J. Agr. Sci. Tech. (2013) Vol. 15: 737-746

Antioxidant Activities of Different Spices on the Lipid Oxidation of Cooked and Uncooked Fillet of Two Fish Species Belonging to the Genus *Puntius*

P. Goswami[1], P. Mandal[2], P. Jha[3], T. Misra[4], and S. Barat[1]*

ABSTRACT

Twenty spices were employed to preserve the cooked and uncooked fillet of *Puntius sarana* (Hamilton) and *Puntius ticto* (Hamilton). IC_{50} values of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) based free radical scavenging activity ranged from 0.1123 µg ml^{-1} in turmeric to 13.035 µg ml^{-1} in roman coriander. Phenol content ranged from 0.365 µg g^{-1} in onion to 5.67 µg g^{-1} in clove. The raw and cooked fillets of *P. sarana*, and the cooked fillet of *P. ticto*, treated with garlic recorded the highest rates of thiobarbituric acid (TBA) reactivity (P< 0.05). For raw *P. ticto*, both the control and garlic treated fillet recorded higher rates of TBA reactivity (P< 0.05). Fillet of both fish species recorded higher TBA reactivity under raw condition, compared to cooked fillet. This condition was similar for the spice treated fillet. The exceptions were garlic, green and black cardamom, roman coriander and onion for *P. sarana* and garlic, cumin, field mustard, black pepper and poppy seed for *P. ticto*, where TBA reactivity was higher in cooked condition. It is recommended that spices with active phenolic antioxidants be used to inhibit the lipid oxidation in *P. sarana* and *P. ticto*. However, application of garlic extract for fillet preservation should be avoided until further documentation.

Keywords: DPPH, Lipid oxidation, Phenol content, *Puntius sarana*, *Puntius ticto*, Spices, Thiobarbituric acid (TBA) reactivity.

INTRODUCTION

Lipid oxidation is an important post-mortem change in fish fillet. The large amount of polyunsaturated fatty acid (PUFA) moieties found in fish lipids make them highly susceptible to oxidation (Huss, 1995; Boran *et al*., 2006). The deleterious effects are considered to be caused by free radicals produced during peroxide formation from fatty acids containing methylene-interrupted double bonds found in the PUFA (Mayes, 2002). The lipid radical reacts very quickly with atmospheric oxygen ultimately resulting in a lipid hydroperoxide and a new lipid radical (Huss, 1995). Lipid oxidation is a chain reaction providing a continuous supply of free radicals that initiate further oxidation (Mayes, 2002). Lipid oxidation of fish not only produces off flavor but also reduces their nutritional value. The hydroperoxides produced in large amounts during propagation are tasteless (Huss, 1995). Besides, they are readily broken down, giving rise to a very broad odor spectrum and in some cases to a yellow discoloration (Huss, 1995). Such deterioration often makes the final product unacceptable for consumption. Peroxidation

[1] Department of Zoology, University of North Bengal, Siliguri 734 013, West Bengal, India.
* Corresponding author; e-mail: fishlab2011@gmail.com
[2] Department of Botany, University of North Bengal, Siliguri 734 013, West Bengal, India.
[3] Department of Zoology, Raiganj Surendranath Mahavidyalaya, Raiganj 733 134, West Bengal, India.
[4] Institute of Plantation Science and Management, University of North Bengal, Siliguri 734 013, West Bengal, India.

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

of lipids exposed to molecular oxygen is not only responsible for rancidity, but also for tissue damage *in vivo*, where it may be a cause of cancer, myopathy, inflammatory diseases, atherosclerosis, aging and others (Sinclair, 1990; Grootverd *et al*., 1998).

Several investigations have been undertaken with the aim to enhance the shelf life, the stability of lipid containing products and food quality. Antioxidants act as radical-scavengers, and inhibit lipid peroxidation and other free radical-mediated processes; therefore, they are able to protect the human body from several diseases attributed to the reactions of radicals (Takao *et al*., 1994). Use of synthetic antioxidants to prevent free radical damage has been reported to involve toxic side effects (Cornwell *et al*., 1998; Juntachote *et al.,* 2006), making attractive the search for natural antioxidants. The antioxidative effect of plants is mainly attributed to phenolic components, which have the ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations (Pietta *et al*., 1998).

There is increasing interest in the antioxidant compounds of herbs and spices because not only they retard the oxidative degradation of lipids but also improve the flavor of food. Phenolic compounds exhibit a wide range of physiological properties, including antiallergenic, antiartherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective and vasodilatory effects (Balasundram *et al*., 2006). Therefore, supplementing a food product with antioxidant plant phenols may play an important role in the prevention of diseases (D'Souza and Ramchandra Prabhu, 2006). In the present investigation, twenty commonly used spices were employed to preserve the cooked and uncooked fish fillet of two species, namely *Puntius sarana* (Hamilton) and *Puntius ticto* (Hamilton), widely distributed in the rivers of the Terai region in West Bengal, India (Shaw and Shebbeare, 1937; Jha *et al*., 2004). The *Puntius* spp. are found in most local markets (Jha *et al*., 2010; Sarkar and Jha, 2011) and the fillet are known to contain high lipid content, compared to other locally available freshwater species (Sen, 2005).

MATERIALS AND METHODS

Raw Material and Processing

Twenty kinds of spices, namely, clove, turmeric, carom seed, cinnamon, green chili, ginger, nutmeg, coriander, garlic, cumin, bay leaf, black mustard, fennel, field mustard, green cardamom, black pepper, black cardamom, roman coriander, onion and poppy seed were collected from a local market in Siliguri town, India and brought to the laboratory where they were crushed in a grinder. Details on the spices and the plant part used in the study are presented in Table 1. Freshly caught/killed *P. sarana* (28.94±1.89 g; n= 5) and *P. ticto* (5.98±0.41 g; n= 10) were randomly collected from a local fish market. The samples were brought to the laboratory within 6-8 hrs in new polyethylene bags, washed, beheaded, eviscerated and muscle from the entire body of the fish was cut apart into very small pieces and thoroughly mixed.

Thiobarbituric Acid Reactive Substances (TBARS) Assessment

One g of each spice (crushed) was dissolved in 10 ml double distilled water, in which 5 g of raw fish muscle was dipped for one hour in amber colored bottles. Contents from some of the bottles were used directly (raw fillet) in the subsequent processing, while others were subjected to cooking for 5 minutes at 100^{o}C temperature (cooked fillet). Both raw and cooked fillet (50% w/v) were then homogenized in cold 10 mM sodium phosphate buffer containing 0.15M sodium chloride (pH 7.2) using a mortar and pestle. Five g of raw and cooked fillet were homogenized in 10 ml of buffer in each case. All unbroken cells and cellular debris were removed by centrifugation at 10,000 rpm for 10 minutes. The supernatants thus

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

Table 1. Phenol content and antioxidant capacity (DPPH [a] based free radical scavenging activity or IC_{50} value) analyzed for the twenty spices.

Spice			Part of plant used[b]	Phenol content ($\mu g\ g^{-1}$)	IC_{50} ($\mu g\ ml^{-1}$)
Scientific name	Common name	Family			
Eugenia caryophyllata Thunberg	Clove	*Myrtaceae*	S	5.670 [a*]	0.4856 [p]
Curcuma longa Linnaeus	Turmeric	*Zingiberaceae*	R	1.920 [c]	0.1123 [s]
Trachyspermum ammi (Linnaeus) Sprague	Carom seed	*Apiaceae*	S	0.910 [g]	3.6590 [j]
Cinnamon zeylanicum Bregn Brown	Cinnamon stick	*Lauraceae*	B	1.680 [d]	1.3000 [o]
Capsicum frutescens (Linnaeus)	Green chili	*Solanaceae*	F	0.443 [m]	5.0690 [g]
Zingiber officinale Roxburgh	Ginger	*Zingiberaceae*	R	1.220 [e]	4.1040 [i]
Myristica fragrans Houttuyn	Nutmeg	*Myristicaceae*	S	1.140 [f]	1.5620 [m]
Coriandrum sativum Linnaeus	Coriander	*Apiaceae*	S	0.598 [k]	7.1500 [e]
Allium sativum Linnaeus	Garlic	*Alliaceae*	B	0.675 [j]	0.3882 [q]
Cuminum cyminum Linnaeus	Cumin	*Apiaceae*	S	0.830 [h]	4.5920 [h]
Cinnamomum tamala (Hamilton) Nees and Ebermaier	Bay leaf	*Lauraceae*	L	2.540 [b]	0.3500 [r]
Brassica nigra (Linnaeus) Koch	Black mustard	*Brassicaceae*	S	0.753 [i]	9.1400 [c]
Foeniculum vulgare Hill	Fennel	*Apiaceae*	S	0.753 [i]	3.3070 [k]
Brassica campestris Linnaeus	Field mustard	*Brassicaceae*	S	0.598 [k]	7.5250 [d]
Elettaria cardamomum (Linnaeus) Maton	Green cardamom	*Zingiberaceae*	S	0.443 [m]	6.8340 [f]
Piper nigrum Linnaeus	Black pepper	*Piperaceae*	S	0.598 [k]	1.5520 [n]
Amomum costatum Roxburgh	Black cardamom	*Zingiberaceae*	S	0.520 [l]	4.5920 [h]
Nigella sativa Linnaeus	Roman coriander	*Ranunculaceae*	S	0.831 [h]	13.0350 [a]
Allium cepa Linnaeus	Onion	*Alliaceae*	B	0.365 [n]	3.2620 [l]
Papaver somniferum Linnaeus	Poppy	*Papaveraceae*	S	0.521 [l]	9.2160 [b]

[a] 2,2'-diphenyl-1-picrylhydrazyl, [b] S: Seed; R: Rhizome; B: Bulb; F: Fruit, L: Leaf.
* Data in the same column with different superscripts are significantly different ($P< 0.05$).

obtained were used in the *in vitro* lipid oxidation study. For control treatments, fish fillet were not treated with the spices and 5 g of raw or cooked (for 5 minutes at 100°C temperature) fillet were directly homogenized in 10 mM sodium phosphate buffer and centrifuged.

Thiobarbituric acid (TBA) ($C_4H_4N_2O_2S$) reactivity in the supernatants was determined using the method of Luotola and Luotola (1985) with some minor modifications. To 1 ml of the supernatant, 2 ml of 20% trichloroacetic acid (TCA) containing 1% w/v 2-TBA was added and kept in boiling water bath for 15 minutes and then cooled to 20°C. Thereafter, the contents were mixed thoroughly and centrifuged at 10,000 rpm for 5 minutes. The rose-pink colored supernatant was carefully collected, cooled to 20°C and measured at 532 nm against appropriate blanks. The amount of TBA reactivity was expressed as nmoles of TBARS g^{-1} of tissue.

Antioxidant Assay by 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH)

The free radical scavenging activities of different concentrations of each spice were assayed using a stable DPPH, according to the method of Blois (1958). Percentage of free radical scavenging activity was expressed as percentage inhibition from the given formula and 50% inhibition concentration (IC_{50}) was determined graphically.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

Absorbance of control

Estimation of Total Phenolics in the Twenty Spices

Total phenol of each spice was estimated using Folin-Ciocalteu reagent and quantified from standard curve equivalent to catechol as per the method of Sadasivam and Manickam (1996).

Statistical Analysis

The data on lipid oxidation (TBA reactivity) of control and spice treated raw or cooked fillet of each fish species were compared using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test using MS Excel and M-Stat computer programs. The differences were considered statistically significant at the probability level $P< 0.05$.

RESULTS AND DISCUSSION

Antioxidant capacity as indicated by the IC_{50} values of DPPH based free radical scavenging activity and phenol content in the twenty spice extracts showed very wide variations (Table 1).

Antioxidant Activity

DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation (Hinneberg *et al*., 2006). Substances, which are able to perform this reaction, can be considered as antioxidants and therefore radical scavengers (Brand-Williams *et al*., 1995). DPPH assay is a well-known method for the evaluation of free radical-scavenging activity (Rasooli, 2007).

All plant extracts were found to be capable of scavenging of DPPH-radicals. IC_{50} values ranged from 0.1123 μg ml^{-1} in turmeric to 13.035 μg ml^{-1} in roman coriander (Table 1). The order of effectiveness of spices in inhibiting free radicals was as follows: turmeric> bay leaf> garlic> clove> cinnamon stick> black pepper> nutmeg> onion> fennel> carom seed> ginger> cumin/black cardamom> green chili> green cardamom> coriander> field mustard> black mustard> poppy seed> roman coriander (Table 1). Khalaf *et al*. (2008) studied the DPPH based free radical scavenging activity of spices in Jordan including some also used in our experiment and found the IC_{50} values for ginger, clove, black pepper, fenugreek and cardamom to be 65.1, 9.9, 144.1, 444.1 and 681.5 μg ml^{-1}, respectively. Khatun *et al*. (2006) observed that clove showed the highest DPPH radical- scavenging activity, followed by allspice, cinnamon, nutmeg, mustard, cumin, ginger, fennel, fenugreek, black pepper, red pepper, mace, coriander, turmeric, cardamom and white pepper. One of the difficulties in comparing the results of different experiments is the differences in the methodology exercised: we applied crushed raw spices in our experiment while some of the earlier workers had used dried spices. Although it could be argued that different spices could have different moisture contents and that difference could affect the results, we preferred to test the effectiveness of raw spices (as available in the market) since if found effective, general public could be more likely to use raw spices instead of dried spices to preserve fish fillet.

Phenol Content

The content of total phenols is expressed as μg g^{-1}. Phenol content ranged from 0.365 μg g^{-1} in onion to 5.67 μg g^{-1} in clove (Table 1). Significantly higher results were found in the order: clove> bay leaf> turmeric> cinnamon stick> ginger> nutmeg> carom seed> cumin/roman coriander> black mustard/ fennel> garlic> coriander/field mustard/black pepper> poppy seed/ black

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

cardamom> green chili/green cardamom> onion (P< 0.05).

Shobana and Naidu (2000) reported the relative antioxidant activities of some spices; the order of the activities was clove, cinnamon, ginger, pepper and onion. Taira *et al*. (1992) reported that the strong DPPH-activities of clove and nutmeg might be related to their phenolic antioxidant components such as eugenol, isoeugenol, etc. Dorman *et al.* (2000) identified 16 - 18 components from clove and nutmeg essential oils, and found that as much as 94% phenylpropanoids were obtained in clove oil; eugenol was the main component of the phenylpropanoids (91%). Twelve major phenolic compounds including flavonoids such as isoquercitrin, kaempferol glycoside and rutin were isolated from fennel (Parejo *et al*., 2004). Quercetin and kaempferol were found in coriander (Justesen and Knuthesen, 2001). Gingerol and shogaol were reported to be responsible for the antioxidant activity of ginger (Kikuzaki and Nakatani, 1993). In addition, five phenolic amides of pepper were also shown to be responsible for antioxidant activity of black pepper (Nakatani, 1996).

Chung *et al*. (1997) identified 3,5-dimethoxy-4-hydroxycinnamic acids as an ester from black mustard. Garlic and onion contain quercetin as major phenolics (Apak *et al*., 2007). Cuminaldehyde, cymene and terpenoids are the major constituents of volatile oils of cumin (Thippeswamy and Naidu, 2005). Vanillic acid, caffeic acid and ferullic acid are found in cinnamon and bay leaf (Muchuweti *et al*., 2007), while gallic acid is found in chilies (Wangcharoen and Morasuk, 2007). Antioxidants present in roman coriander seeds include selenium, DL-α- and DL-γ-tocopherol, all-trans retinol, thymoquinone and thymol (Kanter *et al*., 2006). Mustard seed has retinol palmitate, oryzanol and α-tocopherol while poppy seed concentrate has retinol palmitate and sesamol (Ravikumar Patil *et al*., 2008). Cardamom contains mainly phenolic acids, such as caffeic acid, *p*-coumaric acid, and protocatechuic acid (Variyar *et al*., 1998). Curcumin, (diferuloyl methane) is a natural antioxidant derived from turmeric (Sreejayan and Rao, 1994). Turmerin, the water soluble peptide present in turmeric, acts as a chain breaking antioxidant (Srinivas *et al*., 1992). Polyphenolic flavonoids are the possible candidates that might explain the antioxidant activity of fenugreek (Kaviarasan *et al*., 2004). Until now five different flavonoids namely vitexin, tricin, naringenin, quercetin, and tricin-7-*O*-beta-D-glucopyranoside have been identified in fenugreek seeds (Shang *et al*., 1998). Carom seed contains thymol, a-pinene, p-cymene, limonene and y-terpinene (NBCE, 2006).

TBARS Assessment

TBA reactivity (n moles TBARS g^{-1} tissue) in control and spice treated fillets of the two fish species under raw and cooked conditions are presented in Table 2. The raw and cooked fillet of *P. sarana*, treated with garlic recorded the highest rates of TBA reactivity (P< 0.05). Even in *P. ticto*, the garlic treated fillet recorded the highest rates of TBA reactivity (P< 0.05) under cooked condition (Table 2). For raw *P. ticto*, both the control and the garlic treated fillet recorded higher rates of TBA reactivity (P< 0.05), compared to fillet treated with other spices (Table 2). This is quite surprising, as it was expected that the control treatments would always record the highest levels of lipid oxidation. According to literature available, whole garlic and aged garlic extract exhibit direct antioxidant effects and enhance the serum levels of two antioxidant enzymes, catalase and glutathione peroxidase (Popov *et al*., 1994; Torok *et al*., 1994). Garlic extract and allicin efficiently scavenged exogenously generated hydroxyl radicals in a dose dependent fashion, but their effectiveness was reduced by about 10% by heating to 100^{o}C for 20 minutes (Prasad *et al*., 1995). Other garlic constituents, such as S-allylcysteine, also demonstrated significant antioxidant effects

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

Table 2. Thiobarbituric acid reactivity (n moles TBARS g^{-1} tissue) in control and spice treated fillets of *Puntius sarana* and *Puntius ticto*, under raw and cooked conditions.

Treatment	*Puntius sarana*		*Puntius ticto*	
	Raw fillet	Cooked fillet	Raw fillet	Cooked fillet
Control	9.88 ± 0.74 ^{b}*	6.78 ± 1.04 ^{b}	20.18 ± 0.96 ^{a}	14.42 ± 0.89 ^{b}
Clove	9.54 ± 1.02 ^{bc}	4.73 ± 0.80 ^{c}	11.30 ± 0.94 ^{b}	9.21 ± 0.91 ^{c}
Turmeric	2.50 ± 0.93 ^{klmn}	0.97 ± 0.92 ^{gh}	7.07 ± 1.04 ^{ef}	5.94 ± 1.06 ^{efghi}
Carom seed	8.45 ± 1.06 ^{bcd}	3.65 ± 0.98 ^{d}	9.45 ± 1.11 ^{c}	6.26 ± 0.98 ^{defg}
Cinnamon stick	5.29 ± 0.81 ^{ghi}	3.69 ± 1.02 ^{d}	5.92 ± 1.00 ^{efgh}	5.81 ± 0.93 ^{efghi}
Green chili	6.19 ± 0.81 ^{fg}	1.06 ± 0.65 ^{gh}	9.07 ± 1.02 ^{cd}	5.73 ± 1.02 ^{fghij}
Ginger	7.59 ± 0.85 ^{def}	1.90 ± 1.00 ^{ef}	5.56 ± 0.91 ^{fgh}	3.93 ± 0.92 ^{j}
Nutmeg	6.73 ± 0.87 ^{efg}	0.71 ± 0.08 ^{h}	4.63 ± 0.99 ^{gh}	4.15 ± 0.54 ^{ij}
Coriander	6.10 ± 0.97 ^{fg}	1.51 ± 0.96 ^{fg}	7.26 ± 0.90 ^{ef}	6.37 ± 1.00 ^{defg}
Garlic	17.82 ± 1.04 ^{a}	25.26 ± 0.88 ^{a}	21.26 ± 1.06 ^{a}	23.60 ± 0.93 ^{a}
Cumin	4.21 ± 0.89 ^{hij}	3.75 ± 0.93 ^{d}	7.47 ± 1.02 ^{de}	7.78 ± 0.98 ^{cd}
Bay leaf	3.81 ± 0.81 ^{ijk}	2.30 ± 0.98 ^{e}	10.37 ± 1.00 ^{bc}	7.19 ± 0.94 ^{def}
Black mustard	8.19 ± 0.91 ^{cde}	1.37 ± 0.95 ^{fg}	6.30 ± 1.00 ^{efgh}	5.67 ± 1.00 ^{fghij}
Fennel	5.51 ± 0.86 ^{gh}	2.09 ± 0.98 ^{e}	6.60 ± 1.02 ^{ef}	5.51 ± 1.02 ^{fghij}
Field mustard	2.76 ± 0.98 ^{jklm}	1.90 ± 0.94 ^{ef}	4.53 ± 0.98 ^{h}	4.66 ± 0.98 ^{ghij}
Green cardamom	2.03 ± 0.95 ^{lmn}	3.83 ± 1.23 ^{d}	6.43 ± 0.98 ^{efg}	5.63 ± 1.06 ^{fghij}
Black pepper	2.29 ± 1.02 ^{klmn}	2.18 ± 0.93 ^{e}	11.31 ± 0.94 ^{b}	14.24 ± 1.02 ^{b}
Black cardamom	1.34 ± 0.83 ^{mn}	2.13 ± 1.00 ^{e}	9.37 ± 1.07 ^{c}	6.40 ± 0.95 ^{defg}
Roman coriander	0.82 ± 0.02 ^{n}	7.19 ± 0.99 ^{b}	10.57 ± 1.02 ^{bc}	4.33 ± 0.97 ^{hij}
Onion	1.00 ± 0.94 ^{n}	4.38 ± 0.98 ^{c}	11.63 ± 0.99 ^{b}	5.99 ± 0.90 ^{defgh}
Poppy seed	3.03 ± 0.71 ^{jkl}	1.04 ± 0.53 ^{gh}	7.08 ± 0.18 ^{ef}	7.58 ± 0.18 ^{cde}

* Each value is Mean±SD of triplicate determinations. Data in the same column with different superscripts are significantly different (P< 0.05).

in vitro (Imai *et al*., 1994). In rat liver microsomes, garlic extract prevented formation of TBA reactive substances in cell membranes during lipid oxidation in a dose dependent fashion (Horie *et al*., 1992). Garlic has organosulfur compounds as the main responsible substances of its antioxidant activity (Nuutila *et al*., 2003). In sharp contrast to all literature, garlic tended to increase TBA reactivity in the present experiment. This is difficult to explain, since the IC_{50} values for garlic are quite satisfactory and as such, the quality of the garlic lot purchased for the present set of experiments cannot be questioned. Garlic appears to enhance the synthesis of nitric oxide, which may account, in part, for some of the garlic's antihypertensive and anticoagulant effects; this ability is retained in heat-treated and aged garlic products (Pedraza-Chaverri *et al*., 1998; Das *et al*., 1995, 1996). Increased synthesis of nitric oxide by garlic may activate free radicals and may result in high TBARS concentration by heating at cooking process.

It has been reported earlier that during cooking, heat brings forward mutagenic epoxides, hydroperoxide and unsaturated aldehyde, which are carcinogenic (D'Souza and Ramachandra Prabhu, 2006). Iron salts which are present in fish homogenate can also decompose lipid to give in peroxyl and alkoxyl radicals. Both these radicals can abstract $H^{\cdot}$ and escalate lipid oxidation (D'Souza and Ramachandra Prabhu, 2006). The fish fat gets into the medium by cooking. Cooking can change the physico-chemical nature of the membranes and both the solubility and site of action are favored by having more access to the radical and better activity (Srinivas *et al*., 1992). In the present experiment, the fillet of both species of fish recorded higher TBA reactivity under raw condition, compared to cooked fillet.

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

This condition was similar in spice treated fillet. The exceptions were garlic, green and black cardamom, roman coriander and onion for *P. sarana* and garlic, cumin, field mustard, black pepper and poppy seed for *P. ticto*, where the TBA reactivity was higher in cooked condition (Table 2). It is likely that heating (during cooking) had some effect on the action of the phenolics and other active ingredients in the spices.

Khatun *et al*. (2006) studied the effect of thermal treatment on radical-scavenging activity of some spices and an increase in radical-scavenging activity was found in clove, allspice, fennel, black pepper, mace, coriander, turmeric, cardamom and white pepper due to heating treatment while insignificant changes were observed in cinnamon, mustard, cumin and red pepper. After heating, the solubilities of the active components may increase because of the decomposition of the cell wall and by penetration of solvent into the cell. For this reason, an increase in the radical-scavenging activity of spices might be observed after heating (Khatun *et al*., 2006). Shobana and Naidu (2000) reported that the bound antioxidants might be released due to heat treatment, resulting in the higher antioxidant activity compared with that of fresh spices extract. On the other hand, a significant quantitative loss in the active components of turmeric was found after boiling of mixed spices (Srinivasan *et al*., 1992). Takamura *et al*. (2002) reported a decrease of the radical-scavenging activity of curry paste and cooked curry, possibly due to decomposition or evaporation of the active compounds, since the spices were heated with butter at high temperatures. In the study of Khatun *et al*. (2006), a decrease in the radical-scavenging activity was observed in nutmeg, ginger and fenugreek due to heating.

ConclusionS

Antioxidant activity of different spices was demonstrated in the present experiment. Lipid oxidation of raw or cooked fillet of *P. sarana* and *P. ticto* was restricted by spice treatment, as indicated by the TBA reactivity values. However, garlic tended to increase TBA reactivity, which needs to be studied further. The phenol content in different spices showed a negative correlation with the IC_{50} values of DPPH based free radical scavenging activity. It is recommended that spices with active phenolic antioxidants be used to inhibit the free-radical mediated damage in *P. sarana* and *P. ticto*. However, application of crushed garlic extract for fillet preservation should be avoided until further results are available. Besides, raw spices could be dipped/stirred (in distilled water) for different periods (of longer duration) to standardize a regimen that facilitates better transfer of the biologically active ingredients.

ACKNOWLEDGEMENTS

The authors appreciate the assistance received from Hamilton Library, University of Hawaii, United States in the literature survey.

REFERENCES

1. Apak, R., Guclu, K., Demirata, B., Ozyurek, M., Celik, S. E., Bektasoglu, B., Berker, K. I. and Ozyurt, D. 2007. Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. *Molecules*, **12**:1496-1547.
2. Balasundram, N., Sundram, K. and Samman, S. 2006. Phenolic Compounds in Plants and Agri-industrial by Products: Antioxidant Activity, Occurence, and Potential Uses. *Food Chem*., **99**: 191-203.
3. Blois, M. S. 1958. Antioxidant Determination by the Use of Stable Free Radicals. *Nature*, **181:**1199-1200.
4. Boran, G., Karacam, H. and Boran, M. 2006. Changes in the Quality of Fish Oils Due to Storage Temperature and Time. *Food Chem*., **98**: 693-698.
5. Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Sci. Technol*., **28**: 25-30.

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

6. Chung, S. K., Osawa, T. and Kawakishi, S. 1997. Hydroxyl Radical-scavenging Effect of Spices and Scavengers from Brown Mustard (*Brassica nigra*). *Biosci. Biotechnol. Biochem*., **61**: 118-123.
7. Cornwell, D. G., Jones, K. H., Jiang, Z., Lantry, L. E., Southwell, K. P., Kohar, I. and Donald, E. T. 1998. Cytotoxicity of Tocopherols and Their Quinones in Drugsensitive and Multidrug-resistant Leukemia Cells. *Lipids*, **33**: 295-301.
8. D'Souza, H. P. and Ramachandra Prabhu, H. 2006. *In vitro* Inhibition of Lipid Peroxidation in Fish by Turmeric (*Curcuma longa*). *Indian J. Clin. Biochem*., **21**: 138-141.
9. Das, I., Khan, N. S. and Sooranna, S. R. 1995. Potent Activation of Nitric Oxide Synthase by Garlic: A Basis for Its Therapeutic Applications. *Curr. Med. Res. Opin*., **13**: 257-263.
10. Das, I., Hirani, J. and Sooranna, S. 1996. Arginine Is Not Responsible for the Activation of Nitric Oxide Synthase by Garlic. *J. Ethnopharmacol*., **53**: 5-9.
11. Dorman, H. J. D., Figueiredo, A. C., Barroso, J. G. and Deans, S. G. 2000. *In vitro* Evaluation of Antioxidant Activity of Essential Oils and Their Components. *Flavour Fragr*., **15**: 12-16.
12. Grootveld, M., Atherton, M. D., Sheerin, A. N., Hawkes, J., Blake, D. R., Richens, T. E., Silwood, C. J. L., Lynch, E. and Claxson, A. W. D. 1998. *In vivo* Absorption, Metabolism and Urinary Excretion of *α,β*-unsaturated Aldehydes in Experimental Animals. *J. Clin. Invest*., **102**: 1216-1218.
13. Hinneburg, I., Dorman, H. J. D. and Hiltunen, R. 2006. Antioxidant Activities of eExtracts from Selected Culinary Herbs and Spices. *Food Chem*., **97**: 122-129.
14. Horie, T., Awazu, S., Itakura, Y. and Fuwa, T. 1992. Identified Diallyl Polysulfides from an Aged Garlic Extract which Protects the Membranes from Lipid Peroxidation. *Planta Med*., **58**: 468-469.
15. Huss, H. H. 1995. Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper 348, Food and Agriculture Organization of the United Nations, Rome.
16. Imai, J., Ide, N., Nagae, S., Moriguchi, T., Matsuura, H. and Itakura, Y. 1994. Antioxidant and Radical Scavenging Effects of Aged Garlic Extract and Its Constituents. *Planta Med*., **60**: 417-420.
17. Jha, P., Mandal, A. and Barat, S. 2004. Mahananda Reservoir, West Bengal: Its Ichthyofauna, Fishery and Socio-economic Profile of Fish Production. *Fishing Chimes*, **24(6):** 14-17.
18. Jha, P., Roy, R. P. and Barat, S. 2010. Application of Sensory and Microbial Analysis to Assess Quality of Fish Sold in Siliguri City of West Bengal, India. *J. Environ. Biol*., **31**: 587-594.
19. Juntachote, T., Berghofer, E., Siebenhandl, S. and Bauer, F. 2006. The Oxidative Properties of Holy Basil and Galangal in Cooked Ground Pork. *Meat Sci*., **72**: 446-456.
20. Justesen, U. and Knuthsen, P. 2001. Composition of Flavonoids in Fresh Herbs and Calculation of Flavonoid Intake by Use of Herbs in Traditional Danish Dishes. *Food Chem*., **73**: 245-250.
21. Kanter, M., Coskun, O. and Uysal, H. 2006. The Antioxidative and Antihistaminic Effect of *Nigella sativa* and Its Major Constituent, Thymoquinone on Ethanol-induced Gastric Mucosal Damage. *Arch. Toxicol*., **80**: 217-224.
22. Kaviarasan, S., Vijayalakshmi, K. and Anuradha, C. V. 2004. Polyphenol-rich Extract of Fenugreek Seeds Protect Erythrocytes from Oxidative Damage. *Plant Foods Hum. Nutr*., **59**:143-147.
23. Khalaf, N. A., Shakya, A. K., Al-Othman, A., El-Agbar, Z. and Farah, H. 2008. Antioxidant Activity of Some Common Plants. *Turk. J. Biol*., **32**:51-55.
24. Khatun, M., Eguchi, S., Yamaguchi, T., Takamura, H. and Matoba, T. 2006. Effect of Thermal Treatment on Radical-scavenging Activity of Some Spices. *Food Sci. Technol. Res*., **12**: 178-185.
25. Kikuzaki, H. and Nakatani, N. 1993. Antioxidant Effects of Some Ginger Constituents. *J. Food Sci*., **58**: 1407-1410.
26. Luotola, M. T. and Luotola, J. E. I. 1985. Effects of *a*-tocopherol on the Peroxidation of Cod Liver Oil. *Life Chem. Reports*, **3**: 159-163.
27. Mayes, P. A. 2002. Lipids of Physiologic Significance. In: *"Harper's Biochemistry",* (Eds.): Murry, R. K., Granner, D. K., Mayes, P. A. and Rodwell, V. W.. 25th Edition, McGraw-Hill Education (Asia), New Delhi, PP. 160-171.
28. Muchuweti, M., Kativu, E., Mupure, C. H., Chidewe, C., Ndhlala, A. R. and Benhura,

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

M. A. N. 2007. Phenolic Composition and Antioxidant Properties of Some Spices. *Amer. J. Food Technol*., **2**:414-420.
29. Nakatani, N. 1996. Antioxidants from Spices and Herbs. In: "*Natural Antioxidants: Chemistry, Health Effects and Applications*", (Ed.): Shahidi, F.. Technomic Publishing, Lancaster, PP. 64-75.
30. NBCE. 2006. *The Complete Book on Spices and Condiments (with Cultivation, Processing and Uses)*. National Institute of Industrial Research Board of Consultants and Engineers, Asia Pacific Business Press Inc., Delhi, 880 PP.
31. 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.
32. Parejo, I., Viladomat, F., Bastida, J., Schmeda-Hirschamann, G., Burillo, J. and Codina, C. 2004. Bioguided Isolation and Identification of the Nonvolatile Antioxidant Compounds from Fennel (*Foeniculum vulgare* Mill.) Waste. *J. Agric. Food Chem*., **52**: 1890-1897.
33. Pedraza-Chaverri, J., Tapia, E., Medina-Campos, O. N., Grannados, M. dl. A. and Franco, M. 1998. Garlic Prevents Hypertension Induced by Chronic Inhibition of Nitric Oxide Synthesis. *Life Sci*., **62**: 71-77.
34. Pietta, P., Simonetti, P. and Mauri, P. 1998. Antioxidant Activity of Selected Medicinal Plants. *J. Agric. Food Chem*., **46**: 4487-4490.
35. Popov, I., Blumstein, A. and Lewin, G. 1994. Antioxidant Effects of Aqueous Garlic Extract. 1st Communication: Direct Detection Using the Photochemiluminescence. *Arzneimittelforschung*, **44**: 602-604.
36. Prasad, K., Laxdal, V. A., Yu, M. and Raney, B. L. 1995. Antioxidant Activity of Allicin: An Active Principle in Garlic. *Mol. Cell. Biochem*., **148**: 183-189.
37. Rasooli, I. 2007. Food Preservation: A Biopreservative Approach. *Food*, **1**: 111-136.
38. Ravikumar Patil, H. S., Haraprasad, N., Makari, H. K., Gurumurthy, H., Chetan, D. M. and Anil Kumar, H. S. 2008. Polyphenol Composition of Nutraceutical Concentrate Obtained from Edible Vegetable Oil Seeds. *Electronic J. Environ. Agric. Food Chem.* (*EJEAFChe*) **7**: 3181-3198.
39. Sadasivam, S. and Manickam, A. 1996. *Biochemical Methods*. 2nd Edition, New Age International Publishers, New Delhi, 257 PP.
40. Sarkar, S. and Jha, P. 2011. Application of Sensory Analysis to Assess Quality of Fish Food Sold in Markets of Raiganj, Uttar Dinajpur, Paschimbanga. In: "*Recent Studies in Biodiversity and Traditional Knowledge in India*", (Eds.): Ghosh, C. and Das, A. P. Gour Mahavidyalaya, Malda, PP.353-357.
41. Sen, D. P. 2005. *Advances in Fish Processing Technology*. Allied Publishers Pvt. Ltd., New Delhi, 818PP.
42. Shang, M., Cais, H. J., Li, J., Zhao, Y., Zheng, J., Namba, T., Kadota, S., Tezuka, Y. and Fan, W. 1998. Studies on Flavonoids from Fenugreek (*Trigonella foenum graecum* L). *Zhongguo Zhong Yao Za Zhi*, **23**: 614-639.
43. Shaw, G. E. and Shebbeare, E. O. 1937. The Fishes of North Bengal. *J. Royal Asiatic Soc. Bengal. Sci*., **3**: 1-136.
44. Shobana, S. and Naidu, K. A. 2000. Antioxidant Activity of Selected Indian Spices. *Prostaglandins Leukot. Essent. Fatty Acids*, **62**: 107-110.
45. Sinclair, A. J. 1990. Free Radicals and Antioxidant System in Health and Diseases. *Br. J. Hosp. Med*., **43**: 334-344.
46. Sreejayan, M. N. and Rao, M. N. A. 1994. Curcumnoids as Potent Inhibitors of Lipid Peroxidation. *J. Pharm. Pharmacol*., **46**: 1013-1016.
47. Srinivas, L. V. K., Shalini and Shylaja, M. 1992. Turmerin Water Soluble Antioxidant Peptide from Turmeric. *Arch. Biochem. Biophys*., **292**: 617-623.
48. Taira, J., Ikemoto, T., Yoneya, T., Hagi, A., Murakami, A. and Makino, K. 1992. Essential Oil Phenyl Propanoids. Useful as OH Scavengers? *Free Rad. Res. Commun*., **16**: 197-204.
49. Takamura, H., Yamaguchi, T., Terao, J. and Matoba, T. 2002. Change in Radical-scavenging Activity of Spices and Vegetables during Cooking. In: *"Bioactive Compounds in Foods: Effect of Processing and Storage"*, (Eds.): Lee, T. C. and Ho, C. T.. *American Chemical Society*, Washington, DC, PP. 34-43.
50. Takao, T., Kiatani, F., Watanabe, N., Yagi, A. and Sakata, K. 1994. A Simple Screening Method for Antioxidants and Isolation of

[DOR: 20.1001.1.16807073.2013.15.4.3.6]

Several Antioxidants Produced by Marine Bacteria from Fish and Shellfish. *Biosci. Biotechnol. Biochem*., **58**: 1780-1783.
51. Thippeswamy, N. B. and Naidu, K. A. 2005. Antioxidant Potency of Cumin Varieties - Cumin, Black Cumin and Bitter Cumin– on Antioxidant Systems. *Eur. Food Res. Technol*., **220**: 472-476.
52. Torok, B., Belagyi, J., Rietz, B. and Jacob, R. 1994. Effectiveness of Garlic on the Radical Activity in Radical Generating Systems. *Arzneimittelforschung*, **44**: 608-611.
53. Variyar, P., Bandyopadhyay, C. and Thomas, P. 1998. Effect of Gamma-irradiation on the Phenolic Acids of Some Indian Spices. *Int. J. Food Sci. Technol*., **33**: 533-537.
54. Wangcharoen, W. and Morasuk, W. 2007. Antioxidant Capacity and Phenolic Content of Chilies. *Kasetsart J. (Nat. Sci.)*, **41**: 561-569.

فعالیت آنتی اکسیدانی ادویه‌های مختلف بر روی اکسایش فیله خام و پخته دو نوع ماهی متعلق به جنس *Puntius*

پ. گوسوامی، پ. مندل، پ. جها، ت. میسرا، و س. برات

چکیده

بیست نوع ادویه برای نگهداری فیله‌های خام و پخته *Puntius sarana* (Hamilton) و *Puntius ticto* (Hamilton) مورد استفاده قرار گرفتند. مقادیر IC_{50} فعالیت جذب رادیکال‌های آزاد بر پایه 2,2'-diphenyl-1-picrylhydrazyl (DPPH) از ۰/۱۱۲۳ µg /ml در زردچوبه تا ۱۳/۰۳۵ µg /ml در سیاهدانه متغیر بود. مقدار فنل از ۰/۳۶۵ µg /g در پیاز تا ۶/۵۷ µg /g در میخک متغیر بود. فیله‌های خام و پخته *P. sarana* و فیله پخته *P. ticto* تیمار شده با سیر بیشترین واکنش پذیری اسید تیوباربیتوریک (TBA) را نشان دادند ($P < 0.05$). در *P. ticto* خام، فیله شاهد و تیمارشده با سیر بیشترین نرخ واکنش پذیری TBA را داشتند ($P < 0.05$). فیله‌های هر دو گونه در شرایط خام نسبت به پخته واکنش پذیری TBA بیشتری داشتند. این شرایط مشابه فیله‌های تیمارشده با ادویه بود. موارد استثنا شامل سیر، هل سبز و سیاه، سیاهدانه و پیاز برای *P. ticto* بودند که در آنها واکنش پذیری TBA در شرایط پخته بیشتر بود. توصیه می‌شود که برای جلوگیری از اکسایش چربی در *P. sarana* و *P. ticto* از ادویه‌های حاوی مقادیر بالای آنتی اکسیدان‌های فنولی فعال استفاده شود. با این وجود، تا بررسی‌های بیشتر باید از استفاده از عصاره سیر برای نگهداری فیله اجتناب نمود.

[DOR: 20.1001.1.16807073.2013.15.4.3.6]