver carp fillets. In addition, this study provided greater insight into the potential of grape seed and clove bud extracts as natural and effective sources of antioxidants for fish processing.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of grape seed and clove bud extracts

Grape seeds (Merlot) were obtained from wineries located in Beijing, China, and clove buds were obtained from Xintong Co., Ltd., located in Shandong province, China. Both were dried at 50 ± 2 °C for 8 h and then ground into a fine powder.

Three extracts were obtained: grape seed extract (GSE), clove bud extract (CBE) and 20-fold dilution of CBE (CBE20). For preparation of GSE, 10 g of grape seed powder was added to 100 ml boiled distilled water and left for 3 h at room temperature; the extract was obtained by filtration through Whatman Grade No. 1 filter paper. The extract was collected in a separate bottle, and the residue (9.55 ± 0.25 g) was re-extracted twice under the same conditions as mentioned above. This extract was referred to as GSE. CBE was prepared in the same manner using 10 g clove bud powder (8.25 ± 0.25 g of residue was used for the re-extraction). In the case of CBE20, 1 ml CBE was diluted in 19 ml distilled water. The extract obtained was referred to as CBE20. These extracts were kept at −20 °C prior to analysis. All extracts were analyzed for total phenolic content, 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and Fe^{2+}-chelating ability.

2.1.2. Fish material preparation

A total of 60 silver carp (*H. molitrix*) weighing 274 ± 18 g each were purchased from a market in Beijing, China, and were transported alive to the laboratory. The fresh silver carp were slaughtered, scaled, gutted and washed in water, and each fish was divided into two pieces. The samples were divided into three groups. One group was brined with 1.0% salt (weight of distilled water + weight of fillet) (control); the second group was brined with a mixture of 1.0% salt (weight of distilled water + weight of fillet) + 2.0% GSE (weight of distilled water + weight of fillet) (T1); the third group was brined with a mixture of 1.0% salt (weight of distilled water + weight of fillet) + 2.0% CBE20 (weight of distilled water + weight of fillet) (T2). The fillet to distilled water ratio was 10:1. The fillets were packaged and sealed in polythene bags and stored in refrigerated incubators at 4 ± 1 °C for temperature equalization. Three random samples were taken from each group for analysis at each sampling time (0, 3, 6, 9, 12, 15 and 18 d).

2.2. Methods

2.2.1. Determination of total phenolics

Determination of polyphenol content was performed by the Folin–Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g) (Boligon et al., 2009). A quantity of 0.5 ml of 2 N Folin–Ciocalteu reagent was added to 1 ml of extract. After 5 min, 2 ml of 20% Na_2CO_3 was added to the mixture, which was incubated for 10 min at room temperature. Absorbance was measured at 730 nm.

2.2.2. Determination of chemical compounds in grape seed and clove bud extracts

2.2.2.1. HPLC analysis. The grape seed extract was filtered through a 0.22 μm membrane filter and analyzed using HPLC (Shimadzu, LC-10A Tseries, Japan) equipped with SPD-10A (V) detector, COSMOSIL 5C18-PAQ column (4.6 mm ID × 250 mm). Gradient elution programme was executed by varying the proportion of solvent A (2% acetic acid and 9% acetonitrile) and solvent B (80% acetonitrile). The gradient elution programme: 100% A (0 min) – 100% A (10 min) – 68% A (25 min) – 68% A (35 min) – 100% A (40 min). System was equilibrated 5 min before next analysis. Flow rate was 1 ml/min and column temperature was 30 °C. 50 μl of sample was injected. The peak was detected at 280 nm. Compounds were identified by the retention time of sample chromatographic peaks being compared with standards samples using the same HPLC operating conditions.

2.2.2.2. GC–MS analysis. The clove bud extract was analyzed using a 450-GC (Varian) coupled to a 220-MS (Varian) and equipped with a DB-5 column (30 m × 0.25 mm; 0.25 mm film). The carrier gas was helium, at a flow rate of 1 ml/min. Column temperature was initially 60 °C for 5 min, and then increased to 180 °C at the rate of 3 °C/min, and then to 250 °C at the rate of 10 °C/min. Injector temperature was 250 °C. 1 μl samples was injected by splitting.

2.2.3. Determination of Fe^{2+}-chelating ability

Determination of Fe^{2+}-chelating ability was performed according to the method described by Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, and Fernández-López (2013). The Fe^{2+}-chelating ability was measured in a reaction mixture containing the extract (1.0 ml), 99.5% ethanol (3.7 ml), 2 mM $FeCl_2$ (0.1 ml) and 5 mM ferrozine (0.2 ml). The mixture was left at room temperature for 10 min; absorbance of the resulting solution was measured at 562 nm. (A lower absorbance indicates a stronger Fe^{2+}-chelating ability.) The ability to chelate the ferrous ion was calculated as follows:

$$Fe^{2+}\text{-chelating ability } (\%) = \left(1 - \text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{control}}\right) \times 100$$

2.2.4. Determination of DPPH radical scavenging activity

DPPH assay was performed according to the method of Banerjee et al. (2012). The extract (1.5 ml) and 99.5% ethanol (1.5 ml) were mixed with 0.375 ml of 0.02% (w/v) 2,2-diphenyl-1-picrylhydrazyl in ethanol. The tubes were then incubated at room temperature for 60 min in the dark, and the absorbance was taken at 517 nm. The radical scavenging activity (RSA) was calculated by the following equation:

$$\text{RSA } (\%) = \left(1 - \text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{control}}\right) \times 100\%$$

2.2.5. Sensory evaluation of cooked and raw fish

Each fillet was placed in a plastic bag, which was sealed and steamed for 5 min. After 5 min the fillets were served to panelists and assessed. The samples were evaluated by nine trained panelists and the panelists were trained according to the International Organization for Standardization (1993). Cooked and raw fish