Fishery Technology 53 (2016) : 290 - 300

Effect of Ginger Extract on Lipid Oxidation and Microbial Growth During Frozen Storage of Rohu (*Labeo rohita*) Steaks

Satyendra K. Maurya, Ranendra K. Majumdar, Bahni Dhar* and Deepayan Roy
College of Fisheries (Central Agricultural University), Lembucherra, Tripura - 799210, India

Abstract

Steaks of *Labeo rohita* (Rohu) were treated with aqueous extract of the rhizome of ginger (*Zingiber officinale*) before storage at -16 ± 2°C in domestic refrigerator and sensory, chemical and microbiological changes have been reported. Ginger extract (GE) was used as a source of natural antioxidants and antimicrobials for retarding lipid oxidation and microbial growth during low temperature storage. The proximate composition, phytochemicals and total phenolic content of ginger were determined. Fish steaks were treated with single and double strength GE for one/two minutes. Sampling was done at monthly intervals. Sensory study revealed that the GE-treated samples were acceptable up to 90 and 120 days respectively for treatment of one and two minutes, whereas, shelf-life in control was found to be of 60 days only. In GE-treated samples, the increase of peroxide value and thiobarbituric acid value was found significantly lower than the control. Total plate counts (log cfu g^{-1}) decreased in all the treatments during storage. Total coliform and total psychrophilic count also decreased during storage. Rate of decrease of microbial count was enhanced in treated samples than control. The muscle stiffness as usually experienced in frozen-stored meat products was not perceived in the GE-treated samples by the sensory panel judges.

Keywords: *Labeo rohita*, ginger, antioxidant, antimicrobials, frozen storage of fish

Received 10 October 2014; Revised 21 March 2016; Accepted 07 April 2015

* *E-mail: bahnicof2010@gmail.com*

Introduction

Freezing and frozen storage have largely been employed to retain fish sensory and nutritional properties. The processing and preservation of fresh fish are of utmost importance since fish is highly susceptible to deterioration immediately after harvest and also to prevent economic losses (Okonta & Ekelemu, 2005). The recent trend the world over is the demand for fresh food without any chemical preservative. Many synthetic preservatives, such as butylated hydroxyltolune (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG), are typically used to protect foods from spoilage, although their use is restricted due to possible carcinogenic effects. Their safety, however, is doubtful (Imadia et al., 1983). Therefore, there has been an increasing interest in alternative additives from natural sources, which has gradually provided impetus to eliminating synthetic preservatives in food (Shan et al., 2009). These antioxidants are polyphenol compounds (Yen et al., 2003), which are found in all plants and in all parts of the plants (tree bark, stalks, leaves, fruits, roots, flowers, pods and seeds) (Kim et al., 1997).

Spices act as natural preservatives, besides improving texture and flavour of foods (Nain et al., 2009). Many of them are linked to numerous health benefits, such as anti-inflammatory, antimicrobial, antimutagenic, antioxidant and hypolipidemic activities (Su et al., 2007). Moreover, in recent years, there has been an increasing interest in the food industry towards incorporating ingredients with health beneficial properties (Herrero et al., 2006). Among these ingredients, spices are recognized by their flavoring and coloring potential. Ginger (*Zingiber officinale* Rosc.), a monocotyledon belonging to family Zingiberaceae, is an important spice and medicinal plant originated in South-East Asia

and introduced to many parts of the globe (Park & Pizutto, 2002). Scientific studies have shown that ginger possesses antimicrobial, anti-schistosomal, anti-inuammatory, antipyretic, antioxidative, hypoglycemic hepatoprotective, diuretic and hypocholesterolemic effects (Chrubasik et al., 2005; Ali et al., 2008). Garlic, ginger and carrot have long been documented for their antimicrobial activities against food borne pathogens (Liao, 2007).

Phytochemical studies show that ginger rhizome contains a wide variety of biologically active compounds. The pleasant aroma of ginger is caused by more than 70 active ingredients present in the steam volatile oil (Vasala, 2004). Hori et al. (2003) isolated five sulfonated compounds, namely 4-gingesulfonic acid and shogasulfonic acids A, B, C and D, along with seven earlier reported compounds including 6-gingesulfonic acid from rhizomes and their structures were characterized by spectroscopic analysis. Phytochemical properties of the garlic, ginger and carrot extracts indicated the presence of saponnin, resins, alkaloids, flavonoids, steroids and terpenes in varied proportions (Chukwu et al., 2012). These phytochemicals are documented to be the major biologically active principles, as well as exhibited physiological activity against the tested organisms (Sofowora, 1993). Ginger rhizomes also contain a potent proteolytic enzyme called zingibain. Hydro-ethanolic GE exhibited potent antibacterial activity against Gram-positive and Gram negative bacteria (Mascolo et al., 1989) including *Staphylococcus aureus*, *Streptococcus pyogenes/pneumoniae* and *Haemophilus influenzae* (Akoachere et al., 2002), *Pseudomonas aeruginosa, Salmonella typhimurium* and *Escherichia coli* (Jagetia et al., 2003).

There are several reports regarding antioxidant and antimicrobial effect of ginger and other spices on the shelf life of meat products (Tsai et al., 2012; Naveena et al., 2004; Mansour & Khalil, 2000). But only few studies are reported on the use of ginger and other plant extracts on shelf-life enhancement of fish and fish products (Tironi et al., 2010; Mah et al., 2009). Recent studies have shown that ginger protease could be applied in the tenderization of meat, and improve the quality of meat (Naveena & Mendiratta, 2001; Bhaskar et al., 2006). Literature is scarce on the effect of ginger as a source of natural preservative in fish and fish products. The present study was designed to study the role of GE-treatment on the quality and shelf life of dressed fish during frozen storage.

Materials and Methods

Fresh rohu (*L. rohita*) were procured from the local market within 6 h of landing and brought to the laboratory in ice. Average weight and size were 450 ± 46 g and 31 ± 4.3 cm respectively. The whole fish were washed, beheaded, eviscerated and cut into steaks of approx. 2.5 cm thickness, each weighing about 62–68 g. Chemicals used were of analytical grade procured from E. Merck, India Ltd. and media were procured from Hi-Media Laboratories, Mumbai, India.

Aqueous extraction method was used. About 32 g of the fresh washed ginger was sliced into tiny pieces and blended in an electric blender with 160 ml sterile distilled water. This was then transferred into separate flask and shaken for one hour and filtered with Whatman No. 1 filter paper. The aqueous extract was freeze dried and stored in amber coloured glass container at –20°C. Phytochemical screening for alkaloids, flavonoids, terpenoids, phenols, tannins, resins, steroids, saponins, anthraquinone and glycosides was done following the method described by Trease & Evans (1983).

Total phenolic content and antioxidant activity were determined using freeze-dried aqueous extract (1:5, w/v). The total phenolic content was estimated using Folin–Ciocalteu reagents with analytical grade gallic acid as standard (Kim et al., 2003). The concentration of total phenols was expressed as mg/g of dry extract. The radical scavenging ability was determined as scavenging effect of GE on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) described by Mensor et al. (2001).

GE was prepared following the method suggested by Tsai et al. (2012). Fresh ginger rhizome (*Zingiber officinale*) was washed, peeled and sliced. The ginger slices were immediately homogenized with chilled distilled water (5°C) @ 2:1 (w/v) ratio for 2 min and filtered through two layers of cheese cloth. The filtrate was centrifuged for 10 min at 3000 rpm at 5°C, and the supernatant was collected and saved as the ginger extract (GE) and stored at refrigerator (5°C).

Fish steaks were washed with chilled water to free from blood, mucus and adherent gut contents. They were divided into two batches. One batch was kept as control (CON) without treatment with GE and other batch was treated with equal amount (1:1, w/v) of GE (GET) with different duration of dipping

(one and two minutes) and designated as GET_1 and GET_2 for one and two minutes dipping respectively.

The treated and untreated samples were packed in polypropylene pouches (two steaks per pouch) and sealed and stored at –16 ± 2°C in a freezer chamber of domestic refrigerator. At periodic intervals of 30 days, three pouches were removed randomly from each batch for quality assessment in terms of biochemical, microbiological and sensory methods.

Moisture, ash, total lipid and crude protein analysis was carried out using standard procedures of AOAC (2000). Salt soluble nitrogen (SSN) was estimated following the method of Dyer et al. (1950). Total volatile basic nitrogen (TVBN) was determined by using the Conway's micro-diffusion method (Conway, 1947). Peroxide value (PV) was determined on the chloroform extracts of tissues according to the method suggested by Jacobs (1958). Thiobarbituric acid reactive substances (TBARS) were determined as described by Benjakul & Bauer (2001). The values of three independent experiments were recorded as mean ± SD.

Microbial analysis was done following standard method given in APHA (2001). The fish samples (10 g) from each treatment were aseptically homogenized for 1 min in 90 mL sterile saline in a presterilized poly-pouch using stomacher. Appropriate serial dilutions of the homogenate were placed in petri plates in triplicate. Total plate counts and total psychrophilic counts were determined using plate count agar with incubation period of 24 h at 37°C and 10 days at 7 - 10°C respectively. Total coliform counts were determined using selective Tergitol-7 (T-7) agar by pour plate method and plates were incubated at 37°C for 24 – 48 h. T-7 is a highly selective media for *E. coli* and other coliforms. Bacterial counts were expressed as log cfu g^{-1}.

Sensory characteristics and overall acceptability of with or without GE treated samples during the period of storage were assessed by a panel of ten members belonging to the College of Fisheries, Lembucherra, Tripura on the basis of 5-point scale on each sampling (Ninan et al., 2008). GE treated fish steaks were soaked in water for two minutes before cooking. Fish steaks were kept in glass bowl and steam cooked for 8 minutes in pressure cooker with open vent. Warm steamed fish steaks were served for sensory evaluation. Sensory characteristics study included general appearance, colour, taste, texture, odour/flavour, and overall acceptability of fish. The scores were given as 5 for excellent, 4 for good, 3 for fair and acceptable, 2 for poor and 1 for unacceptable. The mean of the scores given by the panel represented the overall sensory quality. A score below 3 was considered unacceptable.

Data was analyzed using analysis of variance (ANOVA), and when significant differences were found, comparisons among means were carried out by using Duncan's Multiple Comparison Test ($p<0.05$) by Statistical Package for Social Sciences (SPSS, version 11.0 for windows).

Results and Discussion

Biochemical composition of raw material fish *Labeo rohita* (rohu) is given in Table 1. Moisture content was within the ranges observed by other authors (Abii et al., 2007; Islam & Joadder, 2005). Proximate composition of muscle of *L. rohita* was reported by Shekhar et al. (2004) as moisture – 76.5%, protein – 16.4%, ash– 0.89% and total fat– 1.4%. Jabeen & Chaudhry (2011) investigated the biochemical composition of rohu of Indus River and found moisture content of 79.0% and on dry weight basis the crude protein, ash and fat content was 53.78%, 6.4% and 11.25% respectively. In the present investigations, crude protein of 16.5% (wet weight) was similar to the protein levels for carp (16% wet weight; FAO, 2008). Ash contents (1.26% dry weight muscle tissue) observed in this study was within the ranges found by Abii et al. (2007).

Table 1. Biochemical composition and NPN content of cultured *Labeo rohita*

Moisture (%)	79.59 ± 0.3
Ash (%)	1.26 ± 0.05
Crude protein (%)	16.5 ± 0.38
Total lipid (%)	1.66 ± 0.35
Non-protein nitrogen (%)	0.42 ± 0.03

Protein and non-protein nitrogen (NPN) contents were estimated as 16.5% and 0.42% respectively which corroborates with the investigation made by Raju et al. (2010) who reported total nitrogen and non-protein nitrogen of *Labeo rohita* as 16.3 and 0.45% respectively. According to the classification of fish based on the fat content, fish species of this study was classified as lean fish, having fat content less than 5% (Suriah et al., 1995). Moisture and fat

content particularly depend upon the season of the year, type and availability of food besides other unidentified variables and there is an inverse relation between the water and fat content (Nair, 2002).

Proximate composition of local variety of ginger rhizome (*Zingiber officinale*) is presented in Table 2. Moisture, ash, protein, lipid and carbohydrate contents were found as 10.6 ± 1.27, 6.54 ± 0.86, 8.52 ± 1.06, 5.12 ± 0.78 and 68.58 ± 1.05% respectively. Farrell (1985) reported proximate composition of ginger as moisture (9.4%), ash (4.8%), protein (9.1%), fat (6.0%), total carbohydrate (70.8%), fibre (5.9%) and food energy (347 kcal). Quantitative studies have shown that ginger rhizome contains 3 – 6% fatty oil, 9% protein, 60 – 70% carbohydrates, 3 – 8% crude fibre, about 8% ash, 9 – 12% water and 2 – 3% volatile oil (Govindarajan, 1982; Ali et al., 2008). The biochemical composition of ginger grown in Tripura (India) was found to have similarity with the data reported by other authors. pH of the GE was 6.34 ± 0.08, which was in the range (6.3–6.5) of pH reported by Mendiratta et al. (2000).

Table 2. Proximate composition, pH and energy value of Indian ginger (*Zingiber officinale*)

Moisture (%)	10.6 ±1.27
pH	6.34 ± 0.08
Ash (%)	6.54 ± 0.86
Crude Fat (%)	5.12 ± 0.78
Crude Protein (%)	8.52 ± 1.06
Crude Fibre (%)	5.63 ± 0.84
Total carbohydrate	68.58 ± 1.05
Food energy	354 kcal

Phytochemical studies revealed that ginger rhizome contained a wide variety of biologically active compounds. Results of the phytochemical screening are presented in Table 3. Saponins, resins, alkaloids, flavonoids, steroids, terpenoids, phenols and tannins were present in the GE, whereas, glycosides and anthraquinone were found absent. Similar composition of phytochemicals in ginger rhizome was also reported by Chukwu et al. (2012) except tannins. Essential oils and other plant extracts are principally responsible for antimicrobial activities in plants, herbs and spices. Crude ginger contains up to 9% lipids or glycolipids and about 5–8% oleoresin. The pungent principle, accounting for 25% of the oleoresin, consists mainly of gingerols (Chrubasik et al., 2005).

Table 3. Phytochemical properties of Indian ginger (*Zingiber officinale*)

Chemical Compounds	
Alkaloids (Wagner's Test)	+
Saponins	+
Resins	+
Flavonoids	+
Terpenoids	+
Phenols	+
Steroids	+
Tannins	+
Anthraquinone	–
Glycosides	–

+ = Present – = absent

The total phenolic content of the extract was determined to be 848 ± 17 mg g^{-1} dry extract. GE showed a significant effect in inhibiting DPPH, reaching up to 88.2% at concentration 20 µg ml^{-1}. It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals.

Biochemical changes during storage of GE-treated fish steaks at –16°C are presented in Table 4. Moisture content showed a decreasing ($p<0.05$) trend in all the treatments during the storage period. In case of GE-treated samples, the decrease of moisture content was 4.95% and 4.94% in GET_1 and GET_2 respectively. Moreover, on each day of sampling the differences among the moisture contents of all the treatments were found significant ($p<0.05$). This decrease of moisture content may be explained as loss of moisture due to drip loss during thawing. Frozen storage causes freeze denaturation of muscle and dehydration which reduces the water holding capacity. During frozen storage, the moisture content of pink perch surimi decreased from 78.50 ± 0.14 to 76.55 ± 0.02% with storage time because of syneresis and dehydration (Garg et al., 1982). The total lipid content was found to have increased in all the treatments during the storage period and this may be explained as the result of decrease of moisture content. The crude protein

Table 4. Biochemical changes during storage of ginger extract treated Rohu steaks at -16 ± 2° C (mean with SD in parenthesis)

Parameters	Sample	Day 1	Day 30	Day 60	Day 90	Day 120
Moisture (%)	CON	79.25^{dA} (0.08)	78.05^{cA} (0.15)	77.83^{bA} (0.08)		
	GET_1	80.13^{dB} (0.10)	78.91^{cdAB} (1.50)	77.33^{abA} (0.57)	76.16^{aA} (0.69)	
	GET_2	81.03^{fC} (0.20)	80.61^{efB} (0.73)	79.43^{cdeB} (0.07)	79.65^{defB} (0.42)	77.02^{a} (0.41)
Ash (%)	CON	1.21^{aA} (0.07)	1.20^{aA} (0.03)	1.22^{aB} (0.04)		
	GET_1	1.16^{aA} (0.08)	1.19^{aA} (0.02)	1.13^{aA} (0.03)	1.15^{aA} (0.04)	
	GET_2	1.18^{bcA} (0.06)	1.19^{bcA} (0.05)	1.18^{bcAB} (0.03)	1.14^{abcA} (0.05)	1.09^{a} (0.06)
Total Lipids (%)	CON	1.26^{aB} (0.04)	1.42^{cB} (0.03)	1.35^{bB} (0.04)		
	GET_1	1.15^{aA} (0.06)	1.16^{aA} (0.17)	1.22^{aA} (0.06)	1.20^{aA} (0.01)	
	GET_2	1.13^{aA} (0.05)	1.31^{cAB} (0.05)	1.18^{abA} (0.04)	1.19^{abA} (0.03)	1.20^{b} (0.03)
Crude protein (%)	CON	16.90^{bA} (0.10)	16.31^{aA} (0.08)	16.60^{abA} (0.08)		
	GET_1	16.29^{aA} (0.14)	16.58^{abAB} (0.23)	17.24^{bA} (0.32)	16.71^{abA} (0.48)	
	GET_2	16.50^{aA} (0.58)	16.77^{abB} (0.30)	17.01^{abcA} (0.48)	16.94^{abcA} (0.69)	16.94^{abc} (0.11)
PV (mmoles O_2 kg^{-1} lipid)	CON	4.63^{aC} (0.07)	5.57^{cC} (0.02)	7.48^{eC} (0.03)		
	GET_1	3.87^{aA} (0.02)	4.23^{cA} (0.03)	4.47^{eA} (0.02)	5.69^{gB} (0.03)	
	GET_2	3.96^{aB} (0.02)	4.61^{cB} (0.02)	5.35^{eB} (0.03)	5.55^{gA} (0.03)	5.95^{i} (0.03)
TBA (mg malonaldehyde kg^{-1} meat)	CON	0.52^{aB} (0.02)	1.00^{cC} (0.01)	1.25^{eC} (0.03)		
	GET_1	0.56^{bC} (0.01)	0.63^{cB} (0.02)	0.91^{eB} (0.03)	1.10^{gB} (0.03)	
	GET_2	0.58^{aA} (0.02)	0.54^{cA} (0.03)	0.80^{eA} (0.01)	0.97^{gA} (0.01)	1.08^{i} (0.01)
TVBN (mg 100 g^{-1} meat)	CON	11.70^{aC} (0.10)	18.44^{cC} (0.06)	23.52^{eC} (0.09)		
	GET_1	10.06^{aB} (0.18)	14.44^{cB} (0.50)	15.95^{eB} (0.36)	18.37^{gB} (0.24)	
	GET_2	9.75^{aA} (0.10)	11.74^{cA} (0.44)	14.04^{eA} (0.17)	15.62^{fA} (0.05)	17.67^{h} (0.14)

Means with different superscripts of small letters in a row and capital letters in a column differed significantly ($p< 0.05$) with respect to period of storage and treatments respectively

content of fish steaks stored at –16 ± 2°C showed change during the storage period. In control or untreated fish steaks, the crude protein contents slightly decreased (1.77%, $p>0.05$). Whereas, in GET_1, the crude protein content slightly decreased ($p>0.05$) and in GET_2, a slight increase ($p>0.05$) was observed. Such changes in crude protein content during the storage period may be due to changes of moisture content of fish steaks.

Concerning the TVBN values, there was a steady increase in control as well in all the treatments. However, the rate of increase was more in control than the GE-treated samples. The rate of increase ($p<0.05$) of TVBN was 101% in control when the value reached to 23.52 on day-60. Whereas, TVBN increased ($p<0.05$) to 82.6 and 81.2% in GET_1 and GET_2 respectively. TVBN value reached 18.37 (day-90) and 17.67 (day-120) respectively in GET_1 and GET_2. On each sampling day, TVBN values were significantly different ($p<0.05$) between treatments. Accumulation of volatile bases in the muscle post mortem is a result of microbial spoilage and increases with the period of storage and growth of microbial population. It was observed that in all the GE-treated fish steaks the rate of increase of TVBN was lower than the control. This may be due to the effect of antimicrobial compounds present in the ginger which might have exerted some control over the growth of microbial population in the GE-treated samples. However, TVBN value did not cross the upper limit, i.e. 30 mg 100 g^{-1} muscle as suggested by different authors (Reddy et al., 1995; Gokodlu et al., 1998; Lakshmanan, 2002), in any of the treatments up to the day when fish steaks were found unacceptable as per sensory evaluation. The initial low value of TVBN may be because of freshness of fish used for this study.

Peroxide value reduced ($p<0.05$) in GE-treated samples on day-1 and also the rate of increase of PV during the storage period was found higher in control (CON) and the initial value (4.63) increased ($p<0.05$) to 7.48 mmoles O_2 kg^{-1} lipid on day-60. Whereas, in GET_1 and GET_2, PV reached ($p<0.05$) to 5.69 (day-90) and 5.95 mmoles O_2 kg^{-1} lipid (day-120) respectively. Moreover, on each sampling day, the peroxide values were significantly different ($p<0.05$) between the treatments. Mansour & Khalil (2000) concluded that ginger rhizome extract exhibited the highest antioxidant activity and had an activity comparable to commercial antioxidants in their work with beef patties.

Similarly, TBARS values also decreased ($p<0.05$) in the fish steaks immediately after dip treatment with GE. TBARS value increased ($p<0.05$) with storage time for the control (>140.38% increase after 60 days), whereas, TBARS in samples containing active components of ginger were still low on day-60. In control sample, the initial content of TBARS 0.52 increased ($p<0.05$) to 1.25 mg malonaldehyde kg^{-1} meat on day-60. Whereas, in GET_1 and GET_2, TBARS increased ($p<0.05$) from 0.56 to 1.10 mg malonaldehyde kg^{-1} meat (day-90) and from 0.58 to 1.08 mg malonaldehyde kg^{-1} meat (day-120) respectively. TBARS values between the treatments on each day of sampling were found significantly different. The rate of increase of TBARS values in the GE-treated samples was found lower than the control. Retardation of lipid oxidation (in terms of TBARS values) in GE-marinated muscle was also reported by Tsai et al. (2012).

The results of this study, therefore, indicate that GE treatment before storage could slow down the increase in TBARS values, implying a retardation of lipid oxidation in GE-treated samples. These results are in agreement with those obtained by Hettiarachchy et al. (1996) who reported the effectiveness of synthetic and natural antioxidants in controlling lipid oxidation in meat products. Similar results were also found in studies of GE-treated sheep muscle (Mendiratta et al., 2000). This retardation may be due to the presence of polyphenol compounds (such as 6-gingerol and its derivatives) in the GE. GE showed an antioxidant activity comparable with that of BHT in inhibiting the lipid peroxidation (Stoilova et al., 2007). Ginger crude plant material (Kuo et al., 1999) and single constituents such as [6]-gingerol (Ippoushi et al., 2003), [6]-paradol (Chung et al., 2001), phenolic 1, 3-diketones (Patro et al., 2002), zingerone (Krishnakantha & Lokesh, 1993) have been shown to protect against lipid peroxidation in various established models. GE was effective in retarding the development of rancidity in salted pork patties and its effectiveness was directly related to the GE concentration (Lee et al., 1986).

Essential oils and purified components of spices are used as natural products that prevent the growth of food-borne bacteria and molds in food systems, as well as extend the shelf-life of processed foods. The antibacterial principle of ginger was reported by different authors (Mahady et al., 2003; Jagetia et al., 2003). Neveena et al. (2001) found inhibitory

effect of ginger on few pathogenic microorganisms and improving the shelf life of meat and meat products. Total plate counts (log cfu g^{-1}) decreased in all the treatments during the period of storage (Table 5). Even TPC decreased ($p<0.05$) after dip treatment of fish steaks in GE resulting in low initial count in GE treated samples (GE-T). The immediate reduction ($p<0.05$) in TPC after dip treatment was about 8 and 13% in GE-T_1 and GE-T_2 respectively. In control, TPC decreased ($p<0.05$) from 4.39 to 4.16 log cfu g^{-1} (decrease of about 5%) during the storage period of 60 days. In treated samples, a further significant ($p<0.05$) reduction of TPC took place during the storage period of 90 days for GE-T_1 and 120 days for GE-T_2. At the end of storage period, TPC estimated in GE-T_1 and GE-T_2 was 3.60 log cfu g^{-1} (86% of day-1 value) and 3.15 log cfu g^{-1} (81% of day-1 value) respectively.

Negbenebor et al. (1995) reported reduction in psychorotrophic aerobic counts in GE-treated beef patties which had mean bacterial counts of 6.8 log cfu g^{-1} compared to control which had 8.2 log cfu g^{-1} during their storage at 5-7°C for 6 days. The total psychrophilic count ($TP_{sy}C$) recorded a decrease immediately after dip treatment and subsequently during the period of storage. In control, $TP_{sy}C$ decreased ($p<0.05$) from 3.81 to 3.41 log cfu g^{-1} (decrease of about 10.5%) during the storage period of 60 days. Whereas, in case of GET_1 and GET_2, the decrease of $TP_{sy}C$ was estimated as 18.8 and 15.8% respectively from the initial count during the period of storage.

Results of this study suggests that hurdles like low temperature and antimicrobial effect of ginger rhizome might be responsible for restricting the microbial growth in GE treated samples compared to control. Total coliform counts (TCC) showed a decrease in all the treatments during the period of storage. TCC of fish steaks showed an immediate decrease ($p<0.05$) after dip treatment and estimated counts were 4.6 and 11% less in GE-T_1 and GE-T_2 respectively with respect to the control. During the period of storage, the decrease of TCC with respect to the initial value was 13, 15 and 16.6% in CON, GE-T_1 and GE-T_2 respectively.

Ginger oil extracts can inhibit a wide range of microorganisms and volatiles in ginger essential oils can reduce the population of bacteria and yeasts in shredded green papaya (Sa-Nguanpuag et al., 2011).

Table 5. Microbial changes during storage of ginger extract treated Rohu steaks (GE-T) at -16 ± 2°C (mean with SD in parenthesis)

Parameters	Sample	Day 1	Day 30	Day 60	Day 90	Day 120
Total plate count	CON	4.39^{fC} (0.01)	4.27^{dC} (0.02)	4.16^{bB} (0.02)		
	GE-T_1	4.16^{dB} (0.01)	4.09^{cdB} (0.02)	3.36^{aA} (0.53)	3.60^{abB} (0.03)	
	GE-T_2	3.86^{fA} (0.03)	3.69^{eA} (0.05)	3.54^{dA} (0.03)	3.34^{bA} (0.04)	3.15^{a} (0.07)
Total coliform count	CON	3.46^{dB} (0.05)	3.25^{bcB} (0.04)	3.01^{aA} (0.01)		
	GE-T_1	3.38^{dB} (0.04)	3.19^{bcdAB} (0.07)	3.38^{dA} (0.50)	2.87^{abB} (0.01)	
	GE-T_2	3.19^{gA} (0.07)	3.11^{efA} (0.00)	2.94^{dA} (0.02)	2.82^{cA} (0.02)	2.66^{a} (0.02)
Total psychrophilic count	CON	3.81^{eC} (0.02)	3.66^{dC} (0.02)	3.41^{bA} (0.04)		
	GE-T_1	3.66^{dB} (0.02)	3.42^{cdB} (0.06)	3.33^{cdA} (0.49)	2.97^{aB} (0.02)	
	GE-T_2	3.04^{gA} (0.07)	2.98^{fgA} (0.01)	2.86^{eA} (0.02)	2.75^{cA} (0.02)	2.56^{a} (0.04)

Means with different superscripts of small letters in a row and capital letters in a column differed significantly ($p< 0.05$) with respect to period of storage and treatments respectively

The results of antimicrobial effect of ginger in the study are in accordance with most of the reports published regarding ginger antimicrobial activity (Sebiomo et al., 2011; Malu et al., 2009). It was reported that sesquiterpenoids are the main components of ginger which contribute to its antibacterial activity (Malu et al., 2009). Roy et al. (2006) stated that the bioactive compounds of ginger rendering antimicrobial activity are volatile in nature and antimicrobial activity of GE decreases upon storage.

Table 6 depicts the sensory changes during storage of GE-treated Rohu steaks at -16±2ºC. Although the control scored ($p<0.05$) slightly above the border line

Table 6. Sensory changes during storage of ginger extract treated Rohu steaks at -16±2°C (mean with SD in parenthesis)

Parameters	Sample	Day 1	Day 30	Day 60	Day 90	Day 120
General appearance	CON	4.71^{dA} (0.49)	4.29^{cdA} (0.49)	3.71^{bA} (0.49)	3.19^{aA} (0.38)	
	GET_1	5.00^{dA} (0.00)	4.86^{dB} (0.38)	3.86^{bA} (0.38)	4.00^{bB} (0.00)	
	GET_2	5.00^{fA} (0.00)	4.57^{defAB} (0.53)	4.43^{cdeB} (0.53)	4.00^{bcB} (0.00)	3.14^{a} (0.38)
Colour	CON	4.57^{cA} (0.53)	4.43^{cA} (0.53)	3.43^{bA} (0.53)	3.08^{aA} (0.49)	
	GET_1	4.86^{cA} (0.38)	4.57^{cA} (0.53)	3.86^{bA} (0.38)	3.43^{abB} (0.53)	
	GET_2	5.00^{cA} (0.00)	4.43^{bcA} (0.53)	4.43^{bcB} (0.53)	4.00^{bC} (0.58)	3.43^{a} (0.53)
Taste	CON	4.86^{cdA} (0.38)	4.29^{cA} (0.49)	3.57^{bA} (0.53)	2.89^{aA} (0.38)	
	GET_1	5.00^{dA} (0.00)	4.71^{cdA} (0.49)	3.57^{abA} (0.53)	3.57^{abB} (0.53)	
	GET_2	5.00^{dA} (0.00)	4.57^{cdA} (0.53)	4.29^{bcB} (0.49)	4.00^{bB} (0.58)	3.29^{a} (0.49)
Texture	CON	5.00^{cA} (0.00)	4.71^{bcA} (0.49)	3.57^{bA} (0.53)	3.04^{aA} (0.49)	
	GET_1	4.86^{cA} (0.38)	4.43^{cA} (0.53)	3.86^{bA} (0.38)	3.71^{abB} (0.49)	
	GET_2	5.00^{cA} (0.00)	4.71^{cA} (0.49)	4.86^{cB} (0.38)	3.86^{bB} (0.69)	3.14^{a} (0.38)
Odour/Flavour	CON	4.57^{cA} (0.53)	4.57^{cA} (0.53)	3.29^{bA} (0.49)	2.78^{aA} (0.39)	
	GET_1	5.00^{dB} (0.00)	4.29^{cA} (0.49)	3.57^{abA} (0.53)	3.14^{aB} (0.69)	
	GET_2	5.00^{eB} (0.00)	4.57^{deA} (0.53)	4.14^{cdB} (0.38)	3.57^{abB} (0.53)	3.29^{a} (0.49)
Overall acceptability	CON	5.00^{c} (0.00)	4.71^{cA} (0.49)	3.86^{bA} (0.38)	2.91^{aA} (0.53)	
	GET_1	5.00^{e} (0.00)	5.00^{eA} (0.00)	4.00^{cA} (0.00)	3.57^{bB} (0.53)	
	GET_2	5.00^{e} (0.00)	5.00^{eA} (0.00)	4.57^{dB} (0.53)	4.00^{cC} (0.00)	3.00^{a} (0.00)

Means with different superscripts of small letters in a row and capital letters in a column differed significantly ($p< 0.05$) with respect to period of storage and treatments respectively

of rejection (3.0) in respect of all the quality attributes after 90 days of storage life at -16 ± 2°C, the overall acceptability scored below 3.0. Therefore, acceptability of the control was considered to be upto 60 days. In case of GET_1 and GET_2, the fish was acceptable up to the sampling day of 90 and 120 respectively so far as sensory qualities were concerned. In this study, the muscle stiffness as usually experienced in frozen stored meat products was not perceived in the GE treated samples by the panel judges. Rather, smoothness and tenderness of GE treated fish steaks were improved. This may be due to mild proteolytic activity of the GE. Tsai et al. (2012) reported that ginger proteases could extensively degrade several major cytoskeletal/ myofibrillar proteins (titin, MHC, troponin-T, desmin and α-actinin) in GE-marinated duck muscle, which could lead to the breakdown of the integrity of muscle structure and could partially explain why tenderness of GE-treated muscle might be improved. GE showed proteolytic activity, resulting in an increase in collagen solubility and proteolysis in GE-treated spent hen muscle (Naveena & Mendiratta, 2001) and in GE-treated buffalo muscle. (Naveena et al., 2004).

The results of this study indicate that use of aqueous extract of ginger while storing fish steaks at low temperature exerted a marked inhibitory effect on quality loss in terms of both lipid oxidation and microbial growth.

References

Abii, T.A., Afieroho, O.E. and Namdi, F.U. (2007) Comparative assessment of heavy metals in *Oreochromis niloticus* Tilipia (from the Michael Okpara University of Agriculture Umudike) freshwater fish pond in Abia state with those from Uzere Freshwater pond in Delta state of Nigeria. J. Fish Int. 2(3): 226-230

Akoachere, J.F., Ndip, R.N., Chenwi, E. B., Ndip, L.M., Njock, T.E. and Anong, D.N. (2002). Antibacterial effect of *Zingiber officinale* and Garcinia kola on respiratory tract pathogens. East Afr. Med. J. 79: 588-592

Ali, B.H., Blunden, G., Tanira, M.O. and Nemmar, A. (2008) Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem. Toxicol. 46: 409-420

AOAC (2000) Official Methods of Analysis. 15thedn., Association of Official Analytical Chemists, Washington DC, USA

APHA (2001) Compendium of methods for microbiological examination of foods, 4th edn., (Yanderzant, C. and Splittstoesser, D.F., Eds), Chapter 4 and 6, Washington D.C., USA

Benjakul, S. and Bauer, F. (2001) Biochemical and physicochemical changes in catfish (*Silurus glanis* Linne) muscle as influenced by different freeze–thaw cycles. Food Chem. 72: 207-217

Bhaskar, N., Sachindra, N.M., Modi, V.K., Sakhare, P.Z. and Manhendrakar, N.S. (2006) Preparation of proteolytic activity rich ginger powder and evaluation of its tenderizing effect on spent-hen muscles. J. Muscle Foods 17: 174-184

Chrubasik, S., Pittler, M.H. and Roufogalis, B.D. (2005) *Zingiberis rhizoma*: A comprehensive review on the ginger effect and efficacy profiles. Phytomedicine. 12: 684-701

Chukwu, O.O.C. Odu, C.E., Chukwu, I.D., Chidozie, V.N., Onyimba, I.A. and Bala, Z. (2012) Carrot (*Daucus carrota*), garlic (*Allium sativum*) and ginger (*Gingiber officinale*) extracts as bacteria selective agents in culture media. Afr. J. Micro. Res. 6(2): 219-224

Chung, W.Y., Jung, Y.J., Surh, Y.J., Lee, S.S. and Park, K.K. (2001) Antioxidative and antitumor promoting effects of [6]- paradol and its homologs. Mutat. Res. 496: 199-206

Conway, E. J. (1947) Microdiffusion analysis and volumetric error. 4th edn., Van Nostrad Co.Inc., Newyork

Dyer, W. J., French, H.V. and Snow, J.M. (1950) Proteins in fish muscle. I. Extraction of protein fraction in fresh fish. J. Fish Res Board Canada. 7: 585-594

FAO (2008) FAO Fisheries and Aquaculture – Chemical elements of fish. http:// www.fao.org/fishery/topics/ 14820/en

Farrell, K.T. (1985) Spices, Condiments and Seasonings. 2nd edn., pp 15-253, Aspen Publication, Maryland

Garg, D.K., Stephen, J. and Valsan, A.P. (1982) Studies on frozen storage of ghol (*Pseudosciaena diacanthus*). Fish. Technol. 19: 29-31

Gokodlu, N., Ozden, O. and Erkan, N. (1998) Physical, chemical and sensory analysis of freshly harvested sardines (*Sardina pilchardus*) stored at 4ÚC. J. Aquat. Food Prod. Technol. 7: 5-15

Govindarajan, V.S. (1982) Ginger: Chemistry, technology, and quality evaluation: Part 2. Crit. Rev. Food Sci. Nutr. 17: 189-258

Herrero, M., Cifuentes, A., Ibanez, E. (2006) Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food by-products, algae and microalgae: a review. Food Chem. 98: 136-48

Hettiarachchy, N.S., Glenn, K.C., Gnanasambandam, R. and John- son, M.G. (1996) Natural antioxidant extracts from fenugreek (*Trigonella foenumgraecum*) for ground beef patties. J. Food Sci. 61: 516-519

Hori, Y., Miura, T., Hirai, Y., Fukumura, M., Nemoto, Y., Toriizuka, K. and Ida, Y. (2003) Pharmacognostic studies on ginger and related drugs-Part 1: five sulfonated compounds from *Zingiberis rhizome* (Shokyo). Phytochemistry 62: 613-617

Imadia, K., Fukushima, S., Shirai, T., Ohtani, M., Nakanish, K. and Ito, N. (1983) Promoting activities of butylated hydroxytoluene on 2-stage urinary bladed carcinogenesis and inhibition of c-glutamyl transpeptidase- positive foci development in the liver of rats. Carcinogenesis 4: 895-899

Ippoushi, K., Azuma, K., Ito, H., Horie, H. and Higashio, H. (2003) 6-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite induced oxidation and nitration reactions. Life Sci. 73: 3427-3437

Islam, M.N. and Joadder, M.A.R. (2005) Seasonal variation of the proximate composition of freshwater Gobi, *Glossogobius giuris* (Hamilton) from the River *Pamuscle tissuea*. Pakistan J. Biol. Sci. 8(4): 532-536

Jabeen, F. and Chaudhry, A. S. (2011) Chemical Composition and fatty acid profile of three freshwater fish species. Food Chem. 125: 991-996

Jacobs, M.B. (1958) The chemical analysis of foods and food products. pp 393-394, Kreiger Publ. Co. Inc. New York, USA

Jagetia, G.C., Baliga, M.S., Venkatesh, P. and Ulloor, J.N. (2003) Influence of ginger rhizome (*Zingiber officinale* Rose) on survival, gluthathione and lipid peroxidation in mice after whole body exposure to gamma radiation. Radiat. Res. 160: 584-592

Kim, B., Kim, J., Kim, H., and Heo, M. (1997). Biological screening of 100 plants for cosmetic use (II): antioxidative activity and free radical scavenging activity. Int. J. Cosm. Sci. 19: 299-307

Kim, D., Jeond, S. and Lee, C.H. (2003) Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 81: 321-326

Krishnakantha, T.P. and Lokesh, B.R. (1993) Scavenging of superoxide anions by spice principles. Indian J. Biochem. Biophys. 30: 133-134

Kuo, J.M., Yeh, D.B. and Pan, B.S. (1999) Rapid photometric assay evaluating antioxidative activity in edible plant material. Agric. Food Chem. 47: 3206-3209

Lakshmanan, P.T. (2002) Fish spoilage and quality assessment. In: Quality assurance in seafood processing (Iyer, T.S.G., Kandoran, M.K., Thomas, M. and Math, P.T., Eds), pp 28-45, Central Institute of Fisheries Technology (CIFT) and Society of Fisheries Technologists India (SOFTI), Cochin, India

Lee, M.A., Choi, J.H., Choi, S.Y., Kim, H.Y., Kim, H.W., Hwang, K.E., Chung, H.K. and Kim, C.J. (2011) Effect of *kimichi* ethanolic on oxidative stability of refrigerated cooked pork. Meat Sci. 89: 405-411

Lee, Y.B., Sehnert, D.J. and Ashmore, C.R. (1986) Antioxidant property of ginger rhizome and its application in meat products. J. Food Sci. 51: 20-23

Liao, C.H. (2007) Inhibition of Foodborne Pathogenesis by microflora recovered from fresh peeled baby carrot and propagated in culture. J. Food Sci. 72(4): M134-M139

Mah, J.H., Kim, Y.J. and Hwang, H.J. (2009) Inhibitory effects of garlic and other spices on biogenic amine production in *Myeolchi-jeot*, Korean salted and fermented anchovy product. Food Control. 20: 449-454

Mahady, G.B., Pendland, S.L., Yun, G.S., Lu, Z.Z. and Stoia, A. (2003) Ginger (*Zingiber officinale Roscoe*) and the gingerols inhibit the growth of Cag A+ strains of Helicobacter pylori. Anticancer Res. 23: 3699-3702

Malu, S.P., Obochi, G.O., Tawo, E.N. and Nyong, B.E. (2009) Antibacterial activity and medicinal properties of ginger (*Zingiber Officinale*). Global J. Pure Appl. Sci. 15: 365-368

Mansour, E.H. and Khalil, A.H. (2000) Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. Food Chem. 69: 135-141

Mascolo, N., Jain, R., Jain, S.C., Capasso, F., (1989) Ethnopharmacologic investigation of ginger (*Zingiber officinale*). J. Ethnopharmacol. 27: 129-140

Mendiratta, S.K., Anjaneyulu, A.S.R., Lakshmanan, V., Naveena, B.M. and Bisht, G.S. (2000) Tenderizing and antioxidant effect of ginger extract on sheep meat. J. Food Sci. Tech. 37: 565-570

Mensor, L.I., Menezes, F.S., Leitao, G.G., Reis, A.S., dos Santos, T., Coube, C.S. and Leitao, S.G. (2001) Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Res. 15: 127-130

Nain, N., Ahlawat, S.S., Khanna, N. Chhikara, S.K. (2009) Properties and utility of commonly used natural spices – a review. Agric. Rev. 30(2): 108-119

Nair, P.G.V., (2002) Biochemical Composition of fish. In: Textbook of Fish Processing Technology (Gopakumar, K. Ed), pp 18-30, Indian Council of Agricultural Research. New Delhi, India

Naveena, B.M. and Mendiratta, S.K. (2001) Tenderization of spent hen meat using ginger extract. Brit. Poultry Sci. 42: 344-349

Naveena, B., Mendiratta, S.K. and Anjaneyulu, A.S.R. (2004) Tenderization of buffalo meat using plant proteases from *Cucumis trigonus Boxb* (Kachri) and *Zingiber officinale roscoe* (Ginger rhizome). Meat Sci. 68: 363-369

Negbenebor, C., Balamy, Y. and Igene, J. (1995) Yield and storage stability of patties from pre-riggor beef previously dipped in potassium sorbate and ginger solutions. Int. J. Food Sci. Technol. 30(1): 65-70

Ninan, G., Bindu, J. and Joseph, J. (2008) Frozen storage studies of minced based products developed from Tilapia (*Oreochromis mossambicus*, Peters 1852). Fish. Technol. 45(1): 35-42

Okonta, A.A. and Ekelemu, J.K. (2005) A preliminary study of micro-organisms associated with fish spoilage in Asaba, Southern Nigeria. Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria (FISON), 14th-18th November, pp 557-560, Port Harcourt

Park, E.J. and Pezzuto, J.M. (2002). Botanicals in cancer chemoprevention. Cancer Met. Rev. 21: 231-255

Patro, B.S., Rele, S., Chintalwar, G.J., Chattopadhyay, S., Adhikari, S. and Mukherjee, T. (2002) Protective activities of some phenolic 1, 3-diketones against lipid peroxidation: possible involvement of the 1,3-diketone moiety. Chembiochem. 3: 364-370

Raju, C.M., Mathew, S. and Mathew, M. (2010) Comparative Evaluation of Non- Protein Nitrogenous Compound in Fishes of Fresh and Brackish Water Environments. Fish Technol. 47(2): 161-166

Reddy, G.V.S., Srikar, L.N., Khuntia, B.K. and Kumar, N.V. (1995) Effect of pre-process storage in ice on the chemical characteristics of fish mince. J. Food Sci. Technol. 32: 315-319

Roy, J., Shakaya, D.M., Callery, P.S. and Thomas, J.G. (2006) Chemical constituents and antimicrbila activity of a traditional herbal medicine containing garlic and black cumen. Afr. J. Trad. 3: 1-7

Sa-Nguanpuag, K., Kanlayanarat, S., Srilaong, V., Tanprasert, K. and Techavuthiporn, C. (2011) Ginger (*Zingiber officinale*) oil as an antimicrobial agent for minimally processed produce: a case study in shredded green papaya. Int. J. Agric. Biol. 13: 895 901

Sebiomo, A., Awofodu, A.D., Awosanya, A.O., Awotona, F.E. and Ajayi, A.J. (2011) Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. J. Microbiol. Antimicro. 3: 18-22

Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. (2009) Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. J. Sci. Food and Agric. 89(11): 1879-1885

Shekhar, C., Rao, A.P. andAbidi, A.B. (2004) Changes in muscle biochemical composition of *Labeo rohita* (Ham.) in relation to season. Indian J. Fish. 51(3): 319-323

Sofowora, A. (1993) Medicinal Plants and Traditional Medicine in Africa, 289 p, Spectrum Books Ltd. Ibadan, Nigeria

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P. and Gargova, S. (2007) Antioxidant activity of a ginger extracts (*Zingiber officinale*). Food Chem. 102: 764-770

Su, L., Yin, J.J., Charles, D., Zhou, K., Moore, J. and Yu, L. (2007) Total phenolic contents, chelating capacities, and venging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. Food Chem. 100: 990-997

Suriah, R.A., Huah, T.S., Hassan, O. and Daud, N.M. (1995) Fatty acid composition of some Malaysian freshwater fish. J. Food Chem. 54: 45-49

Tironi, V., Tomas, M. and Anon, M.C. (2010) Quality loss during the frozen storage of sea salmon (*Pseudopercis semifasciata*). Effect of rosemary (*Rosmarinus officinalis*) extract. Food Sci. Technol. 43: 263-272

Trease, G.E. and Evans, W.C. (1983) Textbook of Pharmacognosy. 12th Edn., pp 21-22, Bailliere Tindall, London

Tsai, L.L., Yen, N.J. and Chou, R.G.R. (2012) Changes in Muscovy duck breast muscle marinated with ginger extract. Food Chem. 130: 316-320

Vasala, P.A. (2004) Ginger. In: Handbook of Herbs and Spices, Vol 1 (Peter K. V., Eds.), pp 195-206, Woodhead Publishing: Cambridge, UK

Yen, G., Chang, Y. and Su, S. (2003) Antioxidant activity and active compounds of rice koji fermented with *Aspergillus candidus*. Food Chem. 83: 49-54