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Biochemical profile and nutritional quality of Indian squid, *Uroteuthis duvauceli*

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Abstract
This study was designed to find out the biochemical and nutritional profiling of Indian squid. Proximate composition of *Uroteuthis duvauceli* showed a content of 80.47% for moisture, 17.5% for protein, 0.52% for fat and 1.13% for ash, respectively. Amino acid analysis showed a higher content of glutamine, followed by aspartine, tryptopan, leucine, alanine and glycine. Higher concentrations of total amino acid (TAA), total essential amino acid (TEAA), total acidic amino acid (TAAA), total neutral amino acid (TNAA), total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA) were observed. In case of fatty acids, saturated fatty acids, palmitic and stearic acid contributed highest quantity; whereas, DHA, EPA and arachidonic acid were the major unsaturated fatty acids in the sample. Among the macro minerals, potassium showed highest content followed by sodium and calcium. As in the case of micro minerals, magnesium content showed highest proportion and copper showed least quantity. Commendable quantities of biochemical and nutritional content in *U. duvauceli* signify the appropriateness of this moderately exploited resource as an essential nutrients for nutritionally deprived population.

Keywords: *Uroteuthis duvauceli*, proximate composition, amino acids, fatty acids, minerals

1. Introduction
Squids were widely accepted seafood commodity because of its peculiar palatability, sensory properties and better yield percentage of meat for consumption. Marine lipids were mostly accepted by the consumers because of their high content of omega-3 fatty acids and low content of omega-6 fatty acids (Steffens, 1997) [44]. Significant content of omega-3 polyunsaturated fatty acids in squids were essential for growth and maintenance of the body (Ozyurt *et al*., 2006) [30]. Moreover, it had the potential to develop various value added products such as squid gel, surimi and seafood analogues (Sánchez-Alonso *et al.,* 2007) [37]. Generally, squids were marketing as fresh, dried (Kugino *et al*., 1993) [26], chilled (Hurtado *et al.,* 2001) [22] and frozen forms (Paredi and Crupkin, 1997) [31]. However, quality changes especially browning were the major concern for the marketing of squid which was greatly affected by biochemical composition (Caili *et al*., 2006) [9]. Biochemical profiling of squid had remarkably different from fishes. Spitz *et al*., (2010) [43] suggested that ecosystem had significant role for the nutritional quality of an organism. Similarly, metabolic activity had greatly influencing the biochemical composition and body mass of an organism (Schmidt-Nielsen, 1984) [39]. Most of the squids usually showed isometrical metabolism (Seibel, 2007) [40]. According to Roper *et al.,* (1984) [35], squids are beneficial to elderly population because of low fat and high proteins content. Bano *et al.,* (1992) [7] observed high protein and low fat content in squid species. Bano *et al.,* (1992) [7] analyzed and compared the protein and amino acid content of *Sepiella inermis* and *Sympleclotenllies oualaniensis* and found better protein content in *Sthenoteuthis oualaniensis*; whereas, homogenous amino acids content was observed. Amino acids play vital role for the growth and metabolic functions of humans. Amino acid composition and its score determine the nutritional quality of protein (Iqbal *et al.,* 2006) [23]. Generally, seafood are a good source for essential amino acids and aromatic amino acids (Adeyeye, 2009) [3]. Presence of aspartic acid, lysine, glutamic acid, leucine, serine, arginine, cystine, histidine and tryptophan were found in the protein of squid (Bano *et al.,* 1992) [7]. Ali (1987) [4] noticed significant quantity of lysine in squid, which is essential for

growth and development. Alanine, glutamic acid and aspartic acid were the major amino acid in the gelatin of splendid squid (*L. formosana*); whereas tyrosine, phenylalanine, histidine and lysine noticed at lower level (Nagarajan *et al.*, 2012) [27]. There is some report for the biochemical profiling of squid whereas, lack information for nutritional composition of Indian squid (*Uroteuthis duvauceli*). Hence, this study was designed to find out the biochemical and nutritional profiling of Indian squid.

2. Material and materials

2.1 Raw material

Indian squid (*U. duvauceli*) was collected from the fish landing centre at Fort Cochin, Kerala, India. The samples were transported to the laboratory in iced condition. Raw material had an average length of 570±14 mm and weight 445±15 g. Squid mantle was dissected and used for the analysis.

2.2 Chemicals

All reagents and solvents used in this study were of analytical grade. Standards of fatty acid methyl esters, amino acids were purchased from Sigma Aldrich GmbH (Steinheim, Germany).

2.3 Determination of moisture

Moisture was determined according to the AOAC (2000) [5] method by drying. A clean and dry petridish was cooled in desiccators and weighed (W_1). Approximately 10-20g of the finely homogenized samples was evenly spread and weighed (W_2). Petridish with the sample was dried in an oven at 105 °C, cooled in a desiccators and weighed (W_3). The process of heating and cooling were repeated to get a constant value. Results were expressed as percentage of wet weight.

$$\text{Moisture (\%)} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

2.4 Determination of protein

Total protein content in the homogenized samples (0.2 g) was determined using Kjeldahl method (AOAC, 2000) [5] and the protein content was calculated by the following methods,

$$\text{Protein content (g/100g)} = \frac{X \times 0.14 \times 6.25 \times 100}{1000 \times V_1 \times W}$$

Where,
V = Total volume of the digest
V_1 = Volume of the digest for distillation
W = Weight of sample for digestion

2.5 Amino acids analysis

Total amino acid composition was determined using Shimatzu amino acid analyzer (Ishida *et al.*, 1981) [24]. The results were expressed in g/100 g of protein.

2.6 Determination of tryptophan

About 200-250 mg of sample was hydrolyzed with 10 ml of 5% NaOH at 110 ºC for 24 hours in a sealed tube filled with pure nitrogen. The hydrolysate was neutralized to pH 7.0 with 6 N HCl using phenolphthalein indicators. The volume was made up to 100 ml with distilled water. The solution was then filtered through whatman filter paper No.1 and filtrate was used for estimation. To a test tube containing 4 ml of 50% H_2SO_4, 0.1 ml of 2.5% sucrose and 0.1 ml of 0.6% thioglycolic acid were added. These tubes were kept for 5 min in water bath at 45-50 ºC and cooled. The sample was then added to the test tubes. A set of (0.1 to 0.8) standard tryptophan (10µg/ml) was treated similarly. The volume was made up to 5 ml with 0.1 N HCl and allowed to stand for 5 minutes. The absorbance was measured using Shimadzu-UV spectrophotometer at 500 nm. The concentration was obtained by drawing standard graph

$$\text{Tryptophan(g/100g meat)} = \frac{\mu\text{g of Tryptophan} \times \text{Volume made up} \times 100 \times 16}{\text{Sample taken for color development} \times \text{Wt of sample} \times 1000 \times 1000 \times 6.25}$$

2.7 Estimation of quality of dietary protein

Essential amino acid score was calculated using the following formula (FAO/WHO, 1973) [13].

$$\text{Amino acid score} = \frac{\text{Amount of amino acid per test protein (mg/g)}}{\text{Amount of amino acid per protein in reference pattern (egg) (mg/g)}} \times 100$$

Determination of the total essential amino acid (TEAA) to the total amino acid (TAA), *i.e.*, (TEAA/TAA); total sulphur amino acid (TSAA); percentage of cysteine in TSAA (% Cys/TSAA); total aromatic amino acid (TArAA), etc.; the Leu/Ile ratios were calculated while the predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer *et al*.,1974), *i.e.*, P-PER = -0.468 + 0.454 (Leu) - 0.105 (Tyr).

2.8 Determination of crude fat (Soxhelet method)

The estimation of crude fat content was carried out by continuous extraction of fat with petroleum ether according to AOAC (2000) [5]. 2 g (W_1) of dried sample was weighed into a thimble and a cotton plug was kept on top of it. Thimble was placed in a Soxhelet apparatus and extracted with petroleum ether for 16 hours. The apparatus was cooled and the solvent was filtered in to a pre-weighed conical flask (W_2). The flask of the apparatus was washed with small quantities of ether and the washings were added to the above flask. The ether was removed by evaporation and the flask with fat was dried at 80-100 ºC, cooled in a desiccator and weighed (W_3).

$$\text{Fat content (g/100g)} = \frac{(W_3 - W_1)}{(W_1)} \times 100$$

2.9 Extraction of total lipids

The total lipid content of the tissues was estimated by the method of Folch *et al.*, (1957) [15]. A weighed amount of the samples was minced well and subjected to lipid extraction using chloroform-methanol mixture (2:1). The extraction was repeated twice with fresh aliquot of chloroform-methanol

mixture. The lipid extracts were transferred to a separating funnel and added 20% of water into it and left overnight. Next day the lipid extracts were drained through filter paper containing anhydrous sodium sulphate and was collected in round bottom flask and was made up to 10 ml by using chloroform. From this 1.0ml was taken into a pre-weighed vial and allowed to dry in warm temperature to constant weight and total lipid content were calculated from the different in weight. Sample made up to 10 ml was used for the estimation of various lipid components viz, cholesterol (total and free) triglycerides, free fatty acids and phospholipids after evaporating the solvent in air at room temperature.

2.10 Fatty acid analysis
Fatty acids methyl esters (FAMEs) were analyzed by the modified method of Gershbein & Metcalfe (1966) [16]. Individual fatty acids were expressed as a percentage of total fatty acids.

2.11 Determination of ash
Ash content was determined by heating sample for 6 h in a furnace at 600 °C (AOAC, 2000) [5]. Results were expressed as percentage of wet weight.

$$\text{Ash content (\%)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

2.12 Determination mineral analysis
The minerals were analyzed by dissolving the ash (obtained in ash determination) in diluted HCl (6 N) and estimated using atomic absorption spectrophotometer (Spectra AA 220, AAS VARIAN), with Deuterium background correction, acytelene and air supplied in constant ratio for flame and hollow cathode lamp. The wavelengths (nm) of light used for analyzing different minerals are 285.2 for magnesium, 213.9 for zinc, 766.5 for potassium, 328.4 for copper, 279.5 for manganese, 248.3 for iron, 240.7 for cobalt, 670.8 for lithium, 228.8 for cadmium, 217 for lead.

3. Result and Discussion
3.1 Proximate composition
Proximate composition of seafood determines the nutritional and edible characteristics in terms of energy units. Moisture, protein, crude fat and ash content of the sample are presented in Table 1. Proximate composition of *U. duvauceli* showed a content of 80.47% for moisture, 17.5% for protein, 0.52% for fat and 1.13% for ash, respectively. Similar finding was observed in the tentacles of European squid (*L. vulgaris*) (Atayeter and Ercoşkun, 2011) [6]. Santoso *et al.,* (2013) [38] investigated the proximate composition of Japanese common squid (*Todarodes pacificus*) and reported the content of 44.0 g/100 g of fat, 13.5 g/100 g of protein and 2.11 g/100 g of ash in dry matter. Relatively high moisture content was observed in *U. duvauceli* as compared to its total fat content and also revealed better content of protein and ash. Roper *et al.,* (1984) [35] suggested that low fat content and high protein concentrations in cephalopods make them appropriate for human consumption especially for elderly population. Bano *et al.,* (1992) [7] analyzed and compared the protein content of *Sepiella inermis* and *Sympleclotenllies oualaniensis* and found better protein content in *S. oualaniensis*. The present findings were in corroboration with the findings of Thanonkaew *et al*., (2006) [48]. Authors suggested that low fat and high protein content were beneficial in the formulation of protein supplements for targeted populations. Lipid was also essential in diet, which absorb fat soluble vitamin A, D, E and K from food and also regulate cholesterol metabolism. Studies had shown that proximate composition of cephalopods varies with habitat, age of maturation, food and feeding, species and season etc. (Ozogul *et al.,* 2008) [29].

Table 1: Proximate composition of *U. duvauceli*

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
U. duvauceli	80.40±0.23	17.50±0.06	0.52±0.08	1.31±0.15

Values are expressed as mean±SD for three replicates, ND, not detected

3.2 Amino acid composition
Amino acids play vital role for growth and metabolic functions of humans. Amino acid composition and its score determine the nutritional quality of protein (Iqbal *et al.,* 2006) [23]. Total amino acid content in *U. duvauceli* was 12.46 g/100g and amino acid profile of the sample is presented in Table 2. Giménez *et al.,* (2009a) [17] and Gómez-Guillén *et al.,* (1998) [19] revealed 16 to 17% of amino acid in giant squid (*Dosidicus gigas*) gelatin. Present study showed a better content of glutamine, followed by aspartine, tryptopan, leucine, alanine and glycine. Glutamic acid and aspartic acid were the acidic amino acid occupied highest concentrations compared to other amino acids. Nagarajan *et al.,* (2012) [27] reported better content of alanine, glutamic acid and aspartic acid in the gelatin of splendid squid (*L. formosana*); whereas tyrosine, phenylalanine, histidine and lysine noticed at lower level. In the present study leucine and tryptophan were found to be highest essential amino acid (EAA) content in the sample. Nevertheless, amino acid such as phenylalanine, proline, threnone, serine and isoleucine were also observed in commendable quantity. Lysine at a content of 0.15 g/100g was noticed in the sample. Ali (1987) [4] reported significant quantity of lysine in squid, which was essential for growth. Glutamic acid considered as one of the vital amino acid for metabolic activities especially for the synthesis of nucleic acid. Bano *et al.,* (1992) [7] reported the presence of aspartic acid, lysine, glutamic acid, leucine, serine, arginine, cystine, histidine and tryptophan in the protein of squid. Similar findings were reported by Konosu *et al.,* (1958) [25]. Present study was better correspondence with Adeyeye (2009) [3] who reported good source of essential (leucine), non essential (glutamic acid) and aromatic amino acids (phenylalanine) in fishes. This indicates the similarities of amino acid composition of cephalopods with fishes. Aromatic amino acids (tyrosine and phenylalanine) were essential for the synthesis of peptide hormones especially epinephrine and thyroxine (Robinson, 1987) [33]. Glutamic acid, glycine and aspartic acid possess wound healing ability (Chyun and Griminager, 1984) [10]. Aromatic amino acids (i.e., tyrosine, histidine, and phenylanine) and hydrophobic amino acids (i.e., valine, alanine, proline and leucine) were able to scavenge free radicals (Suetsuna *et al*., 2000) [46]. Among the nutrients, supplementation with amino acids, and branched chain amino acids in particular, could reduce fat accretion and maintain lean body mass, and therefore had a fundamental role in maintaining insulin sensitivity and counteracting obesity-induced metabolic syndrome **(**Giuseppe D Antona, 2014**)**[18]. Sulfur-containing amino acids play indispensable roles in biological activities including protein synthesis, methylation, biosynthesis of polyamines and glutathione (Nozaki *et al*.,

2005) [28]. Bano *et al.,* (1992) [7] compared amino acid content of *Sepiella inermis* and *Sympleclotenllies oualaniensis* and found no significant difference for amino acids. However, nitrogen content in the mantle of *I. argentines* varies with sex and maturity (Clarke *et al.,* 1994) [11].

Table 3 shows the concentrations of total amino acid (TAA), total essential amino acid (TEAA), total acidic amino acid (TAAA), total neutral amino acid (TNAA), total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA). Content of TEAA with histidine in *L. duvauceli* was 272.00 mg/gcp and it was reasonably compare with egg reference protein (566 mg/gcp) (Paul *et al*., 1980) [32]. This nutritional profile of sample was par with *Zonocerus variegates*, 351 mg/gcp (Adeyeye, 2005) [2], *S. budgetti*, 389 mg/gcp and *H. fasciatus*, 394 mg/gcp (Abdullahi and Abolude, 2002) [1].

Table 2: Amino acid composition (g/100g of meat) *of U. duvauceli*

Amino acid	*U. duvauceli*
Asp	1.87±0.05
Thr	0.78±0.12
Ser	0.72±0.02
Glu	2.92±0.125
Pro	0.75±0.018
Gly	1.01±0.043
Ala	1.19±0.072
Cys	0.06±0.01
Val	0.18±0.025
Met	0.18±0.031
Ile	0.92±0.045
Leu	1.54±0.01
Tyr	0.39±0.05
Phe	0.76±0.06
His	0.46±0.05
Lys	0.15±0.072
Arg	ND
Try	1.6±0.013

Values are expressed as mean±SD for three replicates, ND, not detected.

Table 3: Concentrations of essential, non-essential, acidic, neutral, sulphur aromatic amino acid composition (mg/g crude protein) of *U. duvauceli*

Amino acid	*U. duvauceli*
TAA	731.23
TNEAA	459.23
%TNEAA	62.80
TEAA with His	272.00
TEAA with out His	248.29
% TEAA with His	37.20
% TEAA with out His	33.96
TNAA	100.59
% TNAA	13.76
TAAA	246.84
% TAAA	33.76
TBAA	16.21
%TBAA	2.22
TSAA	12.35
% TSAA	1.69
% of CYS in TSAA	590.97
TArAA	75.36
% TArAA	10.31
P-PER	3.22
Leu/Ile ratio	1.68

Values are expressed as mean±SD for three replicates.

3.3 Fatty acid composition

Fatty acid profile of *U. duvauceli* is shown in Table 4. Among saturated fatty acids, palmitic and stearic acid contributed highest quantity; whereas, DHA, EPA and aracidonic acid were the major unsaturated fatty acids in the sample. Suzuki *et al.,* (1992) [47] reported better concentration of DHA in the integument of squid *Ommastrephes bartrami.* Studies showed that cephalopod from Mediterranean Sea was rich in biologically beneficial PUFA (Sinanoglou and Miniadis-Meimoroglow, 1998) [41] and DHA and EPA were the dominant PUFA (Sinanoglou and Miniadis-Meimoroglow, 1998) [41]. Significant level of essential fatty acids was observed in the present study (Table 5). Occurrence of significant quantity of fatty acid content in *U. duvauceli*, *L. valgaris* (Salman *et al.,* 2007) [36] and *Sthenoteuthis oualaniensis* (Wang *et al.,* 2008) [49] was reported. According to Ozyurt *et al.,* (2006) [30] squids had commendable quantity of nutritional elements including omega-3 polyunsaturated fatty acids, which were essential for growth and maintenance of body. DHA, EPA, aracidonic acid and palmitic were the dominant fatty acid in cephalopods (Stowasser *et al.,* 2006) [45]. Fatty acid profile of *U. duvauceli* had striking similarity with *Loligo valgaris* (Salman *et al.,* 2007) [36] and egg of *Sthenoteuthis oualaniensis* (Wang *et al.,* 2008) [49]. Generally, fat content of squid species was low; however, majority of fat contains nutritionally important long polyunsaturated fatty acids. PUFA were nutritionally vital for growth and development integral part of metabolic activities in higher animals and their consumption was well appreciated (Haliloğlu *et al.,* 2004) [21]. Prostaglandins and thromboxanes were potent arachidonic derivatives play significant role in blood clotting and healing process (Bowman and Rand, 1980). EPA and DHA had crucial role in preventing cardiovascular problems and side effect caused due to prostaglandin mediated inflammation (Connor, 2000) [12]. Long chain fatty acids were essential for growth and developments of infant. Omega -3 PUFA like DHA plays vital role in developmental stages of brain and retina during pre and post pregnancy (Rogers *et al.,* 2013) [34]. Result of the present study reveals the significance of fatty acid profile of *U. duvauceli*.

Table 4: Fatty acid composition (% of fatty acids in terms of total fatty acids) of *U. duvauceli*

Fatty acid	*U. duvauceli*
C 14:0 (Myristic acid)	2.25±0.13
C16:0 (Palmitic acid)	21.63±0.02
C18:0(Stearic acid)	5.24±0.15
C23:0(Tricosanoic acid)	1.1±0.02
Σ SFA	30.12
$C^{1}8:1$(Oleic acid)	1.39±0.09
C20:1(Eicosenoic acid)	0.59±0.12
Σ MUFA	1.98
C20:4(Arachidonic acid) n-6	7.31±0.03
C20:5(Eicosapentanoic acid)n-3	11.96±0.07
C22:6(Decosahexanoic acid)n-3	48.53±0.16
Σ PUFA	67.9
GRAND TOTAL	100

Values are expressed as mean±SD for three replicate

Table 5: Fatty acid n-3/n-6 and polyunsaturated/saturated fatty acid ratio of *U. duvauceli*

Species	n-3:n-6 ratio	P/S ratio
U. duvauceli	8.27	2.24

P/S, polyunsaturated/saturated fatty acid ratio. n-3 -omega 3 fatty acid n-6- omega 6 fatty acid

3.4 Mineral composition

Minerals were necessary for physicochemical processes as well as metabolic activities of the body (Soetan *et al*., 2010) [42]. Macro and micro minerals profile of the sample is presented in Table 6 and 7, respectively. Among the macro minerals, potassium showed highest content followed by sodium and calcium. Sodium and potassium ATPase was actively involved in sodium and potassium transport across the cells. Calcium and phosphorous were essential for energy metabolism, signal transduction and bone homeostasis (Ha and Bhagavan, 2011) [20]. As in the case of micro minerals, magnesium content showed highest proportion and copper showed least quantity. Santoso *et al.,* (2013) [38] reported better quantity of macro minerals (sodium, potassium, magnesium, and calcium) and trace minerals (iron, zinc, cadmium, and copper) in Japanese common squid (*Todarodes pacificus*). However, trace minerals content in seafood depends on various factors such as nourishment sources, biological differences, seasonal factors and environmental conditions (Fallah *et al*., 2009) [14].

Table 6: Macro minerals profile (wet basis) (g/100g) of *U. duvauceli*

Macro mineral	*U. duvauceli*
Na	0.102±0.05
K	0.189±0.03
Ca	0.015±0.001

Values are expressed as mean±SD for three replicates

Table 7: Micro minerals profile (wet basis) (mg/100g) of *U. duvauceli*

Micro minerals	*U. duvauceli*
Fe	2.885±0.8
Zn	1.032±0.09
Mg	163.019±16.02
Cu	0.121±0.02

Values are expressed as mean±SD for three replicates

4. Conclusion

Biochemical profiling of Indian squid (*U. duvauceli*) revealed significant composition of essential nutrients such as proximate contents (80.47% of moisture, 17.5% of protein, 0.52% of fat and 1.13% of ash), essential amino acids (leucine and tryptophan) and essential minerals (sodium, calcium, magnesium and potassium). Generally, essential fatty acids had great role for the determination of the level of nutritional quality of seafood. In this study essential fatty acids such as DHA, EPA and arachidonic acid were observed at significant level. Moreover, amino acids such as essential amino acid, acidic amino acid, neutral amino acid, sulphur amino acid and aromatic amino acid also indicates its nutritional significance. Commendable quantities of biochemical and nutritional content in *U. duvauceli* signify the appropriateness of this moderately exploited resource as an essential nutrients for nutritionally deprived population.

5. Conflict of interest statement

The authors declare that there are no conflicts of interest.

6. Acknowledgements

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7. References

1. Abdullahi SA, Abolude DS. Investigation of protein quality of some fresh water fish species of Northern Nigeria. Academy Journal of Science and Technology. 2002; 2(1):18-25.
2. Adeyeye EI. Amino acid composition of variegated grasshopper, *Zonocerus variegatus*. Tropical Science. 2005; 45(4):141-143.
3. Adeyeye EI. Amino acid composition of three species of Nigerian fish: *Clarias anguillaris*, *Oreochromis niloticus* and *Cynoglossus senegalensis*. Food Chemistry. 2009; 113(1):43-46.
4. Ali S. Pakistan seafood digest, 1987, 25-27.
5. AOAC. Official methods of analysis (17th 268 ed.): Association of Official Analytical Chemist. Gaithersburg, Maryland, USA, 2000.
6. Atayeter S, Ercoşkun H. Chemical composition of European squid and effects of different frozen storage temperatures on oxidative stability and fatty acid composition. Journal of Food Science and Technology. 2011; 48(1):83-89.
7. Bano ALIA, Shakir SADIOA, Begum ASKARI, Qadri RB. Amino acids content of sea squids. Journal-Chemical Society of Pakistan. 1992; 14:184-184.
8. Bowman WC, Rand MJ. Textbook of Pharmacology (No. 2nd Ed.). Blackwell Scientific Publications.
9. Caili FU, Huan S, Quanhong LI. A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods for Human Nutrition. 2006; 61(2):70-77.
10. Chyun JH, Griminger P. Improvement of nitrogen retention by arginine and glycine supplementation and its relation to collagen synthesis in traumatized mature and aged rats. The Journal of Nutrition. 1984; 114(9):1697-1704.
11. Clarke A, Rodhouse PG, Gore DJ. Biochemical composition in relation to the energetics of growth and sexual maturation in the ommastrephid squid *Illex argentinus*. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 1994; 344(1308):201-212.
12. Connor WE. Importance of n-3 fatty acids in health and disease. The American Journal of Clinical Nutrition. 2000; 71(1):171S-175S.
13. FAO/WHO (Food and Agriculture Organization/World Health Organization). Energy and Protein Requirements. Report of a Joint FAO/WHO Ad Hoc Expert Committee. Technical Report Series No. 552; FAO Nutrition Meetings Report Series 52. World Health Organization, Rome. 1973, 118.
14. Fallah AA, Rahnama M. Determination of copper, zinc and iron levels in edible muscle of three commercial fish species from Iranian coastal waters of the Caspian Sea. Journal of Animal and Veterinary Advances. 2009; 8(7):1285-1288.
15. Folch J, Lees M, Stanely GHS. A simple method for isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry. 1957; 226:497-509
16. Gershbein LI, Metcalfe LD. Gas chromatographic analysis of fatty acids of human hair lipids. The Journal of Investigative Dermatology. 1966; 46(5):477-479.
17. Giménez B, Gómez-Estaca J, Alemán A, Gómez-Guillén MC, Montero MP. Physico-chemical and film forming

properties of giant squid (*Dosidicus gigas*) gelatin. Food Hydrocolloids. 2009a; 23(3):585-592.
18. Giuseppe-D'Antona. Essential Amino Acid Supplementation for the Prevention and Treatment of Obesity Nutrition in the Prevention and Treatment of Abdominal Obesity, 2014, 447-458.
19. Gómez-Guillén MC, Montero P, Solas MT, Borderías AJ. Thermally induced aggregation of giant squid (*Dosidicus gigas*) mantle proteins. Physicochemical contribution of added ingredients. Journal of Agricultural and Food Chemistry. 1998; 46(9):3440-3446.
20. Ha CE, Bhagavan NV. Essentials of medical biochemistry: with clinical cases. Academic Press, 2011.
21. Haliloğlu Hİ, Bayır A, Sirkecioğlu AN, Aras NM, Atamanalp M. Comparison of fatty acid composition in some tissues of rainbow trout (*Oncorhynchus mykiss*) living in seawater and freshwater. Food Chemistry. 2004; *86*(1):55-59.
22. Hurtado JL, Montero P, Borderías J. Behavior of octopus muscle (*Octopus vulgaris*) under a process of pressure-time-temperature combinations. Revista de Agaroquimica y Tecnologia de Alimentos. 2001; 7(3):259-267.
23. Iqbal A, Khalil IA, Ateeq N, Khan MS. Nutritional quality of important food legumes. Food Chemistry. 2006; 97(2):331-335.
24. Ishida YFT, Asai K. New detection and separation method for amino acid by high performance liquid chromatography. J Chromatogr. A. 1981; 204:143-148
25. Konosu S, Akiyama T, Mori T. Muscle extracts of aquatic animals-I. Amino acids, trimethylamine and trimethylamine oxide in the muscle extracts of a squid, *Ommastrephes sloani* pacificus. Nippon Suisan Gakkaishi. 1958; 23:561-564.
26. Kugino M, Kugino K, Wu ZH. Rheological properties of dried squid mantle change on softening. Journal of Food Science. 1993; 58(2):321-324.
27. Nagarajan M, Benjakul S, Prodpran T, Songtipya P, Kishimura H. Characteristics and functional properties of gelatin from splendid squid (*Loligo formosana*) skin as affected by extraction temperatures. Food Hydrocolloids. 2012; 29(2):389-397.
28. Nozaki T, Ali V, Tokoro M. Sulfur-containing amino acid metabolism in parasitic protozoa. Advances in Parasitology. 2005; 60:1-99.
29. Ozogul Y, Duysak O, Ozogul F, Özkütük AS, Türeli C. Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. Food Chemistry. 2008; 108(3):847-852.
30. Ozyurt G, Duysak Ö, Akamca E, Tureli C. Seasonal changes of fatty acids of cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda) in the north eastern Mediterranean sea. Food Chemistry. 2006; 95(3):382-385.
31. Paredi ME, Crupkin M. Biochemical properties of actomyosin from frozen stored mantles of squid (*Illex argentinus*) at different sexual maturation stages. Journal of Agricultural and Food Chemistry. 1997; 45(5):1629-1632.
32. Paul AA, Southgate DAT, Russell J, Mc-Cance RA. First supplement to McCance and Widdowson's The composition of foods; amino acid composition (mg per 100 g food), fatty acid composition (g per 100 g food), 1980.
33. Robinson DS. Food-biochemistry and nutritional value. Longman Scientific and Technical, 1987.
34. Rogers LK, Valentine CJ, Keim SA. DHA supplementation: Current implications in pregnancy and childhood. Pharmacological Research. 2013; 70(1):13-19.
35. Roper CF, Sweeney MJ, Nauen C. Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries, 1984.
36. Salman Y, Salman A, Ozkizilcik S. The fatty acid profile of the marine cephalopod Loligo vulgaris. The Israeli Journal of Aquaculture-Bamidgeh. 2007; 59(3):133-136.
37. Sánchez-Alonso I, Careche M, Borderías AJ. Method for producing a functional protein concentrate from giant squid (*Dosidicus gigas*) muscle. Food Chemistry. 2007; 100(1):48-54.
38. Santoso J, Ishizuka Y, Yoshie-Stark Y. Characteristics of divalent minerals extracted from liver of Japanese common squid (*Todarodes pacificus*) under various experimental conditions. Fisheries Science. 2013; 79(2):293-301.
39. Schmidt-Nielsen K. Scaling: why is animal size so important?. Cambridge University Press, 1984.
40. Seibel BA. On the depth and scale of metabolic rate variation: scaling of oxygen consumption rates and enzymatic activity in the Class Cephalopoda (Mollusca). Journal of Experimental Biology. 2007; 210(1):1-11.
41. Sinanoglou VJ, Miniadis-Meimaroglou S. Fatty acid of neutral and polar lipids of (edible) Mediterranean cephalopods. Food Research International. 1998; 31(6):467-473.
42. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants-A review. African Journal of Food Science. 2010; 4(5):200-222.
43. Spitz J, Mourocq E, Schoen V, Ridoux V. Proximate composition and energy content of forage species from the Bay of Biscay: high-or low-quality food?. ICES Journal of Marine Science. 2010; 67(5):909-915.
44. Steffens W. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture. 1997; 151(1-4):97-119.
45. Stowasser G, Pierce GJ, Moffat CF, Collins MA, Forsythe JW. Experimental study on the effect of diet on fatty acid and stable isotope profiles of the squid *Lolliguncula brevis*. Journal of Experimental Marine Biology and Ecology. 2006; 333(1):97-114.
46. Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. The Journal of Nutritional Biochemistry. 2000; 11(3):128-131.
47. Suzuki S, Kubo A, Shinano H, Takama K. Inhibition of the electron transport system in *Staphylococcus aureus* by trimethylamine-N-oxide. Microbios. 1992; 71(287):145-148.
48. Thanonkaew A, Benjakul S, Visessanguan W. Chemical composition and thermal property of cuttlefish (*Sepia pharaonis*) muscle. Journal of Food Composition and Analysis. 2006; 19(2):127-133.
49. Wang Q, Xue C, Li Z, Xu J. Analysis of DHA-rich phospholipids from egg of squid *Sthenoteuthis oualaniensis*. Journal of Food Composition and Analysis. 2008; 21(4):356-359.