

Characterisation of non-protein nitrogen in the Cephalopods volador (*Illex coindetii*), pota (*Todaropsis eblanae*) and octopus (*Eledone cirrhosa*)

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Abstract

A characterisation of non-protein nitrogen (NPN) was carried out in the muscle of three species of cephalopods: volador (*Illex coindetii*), pota (*Todaropsis eblanae*) and octopus (*Eledone cirrhosa*). These species were classified according to sex, sexual development (immature, mature) and anatomical zone (mantle, arms). There were no major differences in the NPN contents of the three species studied. The anatomical zone clearly marks the difference in NPN content, with the content being greater in the mantle than in the arms for all the species. The trimethylamine oxide–nitrogen levels in volador were greater in the mantle than in the arms and these differences were not observed in the other two species. In pota and octopus, however, it is the stage of sexual development that exhibited differences, with a greater quantity of trimethylamine oxide–nitrogen in the immature specimens than the mature ones. The trimethyl and total volatile basic nitrogen behaviour had no clear trend, the TVB-N levels were probably so low that these differences were not important. The same thing occurred with the pH, where the lowest values were recorded in octopus and the highest in pota. The free amino acid (FAA) compositions varied to a great extent, depending on the species and, to a less extent, depending on the sex, stage of sexual development and anatomical zone. The total FAA content was virtually the same in volador and pota, while it was about 75% lower in octopus. The highest concentrations of FAA in the three species were for proline, arginine and alanine, but in different proportions. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Cephalopods; Characterisation; Non protein nitrogen; Free amino acids

1. Introduction

Cephalopods constitute an important part of the marine resources most suitable for human consumption. They are common in Mediterranean and Far Eastern diets, particularly in Japan. Nowadays, their consumption is also increasing in America. Cephalopods are currently recognised as the most promising resource because of their abundance and rapid stock renewal, since their biological cycle lasts between eight months and just under two years, depending on the species (Guerra, 1996). Despite these advantages, it is estimated that the world catch of cephalopods represents only 10% of the stocks detected (Guerra, 1996). There are several reasons why many species of cephalopods are

not exploited intensively and one of them is that there are not enough scientific studies providing information on their biochemical characterisation. This information would be advantageous for subsequent technological applications and would widen the scope of their use for commercial purposes. Thus, non-protein nitrogenous (NPN) compounds are of major importance and are used as quality parameters for fish, along with sensory aspects, such as smell and taste (Iida, Nakamura, & Tokunaga, 1982). NPN includes different compounds, such as trimethylamine oxide (TMA-O), trimethylamine (TMA), nucleotides, creatine, creatinine, free amino acids, ammonia, and urea. From the point of view of quality, these non-protein nitrogenous substances modify the muscle of cephalopods, both physically and chemically, and have a direct effect on the ice crystallisation processes during freezing by, altering the osmotic pressure, due to their intracellular location. Moreover, most of these substances are indicative of change, since they

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are substrates of contaminant bacterial growth in cephalopod muscle. The determination of these substances is therefore extremely important during the chilled storage of cephalopods. Many of these substances, like TMA-N and total volatile basic nitrogen (TVB-N), are demanded as quality indices by authorities in many countries (Ruiz-Capillas & Moral, 2001a). Other compounds, such as free amino acids (FAA), could also be used as quality indices in species of fish such as hake (Ruiz-Capillas & Moral, 2001b). However, in spite of the importance of these NPN compounds, there is no information on their composition in species such as volador, pota and octopus, and the information is also quite scant for similar species.

Thus the objective of this work was the characterisation of NPN in three species of cephalopods of major importance in Spain (volador, pota and octopus). This characterisation was done according to the sex of the individual specimens, their sexual development and anatomical zone.

2. Materials and methods

2.1. General

The three species of cephalopods used in this study were: volador (*Illex coindetii*, Vérany, 1839), pota (*T. eblanae*, Ball, 1841) and octopus (*Eledone cirrhosa*, Lamarck, 1798). The male and female volador specimens had an average size of 156 mm and 157 mm, respectively. The immature male pota specimens had an average size of 106 mm and the mature ones were 127 mm; the immature females were 103 mm and the mature ones 156 mm. The immature male octopus specimens had an average size of 87 mm and the mature ones 97 mm; the immature females were 93 mm and the mature ones 97 mm. The three species were caught by dragnet from the Galician shelf. After capture, the different species were placed in boxes with ice, with a polyurethane film separating them from the ice, and they were taken in an refrigerated lorry in a day to the Instituto del Frío (Madrid, Spain), where the samples were prepared. They were divided into sexes and stages of sexual development (immature: stages II and III; mature: stages IV and V). They were then gutted, the mantles were separated from the arms and they were skinned and cut into pieces. The samples were from at least 30 specimens. All the determinations were done three times.

2.2. Analyses

pH was determined in a Radiometer model PHM 93 pH-meter at room temperature on homogenates in water in a ratio 1:10 (w/v).

The determination of nonprotein nitrogen (NPN) was done according to the method recommended by the AOAC (1995).

Determination of trimethylamine oxide (TMA-O) was done by reducing to TMA, by adding 15% titanium trichloride (Bystedt, Swenne, & Aas, 1959), and it was determined as TMA.

Trimethylamine nitrogen (TMA-N) was determined by the method of AOAC (1995)

Total volatile basic nitrogen (TVB-N) was determined according to the method of Antonocopoulos and Vyncke (1989) and the distillation was performed in a Tecator model 1002 apparatus.

Free amino acids (FAA) were determined on the sample prepared in accordance with the methodology described in Ruiz-Capillas and Moral (2001b). The free amino acids were separated by means of cation-exchange chromatography, using an amino acid automatic analyser Biochron 20 (Pharmacia LKB, Sweden) with a high-resolution cation-exchange resin column Ultropac ($9 \pm 0.5 \mu$) 200×4.6 mm. The free amino acids were determined and measured using ninhydrin derivative reagent at 570 nm, except for proline and hydroxyproline which were measured at 440 nm.

2.3. Statistical analysis

Two-way analysis of variance (ANOVA) was carried out for difference. The differences (means) between pairs were resolved by means of confidence intervals using a least significance range test at $P \leq 0.05$. The computer program used was Statgraphics (STATC Inc., Rockville, MD).

3. Results and discussion

3.1. pH

The pH values of recently captured volador, pota and octopus fluctuated between 5.9 and 7.0 (Table 1). The pota had higher pH values than the other two species, and the octopus had the lowest (Table 1). The pH value depends on many factors, such as the time that has elapsed since the capture, storage temperature and physiological state of the animal (Moral, 1987). Consequently, the pH is more a quality index than a characterisation parameter, since it changes during chilled storage and tends to be higher than 7 due to the accumulation of alkaline metabolites during microbial spoilage (Hebard, Flick, & Martin, 1982).

3.2. Non-protein nitrogen NPN

The quantity of NPN present in the study of the muscle of the recently captured species and its percentage in

Table 1
pH values in muscle of volador, pota and octopus

Species	Mantle		Arms	
	Male	Female	Male	Female
Volador, immature	6.4±0.1	6.5±0.1	6.4±0.2	6.4±0.3
Pota, immature	7.0±0.0	6.8±0.0	6.9±0.0	6.8±0.0
Pota mature	6.4±0.1	6.6±0.0	6.7±0.0	6.8±0.0
Octopus, immature	6.3±0.0	6.0±0.0	6.2±0.1	6.0±0.0
Octopus mature	6.2±0.0	6.1±0.0	6.0±0.0	5.9±0.0

Immature (phases II–III) and mature (phases IV–V).

Table 2
NPN content (mg /100 g muscle) in muscle of volador, pota and octopus

Species	Mantle		Arms	
	Male	Female	Male	Female
Volador, immature	1161a	1182a	769b	775b
Pota, immature	1146a	1177a	863b	937c
Pota mature	1153a	1149a	890bc	926c
Octopus, immature	1092ab	1178a	966b	937bc
Octopus mature	946bc	1022ab	797cd	650d

Immature (phases II–III) and mature (phases IV–V). Different letters in the same species mean significant difference ($P \leq 0.05$).

comparison with total nitrogen is shown in Table 2. The quantity of NPN nitrogen was significantly higher ($P \leq 0.05$) in the mantle than in the arms. There were no significant differences between the male or female volador specimens for either of the two anatomical zones. Significant differences ($P \leq 0.05$) in the quantity of NPN were not detected in the pota specimens according to the stage of sexual development, and a similar content was observed in immature and mature specimens. Among the pota mantle lots there were no statistical differences by sex. However, the immature females had a greater quantity ($P \leq 0.05$) of NPN in their arms than the immature males, while no significant differences ($P \leq 0.05$) were observed in NPN values in the arms of the male and female mature specimens (Table 2). There were no differences between the quantities of NPN in immature and mature octopus mantles, although the immature specimens had a greater quantity ($P \leq 0.05$) of NPN in their arms than the mature ones, as occurred in the other species.

Therefore, the anatomical zone is the one that clearly marks differences in NPN content in all the species and NPN was higher in the mantles than in the arms. The results for NPN contents of the three species studied were somewhat higher than those found by other authors in similar cephalopod species, such as *Loligo* sp., *Loligo vulgaris*, and *Architeuthis japonica* (Iida, Nakamura, & Tokunaga, 1992; Joseph, Varma, & Venkataraman, 1977; Moral, Tejada, & Borderías, 1983). The variations in NPN contents are attributed to environmental factors (e.g. food, habitat), species variability and also to their high enzyme activity. In our study in

which the analyses was carried out 24–36 h after capture, the increase in NPN could have been caused by the high proteolysis produced during this period, as has been highlighted by other authors (Ayensa, An, Montero, Gómez-Guillén, & Borderías, 1999; Konno & Fukazawa, 1993; LeBlanc & Gill, 1982). The handling of the cephalopods after capture also influences the quantity of NPN present in these species. The direct contact of the cephalopods with ice potentiates the flushing of NPN which dissolves in the water of the melted ice, thereby reducing its concentration (Raghu-nath, 1984). In our study, the species were placed in boxes with ice but the cephalopods were not in direct contact with the ice. This prevented the loss of NPN in the pool of melted ice and would also explain the higher levels of NPN found in this study. However, some of the NPN results obtained in different species of cephalopods, in the literature, were determined in muscle that had previously been flushed with water (Joseph et al., 1977; Moral et al., 1983; Selvaraj, Jasmine, & Jeyachandran, 1991). On the other hand, in esasmobranchii molluscs, NPN values have been detected similar to those obtained for the species in this study. In these molluscs, 33–38% of the total nitrogen present in muscle was made up of non-protein nitrogen (Belitz & Grosch, 1988).

3.3. Trimethylamine oxide nitrogen (TMAO-N)

The TMAO-N values determined in the mantle of volador were higher than those in the arms, although there were no significant differences ($P \leq 0.05$) between

Table 3
TMAO-N content (mg 100 g muscle) in muscle of volador, pota and octopus

Specie	Mantle		Arms	
	Male	Female	Male	Female
Volador, immature	62.6a	61.8a	30.7b	31.6b
Pota, immature	47.9a	45.9b	49.4c	47.5a
Pota mature	32.5d	24.8e	26.7f	26.8f
Octopus, immature	57.7a	59.9ab	60.0b	60.2b
Octopus mature	33.3c	32.5c	36.3d	27.8e

Immature (phases II–III) and mature (phases IV–V). Different letters in the same species mean significant difference ($P \leq 0.05$).

Table 4
TMA-N content (mg /100 g muscle) in muscle of volador, pota and octopus

Species	Mantle		Arms	
	Male	Female	Male	Female
Volador, immature	0.97a	0.86a	0.53b	0.51b
Pota, immature	0.77a	0.54b	0.29c	0.29c
Pota mature	1.32d	0.96e	0.76a	0.75a
Octopus, immature	0.68a	0.81b	1.03c	0.77b
Octopus mature	0.42d	0.51e	0.47de	0.48de

Immature (phases II–III) and mature (phases IV–V). Different letters in the same species mean significant difference ($P \leq 0.05$).

the sexes for either of the anatomical zones (Table 3). These results suggest that the presence of TMA-O could be related to the type of muscle activity and they would coincide with the studies by Reay and Shewan (1949) and Horie and Sekine (1956), who observed that the white muscle of fish had a greater quantity of TMA-O than the red muscle. However, this fact was not observed either in pota or in octopus, where the quantity of TMAO-N was similar in both the mantle and the arms and, although in some instances there are significant differences ($P \leq 0.05$), the trend is not as clear as for volador (Table 3). In pota, however, the difference ($P \leq 0.05$) in TMAO-N was at the stage of sexual development, the immature specimens had a greater quantity of TMAO-N and this level was greater in males than in females and, in the case of the mature pota specimens, higher levels of TMAO-N were observed in the male mantles (Table 3). By anatomical zone, there was a greater quantity of TMAO-N in the mantle of the male specimens, both immature and mature ones, while in the females the quantity of TMAO-N was significantly higher in the arms than in the mantles (Table 3). As with pota, the immature octopus specimens had the greatest quantity of TMAO-N (Table 3). By sex, there were only significant differences ($P \leq 0.05$) between the TMAO-N contents of mature specimens, where the male exhibited a significantly higher TMAO-N level in the arms than the female. The specimens of immature and mature male octopus had higher TMAO-N values in the arms than in the mantle, while the mature female contained more TMAO-N in the mantle than in the arms (Table 3).

In the three species studied, the immature specimens had high TMAO-N values and, in the case of pota and octopus, higher than the mature specimens. Other authors also observed differences in the TMAO-N in fish due to sexual development as well as due to other factors, such as diet, species, habit, season and time of the year (Hebard, Flick, & Martin, 1982; Rasero, González, Castro, & Guerra, 1996). These factors could also be responsible for the fact that the TMAO-N levels found in this study are slightly lower than those obtained by other authors (Iida et al., 1992; Lin & Hurng, 1985; Stanley & Hulting, 1982).

3.4. Trimethylamine nitrogen TMA-N

The TMA-N content in volador was significantly higher ($P \leq 0.05$) in the mantle than in the arms (Table 4), but there were no significant differences according to sex in either of the two anatomical locations studied. In pota, as in volador, a greater quantity ($P \leq 0.05$) of TMA-N was observed in the mantles than in the arms, both in the immature and mature pota (Table 4) and the TMA-N levels were higher ($P \leq 0.05$) in the mature specimens than in the immature ones. The only differences between males and females were detected in the mantle, where the males exhibited a greater proportion of TMA-N than the females, while in the arms the quantity of TMA-N was the same for both immature and mature males and females. Unlike what happened in pota, in octopus the highest TMA-N values were in the immature specimens (Table 4). By sexes, the mantles of the immature and mature females exhibited

Table 5
TVB-N content (mg /100 g muscle) in muscle of volador, pota and octopus

Species	Mantle		Arms	
	Male	Female	Male	Female
Volador, immature	16.9a	15.5a	15.8a	16.8a
Pota, immature	15.8a	14.5b	12.8c	13.9b
Pota mature	17.0d	16.5ad	17.4d	17.4d
Octopus, immature	14.7a	17.8b	13.3cf	13.9ac
Octopus mature	16.3d	12.1ef	13.2cf	11.8e

Immature (phases II–III) and mature (phases IV–V). Different letters in the same species mean significant difference ($P \leq 0.05$).

significantly higher TMA-N values than the respective male samples. There was a greater quantity of TMA-N in the arms of the immature males than the females at the same stage of sexual development (Table 4). The only difference, according to the anatomical zone, was in the immature male octopus, which exhibited more TMA-N in the arms than in the mantle (Table 4). The TMA-N levels found in this study coincide with those of other authors (Stanley & Hulting, 1982; Iida et al., 1992) who indicated that, in most recently captured cephalopods, the TMA-N levels are equal to or lower than 1 mg of N/100 g. These TMA-N levels are indicative of an initial high freshness in these cephalopods, and in spite of the fact that, in some instances, significant differences were observed in the different variables studied (anatomical zone, sex, sexual stage), from the practical point of view these levels are not very important because they were very low. As the TMA-N parameter is a specific measurement (quality index; Hebard et al., 1982; Ruiz-Capillas & Moral, 2001a), it only really provides information when it is used throughout chilled storage of the cephalopods.

3.5. Total volatile basic nitrogen TVB-N

The TVB-N values obtained in volador were similar in all the lots, so no significant statistical differences ($P \leq 0.05$) were detected according to sex or anatomical zone (Table 5). The mature pota specimens had significantly higher ($P \leq 0.05$) levels than the immature ones. This was also true for TMA-N. The differences between sexes was only seen in the immature specimens. The male mantles exhibited significantly higher TVB-N values than the female ones, while the opposite occurred for the arms. The immature male mantles recorded higher TVB-N values ($P \leq 0.05$) than their arms, while, in the rest of the lots there were no significant differences (Table 5). There were no logical relationships among the TVB-N levels found in octopus. By sexes, the immature female mantles had a greater quantity of TVB-N than the male mantles at the same stage, while the mature male mantles had the greatest TVB-N content (Table 5). The mature female arms had a greater quantity ($P \leq 0.05$) of TVB-N than the male ones.

TVB-N behaviour, as also occurred in the case of TMA-N, did not show a clear trend and, although there were significant differences between the different parameters studied, TVB-N levels were so low that these differences were not important. The main modifications in this parameter occur when the cephalopods are stored chilled, when this parameter is used as a quality indicator. Ke, Burns and Woyewoda (1984) proposed a classification to typify the quality of the cephalopods in accordance with TVB-N content, where a limit of < 30 mg for grade A quality and 30–45 mg for grade B is established and higher than 45 mg is considered unacceptable. This classification was done bearing in mind that the method used for the quantification of TVB-N was a distillation method. However, in the Japanese market, a TVB-N value above 15 mg is unacceptable. Probably this limit is severe because of the exceptional quality of the raw material demanded for preparing products such as desiccated, salted, marinated cephalopods, which take up a large part of the capture in Japan. The results of the current study show that the qualities of these three species of cephalopods were very good since they had values around 12–18 mg/100g, and they would belong to the grade A category. They also coincide with the information presented by other authors (Moral et al., 1983) for squid (*Loligo vulgaris*).

3.6. Free amino acids FAA

The FAA composition in muscle of the different species studied, as well as the percentage compared with NPN, is shown in Tables 6 and 7. In these tables it is seen that FAA composition varied to a great extent with the species, and to a less extent with the sex, sexual development and anatomical zone. Thus, while in volador and pota the total content was virtually the same, in octopus it was about 75% lower.

In volador, the FAA: aspartic acid, glutamic acid, alanine, leucine, lysine, arginine and proline, were those found in the greatest amounts in the mantle and arms, representing more than 60% of the total FAA. In this species, the free amino acid composition did not seem to differ according to sex. There was a

Table 6
FAA (mg /100 g muscle) in mantle of volador, pota and octopus

	Volador		Pota				Octopus			
	M	F	M-i	F-i	M-m	F-m	M-i	F-i	M-m	F-m
Threonine*	49	54	25	22	24	25	10	10	11	10
Valine*	50	56	26	20	22	21	10	8	12	11
Methionine*	36	37	25	18	15	17	4	4	4	6
Isoleucine*	39	46	23	16	17	16	9	5	7	8
Leucine*	73	78	52	35	32	37	15	10	14	19
Phenylalanine*	62	66	49	33	26	34	50	79	55	59
Histidine *	11	8	23	17	10	18	8	5	5	8
Lysine*	78	91	45	33	21	31	17	13	15	16
Arginine*	90	90	208	262	276	159	55	52	33	39
Aspartic acid	75	82	40	30	31	30	10	10	15	14
Glutamic acid	122	129	71	58	49	60	31	27	37	37
Serine	63	68	45	37	39	34	15	11	14	15
Glycine	54	56	151	157	130	129	10	8	9	12
Alanine	121	122	118	107	82	97	16	24	28	24
Cysteine	12	13	11	10	10	16	4	4	5	5
Tyrosine	29	27	30	20	15	22	7	5	6	7
Proline	248	263	414	373	178	280	49	49	45	45
Hydroxyproline	0	0	3	4	2	4	4	4	4	2
Total	1212	1286	1359	1252	979	1030	324	328	318	337
% NNP	164	168	233	236	214	233	58	52	55	50

M, male; F, female; i, immature (phases II–III) and m, mature (phases IV–V). (*): essential amino acid.

Table 7
FAA (mg /100 g muscle) in arms of volador, pota and octopus

	Volador		Pota				Octopus			
	M	F	M-i	F-i	M-m	F-m	M-i	F-i	M-m	F-m
Threonine*	36	34	22	23	15	26	10	8	5	6
Valine*	27	31	25	25	13	13	10	10	7	5
Methionine*	25	25	20	20	11	10	5	5	2	3
Isoleucine*	21	24	19	19	9	11	9	4	4	5
Leucine*	48	46	39	40	17	21	7	7	8	6
Phenylalanine*	31	33	38	44	21	32	36	58	36	38
Histidine*	22	20	17	19	10	15	5	4	3	3
Lysine*	56	50	39	37	16	49	17	11	7	8
Arginine*	89	88	159	157	166	147	106	102	68	65
Aspartic acid	48	45	40	42	17	20	17	14	12	9
Glutamic acid	82	85	71	71	29	32	35	30	30	26
Serine	52	54	47	48	26	24	14	10	7	8
Glycine	54	53	158	180	162	119	11	8	4	5
Alanine	115	114	99	111	71	87	17	21	16	11
Cysteine	10	9	10	11	11	10	2	6	4	3
Tyrosine	24	23	23	26	10	11	9	6	6	6
Proline	180	174	361	313	194	326	35	33	53	48
Hydroxyproline	3	3	2	4	2	4	2	4	3	3
Total	923	911	1189	1190	800	957	347	341	275	258
% NNP	206	201	261	241	203	207	91	90	77	87

M, male; F, female; i, immature (phases II–III) and m, mature (phases IV–V). (*): essential amino acid.

greater quantity of free amino acids in the mantle, although the percentage of these free amino acids, compared with NPN, was the same in the two anatomical zones. There were greater concentrations of methionine, arginine, serine, glycine, cysteine, and tyrosine in the arms (Tables 6 and 7).

In pota, approximately 60% of the FAA were represented by four amino acids: glycine, alanine, arginine and proline. Of these amino acids, proline was present in a greater proportion in the immature specimens. FAA concentration did not vary much according to sex since the quantitative differences were low (Tables 6 and 7).

The mantles had a higher total free amino acid value than the arms, and the difference was 8–12%. The immature pota specimens also exhibited about 26% more FAA than the mature specimens.

In octopus, the amino acids: glutamic acid, alanine, phenylalanine, arginine and proline accounted for over 60% of the total FAA. There was no clear trend in FAA composition depending on sex, anatomical zone or stage of sexual development. The only exception was the arginine content which was twice as great in the arms as in the mantles (Tables 6 and 7).

There were three FAA, which always appeared as a large of the total FAA proportion in the three species; these were proline, arginine and alanine. Of these three, the most important, from the quantitative point of view, was proline, particularly in volador and pota. The only essential amino acid was arginine, which is highly important from the nutritional point of view, since the consumption of cephalopods provides a good source of it. This FAA represented 8–15% of FAA in volador and pota, while in octopus the percentage was extremely variable and was about 20%. In volador and pota, the percentage of proline compared with the total FAA was between 20 and 30%, while in octopus it was only 10–15%. Proline is the main component in the connective tissue of cephalopods (Morales, Montero, & Moral, 2000) and it is constantly being renewed, and it is because of this that there is such a high content. Alanine was present in a similar proportion in the three species and varied between 7 and 10% of the total FAA. FAA composition in similar cephalopods, such as *Illex argentinus*, *Octopus vulgaris*, *Ommastrephes sloani pacificus*, *Ommastrephes bartrami*, *Todarodes sagitatus*, differs for some amino acids, such as alanine, arginine, glycine and proline; some authors have attributed these differences to the species (Florkin & Bricteux-Grégoire, 1972; Iida et al., 1992; Suyama & Kobayashi, 1980). Moreover, FAA concentration changes in accordance with physiological conditions such as diet, season of the year, temperature, environment, stress and salinity. (Hirano, Yamaguchi, Shirai, Suzuki, & Suyama, 1992; Suyama & Kobayashi, 1980). Furthermore, the most abundant FAA in these species are proline, arginine, glutamic acid, alanine and glycine, which are primarily responsible for the formation of flavours. It is known that glycine and alanine are free amino acids, characteristic of cephalopods, since they contribute to the inherent sweet taste of these animals (Murata & Sakaguchi, 1986; Otsuka, Tanaka, Nishigaki, & Miyigawa, 1992; Sakaguchi & Michiyo, 1989). Another free amino acid which is important in the development of this sweet taste is proline, the contribution of which, to this sensory characteristic in cephalopods was described by Amano and Bito (1951) and which, as can be seen in Tables 6 and 7, was one of the most abundant free amino acids in the species studied.

4. Conclusion

NPN was more abundant in the mantles than in the arms of the three species. Trimethylamine oxide was greater in the immature pota and octopus specimens than in the mature ones.

Among the TMA-N, pH and TVB-N parameters, no differences were observed concerning sex, anatomical zone and stage of sexual development and the values were very low, indicating the state of freshness of the fish.

FAA compositions varied to a great extent with the species, and to a less extent with the sex, stage of sexual development and anatomical zone. Thus, while the total content was virtually the same in volador and pota, it was about 75% lower in octopus. The FAA present in the greatest amount in the three species were proline, arginine and alanine.

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References

- Amano, K., & Bito, M. (1951). Consequence of free amino acids generated from descomposing fish muscle. *Bulletin. Japanese Society of Scientific Fisheries*, 16, 10–16.
- Antonacopoulos, N., & Vyncke, W. (1989). Determination of volatile basic in fish. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, 189, 309–316.
- AOAC. (1995). *Official methods of analysis*. Association of Official Analytical Chemistry.
- Ayensa, M. G., An, H., Montero, P., Gómez-Guillén, C., & Borderias, J. (1999). Partial protease activity characterization of squid *Thoradopsis eblanae* mantle. *Food Science and Technology International*, 5, 391–396.
- Belitz, H. D., & Grosch, W. (1988). *Peces, ballenas crustáceos y moluscos Fish, Whales, Crustaceans, Molluscs*. Química de los alimentos. Zaragoza, Spain: Editorial Acirbia S.A.
- Bystedt, J., Swenne, L., & Aas, H. W. (1959). Determination of trimethylamine oxide in fish muscle. *Journal of the Science of Food and Agriculture*, 10, 301–304.
- Florkin, M., & Bricteux-Grégoire, S. (1972). Nitrogen metabolism in molluscs. In M. Florkin, & B. T. Scheer (Eds.), *Chemical Zoology, Vol VII. Mollusca*. London: Academic Press.
- Guerra, A. (1996). *Explotación mundial de cefalópodos*. In: *II Jornadas Internacionales sobre Utilización de Cefalópodos: Aspectos Científicos y tecnológicos*. Madrid, Spain: Instituto del Frio.
- Hebard, C. E., Flick, G. J., & Martin, R. E. (1982). Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In R. E. Martin, G. J. Flick, & X. Hebard (Eds.), *Chemistry and biochemistry of marine food products* (pp. 149–304). Westport, Connecticut: Avi. Publishing Company.
- Hirano, T., Yamaguchi, M., Shirai, T., Suzuki, T., & Suyama, M. (1992). Free aminoacids, trimethylamine oxide, and betaines of the raw and boiled meats of mantis shrimp *Oratosquilla oratoria*. *Nippon Suisan Gakkaishi*, 58, 973.

- Horie, S., & Sekine, Y. (1956). Determination method of freshness of fish muscle with trimethylamine. *Journal Tokyo University of Fisheries*, 42, 25–31.
- Iida, H., Nakamura, K., & Tokunaga, T. (1992). Non-protein nitrogenous compounds in muscle extract of oceanic cephalopods. *Nippon Suisan Gakkaishi*, 58, 2383–2390.
- Joseph, J., Varma, P.-R.G., & Venkataraman, R. (1977). Iced and frozen storage of squid. *Fishery Technology*, 14, 13–20.
- Ke, P. J., Burns, B. G., & Woyewoda, A. D. (1984). Recommended procedures and guidelines for quality evaluation of atlantic short fin squid (*Illex illecebrosus*). *Lebensmittel- Wissenschaft und- Technologie*, 17, 276–281.
- Konno, K., & Fukazawa, C. (1993). Autolysis of squid mantle muscle protein as affected by storage conditions and inhibitors. *Journal of Food Science*, 58, 1198–1202.
- LeBlanc, R. J., & Gill, T. A. (1982). Comparative study of proteolysis in short-finned (*Illex illecebrosus*) and long-finned (*Loligo pealei* Lesueur) squid. *Comparative Biochemistry and Physiology*, 13B, 201–210.
- Lin, J. K., & Hurng, D. C. (1985). Thermal conversion of trimethylamine-*N*-oxide to trimethylamine and dimethylamine in squids. *Food and Chemical Toxicology*, 23, 579–583.
- Moral, A. (1987). Métodos físico-químicos de control de calidad de pescados. *Alimentación Equipos y Tecnología*, 5(6), 115–122.
- Moral, A., Tejada, M., & Borderías, A. J. (1983). Frozen storage behaviour of squid (*Loligo vulgaris*). *Revue Internationale du Froid*, 6, 54–57.
- Morales, J., Montero, P., & Moral, A. (2000). Isolation and partial characterization of two types of muscle collagen in some cephalopods. *Journal of Agricultural and Food Chemistry*, 48, 2142–2148.
- Murata, M., & Sakaguchi, M. (1986). Changes in contents of free amino acids, trimethylamine, and nonprotein nitrogen of oyster during ice storage. *Nippon Suisan Gakkaishi*, 52, 1975–1980.
- Otsuka, Y., Tanaka, S., Nishigaki, K., & Miyigawa, M. (1992). Change in the contents of arginine, ornithine, and urea in muscle of marine invertebrates stored in ice. *BioScience Biotechnology and Biochemistry*, 56, 863–866.
- Raghunath, M. R. (1984). Soluble nitrogen losses in squids (*Loligo duvauceli*) during storage in slush ice. *Journal of Food Science and Technology*, 21, 50–52.
- Rasero, M., González, A. F., Castro, B. G., & Guerra, A. (1996). Predatory relationships of two sympatric squid, *Todaropsis eblanae* and *Illex coindetii* (Cephalopoda: Ommastrephidae) in Galician Waters. *Journal Marine Biological Association*, 76, 73–87.
- Reay, G. A., & Shewan, J. M. (1949). The spoilage of fish and its preservation by chilling. *Advances in Food Research*, 2, 343–398.
- Ruiz-Capillas, C., & Moral, A. (2001a). Correlation between biochemical and sensory quality indices in hake stored in ice. *Food Research International*, 34, 441–447.
- Ruiz-Capillas, C., & Moral, A. (2001b). Changes in free amino acids during chilled storage of hake (*Merluccius merluccius*, L.) in controlled atmospheres and their use as a quality control index. *European Food Research and Technology*, 212, 302–307.
- Sakaguchi, M., & Michiyo, M. (1989). Seasonal variations of free aminoacids in oyster whole body and adductor muscle. *Nippon Suisan Gakkaishi*, 55, 2037–2041.
- Selvaraj, P., Jasmine, G. I., & Jeyachandran, P. (1991). Effect of ascorbic acid dip treatment on frozen storage of squid (*Loligo duvaucelii*, Orbigny). *Fishery Technology*, 28, 117–121.
- Stanley, D. W., & Hulting, H. O. (1982). Quality factors in cooked North Atlantic squid. *Canadian Institute of Food. Science and Technology Journal*, 15, 277–282.
- Suyama, M., & Kobayashi, H. (1980). Free amino acids and quaternary ammonium bases in mantle muscle of squids. *Nippon Suisan Gakkaishi*, 46, 1261–1264.