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L-CARNITINE CONTENTS IN SEAFOODS COMMONLY EATEN IN MIDDLE EASTERN COUNTRIES

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ABSTRACT

Beta-hydroxy-gamma-trimethyl amino butyric acid (L-carnitine) content of raw and cooked seafood was determined using high-performance liquid chromatography method. Thirty-one different fish species and nine different crustaceans were used to compare L-carnitine content of raw and cooked seafood. Significant differences in L-carnitine content were found in some species, regardless of the raw or cooked seafood ($P < 0.05$). There were also significant differences between some of the raw and cooked species ($P < 0.05$). The levels of L-carnitine in raw fish samples ranged from 17.98 mg/kg for big-scale sand smelt to 73.07 mg/kg for European conger (*Conger conger*). Squid (*Loligo vulgaris*) and green tiger prawn (*Penaeus semisulcatus*) were found as the best sources of L-carnitine among the tested seafood. Microwave cooking also significantly reduced the L-carnitine content of some seafoods ($P < 0.05$). The study showed that seafoods are an important origin of L-carnitine for covering the daily requirements of humans.

PRATICAL APPLICATION

Beta-hydroxy-gamma-trimethyl amino butyric acid (L-carnitine) has been used as a drug in various diseases such as dislipoproteinemia, anorexia and dyspepsia. Recently, L-carnitine and its derivatives have also been shown to protect cardiac metabolism and function in ischemic heart disease, and other clinical conditions of myocardial ischemia. In this study, L-carnitine level was determined in different fish and crustacean species to assess the presence of L-carnitine in the muscle of seafood. When humans suffer from L-carnitine deficiency, the knowledge of L-carnitine concentrations in seafood is helpful for diet specialists in order to prepare a specific diet.

INTRODUCTION

Meat-based bioactive substances such as L-carnitine have been studied for their potential beneficial effects (Arihara 2006). L-carnitine, beta-hydroxy-gamma-trimethyl amino butyric acid, is a naturally occurring vitamin-like amino acid derivative and has interesting pharmacological and nutritional properties (Mardones *et al.* 1999; Freimuller and Altorfer 2002; Sánchez-Hernández *et al.* 2010a). L-carnitine has a molecular weight of 161.2 and is easily soluble in water (Harpaz 2005). It is important in the transport of long-chain fatty acids (LCFAs) across the mitochondrial membrane before fatty acids are oxidized. Fatty acid partitioned for oxidation undergoes activation to fatty acyl-coenzyme A (CoA), which is then taken up by mitochondria to be oxidized (Galland *et al.* 2001). On the other hand, *n*-3 LCFAs and their CoA derivatives cannot cross the mitochondrial membrane without carnitine, which is synthesized in humans from lysine and methionine (Tanpaichitr and Leelahagul 1993; Galland *et al.* 2001; Reda *et al.* 2003). Therefore, L-carnitine is a key element in fat metabolism.

L-carnitine has been used as a drug since 1960 for the therapy of primary and secondary carnitine deficiency, and in various other diseases such as dislipoproteinemia, anorexia, and dyspepsia (Sánchez-Hernández *et al.* 2010b). L-carnitine and its derivatives have recently been shown to protect cardiac metabolism and function in ischemic heart disease and other clinical conditions of myocardial ischemia (Lango *et al.* 2001). L-carnitine is supplied to the body by two independent mechanisms: an endogenous biosynthesis and food supply (Rigault *et al.* 2008). If the diet is deficient in L-carnitine, a considerable drop soon develops in its plasma concentration

(Rebouche 1992). Minimum supplementation of carnitine in the diet to maintain its body stores constant ranges from 8 to 11 mg/day. It has been proposed that the daily need of L-carnitine for humans is between 2 and 12 μmol/kg body weight/day or 0.3 and 1.9 mg/kg/day (Demarquoy *et al*. 2004). These amounts may be even higher in some people such as athletes or pregnant women (Walter and Schaffhauser 2000). Food of animal origin contains high amounts of L-carnitine, ranging from 3 to 4 mg/serving for cheese, chicken and fish and up to 80 mg/serving for beef steak (Rebouche 1992).

L-carnitine has become an increasingly popular ingredient in dietary supplement (Hathcock and Shao 2006). In previous works, meat products have been reported as the best source for L-carnitine (Demarquoy *et al*. 2004; Knüttel and Harmeyer 2007). Dairy products and seafoods are reported to be relatively low in L-carnitine (Indyk and Woollard 1995; Woollard *et al*. 1999; Knüttel and Harmeyer 2007; Sánchez-Hernández *et al*. 2010a), whereas vegetables are mostly very low in L-carnitine (Demarquoy *et al*. 2004). Rigault *et al*. (2008) evaluated the effects of freezing and of different cooking methods on the L-carnitine content of red meat and fish. They reported that L-carnitine was abundant in all beef products and freezing or cooking did not modify L-carnitine content. Domestic cooking of salmon contained about 12 times less L-carnitine than beef, except in smoked salmon. The objective of this study was to compare the levels of free L-carnitine in raw and cooked seafoods.

MATERIALS AND METHODS

Sample Preparation and Cooking Procedure

Thirty-one different fish species and nine different crustaceans were caught by bottom trawling from the Mediterranean Sea in September 2010. Fish species used are listed in Tables 1 and 2. They were caught by different fishermen on the same day. Fish and crustaceans of marketable size were selected at random from the catch. They were immediately iced in a box and transported to the laboratory within 3 h. Fresh fish species were gutted and washed; then, skin and bones were removed. For crustaceans, carapaces of specimens were removed, and the two largest portions of meat connected to the swimming legs were carefully scraped out with a scalpel. Minimum of 10 from each species were used for the analyses. After that, meat was divided into two groups. First group was minced for determination of carnitine in raw samples and the second group was cooked in microwave at medium power (600 W) by microwave set at a frequency of 300 MHz for 2 min and then minced. Triplicate samples were taken to determine the free L-carnitine content of seafood.

Extraction Procedure

Sample preparation was carried out according to the method of Cao *et al*. (2007) with minor modifications. Briefly, each species were minced and 0.5 g was weighed into a test tube. The 2 mL of saline buffer was added and homogenized for 1 min. The composition of this buffer was: 25 mM sucrose, 1 mM EDTA, 100 mM Tris–HCl buffer and pH 7.5 (Tris, Sucrose, EDTA buffer). The homogenate was centrifuged at 5,000 rpm for 10 min at 4C. The upper layer of the supernatant was taken and diluted twice with saline buffer. The final supernatant was used for L-carnitine determination.

Protein Precipitation

The 500 μL of 0.3 M $Ba(OH)_2$ solution was added to 1 mL of stock solution and vortexed for 2 min. The 300 μL of 10% $ZnSO_4$ solution were then added to each sample and vortexed at high speed for 1 min. The tubes were then centrifuged at 10,000 rpm for 10 min. The supernatant was separated from the precipitate and used for the derivatization reaction.

Derivatization Procedure

The 1-aminoanthracene (fluorescent reagent) was dissolved in acetone (16 mg/mL) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in 0.01 M NaH_2PO_4-H_2O pH 3.5 (160 mg/mL). The 1 mL of the supernatant was mixed with 1 mL of 0.01 M NaH_2PO_4-H_2O (pH 3.5). The 40 μL of 1 M HCl, 200 μL of 1-aminoanthracene solution and 200 μL of EDC solution were then sequentially added to the sample with continuous vortex mixing. The mixture was incubated at 25C for 20 min and the excess reagent was removed by washing the sample with 5 mL of diethyl ether. A 600 μL aliquot of the aqueous phase was then transferred to a plastic tube, and 1,400 μL of 0.01 M Na_2HPO_4-$2H_2O$, pH 9.1 was added to adjust the pH of the samples to about seven and the mixture was washed with 5 mL of chloroform. The 500 μL aliquots of the final aqueous phase were diluted with 500 μL of 0.01 M NaH_2PO_4-H_2O, pH 3.5 and 20 μL of this solution was injected into the high-performance liquid chromatography (HPLC) system.

HPLC APPARATUS AND COLUMNS

HPLC analyses were conducted using a Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (RF-10AXL), two binary gradient pumps (Shimadzu LC-10AT), autosampler (SIL 20AC), column oven (CTO-20AC) and a communication bus module (CBM-20A) with a valve unit (FCV-11AL). The column, purchased from Phenomenex (Macclesfield, Cheshire, UK), was packed with reversed-phase ODS (C18)

TABLE 1. L-CARNITINE CONTENT OF SOME RAW AND COOKED FISH SPECIES (Mg/Kg)

Common names	Raw $\bar{X} \pm S_X$	Cooked $\bar{X} \pm S_X$	Binomial names
Atlantic bonito	$55.63 \pm 2.45^{c,y}$	$45.84 \pm 0.94^{de,x}$	*Sarda sarda*
Atlantic chub mackerel	$73.46 \pm 1.99^{a,y}$	$58.32 \pm 3.68^{b,x}$	*Scomber colias*
Atlantic horse mackerel	$65.58 \pm 4.93^{b,x}$	$58.27 \pm 1.19^{b,x}$	*Trachurus trachurus*
Big-scale sand smelt	$17.98 \pm 1.06^{mn,x}$	$15.77 \pm 0.70^{lm,x}$	*Atherina boyeri*
Bogue	$55.25 \pm 3.77^{c,y}$	$43.53 \pm 0.47^{e,x}$	*Boops boops*
Brown meagne	$35.44 \pm 0.57^{d,y}$	$27.08 \pm 2.06^{fg,x}$	*Sciaena umbra*
Common pandora	$27.35 \pm 1.68^{fghk,x}$	$28.00 \pm 2.02^{fg,x}$	*Pagellus erythrinus*
Common sole	$55.04 \pm 2.40^{c,x}$	$50.59 \pm 3.05^{d,x}$	*Solea solea*
Common two-banded seabream	$52.02 \pm 4.39^{c,x}$	$50.63 \pm 7.46^{cd,x}$	*Diplodus vulgaris*
Corb	$29.47 \pm 2.77^{defgh,y}$	$20.67 \pm 0.69^{hk,x}$	*Umbrina cirosa*
European anchovy	$53.91 \pm 2.81^{c,y}$	$42.46 \pm 0.53^{e,x}$	*Engraulis encrasicolus*
European conger	$73.07 \pm 3.47^{a,y}$	$56.81 \pm 3.64^{b,x}$	*Conger conger*
Filefish	$27.88 \pm 2.05^{efghk,x}$	$23.58 \pm 2.33^{ghk,x}$	*Stephanolepis diaspros*
Goatfish	$33.04 \pm 2.23^{def,y}$	$19.07 \pm 1.71^{hklm,x}$	*Upeneus pori*
Goldband goatfish	$24.28 \pm 2.52^{hkl,x}$	$22.90 \pm 2.16^{ghk,x}$	*Upeneus mollucensis*
John dory	$34.43^{x} \pm 1.33^{de,y}$	$22.63 \pm 1.99^{ghk,x}$	*Zeus faber*
Kluzinger's ponyfish	$31.77 \pm 0.97^{defg,y}$	$24.34 \pm 1.28^{gh,x}$	*Leiognathus kluzingeri*
Lizardfish	$33.10 \pm 1.79^{def,x}$	$31.33 \pm 1.30^{f,x}$	*Saurida undosguamis*
Mackerel	$28.19 \pm 1.64^{efghk,y}$	$22.48 \pm 1.63^{ghk,x}$	*Scomber scombrus*
Red mullet	$52.54 \pm 3.43^{c,y}$	$44.38 \pm 2.95^{e,x}$	*Mullus barbatus*
Sardine	$65.32 \pm 2.36^{b,y}$	$58.35 \pm 4.42^{b,x}$	*Sardinella aurita*
Sea bass	$26.34 \pm 1.25^{ghk,x}$	$23.90 \pm 0.59^{ghk,x}$	*Dicentrarchus labrax*
Sea bream	$22.11 \pm 1.99^{klm,x}$	$19.65 \pm 1.18^{hkl,x}$	*Sparus aurita*
Silver whiting	$50.39 \pm 4.29^{c,x}$	$43.98 \pm 7.74^{e,x}$	*Shillago sihama*
Spotted flounder	$20.50 \pm 1.21^{lm,y}$	$13.52 \pm 0.76^{m,x}$	*Citharus linguatula*
Striped bream	$68.73 \pm 2.45^{b,y}$	$53.38 \pm 0.96^{c,x}$	*Lithognathus mormyrus*
Thinlip grey mullet	$67.55 \pm 6.16^{b,x}$	$64.77 \pm 1.15^{a,x}$	*Liza ramada*
Thornback ray	$24.61 \pm 1.41^{kl,x}$	$20.69 \pm 1.90^{hkl,x}$	*Raja clavata*
Tub gurnard	$50.90 \pm 2.41^{c,x}$	$44.88 \pm 2.45^{e,x}$	*Trigla lucerna*
White grouper	$30.60 \pm 1.93^{defgh,x}$	$27.81 \pm 2.64^{fg,x}$	*Epinephelus aeneus*
Yellowstripe barracuda	$21.68 \pm 0.44^{klm,y}$	$17.67 \pm 0.93^{klm,x}$	*Sphyraena chrysotaenia*

$\bar{X} \pm S_X$: average $\pm$ standard deviation. ($n = 10$) Different lowercase letters $^{(a–n)}$ in a column indicate significant differences ($P < 0.05$) among fish species. Different letters $^{(x,y)}$ in same rows indicate significant differences ($P < 0.05$) between raw and cooked fish species.
L-carnitine, beta-hydroxy-gamma-trimethyl amino butyric acid.

TABLE 2. L-CARNITINE CONTENT OF SOME RAW AND COOKED CRUSTACEA (Mg/Kg)

Common names	Raw $\bar{X} \pm S_X$	Cooked $\bar{X} \pm S_X$	Binomial names
Atlantic blue crab	$30.20 \pm 2.77^{de,y}$	$15.21 \pm 0.86^{f,x}$	*Callinectes sapidus*
Blue swimmer crab	$66.99 \pm 3.91^{b,y}$	$48.72 \pm 3.37^{b,x}$	*Portunus pelagicus*
Common cuttlefish	$33.06 \pm 1.23^{de,y}$	$24.60 \pm 1.89^{de,x}$	*Sepia officinalis*
Green tiger prawn	$73.06 \pm 5.47^{ab,y}$	$59.18 \pm 2.96^{a,x}$	*Penaeus semisulcatus*
Mantis shrimp	$34.30 \pm 2.66^{d,y}$	$29.33 \pm 0.06^{cd,x}$	*Eurogosquilla massavensis*
Mediterranean prawn	$22.57 \pm 2.22^{f,x}$	$21.83 \pm 0.85^{e,x}$	*Penaeus kerathurus*
Octopus	$26.47 \pm 1.36^{ef,y}$	$20.05 \pm 1.18^{ef,x}$	*Octopus vulgaris*
Spiny hands	$49.55 \pm 4.27^{c,y}$	$32.30 \pm 1.53^{c,x}$	*Charybdis helleri*
Squid	$78.90 \pm 2.14^{a,y}$	$60.38 \pm 4.46^{a,x}$	*Loligo vulgaris*

$\bar{X} \pm S_X$: average $\pm$ standard deviation. ($n = 10$) Different lowercase letters $^{(a–n)}$ in a column indicate significant differences ($P < 0.05$) among fish species. Different letters $^{(x,y)}$ in same rows indicate significant differences ($P < 0.05$) between raw and cooked fish species.
L-carnitine, beta-hydroxy-gamma-trimethyl amino butyric acid.

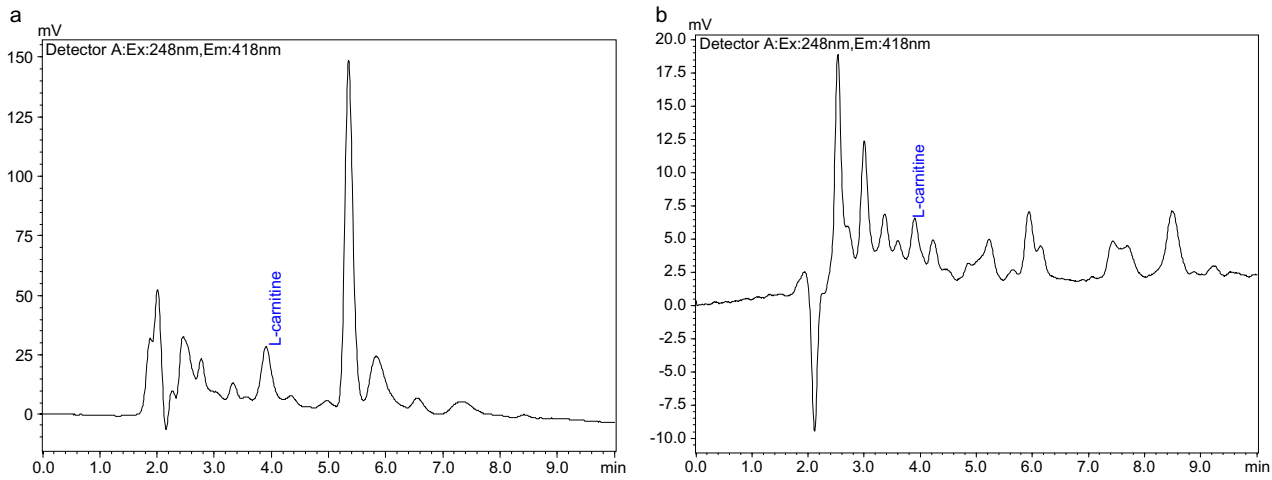

FIG. 1. THE HPLC CHROMATOGRAMS OF STANDARD L-CARNITINE (a) AND ANCHOVY (b)
HPLC, high-performance liquid chromatography; L-carnitine, beta-hydroxy-gamma-trimethyl amino butyric acid.

Hypersil, 5μ, 250 × 4.60 mm. L-carnitine standard was purchased from Sigma-Aldrich (Munich, Germany) and the mobile phase consisted of acetonitrile (30%) and ammonium acetate (70%) for the analysis. Flow rate was 0.5 mL/min.

Statistical Analyses

The data are presented as mean ± 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

L-carnitine is synthesized from the essential amino acids lysine and methionine with the assistance of vitamin C and other secondary compounds produced in the body (Harpaz 2005). To assess the presence of L-carnitine in the muscle of seafood, L-carnitine level was determined in different fish and crustacean species. Table 1 shows L-carnitine content of raw and cooked fish. Figure 1a,b also represent the L-carnitine chromatograms of standard (a) and a fish sample (b). Significant differences in L-carnitine content were found in some species, regardless of whether they are raw or cooked seafood ($P < 0.05$). There were also significant differences between some of the raw and cooked species ($P < 0.05$). Harpaz (2005) reported that red meats were very rich in carnitine with values of 500–1,200 mg/kg, followed by fish, chicken and milk-derived substances, which contain 16–64 mg/kg. Tada *et al.* (1984) reported on the L-carnitine content of some seafoods *(unit: mg/100 g edible portion)*: Japanese little-neck manila clam 8.6 mg, freshwater clam 1.3 mg, shrimp 2.9–3.9 mg, Japanese tiger prawn 8.5 mg, red sea bream 8.1–10.8 mg, shellfish ligament 47.8 mg, ark shell 21.7–134.4 mg, octopus 17.0–27.0 mg, giant Pacific oyster 10.6 mg, Bluefin tuna 1.9–3.5 mg, Japanese yellow horse mackerel 6.0 mg, Pacific saury 12.1 mg and bigfin reef squid 48.1 mg.

In this study, seafoods contained considerable amounts of L-carnitine. The levels of L-carnitine in raw fish samples ranged from 17.98 mg/kg for big-scale sand smelt to 73.46 mg/kg for Atlantic chub mackerel; whereas, its level for cooked fish ranged from 13.52 mg/kg for spotted flounder to 64.77 mg/kg for thinlip grey mullet. Atlantic chub mackerel and European conger had the highest L-carnitine content among the tested seawater fish species, and no significant differences were found between these raw fish species for L-carnitine content. Striped bream, thinlip grey mullet, Atlantic horse mackerel, European conger and sardine had high L-carnitine content (>65 mg/kg). It was reported that among marine products, salmon is one of the major sources for L-carnitine (Rigault *et al.* 2008).

L-carnitine content of raw and cooked crustaceans are given in Table 2. Squid and green tiger prawn were found to be the best sources of L-carnitine among the tested seafood. However, Mediterranean prawn and octopus contained the lowest with values of 22.5 and 26.47 mg/kg, respectively. Compared with crab species, blue swimming crab had approximately twofold higher L-carnitine content than Atlantic blue crab. Similar results were obtained for Mediterranean prawn, which contained more than threefold L-carnitine compared with green tiger prawn. Rigault *et al.* (2008) reported that cooking did not alter the L-carnitine content of salmon and no difference was found between fresh and frozen salmon for L-carnitine content. Smoked salmon showed a reduction in L-carnitine content compared with fresh salmon. On the other hand, in this study, generally L-carnitine content of most of the seawater fish species was

affected by the cooking process. This could be due to the water loss during cooking. For example, L-carnitine contents of john dory, kluzinger ponyfish, mackerel, Atlantic bonito, corb and yellowstripe barracuda decreased with the cooking process ($P < 0.05$). Among the cooked seafood, the highest L-carnitine amount was found for thinlip grey mullet (64.7 mg/kg), whereas cooked big-scale sand smelt and spotted flounder contained the lowest level of L-carnitine. There were no significant differences in L-carnitine content among cooked Atlantic horse mackerel, Atlantic chub mackerel, sardine and European conger.

Microwave cooking significantly reduced L-carnitine content of crustaceans, apart from Mediterranean prawn ($P < 0.05$). Cooked squid and green tiger prawn, and blue swimmer crab contained the higher amount of L-carnitine (Table 2) than the other crustaceans (<33 mg/kg). The distribution of carnitine between the different species was not similar. Demarquoy *et al.* (2004) measured the L-carnitine content of raw and cooked fish, ranging from 7 to 58 mg/kg, which are lower values than those obtained in our study. Similar results (19–55.5 mg/kg) were obtained from raw, frozen, cooked and smoked salmon (Rigault *et al.* 2008). The L-carnitine content in this study ranged between 17.98 and 73.07 mg/kg for raw fish, between 22.57 and 78.90 mg/kg for raw crustaceans, between 13.52 and 64.77 mg/kg for cooked fish species, and between 15.21 and 60.38 mg/kg for cooked crustaceans. Zhang *et al.* (2006) found that endogenous L-carnitine was quite variable in live foods and depends on their physiological status. L-carnitine in microalgae used as live foods for aquaculture was much higher (161–504 mg/kg) than that of seafood. The different level of L-carnitine could be due to differences in the samples such as tissues, age, gender and water content, season, origin of fish in the sample preparations, and also due to different methods of L-carnitine determination.

Infants are particularly susceptible to L-carnitine depletion, which affects their normal growth (Bremer 1983; Lohninger *et al.* 1987; Harpaz 2005). In carnitine deficiency, fatty-acid oxidation is reduced, resulting in mitochondrial failure. The addition of sufficient quantities of L-carnitine to the diet prevents these problems (McDowell 1989; Harpaz 2005). In the present study, L-carnitine content of seafood is found to be above the daily requirements for humans. In addition, cooked seafood species as well as raw fish generally seemed to be good sources of L-carnitine and meet the proposed daily need for L-carnitine (0.3–1.9 mg/kg). When humans suffer from L-carnitine deficiency, the knowledge of L-carnitine concentrations in seafood could be helpful to nutritionists for making dietary recommendations.

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