Biochemical and Bacteriological properties of fresh and frozen sold cephalopods in the Egyptian market.

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ABSTRACT

Various cephalopods are available for seafood consumers in the Suez Canal area either caught from the Suez Canal and stored in ice during transporting and marketing or imported and sold frozen in retail market. The lower price of imported cephalopods compared with the fresh ones makes it more demanded by consumers, restaurants and hotels. The objective of this study is to evaluate the quality and safety of fresh/chilled squid (*Uroteuthis duvauceli*) and cuttlefish (*Sepia pharaonis*) compared to frozen imported squid in the Egyptian market through chemical, biochemical and bacteriological analyses. The chemical analysis revealed that the mantle tissue pH, volatile base nitrogen (VBN) and trimethylamine (TMA) in frozen squid were significantly higher ($P < 0.05$) than that in fresh/chilled samples. The biochemical analysis showed that the crude protein content (% dry weight) was significantly lower ($P < 0.05$) (14.2±0.73%) in frozen samples than that in fresh squid and cuttlefish (17.25±0.41% & 19.95±0.55% respectively). The total lipid content was generally low, ranging from 0.39 – 1.77% in all samples. Most of saturated fatty acid content of the three samples was presented as C16:0, monounsaturated fatty acid content as C18:1 and polyunsaturated fatty acid (PUFA) content as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3). EPA and DHA contents were significantly lower ($P < 0.05$) in frozen squid than in fresh samples. The major essential amino acids (EAA) in the three samples were lysine, arginine, and leucine. The major non-essential amino acids (NEAA) were glutamic acid, aspartic acid and glycine. EAA and NEAA values were significantly lower ($P < 0.05$) in frozen squid than in fresh cuttlefish. Bacteriological results suggest that hydrogen sulphide producing bacteria constituted a significant proportion of the total spoilage flora in frozen samples, while *Pseudomonas* sp. constituted a major part of the spoilage flora of fresh samples. An overall look into the data obtained showed that there might be significant health hazards to humans from consuming imported frozen squid besides its much lower nutritional value than fresh samples.

Keywords: frozen squid, fresh/chilled squid, cuttlefish, chemical analysis, biochemical analysis, bacterial count.

INTRODUCTION

Cephalopods represent important economic seafood for human consumption as they contribute 14% of the world fisheries, according to FAO (2004). Of the species explored worldwide, roughly 41% belong to the genera *Loligo*, *Sepia* or *Octopus*, which are predominantly found on the continental shelf (Guerra, 1996). Cephalopods, like fishes and crustaceans, are rapidly perishable products; their sensory quality decrease quickly after capture. However, the shelf-life of cephalopods is much shorter (approximately between 50% and 80%) than for most fish species stored in similar temperatures and conditions (Kreuzer, 1984). The rejection time for cephalopods products is always around 10 days for cuttlefish and 9 days for squid (Vaz-Pires *et al*., 2008). This indicates that the spoilage process in cephalopods is different from fish due to many reasons, including their thinner and fragile skin, nutritional composition that is more liable to enzymatic degradation, shorter and less pronounced rigor mortis and initial autolytic degradation for a longer period (Vaz-Pires & Seixas, 2006).

In Egypt, particularly in Suez Canal area, the squid *Uroteuthis duvauceli*, and the cuttlefish *Sepia pharaonis* are the most commonly-consumed cephalopods in the fish market. They are sold as fresh product covered with ice to increase their shelf-life. Recently during the last few years, the consumption of frozen imported seafood including cephalopods mainly the squid has increased in Egypt. The mantle is the only body part that is sold without head, skin and viscera to consumers. Despite frozen and thawed products have a lower quality and poor flavor than fresh ones (Kreuzer, 1984), there is a growing tendency of consumers, restaurants and hotels for their consumption due to their marked lower price.

Meanwhile, the topic of seafood safety and quality assurance is one of the most important and timely issues directly linking the oceans and human health considerations (Tyson *et al*., 2004). Seafood is generally the main contributor of n−3 PUFA in the human diet. Lipids of marine fish species are generally characterized by high levels of long-chain n−3 polyunsaturated fatty acids (Ackman, 1989). Among the polyunsaturated fatty acids, EPA (eicosapentaenoic acid, C20:5 n−3) and DHA (docosahexaenoic acid, C22:6 n−3) are the dominant n−3 fatty acids in marine fish (Ackman, 1989). These fatty acids are of great importance to humans for the prevention of coronary heart disease (Mozaffarian *et al*., 2005). Although cephalopods contain low levels of fat, it is rich in n−3 fatty acids (Zlatanos *et al*., 2006). While, seafood can be a highly nutritious component of human diet, the contaminated, imported or domestic seafood can cause foodborne illnesses, with problems ranging from mild gastrointestinal discomfort to neurological damage (GAO, 2001). The storage method seems to influence the quality characteristics of seafood especially cephalopods (GAO, 2001). Although frozen storage can inhibit microbial spoilage, tissue proteins undergo a number of changes, which modify

their structural and functional properties (Mackie, 1982). These changes include protein insolubility, formation of aggregates, mechanical damage and enzymes and other components are released (Nilsson & Ekstrand, 1993).

To our knowledge, no studies on commercial cephalopods quality and safety are so far reported in Egypt. However, in general few studies on cephalopods quality were directed mostly to the squid (Ruíz-Capillas *et al*., 2002a&b; Albaneses *et al*., 2005) and *Octopus* (Lougovois *et al*., 2007; Atrea *et al*., 2009). Therefore, from the nutritional point of view, the objectives of this research are: 1- Comparison of the chemical (pH, trimethylamine and volatile base nitrogen) and biochemical composition of the most commonly consumed fresh chilled cephalopods in the Suez Canal area with the imported frozen squid in the market. 2- Check the microbiological status to detect pathogenic bacteria which are possible indications of contamination or poor processing practices.

MATERIALS AND METHODS

Samples

Fresh chilled squid and cuttlefish were purchased from local fish market. They were caught along the coast of Suez Canal. Frozen imported squids were bought from a supermarket. A total of 8 individuals from each sort were used for all analyses. Mean values of mantle length and body weight of all the samples were 23.7 ± 2.71 cm and 144 ± 10.2 g for *Uroteuthis duvauceli*, 15.3 ± 4.38 cm and 220 ± 14.62 g for *Sepia pharaonis*, and 30 ± 1.11 cm and 220 ± 4.11 g for frozen squids. All the samples were stored in the Lab under the same conditions of market storage. The fresh samples were kept among ice cubes in a box with perforated bottom and new ice was added as needed, while the frozen squid were stored at - 20 °C until analysis. All the sample analyses were done within two days of arrival to the Lab on only the mantle part, which is the main edible portion of cephalopods. The analyses were performed on two replicates.

Chemical Analysis

pH measurement

Ten gram of each sample were blended with 20 ml distilled water in a blender for 30 s and pH value of each sample homogenate was measured by a digital pH-meter standardized at pH 4 and 7.

Trimethylamine (TMA) and volatile base nitrogen (VBN)

Mantle extracts for determination of trimethylamine (TMA) and volatile basic nitrogen (VBN) that includes TMA, other volatile amines and ammonia were prepared by homogenizing 10 g of minced mantle with 20 ml 5% trichloroacetic acid (TCA) for 1 min using an Ultra-Turrax homogenizer. The homogenate was centrifuged (1200 x g, 4 min, 18 °C) and the extract filtered in filter paper. The precipitate was washed twice with 10 ml 5% TCA, centrifuged and filtered. The extracts were 10 ml 5% TCA, centrifuged and filtered. The extracts were collected and diluted to 50 ml with 5% TCA in a volumetric flask and kept refrigerated at 4 °C until required. Contents of trimethylamine and

volatile base nitrogen were determined in these muscle extracts according to the procedure of Howgate (1976).

Biochemical analysis

Proximate composition

Moisture, total protein, total lipid, total carbohydrates and ash were determined as percentage: (wet weight of biochemical content (g) per g of mantle tissue) x 100 according to the procedure of AOAC (1998).

Fatty acids analysis

Fatty acids in the mantle samples were determined using the procedure of Cohen *et al.* (1988). Fatty acid methyl esters were analyzed using a gas liquid chromatography on a Hewlett Pachard 6890 series fitted with a flame ionization detector and a split–splitless injector. The separation was carried out with helium as the carrier gas in an INNO wax capillary column, programmed from 150°C to 200°C at 4°C min^{-1}, held for 10 min and heated to 210 °C, for 15 min, then increased by 1°C min^{-1} up to 220°C and finally increased by 10°C min^{-1} up to 240°C. Fatty acid methyl esters were identified by comparison of their retention time with those of chromatographic Sigma standards. Peak areas were determined using the Varian software.

Amino acids analysis

Amino acids in the mantle samples were determined according to the method described by Blackburn (1986). Proteins were hydrolyzed with 6 N hydrochloric acid (containing 0.1% phenol) in a MLS-1200 Mega Microwave System (Milestone), at 800 W, 160 °C for 10 min. The hydrolysis was performed under inert and anaerobic conditions to prevent oxidative degradation of amino acids. The hydrolysates were filtered and dissolved in sodium citrate buffer (pH 2.2). Amino acids were separated by ion exchange liquid chromatography in an automatic analyzer Biochrom 20 (Amersham Biosciences), equipped with a column filled with a polysulfonated resin (250x4.6 mm), using three sodium citrate buffers (pH 3.20, 4.25, and 6.45; Amersham Biosciences) and three temperatures (50 °C, 58 °C, and 95°C). The detection of amino acids was done at 440 nm and 570 nm after reaction with ninhydrin (Amersham Biosciences). Amino acids were identified by comparison of their retention time with those of specific standards (Sigma) and were quantified with the software EZChrom Chromatography Data System, version 6.7 (Scientific Software) using norleucine (Sigma) as internal standard.

Bacteriological Analysis

Two replicates from each sample were used exclusively for the microbiological examination. Samples were analyzed as recorded in Standard Methods for Examination of Water and Wastewater (APHA, 1992). Individual areas of around 10 cm^2 of each mantle were cut and homogenized by a blinder in sterile peptone water (0.1%) and from which surface inoculations were made using the 20 µl drop method in five solid medium: Plate count agar for total viable bacteria (TVB), *Pseudomonas* selective agar media for isolation of

Pseudomonas species (Ps.), T.C.B.S agar medium (Scharlau) for *vibrio* sp., Triple sugar iron agar for H_2S-producing bacteria and Endo Agar Base for *E.coli*, culture media were adopted from the Oxoid Manual (1985), Identification of well-isolated pure colonies proceeded to the generic level based on biochemical tests according to Bergey's Manual (1994). Plates were incubated at 25°C for 3 days and black colonies (H_2S-producing bacteria) formed were counted.

Statistical Analysis

Data were analyzed using an ANOVA to investigate the presence of significant differences in parameters between the fresh/chilled samples and the frozen samples. Significance of differences was defined as $P < 0.05$. Statistics were performed using the commercially available software programs "Statistica" and "MS Excel".

RESULTS

Chemical quality

Mantle tissue pH was significantly higher ($P < 0.05$) in frozen squid (8.0±0.01) than that in fresh/chilled squid (6.85±0.01) and cuttlefish (6.51±0.01). There is also significant increase ($P <0.05$) in volatile base nitrogen (VBN) and trimethylamine (TMA) in frozen squid (15.93± 0.01 & 3.2± 0.01 mg/100g respectively) than that in fresh/chilled squid (13.78± 0.10 & 2.0± 0.02 mg/100g respectively) and in fresh/chilled cuttlefish (10.52± 0. 20 & 1.7± 0.02 mg/100 g respectively) (Table 1).

Table 1: Means ± S.D of pH, volatile base nitrogen (VBN) and trimethylamine (TMA) in the mantle tissue of frozen and fresh/chilled cephalopods collected from the Egyptian market.

Name	pH	VBN (mg/100g)	TMA (mg/100g)
frozen squid	8.00±0.01	15.93 ± 0.01	3.2± 0.01
fresh/chilled squid	6.85±0.01	13.78 ± 0.10	2.0± 0.02
fresh/chilled cuttlefish	6.51±0.01	10.52 ± 0.20	1.7± 0.02

Proximate composition

Mantle proximate compositions of frozen and fresh/chilled samples are presented in Table 2. All the proximate composition values of the three samples were found to be significantly different ($p < 0.05$). Frozen squids were found to have higher water content (82.1±0.20%) than in fresh squids (78.65±0.04%) and fresh cuttlefish (75.74±0.18%). In contrast, the protein content was lower in frozen squids (14.20±0.73%) than in fresh squids (17.25±0.41%) and fresh cuttlefish (19.95±0.55%). The lipid contents of the three samples were found to be very low.

The highest lipid content was recorded in fresh squids (1.77±0.13%) followed by frozen squid (1.41±0.15%) and fresh cuttlefish (0.39±0.02%).

Table 2: Means ± S.D of proximate composition (wet weight %) in the mantle tissue of frozen and

fresh/chilled cephalopods collected from the Egyptian market.

Composite					
Name	**Moisture**	**Protein**	**Lipid**	**Carbohydrate**	**Ash**
Frozen squid	82.10± 0.20	14.20± 0.73	1.41± 0.15	1.09± 0.55	1.20± 0.04
Fresh/chilled squid	78.65± 0.04	17.25± 0.41	1.77± 0.13	1.78± 0.51	0.55± 0.01
Fresh/chilled cuttlefish	75.74± 0.18	19.95± 0.55	0.39± 0.02	2.46± 0.60	1.46± 0.1

Meanwhile, the highest carbohydrate content was recorded in fresh cuttlefish (2.46± 0.60%) followed by fresh squids (1.78± 0.51%) and frozen squids (1.09± 0.55%). Ash formed the highest value in fresh cuttlefish (1.46± 0. 1%) followed by frozen squids (1.20± 0.04%) and fresh squids (0.55± 0.01%).

Fatty acid profiles

A wide variety of fatty acids were detected among the lipids in the mantle of the three samples (Table 3). Most of the saturated fatty acid content was represented by palmitic acid (C16:0), monounsaturated fatty acid content by oleic (C18:1) and polyunsaturated fatty acid (PUFA) content as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3).

Table 3: Saturated and unsaturated lipids {percentage = [weight (g) of fatty acid per one gram of mantle wet weight] x100} in the mantle tissue of frozen and fresh/chilled cephalopods collected from the Egyptian market.

The values are expressed as means±standard deviation, n = 2.
nd: not detected

			Frozen squid	**Fresh/chilled squid**	**Fresh/chilled cuttlefish**
Degree of Saturation	**Acid**	**Symbol**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Saturated Fatty Acids	**Lauric**	**C12:O**	**2.83 ± 0.21**	**1.14 ± 0.32**	**0.15 ± 0.18**
	Myristic	**C14:O**	**6.57 ± 0.12**	**6.21±1.20**	**01.80 ± 0.20**
	Palmitic	**C16:O**	**33.70 ± 2.10**	**32.61±1.30**	**24.56 ± 1.10**
	Stearic	**C18:O**	**6.57 ± 0.59**	**4.20 ± 0.08**	**05.10 ± 0.72**
	Arachidic	**C20:O**	**nd**	**0.31 ± 0.01**	**01.45 ± 0.03**
	Behenic	**C22:O**	**nd**	**0.15 ± 0.00**	**0.27 ± 0.01**
Total Saturated Acids			**49.68**	**44.63**	**33.34**
Mono-Unsaturated Fatty Acids	**Palmiboleic**	**C16:1**	**nd**	**0.40 ± 0.01**	**5.1 ± 0.19**
	Oleic	**C18:1**	**19.27 ± 0.13**	**5.30 ± 0.21**	**9.00 ± 0.14**
	Cis-Eicosenic	**C20:1**	**5.75 ± 0.22**	**1.00 ± 0.01**	**3.51 ± 0.18**
Total Monounsaturated Acids			**25.03**	**6.70**	**17.61**
Poly-Unsaturated Fatty Acids	**Omega 6 Linoleic**	**C18:2 w 6**	**4.08 ± 0.01**	**1.21 ± 0.04**	**1.75 ± 0.95**
	Omega 3 Linolenic	**C18:3 w 3**	**5.41 ± 0.30**	**0.06 ± 0.01**	**0.11 ± 0.02**
	(EPA) Omega 3 Eicosapentaenoic	**C20:5 w 3**	**5.61 ± 0.11**	**12.20 ± 0.11**	**16.3 ± 0.04**
	(DHA) Omega 3 Docosahexaenoic	**C22:6 w 3**	**10.20 ± 0.24**	**35.20 ± 0.12**	**30.89 ± 0.10**
Total Poly-Unsaturated Acids			**25.29**	**48.67**	**49.05**
Total Unsaturated Fatty Acids			**50.32**	**55.37**	**66.66**

The levels of saturated and unsaturated fatty acids of the three samples differ significantly ($P < 0.05$). The lowest mean value of saturated fatty acid fraction was in the fresh cuttlefish (33.34%), whereas the highest mean value (49.68%) was reported in the frozen squids. In contrast, the lowest mean level of unsaturated fatty acids was found in the frozen squids (50.32%) and the highest

value (66.66%) was in the fresh cuttlefish. EPA content was higher in the fresh squids and fresh cuttlefish (12.20±0.11 & 16. 3±0.04% respectively) than that in frozen squids (5.61± 0.11%). DHA comprised the highest percentage levels about 35.20±0.12 & 30. 89±0.10% in fresh squids and fresh cuttlefish respectively compared to 10.20± 0.24 in frozen squids (Table 3).

Amino acid profiles

The amino acid composition of the three cephalopod samples (% of total protein) is shown in Table 4. The major essential amino acids (EAA) were lysine, arginine, and leucine. The major non-essential amino acids (NEAA) were glutamic acid, aspartic acid and glycine. Generally, EAA and NEAA values were significantly higher ($P < 0.05$) in fresh cuttlefish than in fresh and frozen squids. The lowest mean value of EAA (45.13%) was in the frozen squids whereas the highest mean value (58.25%) was in the fresh cuttlefish followed by fresh squids (45.86%). Similarly, the lowest mean value of NEAA was in the frozen squids (29.51%), whereas the highest mean value (39.76 %) was in the fresh cuttlefish followed by fresh squids (32.41%).

Table 4: Amino acid composition (% dry weight) in the mantle tissue of frozen and fresh/chilled cephalopods collected from the Egyptian market.

Essential (EAA)	Frozen squid	Fresh/chilled squid	Fresh /chilled cuttlefish
Threonine	2.89± 0.09	2.18± 0.02	2.89± 0.05
Valine	3.97± 0.09	3.4± 0.03	3.81± 0.05
Methionine	0.43± 0.01	nd	1.00± 0.10
Isoleucine	3.67± 0.03	4.9± 0.06	4.95± 0.08
Leucine	5.55± 0.02	5.4± 0.07	7.04± 0.11
Phenylalanin	4.48± 0.02	4.82± 0.06	5.78± 0.10
Lysine	10.16± 0.15	10.87± 0.04	13.54± 0.10
Histidine	3.06± 0.10	2.58± 0.09	3.63± 0.09
Arginine	5.98± 0.01	6.55± 0.11	8.86± 0.11
Tyrosine	4.94± 0.09	5.16± 0.08	6.75± 0.11
Sum EAA	45.13	45.86	58.25
Non-essential (NEAA)			
Glutamic acid Glutamic acid	643±006 6.43± 0.06	9±009 9± 0.09	11±015 11± 0.15
Alanine	6.38± 0.11	4.53± 0.05	6.48± 0.15
Glycine	7.26± 0.10	6.55± 0.13	7.83± 0.11
Serine	2.22± 0.02	1.67± 0.01	2.22± 0.02
Asp	6.29± 0.06	9.65± 0.03	10.27± 0.14
Prolin	0.51± 0.07	0.77± 0.01	1.02± 0.03
Cystine	0.42± 0.01	0.24± 0.01	0.94± 0.02
Sum NEAA	29.51	32.41	39.76
Sum TAA	74.64	78.27	98.01

The values are expressed as means±standard deviation,
n = 2.nd: not detected

Bacteriological analysis

Results from bacteriological analysis are presented in Fig.1. TVB and H_2S bacteria were massively increased in the frozen squids (14×10^5 & 3×10^5 cfu/ ml respectively). Meanwhile, a relatively low numbers of TVB (1264×10^3 & 888×10^3 cfu/ml) and H_2S bacteria (1612×10 & 1×10^3 cfu/ml) were recorded in fresh squids and fresh cuttlefish respectively. In contrast, Ps and vibrio were detected in low numbers in frozen squids (8×10^3 & 872 x10 cfu/ ml) and a relatively high number in fresh squids (392×10^2 and 2036 x 10 cfu/ ml) and

fresh cuttlefish (704 x 10^2 and 1672 x 10 cfu/ ml). *E. coli* was not detected in all the tested samples.

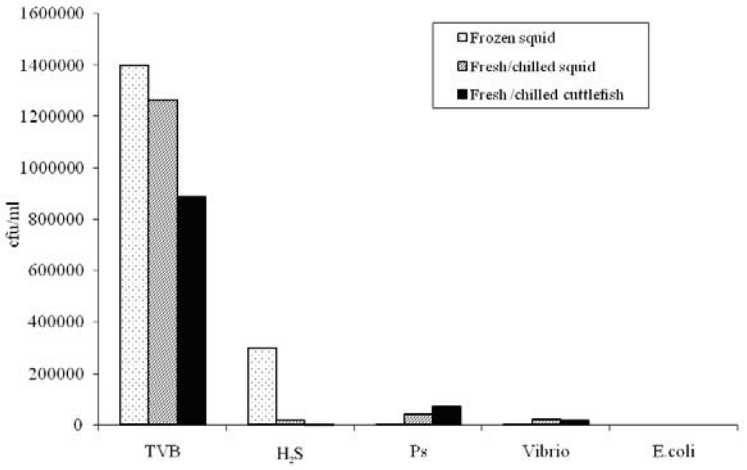

Fig. 1: Microbiological results in the mantle tissue of fresh/chilled and frozen cephalopods collected from the Egyptian market.
Values are means of two replicates measurements.

DISCUSSION

Chemical quality

Chemical indices for freshness evaluation of fish, crustaceans and molluscs are mainly based on changes of pH, non-protein nitrogen (NPN) components during the storage, such as trimethylamine (TMA) and volatile basic nitrogen (VBN) that includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage (Lapa-Guimaraes *et al*., 2005). In the present study, the fresh chilled squids and cuttlefish pH was slightly higher (6.85±0.01& 6.51±0.01 respectively) than 5.8, which is a typical value in very fresh products (Sykes *et al*., 2009). Meanwhile, a significant increase ($P < 0.05$) was recorded in pH of frozen squids (8.0±0.01). This value was within the observed range in other studies by Albanese *et al*. (2005) and Lougovois *et al*. (2007) who noticed that freezing caused a significant increase of pH in different cephalopods. They attributed that to the rapid spoilage of the product and the formation of alkaline compounds of autolysis and bacterial metabolites in the muscles.

VBN mean values in fresh chilled squids and cuttlefish (13.78 ± 0.1 & 10.52 ± 0.2 mg/100 g respectively) indicate that these samples were of good quality according to Ruíz-Capillas *et al*. (2002 b) who detected 20 mg/100 g VBN in the mantle of freshly caught cephalopods. Meanwhile, the present

values were remarkably higher than those detected by Vaz-Pires *et al*. (2008) in other species of fresh squids and cuttlefish (9.9 and 7.7 mg/100 g muscle respectively). On the other side, the production of VBN was more intense in frozen squids (15.93 ± 0.01mg/100g) than in fresh squids and cuttlefish. This value was lower than those in spoiled squids and cuttlefish (26.9 and 21.9 mg/100 g respectively) detected by Vaz-Pires *et al*. (2008). All the present VBN values were below the critical limits of 25, 30 and 35 mg /100g specified by the European Commission guidelines (EC, 1995) for different groups of fish species.

Similar to VBN, TMA value detected for the frozen squids was significantly higher (3.2 ± 0.01 mg /100 g) than that in fresh squids and fresh cuttlefish (2.0 ± 0.02 and 1.7 ± 0.02 mg /100 g respectively) but did not exceed the standard limit of 10 mg/100 g muscle (EC, 1991). Vaz-Pires *et al*., (2008) reported a TMA value of 0.1 and 0.3 mg/100 g for fresh shortfin squid and cuttlefish respectively; while they detected 8.4 and 10 mg/100 g in spoiled squids and cuttlefish after 10 days in ice storage. Although, the levels of VBN & TMA in frozen squids are below the standard limits, yet the standard limits were found to vary within different species, age and sex of animals and season of harvesting (Kilinc & Cakli, 2005).

Nutritional characterization

The highest percentage of protein was detected in the fresh samples. Results obtained for proximate composition were within the levels for 21 species of cephalopods reported by Lee (1994). He reported that the cephalopod proximate composition (g/100 g) is composed of 18 g protein and 79 g moisture, thus leaving only 3 g of body mass for other biochemical compounds needed for life. The significant decrease in protein coincided with the significant increase ($P < 0.05$) in moisture of frozen sample in agreement with Sykes *et al*., (2009) who found a significant decrease in protein of *S. officinalis* after 13 days of spoilage in ice. The high autoproteolytic activity of proteinases produce an increment on the water fraction that led to a loss in protein content by washing effect (Sykes *et al*., 2009).

Fatty acid composition significantly differed ($P<0.05$) between frozen squids, fresh squids and fresh cuttlefish. Unsaturated fatty acids formed the highest proportion that ranged between 55.37 -66.66 % in fresh samples and formed 50.32 % in frozen samples. Such results are approximately within the range of data published for other cephalopods by Sinanoglou & Miniadis-Meimaroglou (1998) who observed that the Mediterranean cephalopods contain large amounts of unsaturated fatty acids (52.89–56.25% of total fatty acids). The most characteristic PUFAs are eicosapentaenoic acid (EPA; 20:5$n-3$, 20%) and docosahexaenoic acid, (DHA; 22:6$n-3$, 36%), which agree with the results of Kilada & Riad (2008). The slight differences in values can be explained by well known variations within aquatic species and within individuals of the same

species, and also by diverse geographical origin and subsequently different biological and non biological factors (Rosa *et al*., 2000).

EPA and DHA in the fresh samples were approximately three times higher than that in frozen squids. This may emphasize the high quality of fresh cephalopods from the health perspective (Mozaffarian *et al*., 2005). The remarkable decrease in EPA & DHA of frozen squids might have resulted from lipid oxidation caused by freezing (Decker & Hultin, 1992). Lipid oxidation processes lead to discoloration, drip losses, off-flavor development and production of potentially toxic compounds (Decker & Hultin, 1992). Furthermore, lipid oxidation in fish muscle during frozen storage showed a detrimental effect on protein structure and functionality (Saeed & Howell, 2002).

The low percentage of EAA in frozen squids indicates that the quality of their protein might be lower than that in fresh squids and fresh cuttlefish. High percentage of H_2S-producing bacteria in frozen squid can account for the change in amino acids composition, since they degrade sulfur-containing amino acids and produces volatile sulfides including H_2S (Brettar *et al*., 2002).

Bacteriological analysis

Total viable bacteria count (TVB) is the most common method for determination of the bacteriological quality of seafood. This measurement is seldom a good indicator of the sensory quality or expected shelf life of the seafood product (Huss *et al*., 1997), but it is taken as an indicator of the hygiene status of the product. The present analysis of TVB revealed that bacterial flora in frozen squids is significantly higher than that in fresh/chilled squids and cuttlefish. However, the numbers detected in all samples are relatively lower ($14x10^5$, $1264\ x10^3$, $888x10^3$ cfu/ml in frozen squids, fresh squids and fresh cuttlefish respectively) than the number recorded at the rejection point which is around 10^7 and 10^9 cfu/g for many fish and fish products (Huss *et al*., 1997).

Counts of the black colonies, which represent the H_2S-producing bacteria, follow the same pattern of TVB. They were significantly higher in frozen squids than that in the fresh/chilled squids and cuttlefish. The number detected in the current study ($3x\ 10^5$ cfu/ml) was much higher than that recorded in other spoiled squids 10^4 cfu/cm^2 at sensory rejection time (Vaz-Pires *et al*., 2008). These bacteria usually constitute only a small fraction of the initial flora on newly caught fish but constitute a significant, sometimes dominant, part of the microbiota during chilled storage, and their numbers determine the shelf life of the product (Vogel *et al*., 2005). *Shewanella putrefaciens* has been considered the most common sulphide producer on fish tissues during iced storage (Brettar *et al*., 2002). All the *Shewanella* strains are able to reduce TMAO to trimethylamine (TMA) and this explains its importance in spoilage of fish stored at low temperatures where the "fishy" off-odor of spoiling fish is caused by the production of TMA (Brettar *et al*., 2002). Thus, the high count of H_2S-producing bacteria explains the high content of TMA in frozen squid.

The majority of the spoilage flora in fresh cuttlefish and fresh squids seems to be composed by *Pseudomonas*. The number of these bacteria was much higher than H_2S-producing bacteria (392 x 10^2 versus 1612 x 10 in squids and 704 x 10^2 versus 1 x 10^3 in cuttlefish cfu/ml). These values were much higher than that recorded in spoiled squids 10^4 cfu/cm^2 at sensory rejection time (Vaz-Pires *et al*., 2008) indicating low quality of the fresh/chilled samples. *Pseudomonas* is one of the specific spoilage organisms (SSOs) which produce metabolites, causing off-flavors or off-odors and consequently cause consumer food rejection (Gram & Dalgaard, 2002).

CONCLUSION

The food quality of cephalopods was found to vary significantly depending on whether it was frozen or chilled before using. Frozen squids proved to have much lower quality than chilled. This conclusion was supported by the presence of high level of VBN and TMA in frozen squids compared to the chilled squids and cuttlefish. Besides these two parameters, the decrease in total protein content in the frozen squids indicated its lower food quality than chilled squid. The present study also showed that frozen squids may be spoiled due to the presence of high number of H_2S-produceing bacteria that exceeds the number recorded at the rejection point for other cephalopods (Vaz-Pires *et al*., 2008).

On the other hand, the EPA, DHA and EAA were significantly higher in chilled squids and cuttlefish compared to frozen squids. This may increase the suitability of the former as better food although it may be more expensive. However, the consumer should not consume any fresh/chilled cephalopods after 10 days shelf life (Vaz-Pires *et al*., 2008). Future research should focus on cephalopods shelf-life under different storage conditions. The storage in noncontact ice may maintain the fresh cephalopods at grade A quality for a longer time and avoid leaching of proteins and minerals (Ke *et al*., 1991).

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