Original Paper

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Effect of dietary supplementation of garlic and vitamin E on lipid and protein oxidation in common carp meat during different storage times

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Keywords

Common carp, garlic, lipid oxidation, protein oxidation, vitamin E

Abstract

This study was conducted to investigate the effects of dietary garlic powder and vitamin E supplement for 6 weeks on lipid and protein oxidation markers in non-frozen and frozen carp meat at various time intervals. Fish were divided into four groups: Group 1 served as control; Group 2 and 3 received 25 and 50 g/kg diet garlic powder, respectively; Group 4 received diet supplemented with 150 mg/kg vitamin E. Based on the results, meat malondialdehyde (MDA) concentrations showed a significant ($p < 0.05$) decrease after 12, 48, 72 and 96 hours storage at 4 °C and after a storage period of 1 and 3 months at -20 °C in all treatment groups as compared to the control group. Carbonyl groups accumulation was found to be significantly reduced at 6 hours (in group 4), at 12 hours (in groups 3 and 4) and at 24, 48, 72 and 96 hours (in all treatment groups) after storage at 4 °C compared to the control group. Alpha-tocopheryl acetate supplementation resulted in the lowest MDA and protein carbonyl contents relative to garlic supplementation. It can be concluded that both studied compounds are notably effective against lipid and protein oxidation of carp meat during storage.

Abbreviations

MDA: Malondialdehyde
PUFAs : Poly Unsaturated Fatty Acids
DNPH : 2,4-dinitrophenylhydrazine
TBA : 2-Thiobarbituric Acid
ROS : Reactive Oxygen Species
TBARS : Thiobarbituric Acid Reactive Substances

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Introduction

Oxidative processes in meat are the most important factors responsible for quality deterioration which can influence shelf-life of meat and meat products. The oxidative stability of meat depends on the balance between antioxidants and pro-oxidants and the content of oxidation substrates including polyunsaturated fatty acids (PUFAs), cholesterol, proteins and pigments (Petron et al., 2007). Lipid and protein oxidation is considered to be a deteriorative process in muscle-based foods during processing and storage and is a major cause of quality deterioration and off-flavor development in muscle foods (Kanner et al., 1991, Pazos et al., 2011). Besides high amounts of polyunsaturated fatty acids, the presence of heme pigments and trace amounts of metallic ions makes the fish meat, compared with other meats, highly susceptible towards oxidative processes (Chaijan, 2008, Hsieh and Kinsella, 1989). Refrigeration of meat products and use of adequate packaging technologies may delay the onset of oxidation. Moreover, antioxidants may also be used to protect food quality by preventing oxidative deterioration of its components. Therefore, antioxidants play an important role in the manufacture, packaging, and storage of meat foods (He and Shahidi, 1997). On the other hand, the antioxidant defense abilities of cultured fish have been found to be insufficient, so increasing antioxidant defense abilities of fish is very important (Nakano et al., 1999). Some synthetic antioxidants have been used to decrease oxidative deterioration during the processing and storage of fish and fish products (Boyd et al., 1993). However, the use of synthetic antioxidants has raised questions regarding food safety and toxicity. Therefore, the use of natural antioxidants is emerging as an effective methodology for controlling oxidative deterioration and limiting its deleterious consequences (Maqsood and Benjakul, 2010). It has been reported that dietary vitamin E supplementation increases the amount of α-tocopherol deposited in muscle and fat tissues (Jensen et al., 1998) and its deposition is effective for preventing lipid and pigment oxidation (Higgins et al., 1998). Vitamin E supplementation appears to be an efficient way to improve the color and lipid stability of cattle (Faustman et al., 1998), pork (Hoving-Bolink et al., 1998), poultry (Morrissey et al., 1997, Sheldon et al., 1997) and rabbit meat (Corino et al., 1999). Garlic (*Allium sativum*) is one of the medicinal plants with known antioxidant properties (Tsai et al., 2012). Studies carried out on garlic have reported the presence of two main classes of antioxidant components, namely flavonoids and sulfur-containing compounds (diallyl sulfide, trisulfide and allyl-cysteine) (Sharma et al., 2010). Garlic has been shown to have antioxidant activity in various meat types including poultry (Kim et al., 2009, Tang and Cronin, 2007), beef (Yin and Cheng, 2003), pork (Park et al., 2008), camel (Gheisari and Ranjbar, 2012), fish (Kumar et al., 2009, Metwally, 2009) and oyster (Bunruk et al., 2013). Little is known about the influence of varying nutritional antioxidant status on oxidative stability of fish flesh. Therefore, the aim of the present study was to investigate the effect of dietary vitamin E and garlic supplementation on the levels of oxidation biomarkers of lipids and proteins in common carp meat during storage time at different temperatures.

Materials and methods

Chemicals

2,4-dinitrophenylhydrazine (DNPH) and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Vitamin E as α-tocopheryl acetate was obtained from National Vitamin Company (USA). The rest of the utilized chemicals were of analytical grade and were supplied by Sigma (St Lewis, MO, USA) or Merck (Darmstadt, Germany).

Preparation of garlic powder

Garlic bulbs were purchased from a local market in Mashhad, Iran. The cloves were peeled, sliced, and dried in the oven in 50 °C, then ground to powder.

Experimental design and sampling

Common carp (n=100), weighing 60 ± 10 g, were obtained from a local commercial farm. They were held in four glass aquaria, each containing 250 l fresh water. Fish were acclimatized for 7 days before the commencement of the experiment and were fed daily with commercial fish food at 2.5% total body weight at a fixed time. Physicochemical conditions of the water during the experimental period were dissolved oxygen, 5.5–6 ppm; temperature, 25±1 °C; pH, 7±0.5. Photoperiod was a 12:12 light–dark cycle. The water in the aquariums was renewed every 48 h. Fish were divided randomly into four groups of 25 each. Group 1 fish were fed with basic diet; served as the control. Fish in group 2 and 3 were fed the basic diet supplemented with 25 and 50 g/kg garlic powder, respectively. Fish in group 4 were fed the basic diet supplemented with 150 mg/kg α-tocopheryl acetate. At the end of the experimental period (6 weeks), 20 fish of every aquarium were selected and randomly divided into two equal sub groups. Meat samples were taken from the middorsal region below the major dorsal fin and wrapped in oxygen-permeable polyethylene films. Meat samples of one sub group were stored at 4 °C until 4 days and were assayed for lipid and protein oxidation after 6, 12, 24, 48, 72, and 96 hours. Meat samples of the other sub group were assayed for lipid and protein oxidation after 1 and 3 months of storage at −20 °C.

Analyses

Tissue samples were homogenized in 10 volumes (w/v) of ice-cold 0.05 M phosphate buffer (pH 7.4) for 5 min, and centrifuged at 4000×g for 15 min at 4 °C and the su-

pernatant was kept in ice until assayed. Determination of malondialdehyde (MDA) concentration was based on spectrophotometry of the pink-colored product of thiobarbituric acid reactive substances, as described by Latha and Pari (2003). The concentration of MDA was calculated using a molar extinction coefficient value of 156,000/M cm.

Carbonyl groups of proteins were detected by reaction with 2,4-dinitrophenylhydrazine, which leads to the formation of a stable 2,4-dinitrophenyl hydrazone product, as described by Jiang et al. (2010). Resulting 2,4-dinitrophenylhydrazones were quantified spectrophotometrically at 370 nm using a molar extinction coefficient of 22,000 /M cm. In order to express the carbonyl groups as nmol/mg protein, protein concentration was calculated at 280 nm using bovine serum albumin as standard (Mercier et al., 1998).

Statistical analysis

All experimental values have been represented as mean±standard error of the mean (SEM). All results were analyzed using repeated measure ANOVA followed by Bonferroni post hoc test for multiple comparisons. The level of significance was set at $p < 0.05$. All calculations were performed using SPSS/PC software.

Results

The effects of dietary garlic and vitamin E supplementation on the levels of measured oxidative stress-related markers in meat of common carp are presented in Figures 1, 2, 3 and 4. As shown in Figure 1, dietary garlic and vitamin E supplementation had no significant effect on MDA concentrations of non-frozen meat samples after 6 and 24 hours storage as compared to the control group. MDA values showed a significant (p<0/05) decrease after 12, 48, 72 and 96 hours storage in all treatment groups as compared to the control group. Compared with all other treatments, α-tocopheryl acetate supplementation resulted in the lowest MDA concentrations, although the decrease was only significant after 72 and 96 hours storage relative to other groups (Figure 1).

After a storage period of 1 and 3 months at -20 °C, significantly lower MDA values were observed for all treatments in comparison to control group (Figure 2). Furthermore, 50 g/kg dietary garlic supplementation in group 3 reduced MDA concentrations more efficiently than 25 g/kg in group 2, and vitamin E supplementation resulted in significantly lower MDA levels than group 3 (Figure 2).

Figure 3 represents the levels of protein carbonyl contents in non-frozen meat samples. Carbonyl contents showed a significant decrease at 6 hours after storage only in the group treated with vitamin E compared to the control group. Carbonyl groups accumulation was found to be significantly reduced at 12 hour after storage in the groups 3 and 4 compared to the control group. Meat protein carbonyl contents showed also significant decline following garlic and vitamin E consumption at 24, 48, 72 and 96 hours after storage at 4 °C in comparison to the control group. Mean protein carbonyls of non-frozen samples were declined in group 3 in comparison to group 2, although this decrease was only significant at 72 hours after storage. Additionally, vitamin E supplementation reduced protein carbonyls of non-frozen samples more notably as compared to the other treatments (Figure 3).

Protein carbonyl groups of frozen meat samples showed significant decline in all treated groups relative to the control group after both 1 and 3 months of storage. Indeed, 50 g/kg dietary garlic supplementation in group 3 reduced protein carbonyls more efficiently than 25 g/kg in group 2, and vitamin E supplementation resulted in lower protein carbonyls than group 3 (Figure 4).

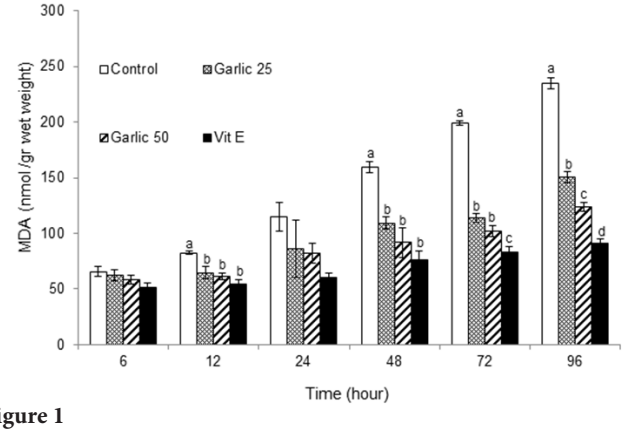

Figure 1
Effect of dietary garlic and vitamin E supplementation on malondialdehyde concentration in carp meat during storage at 4 °C. Data are means ± S.E.M (n=10 in each group). Garlic 25: 25 g/kg dietary garlic; Garlic 50: 50 g/kg dietary garlic; Vit E: 150 mg/kg dietary α-tocopheryl acetate. Values with no common superscript differ significantly ($p < 0.05$).

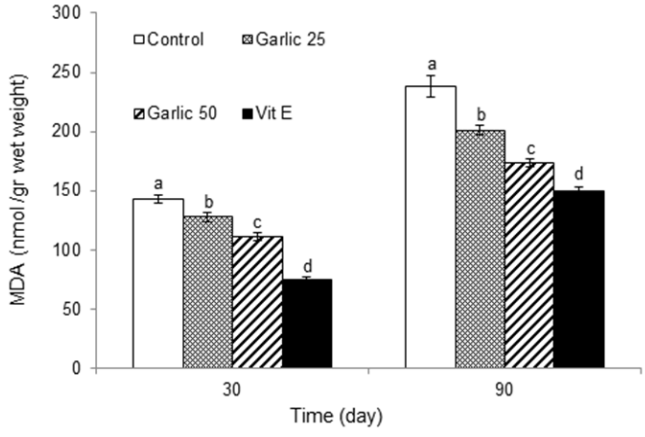

Figure 2
Effect of dietary garlic and vitamin E supplementation on malondialdehyde concentration in carp meat during storage at −20 °C. Data are means ± S.E.M (n=10 in each group). Garlic 25: 25 g/kg dietary garlic; Garlic 50: 50 g/kg dietary garlic; Vit E: 150 mg/kg dietary α-tocopheryl acetate. Values with no common superscript differ significantly ($p < 0.05$).

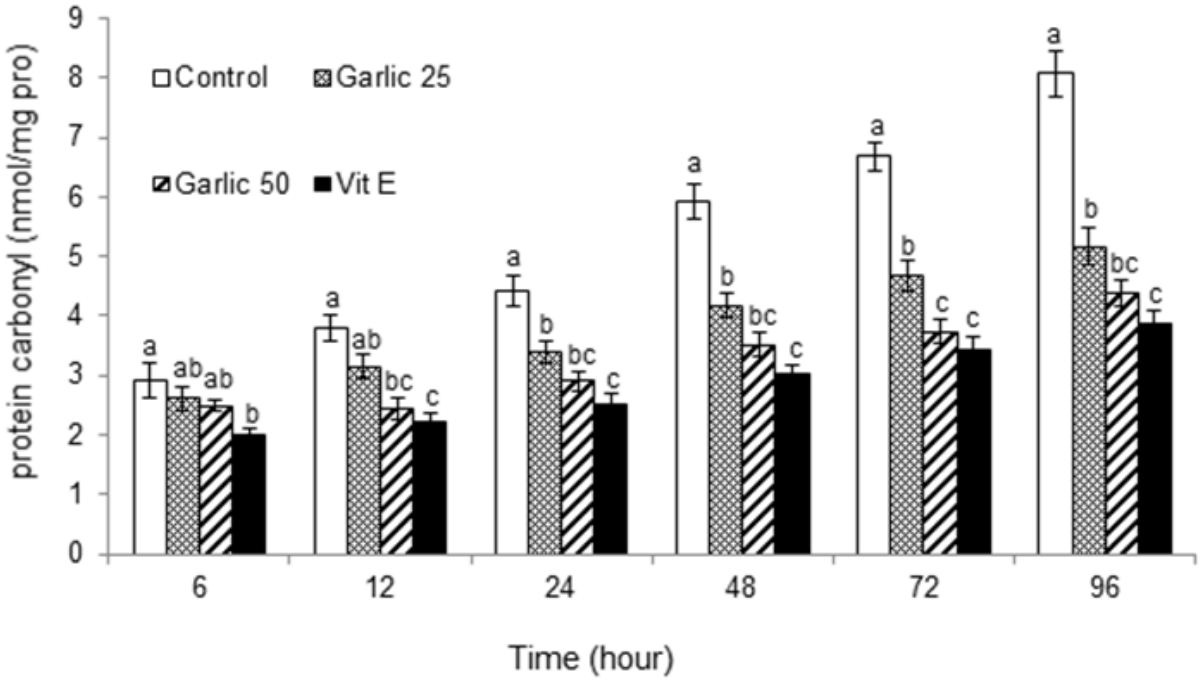

Figure 3
Effect of dietary garlic and vitamin E supplementation on protein carbonyl contents in carp meat during storage at 4 °C. Data are means ± S.E.M (n=10 in each group). Garlic 25: 25 g/kg dietary garlic; Garlic 50: 50 g/kg dietary garlic; Vit E: 150 mg/kg dietary α-tocopheryl acetate. Values with no common superscript differ significantly ($p < 0.05$).

Discussion

In both mammals and fish, insufficient dietary antioxidants have been followed by a decrease in antioxidant defense and increased susceptibility to oxidative stress (Welker and Congleton, 2009, Sie et al., 2005). Indeed, many efforts have been directed to the employment of antioxidants especially endogenous-type antioxidants or natural antioxidants to retard the oxidation of meat components, which can improve meat quality and health and also enhance its shelf life (Ortiz et al., 2009). The oxidative susceptibility of muscle from some meat animals is in the order of beef<pork<poultry<fish which is related to the unsaturated fatty acid content of the muscle lipids (Chan and Decker, 1994).

Reactive oxygen species react with various cellular macromolecules leading to enzyme inactivation, membrane damage and impaired cell viability. A number of analytical techniques have been developed to measure the oxidation products directly (e.g., carbonyl groups of oxidized proteins) or the resultant degradation products (e.g., malondialdehyde for lipid peroxidation) (Halliwell and Whiteman, 2004). Based on the present study results, muscle MDA concentrations were significantly decreased following dietary garlic and vitamin E supplementation at various time intervals following storage at both 4 °C and -20 °C relative to the control group. In agreement with these results, many workers have observed that dietary supplementation of vitamin E decreases lipid peroxidation during meat storage at different conditions in some meat animals (Gatellier et al., 2000). Moreover, the effect of vitamin E consumption on oxidative stability of lipids in trout fillets has been reported (Frigg et al., 1990, Kamireddy, 2011). Similarly, the findings of Yildiz et al. (2006) indicated that increasing the levels of α-tocopherol acetate in the diets can slow down the level of lipid oxidation in fish fillets during refrigerated storage at 1 ± 0.3 °C for 9 days.

Moreover, reduction in fillets lipid oxidation values following increased dietary vitamin E levels have been reported in *Ictalurus punctatus* (Gatlin lii et al., 1992), *Scophthahus maximus* (Stephan et al., 1995), *Dicentrarchus labrax* (Gatta, 2000), *Sparus macrocephalus* (Zhang et al., 2007), and tilapia (Fogaca et al., 2009, Huang and Huang, 2004). The results of Jasour et al. (2011) showed that both dietary and direct surface application (after slaughtering) of α-tocopherol acetate can improve the oxidative stability of fish lipid during storage. It has been also reported that dietary garlic can improve the antioxidant status of rainbow trout (Mohebbi et al., 2012), mirror carp (Tang and Xu, 2010, Xu et al., 2010) and common carp (Naeiji et al., 2013) as evidenced by decreased TBARS in serum and tissues. Indeed, it has been reported that antioxidant properties of fresh garlic can be effective in extending the shelf life of hot-smoked catfish Clarias gariepinus (Kumolu-Johnson and Ndimele, 2011, Kumolu-Johnson et al., 2013).

Based on the current findings, garlic and vitamin E supplementation were effective in decreasing muscle protein carbonyl contents during different storage times at both 4 °C and -20 °C compared to the control group. In line

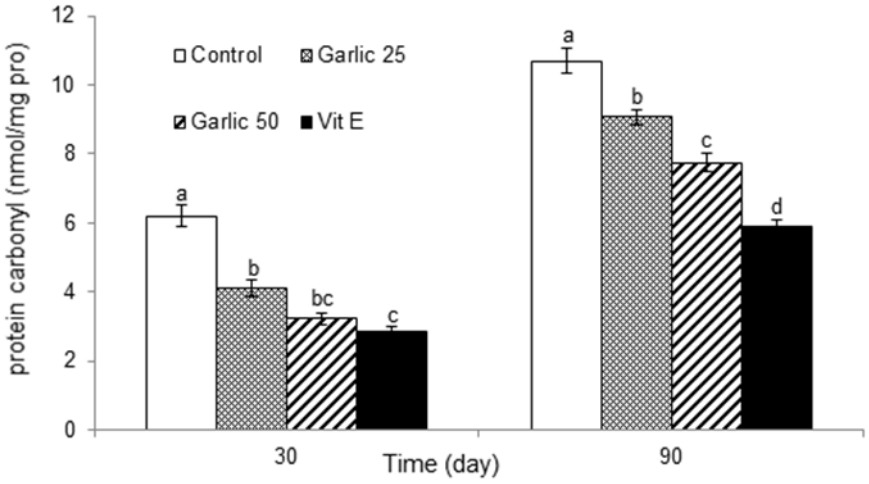

Figure 4
Effect of dietary garlic and vitamin E supplementation on protein carbonyl contents in carp meat during storage at −20 °C. Data are means ± S.E.M (n=10 in each group). Garlic 25: 25 g/kg dietary garlic; Garlic 50: 50 g/kg dietary garlic; Vit E: 150 mg/kg dietary α-tocopheryl acetate. Values with no common superscript differ significantly ($p < 0.05$).

with these results, Mercier et al. (1998) and Batifoulier et al. (2002) revealed that vitamin E can decrease protein oxidation in turkey muscle. Vitamin E supplementation also showed substantial protection against both resting and exercise-induced protein oxidation in skeletal muscle of rats (Reznick et al., 1992). Xiao et al. (2011) reported dietary treatment of vitamin E is an excellent method to protect proteins from chemical and functional damages during processing and storage of chicken meat. Indeed, garlic administration prevented the gentamicin-induced increase in renal levels of protein carbonyl groups (Maldonado et al., 2003).

As the results of the present work show, dietary vitamin E supplementation decreased MDA and protein carbonyl contents more efficiently than garlic supplementation. In agreement with these results, Iranloye and Oludare (2011) reported that vitamin E has a more potent antioxidant activity than garlic juice in preventing nicotine induced oxidative damage in rat. Gokoglu et al. (2012) showed that tomato (which is an important source of antioxidants such as α-tocopherol, lutein and the carotenoids) was more effective than garlic extract in retarding lipid oxidation in marinated anchovy. Similarly, Caputi jambrenghi et al. (2005) observed that vitamin E supplementation is more effective than garlic supplementation on colour stability in lamb meat. On the contrary, Yin and Cheng (2003) found that the addition of four garlic-derived compounds to ground beef was more effective than α-tocopherol in delaying oxymyoglobin and lipid oxidation. It has been noted that some differences in the reported results may be attributed to differences in examined species, utilized antioxidant dose, and experimental procedures.

In summary, the results of the present study indicated that garlic powder and vitamin E are notably effective against lipid and protein oxidation of carp meat during storage. Additionally, dietary vitamin E was more effective than garlic supplementation in retarding lipid and protein oxidation, suggesting its higher antioxidant activity. On the other hand, the destructive oxidative processes of carp meat which causing decreased nutritional value and shelf-life can be delayed by these studied natural safe food additives. However, further studies on the relation of meat oxidation processes and dietary factors in fish species would be of interest.

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