

# TOCOPHEROL CONTENT OF COMMERCIAL FISH SPECIES AS AFFECTED BY MICROWAVE COOKING

ABDURRAHMAN POLAT, YESIM ÖZOGUL<sup>1</sup>, ESMERAY KULEY, FATİH ÖZOGUL, GÜLSÜN ÖZYURT and AYŞE ŞİMŞEK

Department of Seafood Processing Technology, Faculty of Fisheries, University of Cukurova, Balcali, 01330 Adana, Turkey

<sup>1</sup>Corresponding author. TEL: (90)-322-3386084  
Ext: 2961; FAX: (90)-322-3386439; EMAIL:  
ozogul@cu.edu.tr

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## ABSTRACT

Alpha ( $\alpha$ -), beta ( $\beta$ -), gamma ( $\gamma$ -) and delta ( $\delta$ -)tocopherol in commonly consumed fish species and also the effects of microwave cooking on tocopherol content of fish were investigated.  $\alpha$ -Tocopherol was found to be the major form of vitamin E in fish muscle. Golband goatfish (47.81 mg/kg), anchovy (40.84 mg/kg), striped sea bream (34.31 mg/kg) and bogue (33.86 mg/kg) had the highest tocopherol content among the samples, whereas the lowest tocopherol levels were observed for silver fish (16.06 mg/kg) and gurnard (18.01 mg/kg). The total tocopherol contents of the cooked fish ranged from 67.78 to 72.875 mg/kg due to the loss of water content in fish muscle. The result of this study showed that fish species had good tocopherol levels for raw and cooked samples. Stability of tocopherol content to microwave cooking in fish muscle was observed.

## PRACTICAL APPLICATIONS

It is important to achieve a successful determination of the vitamin E constituents that are quantitatively extracted from the food. Although the tocopherol contents of fish vary with different species,  $\alpha$ -tocopherol is the major form of vitamin E in fish muscle. However, various storage temperatures, cooking process and storage time may affect vitamin E levels of fish muscle. Thus, the contents of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol of commercially important fish species were investigated. In addition, the effects of microwave cooking on the tocopherol content of different fish species were investigated. The research findings are important in order to know which fish species has high content of tocopherol and to consider whether cooking process affects tocopherol content in fish.

## INTRODUCTION

Vitamin E is a general term for tocopherols and tocotrienols (Traber and Arai 1999). Naturally occurring vitamin E exists in eight chemical forms ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol and  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocotrienol), which have varying levels of biological activity (Traber 2006). Of these,  $\alpha$ -tocopherol has been the most studied form as it has the highest bioavailability.  $\alpha$ -Tocopherol, the most important lipid-soluble antioxidant, protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas 2001; Traber and Atkinson 2007). This removes the free radical intermediates and prevents the oxidation reaction from continuing. Free-radical

mechanisms have been implicated in the pathology of several human diseases, including cancer, atherosclerosis, malaria, rheumatoid arthritis, and neurodegenerative diseases (Aruoma 1998).

The contents of vitamin E in the edible parts of fish range from 0.2 to 270 mg/100 g wet weight (Sikorski *et al.* 1989). Ozyurt *et al.* (2009) evaluated vitamin E contents of freshwater species and they reported 0.94, 0.46 and 0.80 mg/100 g of vitamin E for pike perch, common carp and European catfish, respectively. There is also some information on  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol contents of seafood (Syvaaja *et al.* 1985; Frigg *et al.* 1990; Passi *et al.* 2002; Gotoh *et al.* 2009; Matsushita *et al.* 2010). The effects of various storage temperature and storage time on vitamin E levels of fish muscle were

also observed (Arslan *et al.* 1997). A new vitamin E ( $\alpha$ -tocomonoenol) from the eggs of Pacific salmon was investigated by Yamamoto *et al.* (1999). The principal objectives of this study were to determine the contents of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol of commercially important fish species using high-performance liquid chromatography (HPLC) and to evaluate the effects of microwave cooking on the tocopherol content of different fish species.

## MATERIALS AND METHODS

### Preparation of Samples

Fish species caught by trawl net or purse seine were obtained from a local fish market during summer (June and July 2010). The duration of time between harvesting and arrival of the fish at the laboratory was 7 h, where they were always kept in ice during transportation. Upon arrival, the whole fish were washed under running tap water, filleted and kept at  $-18^{\circ}\text{C}$  until analyses. Fish fillets without skin were thawed in a refrigerator overnight. The fillets of each species were ground with Moulinex mixer (Moulinex, Societe Anonyme, Bagnolet, France). A minimum of 10 individual fish for each species was used for the analyses. These species were as follows: sea bass (*Dicentrarchus labrax*), anchovy (*Engraulis encrasicolus*), sardine (*Sardina pilchardus*), striped seabream (*Lithognathus mormyrus*), goldband goatfish (*Upeneus moluccensis*), petersfish (*Zeus faber*), derbio (*Trachinotus ovatus*), white seabream (*Diplodus sargus*), golden grey mullet (*Liza aurata*), bogue (*Boops boops*), tub gurnard (*Chelidonichthys lucerna*), silver fish (*Chalcalburnus mosullensis*) and striped red mullet (*Mullus surmuletus*).

### Cooking Method

Only microwave cooking method was applied to fresh or thawed fish samples, which were cooked at medium power by microwave set at a frequency of 300 MHz for 2 min. Triplicate samples were taken to determine the tocopherol content of selected fish species, which were red mullet (*Mullus barbatus*), two banded bream (*Diplodus vulgaris*), sand smelt (*Atherina hepsetus*), corb (*Umbrina cirrosa*), tub gurnad (*Trigla lucerna*), common sole (*Solea solea*), sea bream (*Sparus aurata*), whiting (*Merlangius merlangus*), pike (*Esox lucius*), Atlantic salmon (*Salmo salar*) and common pandora (*Pagellus erythrinus*).

### Tocopherol Analysis

Basic extraction procedure and fast HPLC method were used according to Nirungsan and Thongnopnua (2006) for determining the level of tocopherol in freshwater and marine fish species.

### Extraction of Fish Samples

Extraction procedure of tocopherol analyses was carried out according to Nirungsan and Thongnopnua (2006) with modifications. For tocopherol determination, 1 g of dorsal part of fish muscle without skin was chopped, weighed and transferred to a Falcon tube (Isolab, Wehrheim, Germany) wrapped with aluminum foil. After that, 3 mL of acetonitrile, 1 mL of isopropyl alcohol and 1 mL of 0.1% methanolic butylated hydroxytoluene were added into each tube. The mixture was homogenized with an Ultra-Turrax homogenizer (T 25 basic IKA-WERKE, Staufen, Germany) for 2 min at 0C. The aliquot was then transferred to a centrifuge tube wrapped with aluminum foil and centrifuged at 18,000 rpm for 10 min at 4C. One milliliter of the upper phase was taken and filtered using 0.45- $\mu\text{m}$  filter. After that, 2  $\mu\text{L}$  of aliquot was injected into the HPLC for the tocopherol analysis.

### Apparatus and Columns

HPLC analyses were conducted using a Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a RF-10AXL fluorescence detector and two binary gradient pumps (Shimadzu LC-10AT), autosampler (SIL 20AC), column oven (CTO-20AC) and a communication bus module (CBM-20A) with valve unit FCV-11AL was used. The column, purchased from Phenomenex (Macclesfield, Cheshire, UK), was packed with reversed-phase Develosil 5 $\mu$  RPAQUEOUS (150  $\times$  4.60 mm) (Phenomenex).

Tocopherol standards, which are  $\alpha$ -tocopherol (Calbiochem, KPS101),  $\beta$ -tocopherol (Calbiochem, KPS102),  $\gamma$ -tocopherol (Calbiochem, KPS5103) and  $\delta$ -tocopherol (Calbiochem, KPS104), were purchased from Merck (Darmstadt, Germany). The mobile phase consisted of acetonitrile and methanol. Chromatographic separation was carried out using continuous isocratic elution with HPLC-grade acetonitrile (eluent A) and methanol (eluent B). HPLC isocratic profile was 50% acetonitrile and 50% methanol, and the flow rate was 1.0 mL/min throughout the whole separation. The total separation time was 12 min and the isocratic elution was run for 15 min to ensure full separation. The injection volume was 2  $\mu\text{L}$  and detection was monitored with a fluorescence detector at wavelengths of 295 and 330 nm for excitation and emission, respectively. The regression coefficient of eight replicate standard injections was 0.9999 in the concentrations of 0.01, 0.1, 1 and 10  $\mu\text{g}/\text{mL}$  for each tocopherol, respectively. The total recoveries were 99.7, 99.9, 99.8 and 99.7% for  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol, respectively. The limit of detection was 9.4 ng/mL. The lowest and highest limits of quantification were 30 and 79,000 ng/mL.

**TABLE 1.** TOCOPHEROL LEVELS OF FISH SPECIES (mg/kg)

	Delta ( $\delta$ )	Gamma ( $\gamma$ )	Beta ( $\beta$ )	Alpha ( $\alpha$ )	Total tocopherol
Sea bass ( <i>Dicentrarchus labrax</i> )	0.05 $\pm$ 0.00 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>b</sup>	0.05 $\pm$ 0.00 <sup>b</sup>	26.03 $\pm$ 2.79 <sup>c</sup>	26.23
Anchovy ( <i>Engraulis encrasicolus</i> )	0.13 $\pm$ 0.04 <sup>b</sup>	0.10 $\pm$ 0.00 <sup>b</sup>	0.08 $\pm$ 0.03 <sup>c</sup>	40.53 $\pm$ 2.93 <sup>e</sup>	40.84
Sardine ( <i>Sardina pilchardus</i> )	0.23 $\pm$ 0.08 <sup>bc</sup>	0.10 $\pm$ 0.00 <sup>b</sup>	0.15 $\pm$ 0.04 <sup>cd</sup>	21.0 $\pm$ 2.76 <sup>b</sup>	21.48
Striped seabream ( <i>Lithognathus mormyrus</i> )	0.30 $\pm$ 0.05 <sup>c</sup>	0.08 $\pm$ 0.04 <sup>ab</sup>	0.10 $\pm$ 0.00 <sup>c</sup>	33.83 $\pm$ 1.59 <sup>d</sup>	34.31
Golband goatfish ( <i>Upeneus moluccensis</i> )	0.25 $\pm$ 0.08 <sup>bc</sup>	0.13 $\pm$ 0.04 <sup>b</sup>	0.05 $\pm$ 0.00 <sup>b</sup>	47.38 $\pm$ 2.16 <sup>f</sup>	47.81
Petersfisch ( <i>Zeus faber</i> )	0.15 $\pm$ 0.04 <sup>b</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>c</sup>	25.20 $\pm$ 1.70 <sup>c</sup>	25.48
Derbio ( <i>Trachinotus ovatus</i> )	0.05 $\pm$ 0.00 <sup>a</sup>	0.08 $\pm$ 0.03 <sup>ab</sup>	0.05 $\pm$ 0.00 <sup>b</sup>	32.55 $\pm$ 2.19 <sup>d</sup>	32.73
White seabream ( <i>Diplodus sargus</i> )	0.05 $\pm$ 0.00 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>b</sup>	29.83 $\pm$ 2.09 <sup>d</sup>	29.98
Golden grey mullet ( <i>Liza aurata</i> )	0.05 $\pm$ 0.00 <sup>a</sup>	0.08 $\pm$ 0.04 <sup>ab</sup>	0.08 $\pm$ 0.02 <sup>c</sup>	25.98 $\pm$ 0.53 <sup>c</sup>	26.19
Bogue ( <i>Boops boops</i> )	0.15 $\pm$ 0.00 <sup>b</sup>	0.83 $\pm$ 0.19 <sup>c</sup>	0.18 $\pm$ 0.01 <sup>d</sup>	32.70 $\pm$ 4.31 <sup>d</sup>	33.86
Tub gurnard ( <i>Chelidonichthys lucerna</i> )	0.05 $\pm$ 0.00 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	0.03 $\pm$ 0.00 <sup>a</sup>	17.88 $\pm$ 1.59 <sup>a</sup>	18.01
Silver fish ( <i>Chalcalburnus mosullensis</i> )	0.23 $\pm$ 0.02 <sup>bc</sup>	0.10 $\pm$ 0.00 <sup>b</sup>	0.05 $\pm$ 0.00 <sup>b</sup>	15.68 $\pm$ 3.29 <sup>a</sup>	16.06
Striped red mullet ( <i>Mullus surmuletus</i> )	0.05 $\pm$ 0.01 <sup>a</sup>	0.10 $\pm$ 0.00 <sup>b</sup>	0.05 $\pm$ 0.00 <sup>b</sup>	27.15 $\pm$ 4.67 <sup>c</sup>	27.35

Different letters (a–f) in the same column indicate significant differences ( $P < 0.05$ ).

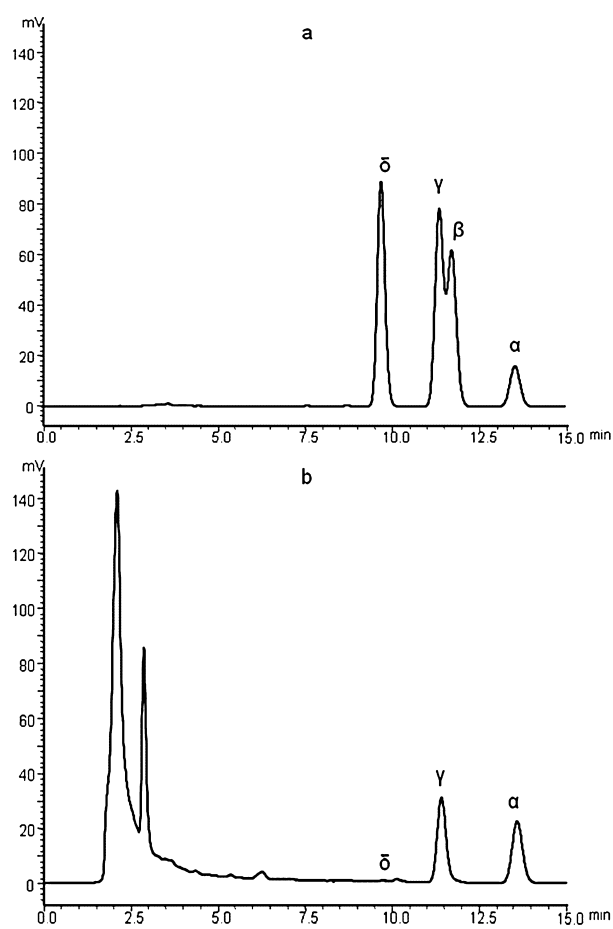
## Statistical Analyses

The data are presented as mean  $\pm$  standard deviation. Statistical significance was determined by using the  $t$ -test; differences were considered significant at a value of  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Tocopherol Content of Raw Fish Species

Alpha ( $\alpha$ )-, beta ( $\beta$ )-, gamma ( $\gamma$ )- and delta ( $\delta$ )-tocopherol of different fish species are shown in Table 1. Figure 1 illustrates HPLC chromatogram for a standard mixture of tocopherol (10 mg/mL). There were significant differences ( $P < 0.05$ ) in the level of each content among fish species. The highest content of  $\delta$ -tocopherol was obtained from golband goatfish and pike (0.25 mg/kg), followed by silver fish and sardine (0.23 mg/kg). The lowest  $\gamma$ -tocopherol contents were obtained from corb, tub gurnard, derbio, white seabream and seabass (0.05 mg/kg), whereas bogue gave the highest  $\gamma$ -tocopherol content (0.83 mg/kg). Beta ( $\beta$ -) tocopherol content of fish ranged from 0.03 mg/kg for tub gurnard to 0.18 mg/kg for bogue.  $\alpha$ -Tocopherol was found to be the major form of vitamin E in fish muscle, ranging from 15.68 mg/kg for silver fish to 47.38 mg/kg for goldband goatfish, followed by anchovy (40.53 mg/kg) and striped seabream (33.83 mg/kg). The levels of total tocopherol in all samples ranged from 16.06 to 47.81 mg/kg. Golband goatfish (47.81 mg/kg), anchovy (40.84 mg/kg), striped sea bream (34.31 mg/kg) and bogue (33.86 mg/kg) had the highest tocopherol contents among the samples, whereas the lowest tocopherol levels were observed for silver fish (16.06 mg/kg) and gurnard (18.01 mg/kg).



**FIG. 1** HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY CHROMATOGRAM FOR A STANDARD MIXTURE OF TOCOPHEROL (10 mg/kg) AND ATLANTIC SALMON

Nutritious foods with polyunsaturated fatty acid (PUFA) include minimum of 0.6 mg tocopherol in each gram PUFA (Sikorski and Kolakowski 2000). Syvaola *et al.* (1985) found that fish with high or medium fat contained an average of 20 mg/kg tocopherol, whereas fish with low fat contained 10 mg/kg tocopherol.  $\alpha$ -Tocopherol content in the fish muscle lipid ranges from 10 to 750 mg/kg in oil (Kinsella 1988). It was reported that anchovy, tuna fish, tench and carp have 5–8 mg/kg tocopherol levels, and canned tuna and som have 5.3–7 mg/kg tocopherol content, respectively (Hogarty *et al.* 1989; Frigg *et al.* 1990; Arslan *et al.* 1997). Riarova *et al.* (2003) reported that zander, trout, mackerel, carp and sprat have 26.2, 15.9, 4.8 and 3.5 mg/kg tocopherol levels in fish muscle, respectively. Passi *et al.* (2002) determined 5.8 mg/kg tocopherol, whereas Bhadra *et al.* (2004) reported 16 mg/kg tocopherol for sardine muscle. These values are lower than our determined value (21 mg/kg) for sardine muscle. It was determined that tocopherol contents of all fish species investigated in this study were higher than those of the other studies. Sigurgisladdottir *et al.* (1994) reported that tocopherol contents of fish depend on fish size, fodder content, feed occasions and water properties. The tocopherol contents of fish were also reported to vary with different species, even with same species. Tocopherol content of marine fish was also reported to be higher than freshwater species (Syvaola *et al.* 1985).

There are marked differences in *per capita*  $\alpha$ -tocopherol supply among different countries varying from roughly

8–10 mg/head/day (e.g., Iceland, Finland, New Zealand and Japan) to 20–25 mg/head/day (e.g., France, Greece and Spain) (Bellizzi *et al.* 1994). Epidemiological studies have reported an inverse relationship between the incidence of coronary heart disease (CHD) and vitamin E status using a variety of methods. A descriptive correlation study of 24 developed countries showed that the supply of tocopherol was strongly related to CHD and explained the low rates of heart disease in some European countries (Bellizzi *et al.* 1994). For example, Spain, with low rates of CHD, has estimated intakes of 18–25 mg/day of vitamin E, whereas in the UK, where the number of deaths from CHD is one of the highest, the intake of vitamin E is only 4.7–11.9 mg/day (Northrop-Clewes and Thurnham 2002). The daily requirements of vitamin E are 3–4 mg  $\alpha$ -tocopherol for infants, 6–7 mg for children and about 10 mg for adolescents and adults (Chow 2000). In the result of our study, daily tocopherol requirement can be supplied by the consumption of 100–200 g of fish meat of these species.

### Effect of Microwave Cooking on Tocopherol Content of Some Selected Fish Species

Table 2 shows tocopherol content of raw and cooked fish by microwave. Significant differences in each total tocopherol content were found between raw and cooked fish species ( $P < 0.05$ ). Cooking process generally had a significant effect on the total tocopherol content of fish ( $P < 0.05$ ).

**TABLE 2.** TOCOPHEROL CONTENTS OF RAW AND COOKED FISH (mg/kg)

Fish species	Delta	Gamma	Beta	Alpha	Total tocopherol	Treatment
Red mullet ( <i>Mullus barbatus</i> )	0.05 ± 0.00 <sup>a</sup>	0.08 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	23.75 ± 0.49 <sup>a</sup>	23.93 ± 0.49 <sup>a</sup>	Raw
	0.13 ± 0.01 <sup>b</sup>	0.08 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	33.95 ± 2.69 <sup>b</sup>	34.20 ± 2.69 <sup>a</sup>	Cooked
Two banded bream ( <i>Diplodus vulgaris</i> )	0.55 ± 0.04 <sup>a</sup>	0.55 ± 0.03 <sup>a</sup>	0.08 ± 0.00 <sup>a</sup>	16.73 ± 0.81 <sup>a</sup>	17.90 ± 0.88 <sup>a</sup>	Raw
	1.38 ± 0.11 <sup>b</sup>	1.85 ± 0.07 <sup>b</sup>	3.40 ± 0.28 <sup>b</sup>	47.08 ± 1.24 <sup>b</sup>	53.70 ± 1.34 <sup>b</sup>	Cooked
Sand smelt ( <i>Atherina hepsetus</i> )	0.23 ± 0.01 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	15.18 ± 1.17 <sup>a</sup>	15.55 ± 1.16 <sup>a</sup>	Raw
	0.28 ± 0.01 <sup>b</sup>	0.45 ± 0.04 <sup>b</sup>	0.20 ± 0.01 <sup>b</sup>	19.75 ± 1.48 <sup>b</sup>	20.6 ± 1.51 <sup>b</sup>	Cooked
Corb ( <i>Umbrina cirrosa</i> )	0.05 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	21.15 ± 0.92 <sup>a</sup>	21.30 ± 0.92 <sup>a</sup>	Raw
	0.10 ± 0.01 <sup>b</sup>	0.05 ± 0.00 <sup>a</sup>	0.10 ± 0.00 <sup>b</sup>	19.80 ± 1.56 <sup>a</sup>	20.05 ± 1.57 <sup>a</sup>	Cooked
Tub gurnad ( <i>Trigla lucerna</i> )	0.05 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	0.03 ± 0.00 <sup>a</sup>	14.88 ± 1.38 <sup>a</sup>	15.00 ± 1.21 <sup>a</sup>	Raw
	0.05 ± 0.00 <sup>a</sup>	0.18 ± 0.01 <sup>b</sup>	11.28 ± 0.67 <sup>b</sup>	37.85 ± 1.70 <sup>b</sup>	49.35 ± 2.36 <sup>b</sup>	Cooked
Common sole ( <i>Solea solea</i> )	0.10 ± 0.01 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	17.38 ± 0.95 <sup>a</sup>	17.37 ± 0.74 <sup>a</sup>	Raw
	0.53 ± 0.04 <sup>b</sup>	1.83 ± 0.04 <sup>b</sup>	0.10 ± 0.01 <sup>b</sup>	30.58 ± 2.86 <sup>b</sup>	33.03 ± 2.81 <sup>b</sup>	Cooked
Sea bream ( <i>Sparus aurata</i> )	0.08 ± 0.00 <sup>a</sup>	0.06 ± 0.00 <sup>a</sup>	0.04 ± 0.00 <sup>a</sup>	25.69 ± 2.17 <sup>a</sup>	25.86 ± 2.1 <sup>a</sup>	Raw
	0.90 ± 0.03 <sup>b</sup>	0.20 ± 0.01 <sup>b</sup>	0.13 ± 0.01 <sup>b</sup>	28.95 ± 1.98 <sup>a</sup>	30.18 ± 2.02 <sup>b</sup>	Cooked
Whiting ( <i>Merlangius merlangus</i> )	0.13 ± 0.01 <sup>a</sup>	0.13 ± 0.00 <sup>a</sup>	0.08 ± 0.00 <sup>a</sup>	9.45 ± 0.14 <sup>a</sup>	9.78 ± 0.14 <sup>a</sup>	Raw
	0.40 ± 3.04 <sup>b</sup>	0.00 ± 0.00 <sup>b</sup>	4.85 ± 0.21 <sup>b</sup>	21.93 ± 0.11 <sup>b</sup>	27.18 ± 2.93 <sup>b</sup>	Cooked
Pike ( <i>Esox lucius</i> )	0.25 ± 0.01 <sup>a</sup>	0.10 ± 0.00 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	17.53 ± 0.60 <sup>a</sup>	17.93 ± 0.6 <sup>a</sup>	Raw
	0.38 ± 0.02 <sup>a</sup>	0.08 ± 0.0 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	26.48 ± 0.95 <sup>b</sup>	26.98 ± 0.98 <sup>b</sup>	Cooked
Atlantic salmon ( <i>Salmo salar</i> )	0.50 ± 0.03 <sup>a</sup>	17.98 ± 1.03 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	54.40 ± 2.62 <sup>a</sup>	72.88 ± 3.67 <sup>a</sup>	Raw
	0.60 ± 0.04 <sup>b</sup>	18.23 ± 1.66 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	48.95 ± 1.20 <sup>b</sup>	67.78 ± 0.42 <sup>b</sup>	Cooked
Common pandora ( <i>Pagellus erythrinus</i> )	0.24 ± 0.02 <sup>a</sup>	0.05 ± 0.00 <sup>a</sup>	0.03 ± 0.00 <sup>a</sup>	24.11 ± 1.82 <sup>a</sup>	24.43 ± 1.80 <sup>a</sup>	Raw
	0.05 ± 0.00 <sup>a</sup>	2.73 ± 0.25 <sup>b</sup>	1.03 ± 0.04 <sup>b</sup>	31.21 ± 0.62 <sup>b</sup>	35.01 ± 0.84 <sup>b</sup>	Cooked

Different letters (a–b) in the same column between raw and cooked of same species indicate significant differences ( $P < 0.05$ ).

There were no differences ( $P > 0.05$ ) in the levels of  $\delta$ -tocopherol between raw and cooked common pandora and pike and also tub gurnad. Significant differences were observed ( $P < 0.05$ ) in the  $\gamma$ -tocopherol content of raw and cooked fish except for striped mullet, corb, pike and Atlantic salmon. As for  $\beta$ -tocopherol contents, there were significant differences ( $P < 0.05$ ) between raw and cooked fish apart from striped mullet and pike species.  $\beta$ -Tocopherol was not detected in Atlantic salmon. Significant differences were observed in the levels of  $\alpha$ -tocopherol of raw and cooked samples apart from corb and sea bream (Table 2).

$\alpha$ -Tocopherol is generally the only tocopherol present in oils from marine fish, and its concentration is low (Kulas *et al.* 2002). However, other tocopherol forms besides  $\alpha$ -tocopherol have recently been considered to be of biological importance (Wagner *et al.* 2004; Kornsteiner *et al.* 2006). Total tocopherol contents of the fish ranged from 9.75 to 72.88 for raw fish and from 20.05 to 67.78 mg/kg for cooked fish. In the case of raw fish species, the highest tocopherol concentration was observed for Atlantic salmon (Fig. 1), sea bream, common pandora and striped mullet, whereas tub gurnad, sand smelt and common sole had the lowest tocopherol content.  $\alpha$ -Tocopherol content of Atlantic salmon was 54.40 mg/kg. Refsgaard *et al.* (1998) reported lower  $\alpha$ -tocopherol content (35.8 mg/kg) for cultivated salmon.

Jittinandana *et al.* (2006) reported that  $\alpha$ -tocopherol was lost after oven-cooking 7-day refrigerated, raw trout fillets from fish fed with high levels of dietary vitamin E. However, hot smoking did not affect the  $\alpha$ -tocopherol content of smoked products compared with raw fillets. Similarly, Gotoh *et al.* (2011) found that heating process such as boiling, grilling and frying significantly decreased the  $\alpha$ -tocopherol contents of fish meat. Isnardy *et al.* (2003) indicated that tocopherols decreased during increasing oxidative stress. Losses in vitamin E content seem to be related to the lipid degradation and factors affecting this degradation include cooking temperatures, time, and exposure to light and oxidative conditions (Wyatt *et al.* 1999). However, cooking process significantly increased the total tocopherol content of fish ( $P < 0.05$ ). This could be attributed to the loss of water during the cooking process. Vitamin profiles differed significantly between fish species. Raw horse mackerel had higher vitamin E content (7.4 mg/kg) than fried (1.8 mg/kg) and grilled fish (1.8 mg/kg), while cooked seabass and hake contained considerable higher vitamin E content than raw fish (Dias *et al.* 2003). Salmon fillets were steamed, or pan-fried without oil, with olive oil, with corn oil, or with partially hydrogenated plant oil (Al-Saghir *et al.* 2004). They found that tocopherol levels remained almost stable and were not affected by the oxidation process.

However, Ruiz *et al.* (1999) studied the content of vitamin E in raw and cooked broiler chicken and found lower

vitamin E levels in the cooked samples compared with raw meat, suggesting that cooking could partially destroy  $\alpha$ -tocopherol (Pearson *et al.* 1977). Total tocopherol content of fish snack products was investigated by Suknark *et al.* (2001). They also found that total tocopherol content increased from 12.79 mg/100 g for raw material to 42.30 mg/100 g for fried products. In another study conducted by Devoli *et al.* (2009), nutritional value of traditional Italian meat-based dishes was influenced by cooking methods. They reported that vitamin E in the products was found to be high and they attributed this to the use of olive oil containing vitamin E source. Erkan *et al.* (2010) found that grilled and steamed horse mackerel had lower vitamin E content (4.5 and 3.5 mg/kg) than raw fish (7.7 mg/kg). The effect of two dietary treatments (50 as opposed to 200 mg/kg of  $\alpha$ -tocopheryl acetate) on rabbit meat, cooked by different procedures (boiling, frying and roasting), were evaluated (Dal Bosco *et al.* 2001). On the other hand, cooking caused a reduction in the  $\alpha$ -tocopherol level; boiling showed a reduction of 39% in the control fed group and 41% in the group fed with supplemented vitamin E; frying showed reductions of 12 and 21%; roasting showed reductions of 14 and 22%, respectively. The good resistance of vitamin E to heat was found for fried and roasted meat.

Tocopherols are antioxidants and  $\alpha$ -tocopherol was found to be the major form of vitamin E in fish muscle. Results of our study showed that fish generally was a good source of tocopherol, which was quite stable under microwave cooking conditions.

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