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Proximate composition, fatty acid analysis and protein digestibility-corrected amino acid score of three Mediterranean cephalopods

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Proximate composition, fatty acid analysis and protein digestibility-corrected amino acid score (PDCAAS) in three commercially important cephalopods of the Mediterranean sea (cuttlefish, octopus and squid) were determined. The results of the proximate analysis showed that these species had very high protein:fat ratios similar to lean beef. Docosahexaenoic, palmitic and eicosipentaenoic acid were the most abundant fatty acids among analyzed species. The amount of *n*-3 fatty acids was higher than that of saturated, monounsaturated and *n*-6 fatty acids. Despite the fact that cephalopods contain small amounts of fat they were found quite rich in *n*-3 fatty acids. Finally, PDCAAS indicated that these organisms had a very good protein quality.

Keywords: Amino acids / Cephalopods / *n*-3 fatty acids / PDCAAS

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1 Introduction

Cephalopods are among the most important marine foods in Greece. The three most popular cephalopods from the Greek bays are the common octopus, *Octopus vulgaris* (Mollusca, Cephalopoda, Octopodidae), the European squid, *Loligo vulgaris* (Mollusca, Cephalopoda, Loliginidae) and the common cuttlefish, *Sepia officinalis* (Mollusca, Cephalopoda, Sepiidae). According to the Food and Agriculture Organization of the United Nations (FAO) [1], the catches of these organisms in Greece during 2003 was 2697, 671, and 2816 metric tons, respectively.

In general, seafood is a potential source of the essential *n*-3 fatty acids. Consumption of these fatty acids protects against various diseases, *e.g.*, ishemic heart disease, cancer, inflammatory diseases, *etc.* [2, 3]. Cephalopods contain small amounts of fat [4, 5], which, however, is rich in *n*-3 fatty acids [4–7]. They also contain lower amounts of cholesterol than fish and crustaceans [7–10] and can be used as a good source of the *n*-3 fatty acids with low cholesterol.

Other parameters that indicate the nutritional value of foods are the proximate composition and protein quality. The FAO/World Health Organization (WHO) [11] expert committee suggested that the protein digestibility-corrected amino acid score (PDCAAS) should be used for the evaluation of the protein quality of foods. The PDCAAS is determined from the amino acid composition of the food and corrected by a factor indicating the digestibility of proteins. The digestibility factor can be found in pertinent databases. However, no such data have been published about the protein digestibility of mollusks, especially cephalopods.

In the present study, the nutritional value of three cephalopods was investigated. In particular, the proximate and fatty acid composition of the octopus, squid, and cuttlefish were determined. In addition, the PDCAAS of the organisms were evaluated.

2 Materials and methods

Approximately 1000 g (three to five specimens) of each cephalopod were bought from the fish market of Thessaloniki during September 2002. After the separation of the non-edible parts, the flesh of the organisms was washed and divided in three samples. Each sample contained one or two specimens and every specimen was used only in one sam-

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Abbreviations: AAS, amino acid score; **DHA,** docosahexaenoic acid; **EPA**, eicosapentaenoic acid; **PDCAAS,** protein digestibility-corrected amino acid score ple. The samples were at first cut in small pieces and then homogenized in a multi-purpose cutter. Two portions (10 g) of each sample were divided. One was set in a thermostatic controlled oven (102°C) and the other in a furnace (550°C). The portions were held until constant weight for moisture and ash determination, respectively.

The rest of the samples were freeze-dried. The freeze-dried samples were put in plastic bags under nitrogen atmosphere and were stored for short time in a static dryer that was filled with silica gel. The freeze-dried samples were used for lipid extraction, amino acids analyses and nitrogen content determination as described below.

Fat was extracted according to the method described by Bligh and Dyer [12]. The total nitrogen content was determined by the Kjeldahl method and was converted to crude protein content by multiplying by 6.25. The fat was methylated using sodium methanolate [13]. Margaric acid (C17 : 0) was added as internal standard. The determination of fatty acids was conducted in a gas chromatograph (Perkin Elmer Sigma 2B), equipped with split injector in a 1 : 50 ratio, FID and a 60 m J & W Scientific (model DB-3) capillary column (film 0.25 μm, diameter 0.32 mm). The temperature of the injector port and detector was held at 250°C and 300°C, respectively. The injected volume was 3 μL. The temperature of the column was held at 80°C for 5 min, raised to 180°C at 5°C/min, held at 180°C for 16 min, raised again to 220°C at 5°C/min and finally held at 220°C for 20°min. Identification of fatty acids was based on the comparison of the retention times with the retention times of fatty acid methyl ester standards. The standards were purchased from Sigma.

For the evaluation of the amino acid content, proteins were hydrolyzed with 6 M HCl. Freeze-dried sample (300 mg) was added in a small test tube. After the addition of 20 μL internal standard solution (117.15 mg DL-Norvalin/ 100 mL) 1.5 mL 6 M HCl were added. The oxygen was removed initially using a vacuum and then with a nitrogen stream. Hydrolysis took place for 22 h at 120°C. After removing the HCl in a rotary evaporator, the hydrolized sample was diluted in 2 mL of a solution containing 9.4 g tri-lithium citrate, 7.4 g citric acid, 20 mL thiodiethanol and ca. 5.5 ml 37% HCl, diluted to 1 L with HPLC grade water; final pH 2.20. The sample was then placed in the sampler of the amino acid analyzer (Biotronik, LC5001) and measured.

For the estimation of the amino acid score (AAS), amino acid ratios for the essential amino acids were calculated according to the formula:

$$\text{Amino acid ratio} = \frac{\text{the amount in mg of an essential amino acid in 1 g protein of the sample}}{\text{the amount in mg of same essential amino acid in 1 g of a reference pattern}} \quad (1)$$

The lowest amino acid ratio was termed AAS. As reference pattern, the amino acid requirements for pre-school children as they were suggested in 1985 by FAO/WHO/UNU were used [11].

The *in vitro* digestibility of the proteins was determined using the three-enzyme pH-drop method described by Hsu *et al*. [14], the four-enzyme pH-drop method described by Saterlee *et al.* [15], as well as the three-enzyme pH-stat method described by McDonough *et al*. [16]. PDCAAS was calculated by multiplying the AAS by the *in vitro* protein digestibility [11]. If the AAS or the PDCAAS exceeded 100, then the scores were given as 100, *i. e*., the protein can be totally utilized by the organism. However, to calculate the PDCAAS, the initial value of the AAS should be used even in the case that this exceeds the value 100 [11].

3 Results and discussion

The proximate composition of the analyzed cephalopods is shown in Table 1. The results indicate that these organisms are excellent protein sources and contain only small amounts of fat. According to food databases, only lean beef contains such high protein : fat ratios [17]. Fish from the same geographical area contain similar amounts of protein but higher amounts of fat [18]. On the other hand, crustaceans have comparable protein:fat ratios [19], as presented in Fig. 1.

The results of the fatty acid analysis (Table 2) reveal that cephalopods are quite rich in *n*-3 fatty acids. In fact, the most abundant fatty acid in squid and cuttlefish was docosahexaenoic acid (DHA) followed by palmitic acid and eicosapentaenoic acid (EPA). On the other hand, in octopus, palmitic acid was slightly higher than DHA and the third most abundant fatty acid was again EPA. The sum of EPA and DHA varied from 33.69% to 46.30% of the total fatty acids. According to Zlatanos and Sagredos [13], the corre-

Table 1. Proximate composition of cephalopod mollusks studied[a)]

	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
Cuttlefish	81.2 ± 2.0	1.6 ± 0.4	15.5 ± 2.2	2.1 ± 0.6
Octopus	80.4 ± 1.5	1.2 ± 0.5	15.8 ± 3.4	1.3 ± 0.3
Squid	78.3 ± 2.2	0.9 ± 0.2	18.0 ± 4.6	1.5 ± 0.2

a) Values are mean ± SD from the analysis of three samples.

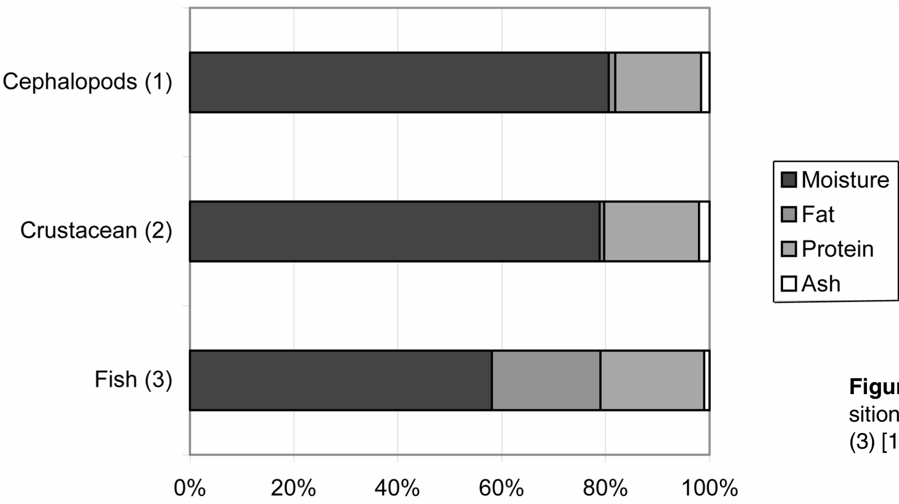

Figure 1. Comparison of proximate composition in seafood. (1) Present study; (2) [19]; (3) [18]

Table 2. Fatty acid analysis of cephalopod mollusks studied (% of total fatty acids)[a)]

	Cuttlefish	Octopus	Squid
C14:0	2.67 ± 0.35	1.02 ± 0.12	3.99 ± 0.20
C15:0	1.09 ± 0.21	0.53 ± 0.04	0.73 ± 0.09
C16:0	23.3 ± 1.21	21.04 ± 0.87	29.67 ± 1.18
C16:1*n*-7	1.35 ± 0.15	1.11 ± 0.07	1.00 ± 0.22
C16:1*n*-9	0.30 ± 0.04	0.09 ± 0.01	0.08 ± 0.01
C16:1*n*-7t	0.66 ± 0.05	0.41 ± 0.01	0.26 ± 0.02
C18:0	8.53 ± 0.41	8.54 ± 0.33	4.09 ± 0.18
C18:1*n*-9	3.51 ± 0.27	2.63 ± 0.18	2.72 ± 0.22
C18:2*n*-6	0.15 ± 0.01	0.14 ± 0.02	0.11 ± 0.01
C18:3*n*-3	0.65 ± 0.11	2.02 ± 0.16	0.19 ± 0.1
C20:1*n*-9	0.33 ± 0.04	2.64 ± 0.37	2.57 ± 0.26
C20:2*n*-6	0.49 ± 0.08	0.58 ± 0.19	0.18 ± 0.05
C20:4*n*-6	3.15 ± 0.31	8.51 ± 2.13	0.82 ± 0.22
C20:5*n*-3	14.97 ± 1.56	13.59 ± 0.83	13.96 ± 1.24
C22:1*n*-9	0.48 ± 0.07	0.74 ± 0.09	0.22 ± 0.04
C22:4*n*-6	0.48 ± 0.16	1.25 ± 0.18	0.08 ± 0.01
C22:5*n*-3	1.8 ± 0.41	1.95 ± 0.32	0.62 ± 0.06
C22:6*n*-3	23.74 ± 2.24	20.1 ± 3.12	32.34 ± 3.52

a) Values are mean ± SD from the analysis of three samples.

Table 3. Amino acid content of cephalopod mollusks studied (g/100 g freeze-dried sample)[a)]

	Cuttlefish	Octopus	Squid
Asp	6.6 ± 0.4	6.7 ± 0.5	6.3 ± 0.4
Thr	2.8 ± 0.2	3.7 ± 0.2	3.8 ± 0.3
Ser	2.8 ± 0.1	2.9 ± 0.2	3.1 ± 0.2
Glu	10.2 ± 0.6	12.5 ± 0.7	11.7 ± 0.7
Pro	4.9 ± 0.3	4.4 ± 0.5	3.7 ± 0.4
Gly	3.9 ± 0.2	3.1 ± 0.2	3.7 ± 0.2
Ala	3.7 ± 0.1	3.9 ± 0.4	4.9 ± 0.3
Val	2.7 ± 0.2	3.3 ± 0.3	2.8 ± 0.3
Cys	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.2
Met	1.8 ± 0.2	1.7 ± 0.2	2.3 ± 0.3
Ile	2.8 ± 0.1	3.0 ± 0.4	2.9 ± 0.3
Leu	4.9 ± 0.4	4.8 ± 0.3	5.1 ± 0.4
Tyr	2.0 ± 0.1	2.1 ± 0.2	2.6 ± 0.2
Phe	2.6 ± 0.2	3.3 ± 0.3	3.2 ± 0.2
Lys	5.4 ± 0.3	6.0 ± 0.4	6.2 ± 0.4
His	1.3 ± 0.1	1.6 ± 0.1	1.5 ± 0.2
Arg	5.3 ± 0.4	5.2 ± 0.5	4.1 ± 0.5

a) Values are mean ± SD from the analysis of three samples.

sponding percentage in fish from the same geographical area is between 5.7% and 36.5%. Moreover, only 3 species among the 16 analyzed in that study had percentages of these fatty acids higher than 30%.

Despite the fact that cephalopods contain small amounts of fat, these organisms are good sources of *n*-3 fatty acids because of the aforementioned high EPA and DHA content. In this study, the analyzed specimens contained on average, only 1.23 ± 0.35 g fat per 100 g edible part. In fish from the same area, the respective value was 3.45 ± 2.94 g [13]. Therefore, the average concentration of EPA and DHA is 0.48 ± 0.08 g/100 g edible part of the cephalopods and 0.65 ± 0.54 g/100 g edible part of the fish.

The amino acids content in cephalopods are presented in Table 3. The crude protein content for the investigated species (cuttlefish 82.4%, octopus 80.6%, squid 83.0% dry basis) was higher than the sum of the protein amino acids (cuttlefish 65.1%, octopus 69.3%, squid 69.2% dry basis). Salo-Vaananaen and Koivistoinen [20] reported that fish shows the highest differences between crude and net protein amounts among all other foods. These investigators suggest that the net protein content of fish can be calculated from the nitrogen content using a factor of 4.94. By applying this factor to the cephalopods, values close to the sum of the protein amino acids were determined (cuttlefish 65.2%, octopus 63.7%, squid 65.6% dry basis).

The PDCAAS was estimated using the AAS and the FAO/WHO pattern for pre-school children [11]. When using this pattern, it is recommended that the content of each essential amino acid is converted to an amino acid:total protein ratio. If the AAS is determined using the crude protein ratio con-

Table 4. Estimated AAS of cephalopod mollusks studied based on the crude and the net protein content

Amino acid	FAO/WHO pattern for pre-school children [11]	Cuttlefish		Octopus		Squid	
		Crude	Net	Crude	Net	Crude	Net
His	1.9	83[a)]	105	104	132	95	120
Ile	2.8	121	153	133	168	125	158
Leu	6.6	90	114	90[a)]	114[a)]	93[a)]	118
Lys	5.8	113	143	128	162	129	163
Met and Cys	2.5	155	196	138	176	174	220
Phe and Tyr	6.3	89	112	106	135	111	140
Thr	3.4	100	126	135	171	135	170
Val	3.5	94	118	117	148	96	122

a) Limiting amino acid

Table 5. Digestibility of protein determined by three different methods

	Cuttlefish	Octopus	Squid
3 enzyme pH-drop	76.2	80.5	78.5
4 enzyme pH-drop	74.9	77.6	76.2
3 enzyme pH-stat	85.4	86.2	87.5

Table 6. Estimated PDCAAS of cephalopod mollusks studied based on the crude and the net protein content

	Cuttlefish		Octopus		Squid	
	Crude	Net	Crude	Net	Crude	Net
AAS	83	100	90	100	93	100
Digestibility	85.4	85.4	86.2	86.2	87.5	87.5
PDCAAS	70.9	89.7	77.6	98.3	81.4	100

tent, as recommended by the FAO/WHO, the AAS is underestimated. For this reason, we determined the AAS by using both the crude protein content and the net protein content of the analyzed organisms (Table 4).

In the present investigation, the digestibility of the organisms was measured by three different methods. The pH-stat method yielded higher digestibility values than the two pH-drop methods (Table 5). El and Kavas [21] have found similar differences between the methods in a study on rainbow trout. It has been claimed that the pH-drop methods, which have been developed mainly for the determination of the digestibility of plant proteins, underestimate the digestibility of animal proteins. Therefore, the value from the pH-stat method was used to estimate the PDCAAS.

The estimated PDCAAS of the investigated species are presented in Table 6. According to these data the protein quality of the cephalopods was not so high as that of beef [11] and fish [21]. However, if the AAS were calculated using the net instead of the crude protein, the PDCAAS of the Cephalopods showed similar high protein quality to fish and beef.

4 Concluding remarks

Cephalopods are food with a high protein:fat ratio; also their PDCAAS indicates that their protein content has a very good nutritional value; therefore, cephalopods can be used as alternative to lean meat. In addition, the fat of cephalopods contains high amounts of *n-3* fatty acids. Since most of the modern diets are poor in *n-3* fatty acids and contain high amounts of *n-6* and saturated fatty acids, cephalopods can be used to enrich these diets in *n-3* fatty acids.

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