Shelf Life Extension of Tuna Fillets using Natural Preservatives Isolated from Garlic

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Abstract

In order to develop a natural preservative for improving the shelf life of fishery products, solvent-free extracts were prepared from raw, water bath boiled (WBB) and microwave-boiled (MOB) garlic (*Allium sativam*). Garlic juices showed antioxidant activity as determined by DPPH radical scavenging method and FRAP antioxidant power assay and antibacterial activity against all the 13 fish spoilage and fish-borne bacteria tested by agar well diffusion method. Raw garlic juice showed significant ($p<0.05$) radical scavenging effect ($96.81\pm0.11\%$) followed by WBB juice ($58.70\pm2.51\%$) and MOB juice ($50.33\pm0.22\%$) at 20% concentration. The IC_{50} value of raw, WBB and MOB garlic juices were respectively 7.63, 14.86 and 18.76 $\mu g\ ml^{-1}$. Among the three garlic juices, the raw garlic juice showed the strongest antibacterial activity against both gram-positive and gram-negative bacterial strains. Highest zone of inhibition was against gram-positive bacteria *Bacillus licheniformis* (55.0 ± 0.57 mm) followed by gram-negative bacteria *Yersinia enterocolitica* (44.0 ± 0.57 mm) indicating its potential as a natural preservative. The natural preservative isolated from garlic significantly delayed the rate of microbial spoilage and extended the shelf life of tuna fillets by six days during refrigerated storage demonstrating its potential as an excellent natural antioxidant and antibacterial agent which can be used as an effective alternative to synthetic antioxidant and antibacterial agents.

Keywords: Antioxidant activity, antibacterial activity, garlic juice, solvent-free extraction, tuna fillet

Introduction

Fish is a highly perishable food item and spoilage occurs soon after its death. Different mechanisms are responsible for fish deterioration during refrigerated or low temperature storage (Howgate, 2006) especially due to lipid oxidation and microbial spoilage (Pereirade Abreu et al., 2010). Lipid oxidation is a critical problem during food processing, distribution, storage and consumption as it decreases food quality, stability, safety and nutritive value. Antioxidants may suppress the lipid peroxidation by reducing the availability of metal catalysts and quenching the radicals in the system, leading to the termination of oxidative radical chain reactions (Athukorala et al., 2003; Matthaus, 2002). The most widely used synthetic antioxidants in food are butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), propyl gallate (PG) and tertiary butyl hydro quinone (TBHQ). However, due to their unstable nature and possible adverse side effects, the demand for novel natural antioxidant sources has greatly increased (Aligiannis et al., 2003).

Food poisoning occurs when food gets contaminated with bacteria or other pathogens due to improper food processing, handling, preparation and storage. Synthetic chemicals and antibiotics are widely used against these microorganisms, but bacteria develop resistance against many antibiotics due to the indiscriminate use of commercial antibiotics (Mukherjee et al., 2002). In addition, sometimes these antibiotics cause allergic reaction and immunity suppression. Consumers are also concerned about the safety of foods containing synthetic preservatives. Hence, there has been increasing interest towards the development of new types of effective, nontoxic, natural antibacterial compounds, such as juices of spices and herbs for food preservation (Smid & Gorris, 1999).

Several reports are available on antioxidant and antimicrobial activity of garlic. However, many of them are based on its organic solvent extracts and essential oil (Ameen et al., 2003; Shin & Kim, 2004) which may pose residue problem and difficulty in incorporation into aqueous food systems. Further, many such reports are based on *in-vitro* studies and very few studies are applied in real food systems. Ethanolic extracts of garlic have been reported to cause detrimental effect and residue problem. Use of essential oils is still limited because of their susceptibility to oxidation, volatility and/or thermal instability and immiscible nature with aqueous system (Debberma et al., 2012). In order to avoid such problems, we followed different methods for extraction of garlic without adding any organic solvent. Tuna fillets are one of the commercially important fishery products with high economic value and growing demand in international markets because of their nutritional value. Hence, the present study was aimed at evaluating the antioxidant and antibacterial activities of garlic juice and its effect on extension of shelf life of tuna fillets during storage.

Materials and Methods

Garlic (*Allium sativum*) was purchased from local market, peeled and washed in distilled water. 100 g of garlic was ground for 2 min without adding water or organic solvents. Three different types of garlic juice were prepared from the ground mixture (i) raw was obtained by simple sieving/filtering of the ground mixture through a fine cotton cloth; (ii) water bath boiled juice was obtained by boiling the ground mixture for 30 min at 100°C and sieved (iii) micro oven boiled (MOB) juice was obtained by cooking 100 g of ground mixture in microwave oven at 900 watts for 2 min and sieved. The final quantity of the Raw, WBB, MOB juices were 40, 20, 20 ml respectively. The three types of juices were stored at 4°C for further analysis. All the chemicals and media used in the study were of analytical grade and purchased from various companies *viz*., Sigma, Qualigens, Merck and HIMEDIA.

Antioxidant activity of garlic juices was measured in terms of hydrogen-donating or radical scavenging ability, using the stable radical 2, 2 -Diphenyl-1-picrylhydrazyl (DPPH). A volume of 150 µl of garlic juice of five different concentrations (4, 8, 12, 16 and 20%) was made by diluting with distilled water and put into screw cap test tubes, and 2 ml 6×10^{-5} M

methanolic solution of DPPH was added. The percentage inhibition of the DPPH radical was calculated according to the method of Yen & Duh (1994).

Ferric Reducing Antioxidant Power (FRAP) assay was carried out according to the procedure of Benzie & Strain (1996) with some modifications. A volume of 20 µl of juices with different concentration (20, 40, 60, 80 and 100%) of juices and 2 ml of the FRAP reagent was added into the tubes and mixed well. The absorbance of the reaction mixture was then recorded using UV-spectrophotometer at 593 nm after 4 min. The results were expressed as µ mol $Fe(II)g^{-1}$ dry weight of garlic.

Total phenolic content in the garlic juices was estimated by the method of Singleton & Rossy (1965). The amount of total phenolics was expressed as catechol equivalents in mg phenols 100 g^{-1} of garlic juices.

Standard cultures of bacterial strains were procured from Microbial Type Culture Collection (MTCC), Chandigarh (India) and few bacterial strains (*Aeromonas hydrophila, Bacillus cereus, Bacillus pumilus* and *Micrococcus* sp.) were isolated from fish and they were maintained on Nutrient Agar (NA) slants at 4°C. Standard bacterial strain used were *Pseudomonas aeruginosa* (MTCC-4676), *Escherichia coli* (MTCC-40), *Salmonella* Typhi (MTCC-3220), *Yersinia enterocolitica* (MTCC-859), *Vibrio cholerae* (MTCC-3904), *Bacillus subtilis* (MTCC-121), *Staphylococcus aureus* (MTCC-87), *Listeria monocytogenes* (MTCC-657) and *Bacillus licheniformis* (MTCC-429).

Agar well diffusion method (NCCLS, 1993) was used for antibacterial assay. A suspension (containing approximately 10^6 cfu ml^{-1}) was swabbed with a cotton swab in three directions on MHA plates. Sterile cut pipette tips of 6 mm diameter were used to make wells, 50 µl and 100 µl of garlic juices were added into the wells. Ampicillin 1% (100 µl well^{-1}) and 10% DMSO (100 µl well^{-1}) were used as the positive and negative controls respectively. The plates were then incubated for 24 h at 37°C. The results were recorded by measuring the diameter of the growth inhibition zones surrounding the loaded agar well. Tests were carried out in triplicate.

Tuna fillets were prepared from long tail tuna (*Thunnus tonggol*) fish procured from Versova fish market, Mumbai. The mean weight of the fillets was about 100-150 g. The fillets were dipped in 10 and

20% of raw garlic juices and kept at 4°C for 30 min. After draining off the excess liquid, the fillets were placed in sterile polythene bags and stored at refrigerated temperature (4°C). Periodically (0, 4, 7, 10 and 13 days), samples in triplicates were randomly removed from each treatment group, and the chemical, microbial and sensory characteristics were determined.

Total plate count was estimated by spread plate technique (BAM, 2001). The average counts of the triplicates were taken and the counts were calculated as cfu g^{-1} of the sample. TBA of fish sample was estimated by standard procedure as given by Tarladgis et al. (1960). TBA value is expressed as mg malonaldehyde (MDA) kg^{-1} sample.

Tuna fish fillets were subjected to sensory evaluation based on characterization and differentiation of the various sensory characteristics such as appearance, odour, flavour, taste, consistency and overall acceptability during storage days. The panellists were asked to assign a score of 1-9 as prescribed by Meliguard et al. (1999). An overall acceptance score was calculated as an average of all scores and score of 6 and above was considered as acceptable.

To verify the statistical significance of the selected parameters, means±SE of analyzed samples were (n=3) used. For the assessment of antioxidant and antibacterial activity, one-way ANOVA was used. The P values of <0.05 were considered significant.

Results and Discussion

Table 1, shows the effective concentrations of each juice required to scavenge the DPPH radical and the scavenging values as inhibition (%). It can be seen that the juices exhibited varying degrees of scavenging capacities. Raw garlic juice showed the strongest

(p<0.05) radical scavenging effect (96.81±.11%) followed by WBB juice (58.70±2.51%) and MOB juice (50.33±0.22%) at 20%. The IC_{50} value of raw, WBB and MOB garlic juices were respectively 7.63, 14.86 and 18.76 µg ml^{-1}. It could be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (Sawa et al., 1999) and the results (Table 1) showed that there is a large difference in inhibition activity of raw juice and WBB and MOB garlic juices at 20%.

FRAP assay, which is a measure of the antioxidant potential, was estimated based on the ability of garlic juices to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex due to antioxidants. The reducing power of juices serves as a significant indicator of its antioxidant activity. Among all the juices, raw garlic juice showed the highest FRAP value, followed by WBB and MOB garlic (249.79±2.48, 169.45±7.17, 115.79±1.01 µ mol Fe(II) g^{-1} dry wt respectively) as shown in Table 2. The antioxidant activities of phenolic compounds are mainly of redox properties, including free radical scavenging, hydrogen donating and singlet oxygen quenching (Mayachiew & Devahastin, 2008). Pulido et al. (2000) reported the reducing capacity of polyphenols, as determined by the FRAP assay.

Among the juices, raw garlic juice contained a significantly higher amount of total phenolics (138.33±5.74 mg phenols 100 g^{-1}) followed by WBB juice (86.58±1.12 mg phenols 100 g^{-1}) and MOB juice (82.41±1.90 mg phenols 100 g^{-1}) respectively and were significantly different p<0.05 (Table 3). Many studies observed correlations between the antioxidant activity and content of phenolic compounds (Nuutila et al., 2003; Parejo et al., 2002). In the bivariate correlation of the present study, the correlation coefficient of changes of antioxidant capacity (%) with total phenolic contents (TPC)

Table 1. Antioxidant activity of different types of garlic juice at different concentrations measured by the DPPH method

Sample/ Concentration (%)	Inhibition (%)					IC $_{50}$
	4	8	12	16	20	
Garlic raw	27.82±0.89^{aC}	45.52±1.00^{bC}	61.36±1.58^{cB}	81.70±1.27^{dB}	96.81±0.11^{eC}	7.63
Garlic WBB	22.46±0.8^{aB}	30.95±0.34^{bB}	41.28±0.47^{cA}	51.08±2.82^{dA}	58.70±2.51^{dB}	14.86
Garlic MOB	14.83±0.59^{aA}	24.82±0.87^{bA}	38.23±0.68^{cA}	45.18±0.65^{dA}	50.33±0.22^{eA}	18.77

IC_{50}, concentration (g l^{-1}) for a 50% inhibition
Results are mean ± standard error (n=3); values with different letters within a row (a-e) and values with different letters within a column (A-C) are significantly different (p<0.05) in one way ANOVA followed by Tukey HSD test

Table 2. Antioxidant activity of different types of garlic juice at different concentrations measured by FRAP method

Sample/ Concentration (%)	μ mol Fe (II) g^{-1} dry wt				
	20	40	60	80	100
Garlic raw	45.45±2.48[aA]	93.45±8.27[bB]	111.2±9.92[bC]	157.12±1.66[cB]	249.79±1.9[dC]
Garlic WBB	45.87±3.87[aA]	78.37±2.94[aB]	80.04±5.44[aB]	131.45±17.97[bB]	169.45±7.17[bB]
Garlic MOB	36.45±0.46[aA]	41.45±1.47[aA]	48.20±0.87[abA]	68.54±510.1[bA]	115.79±1.01[cA]

Results are mean ± standard error (n=3); values with different letters within a row (a-e) and values with different letters within a column (A-C) are significantly different (p<0.05) in one way ANOVA followed by Tukey HSD test

showed that DPPH values and FRAP values were highly correlated with TPC in all the garlic juices. In DPPH method, raw garlic (R^2=0.9935, p<0.01), WBB garlic (R^2=0.9946, p<0.01) and MOB garlic (R^2=0.9331, p<0.05) juices showed high positive linear correlation with total phenolic contents. In FRAP assay also, raw garlic juice (R^2=0.9761, p<0.01), WBB garlic (R^2=0.9783, p<0.01) and MOB garlic (R^2=0.9582, p<0.05) showed similar correlation with total phenolic contents indicating phenolic compounds as responsible for their antioxidant capacity. It is well known that phenolic compounds contribute directly to the antioxidant activity of spice extracts (Rice-Evans et al., 1996). Much of the total antioxidant activity of fruits and vegetable is related to their phenolic content, not only to their vitamin C content, also a correlation exists between the polyphenol content and antioxidant activities (Ramakrishnan et al., 2010).

From these results it can be observed that raw garlic juice shows excellent antioxidant activity due to high amount of total phenolic content compared to other two boiled juices, revealing that the boiling of garlic reduces the total phenolic content. The decrease in antioxidant capacity of boiled garlic at 100°C (Jastrzebski et al., 2007; Wangcharoen & Morasuk, 2009) and escape of some volatile sulphur-containing compounds at 65°C (Yin et al., 2002) have been observed earlier.

Inhibition zone of raw garlic juice against 13 fish-borne pathogenic and fish spoilage bacteria showed that garlic juice at different doses had substantial inhibitory effect against all the tested bacterial strains (Table 4). Among all the tested juices, raw garlic juice exhibited the strongest antibacterial activity against both gram-positive and gram-negative bacterial strains. The highest inhibition zone was shown against gram-positive bacteria

B.licheniformis (55.00±0.57 mm) at 100 µl followed by gram-negative bacteria *Y. enterocolitica* (44.00±0.57 mm). At 100 µl dose of raw garlic juice, *P. aeruginosa*, *E. coli*, *S.* Typhi, *V. cholerae*, *B. subtilis*, *S. aureus*, *L. monocytogenes*, *B. licheniformis*, *B. cereus*, and *B. Pumilus* were found more susceptible compared to 1% Ampicillin (Table 4), which clearly revealed that the raw garlic juice exhibits greater antibiotic action against bacteria than synthetic antibiotic.

WBB garlic juice showed different degrees of inhibition zone against all tested bacterial strains (Table 4). WBB garlic juice showed high activity against gram-positive bacteria such as *Micrococcus* sp, *B. cereus* and *B. subtilis*, with their respective zone of inhibition of 30.00±0.57 mm; 29.33±0.88 mm and 28.66±0.33 mm at 100 µl of juice (Table 4). MOB garlic juice showed the highest antibacterial activity against gram-positive bacteria *B. subtilis* with high inhibition zone (45.66.±0.33 mm) at 100 µl (Table 4). The garlic WBB and MOB juices also showed strong antibacterial activity against gram-positive bacteria than gram-negative bacteria.

The results revealed clear differences in the sensitivity of different bacteria to garlic juices, suggesting that mechanisms of action are different in different organisms. This may be due to the lipid content of the membranes of different microorganisms and the permeability of bioactive constituents. The antibacterial activity of garlic has been ascertained to the action of bioactive molecules such as allicin, diallylthiosulphinic acid and diallyl disulphide (Avato et al., 2000). It was also observed that WBB and MOB garlic juices showed less antibacterial activity for certain bacteria than raw garlic juice. The loss of antibacterial activity by heating at 100°C and 900 Watts in WBB and MOB juices may be due to loss of volatile molecules and/or the physical and chemical changes that take place during heating. It

Table 3. Total phenolics contents of different types of garlic juice at different concentrations

Sample/ Concentration (%)	mg phenols 100 g^{-1}				
	20	40	60	80	100
Garlic raw	5.16±1.58^{aA}	42.16±0.84^{bC}	72.08±1.37^{cC}	96.5±2.50^{dB}	138.33±5.74^{eB}
Garlic WBB	2.66±0.96^{aA}	28.08±1.97^{bB}	42.08±4.29^{cB}	69.75±1.32^{dA}	86.58±1.12^{eA}
Garlic MOB	4.16±0.60^{aA}	13.25±0.80^{aA}	27.08±0.50^{bA}	57.83±5.21^{cA}	82.41±1.90^{dA}

Results are mean ± standard error (n=3); values with different letters within a row (a-e) and values with different letters within a column (A-C) are significantly different (p<0.05) in one way ANOVA followed by Tukey HSD test

Table 4. Diameter of inhibition zone (mm) of garlic raw juice against fish-borne pathogen and spoilage bacteria using well diffusion method

Name of Bacteria/ Sample/ Concentration (µl)	Garlic raw juice		Garlic WBB juice		Garlic MOB juice		Standard references	
							Positive Control	Negative control
	50	100	50	100	50	100	Ampicillin (1%)	10% DMSO
Pseudomonas aeruginosa	15.33±0.33^{ab}	23.33±0.33^{d}	11.33±0.33^{a}	12.66±0.33^{b}	21.66±0.33^{b}	26.00±0.57^{c}	11.33±0.33^{a}	—
Aeromona shydrophila	32.00±0.00^{b}	38.66±0.33^{d}	14.00±0.00^{b}	20.33±0.66^{d}	19.66±0.66^{b}	22.00±0.00^{c}	41.66±0.33^{e}	—
Escherichia coli	13.33±0.33^{b}	21.66±0.33^{c}	15.66±0.33^{b}	18.66±0.33^{c}	20.33±0.33^{b}	25.33±0.33^{d}	13.00±0.33^{a}	—
Salmonella Typhi	18.66±0.33^{c}	21.33±0.33^{d}	10.66±0.33^{b}	12.66±0.33^{c}	18.66±0.33^{b}	22.00±0.00^{d}	11.00±0.57^{a}	—
Yersinia enterocolitica	34.66±0.33^{a}	44.00±0.57^{b}	14.33±0.33^{b}	19.66±0.66^{c}	17.66±0.33^{a}	23.00±0.00^{b}	45.66±2.18^{b}	—
Vibrio cholerae	25.66±0.33^{b}	31.33±0.33^{d}	11.00±0.00^{ab}	13.33±0.33^{c}	22.66±0.33^{b}	26.00±0.57^{c}	21.00±0.57^{a}	—
Bacillus subtilis	16.00±0.33^{b}	20.66±0.33^{c}	23.66±0.33^{b}	28.66±0.33^{d}	35.00±0.57^{b}	45.66±0.33^{d}	11.66±0.33^{a}	—
Staphylococcus aureus	17.33±0.33^{b}	20.33±0.33^{c}	10.33±0.33^{a}	14.00±0.00^{b}	16.33±0.33^{b}	19.66±0.33^{c}	12.00±0.57^{a}	—
Listeria monocytogenes	16.33±0.33^{c}	21.66±0.33^{d}	13.33±0.33^{b}	21.00±0.00^{d}	28.66±0.33^{b}	33.66±0.66^{c}	11.00±0.57^{a}	—
Bacillus licheniformis	41.00±0.57^{b}	55.00±0.57^{e}	16.00±0.00^{b}	19.00±0.00^{d}	15.33±0.33^{b}	19.00±0.57^{d}	48.33±0.33^{c}	—
Bacillus cereus	30.33±0.33^{c}	39.33±0.33^{e}	25.33±0.33^{b}	29.33±0.88^{c}	20.66±0.33^{b}	28.00±0.00^{d}	20.33±0.33^{a}	—
Bacillus pumilus	26.00±0.00^{b}	30.00±0.00^{d}	18.66±0.66^{b}	21.00±0.00^{c}	20.00±0.00^{b}	23.00±0.00^{d}	25.33±0.33^{b}	—
Micrococcus sp.	34.00±0.00^{a}	38.66±0.33^{c}	21.00±0.00^{b}	30.00±0.57^{d}	20.66±0.33^{b}	28.00±0.00^{d}	41.66±0.33^{d}	—

Results are mean ± standard error (n=3), values within a row with different superscript letters are significantly different (p<0.05) in one way ANOVA followed by Tukey HSD test

has been reported that the antibacterial activity of garlic decreased as the heating temperature increased and the activity of garlic extract by heating at 80°C to 90°C for 5 min was completely destroyed (Dababneh & Al-Delaimy, 1984).

Based on the antioxidant and antibacterial activity results of different garlic juices (Tables 1-4), raw garlic juice was selected to evaluate its preservative effect on tuna fish fillets during storage studies.

TBA value of untreated fish fillet progressed significantly as storage days increased but raw garlic juice treated fillets retarded the increase in TBA values (Fig. 1). The initial TBA value of tuna fillets was 0.82 ± 0.01 mg MDA kg^{-1} muscle. A rapid lipid oxidation occurred in the control, by which TBA value increased to 2.75 ± 0.03 and 7.67 ± 0.01 mg MDA kg^{-1} muscle on Day 4 and Day 10, respectively. The fillets treated with garlic juices 10 and 20% had significantly ($p< 0.05$) lower TBA values throughout the storage period of 13 days, indicating antioxidant activity of garlic juice in retarding lipid oxidation. At the end of storage period, the most profound differences were noticed for samples treated with raw garlic juices, where the TBA values were well below the threshold level of 3 mg MDA kg^{-1} fish muscle (Connell, 1990) throughout the storage period. Whereas, the TBA of control fillets crossed 3 mg of MDA kg^{-1} on 7th day of storage indicating early spoilage of fillets. Hence, it is clearly evident that the garlic juices extended the shelf life of fish fillets by six days compared to control samples.

Changes in the total bacterial counts during storage are shown in Fig. 2. The initial TPC counts (4.12 ± 0.00 log cfu g^{-1}) of all fillet samples indicate acceptable quality, considering the proposed upper limit for aerobic plate count of 5.70 log cfu g^{-1} for fresh fish (ICMSF, 1986). The microbiological condition of fish muscle is directly related to fishing ground and environmental factors (Ludorff & Meyer, 1973). During the storage period, the TPC counts gradually increased as storage time increased. TPC count of fish fillets dipped in 10 and 20% of raw garlic juice increased from the initial level of 4.12 ± 0.00 log cfu g^{-1} to 7.45 ± 0.00 log cfu g^{-1}, 7.20 ± 0.00 log cfu g^{-1} respectively on 13th day of storage period, whereas

TPC of control has reached 8.97 ± 0.00 log cfu g^{-1}. The control fish fillet reached the TPC count 7.45 ± 0.00 log cfu g^{-1} at 7th day of storage, whereas fish fillet treated with 20% raw garlic juice, TPC count reached 7.20 ± 0.00 log cfu g^{-1} at 13th day of storage. This revealed that the raw garlic juice significantly delayed the rate of microbial spoilage and extended the shelf life of fish fillet by 6 days during storage. In sensory evaluation, the overall acceptability of control and treated tuna fillets in 10 and 20% of garlic juices gradually decreased during storage (Fig. 3). The overall acceptance score of tuna fish fillets dipped in 10 and 20% of raw garlic juices got more than the acceptable level (>6), while control tuna fish fillet became unacceptable (5.38 ± 0.11) after a storage period of 13 days (Fig. 3), which clearly showed that raw garlic juices effectively extended the shelf life of tuna fillet during refrigerated storage at 4°C.

It can be concluded from this study that garlic juice has excellent antioxidant and antibacterial activities against all 13 fish spoilage and fish-borne bacteria. The raw garlic juice has higher antioxidant activity because of its high phenolic content, but water bath-boiled and microwave oven boiled extracts showed reduced antioxidant activity with reduced phenolic contents. Similarly, raw garlic juice showed the strongest antibacterial activity against both gram-positive and gram-negative bacterial strains. The extension of shelf life of tuna fish fillets by dip treatment in raw garlic juice (at 10 and 20%) reveals that raw garlic juice can be used as a natural preservative to improve the shelf life of fishery products.
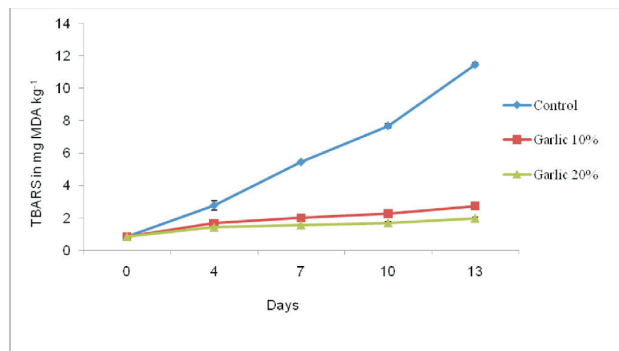

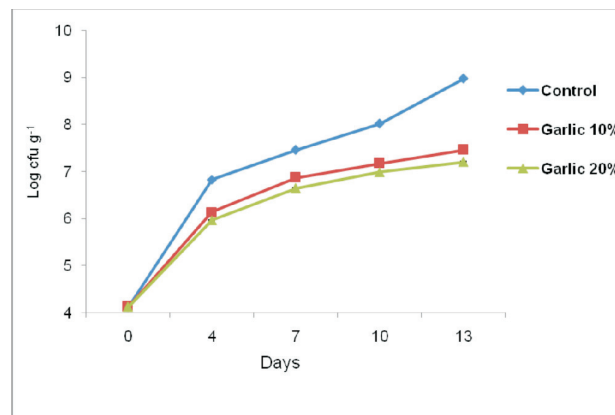Fig. 1. Changes in the TBA in tuna fillets dipped in raw garlic juices during storage

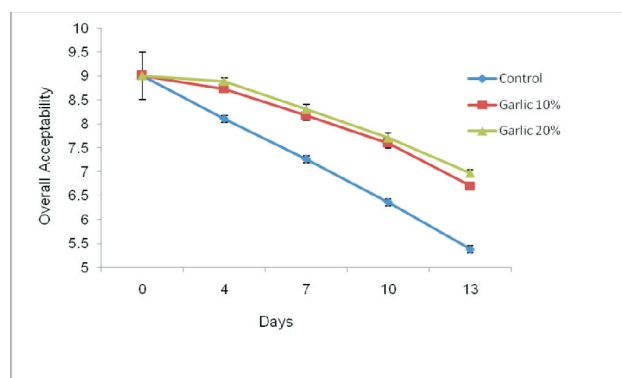Fig. 2. Changes in TPC of tuna fillets dipped in raw garlic juices during storage

Fig. 3. Changes in over all acceptability of tuna fillets dipped in 10 and 20% raw garlic juices during storage

Acknowledgements

References

Aligiannis, N., Mitaku, S., Tsitsa-Tsardis, E., Harvala, C., Tsaknis, I., Lalas, S. and Haroutounian, S. (2003) Methanolic extract of *Verbascummacrurum* as a source of natural preservative against oxidative rancidity. J. Agric. Food Chem. 51: 7308-7312

Ameen, M., Musthapa, M. S., Abidi, P., Ahmad, I. and Rahman, Q. (2003) Garlic attenuates chrysotile-mediated pulmonary toxicity in rats by altering the phase I and phase II drug metabolizing enzyme system. J. Biochem. Mol.Toxicol. 17 (6): 366-371

Athukorala, Y., Lee, K. W., Shahidi, F., Heu, M. S., Kim, H. T., Lee, J. S. and Jeon, Y. J. (2003) Antioxidant efficacy of extracts of an edible red alga, (*Grateloupia filicina*) in linoleic acid and fish oil. J. Food Lipids. 10: 313-327

Avato, P., Tursil, E., Vitali, C., Miccolis, V. and Candido, V. (2000) Allylsulfide constituents of garlic volatile oil as antibacterial agents. Phytomedicine. 7(3): 239-243

BAM (2001) Aerobic plate count. In: Bacteriological Analytical Manual, Chapter 3; United States Food and Drug Administration

Benzie, I. F. F. and Strain, J. J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239: 70-76

Chung, L. Y. (2006) The antioxidant property of garlic compounds: Allyl cysteine, alliin, allicin and allyl disulphide. J. Med. Foods. 9: 205-213

Connell, J. J. (1990) Methods of assessing and selecting for quality, In: Control of Fish Quality (Connell, J. J., Eds) pp 122–150, Fishing News Books, Oxford, UK

Dababneh, B. F. A. and Al-Delaimy, K. S. (1984) Inhibition of *Staphylococcus aureus* by garlic extract. Lebensm. Wiss. Technol. 17: 29-31

Debbarma, J., Kishore, P., Nayak, B. B., Kannuchamy, N. and Gudipati, V. (2012) Antibacterial activity of ginger, eucalyptus and sweet orange peel essential oils on fish-borne bacteria. J. Food Process. doi: 10.1111/j.1745-4549.2012.00753.x

Howgate, P. (2006) A review of the kinetics of degradation of inosine monophosphate in some species of fish during chilled storage. Int. J. Food Sci. Tech. 41: 341-353

ICMSF (1986) Microorganisms in Foods, Book 2. Sampling for Microbiological Analysis: Principles and Specific Applications 2nd edn., University of Toronto, Toronto

Jastrzebski, Z., Leontowicz, H., Leontowicz, M., Namiesnik, J., Zachwieja, Z., Barton, H., Pawelzik, E., Arancibia-Avila, P., Toledo, F. and Gorinstein, S. (2007) The bioactivity of processed garlic (*Allium sativum L.*) as shown In Vitro and In Vivo studies on rats. Food Chem. Toxicol. 45: 1626-1633

Ludorff, W. and Meyer, V. (1973) Fische und Fischerzeugnisse.Verlag Paul Parey in Hamburg und Berlin. ISBN: 3 489 71914 X

Matthaus, B. (2002) Antioxidant activity of juices obtained from residues of different oil seeds. J. Agric. Food Chem. 50: 3444-3452

Mayachiew, P. and Devahastin, S. (2008) Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. Food Sci. Tech. 41: 1153-1159

Meilguard, M., Civille, G. V. and Carr, B. T. (1999) Sensory evaluation techniques, 387p, Boca Raton, Fla: CRC Press

Mukherjee, P. K., Saritha, G. S. and Suresh, B. (2002) Antimicrobial potential of two different Hypericum species available in India. Phytotherapy Res. 16: 692-695

Nuutila, A. M., Puupponen-Pimia, R., Aarni, M. and Oksman-Caldentey, K. M. (2003) Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical-scavenging activity. Food Chem. 81: 485–493

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J. and Codina, C. (2002) Comparison between the radical scavenging activity and antioxidant activity of six distilled and non distilled Mediterranean herbs and aromatic plants. J. Agric. Food Chem. 50: 6882–6890

Pereira de Abreu, D. A., Paseiro Losada, P., Maroto, J. and Cruz, J. M. (2010) Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar* L.). Food Res. Int. 43: 1277-1282

Pulido, R., Bravo, L. and Calixo, F. S. (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 4: 3396-3402

Ramakrishnan, K., Narayanan, P., Vasudevan, V., Muthukumaran, G. and Antony, U. (2010) Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. J. Food Sci. Tech. 47 (1): 27-33

Rice-Evans, C., Miller, N. J. and Paganga, G. (1996) Structure antioxidant activity relationships of ûavonoids and phenolic acids. Free Radical Biol. Med. 20: 933-956

Sawa, T., Nakao, M., Akaike, T., Ono, K. and Maeda, H. (1999) Alkyl peroxyl radical scavenging activity of various flavonoids and other phenolic compounds: implications for the antitumor-promoter effect of vegetables. J. Agric. Food Chem. 47: 397-402

Shin, S. H. and Kim, M. K. (2004) Effect of dried powders or ethanol extracts of garlic flesh and peel on lipid metabolism and antithrombogenic capacity in 16-month-old rats. Hanguk Yongyang Hakhoechi, 37 (7): 515-524

Singleton, V. L. and Rosy, J. A. (1965) Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. American J. Enol. Viticul. 16: 144-158

Smid, E. J. and Gorris, L. G. M. (1999) Natural antimicrobials for food preservation In Handbook of Food Preservation, (Rahman, M. S., Ed) pp 285-308 Marcel Dekker. New York

Tarladgis,G. B., Watts, M. B and Younathan, T. M. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J Am Oil Chem Soc. 37: 44-50

Wangcharoen, W. and Morasuk, W. (2009) Effect of heat treatment on the antioxidant capacity of Garlic. Maejo Int. J. Sci. Tech. 3:60-70

Yen, G. C. and Duh, P. D. (1994) Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. J. Agric. Food Chem. 42: 629-632

Yin, M. C., Hwang, S. W. and Chan, K. C. (2002) Nonenzymatic activity of four organosulfur compounds derived from garlic. J. Agric. Food Chem. 50: 6143-6147