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Quality aspects and species identification of cephalopod products on the German market

Qualität und Speziesidentifizierung von Cephalopodenprodukten auf dem deutschen Markt

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Summary

Commercial deep-frozen products of cuttlefish (labelled as *Sepia* spp, *Sepiella* spp.), squid (*Illex* spp., *Loligo* spp., *Todarodes* spp.), and octopus (*Octopus* spp., *Eledone* spp.) were analysed to give an overview of the current product quality on the German market and to see if the contents of total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), trimethylamine oxide nitrogen (TMAO-N), ammonia, and pH are appropriate indicators to confirm the sensory data of such products. All cuttlefish, octopus, and squid tubes had TVB-N contents ranging between 1.6 and 16.2 mg/100 g which agreed with a satisfactory sensory quality. Only a few products made from whole and skinned squid had elevated TVB-N values up to 27.8 mg/100 g muscle tissue. TMA-N and TMAO-N levels were low in all samples (≤1.0 and <15 mg/100 g, respectively). Measurement of pH and ammonia content were not useful in assessing quality deterioration. The spoilage potential of defrosted products kept at 4 °C in a refrigerator was followed by chemical and sensory assessment. The increase of cadaverine, putrescine, and tyramine in the products varied. Free amino acids seemed notable as precursors of biogenic amines and due to nutritional aspects. Declaration of cephalopod species was investigated by PCR-based DNA analysis. A DNA segment of 208 base pairs from the cytochrome b gene was amplified and sequenced. Mislabelling of species was detected in a number of products.

Keywords: squid, cuttlefish, octopus, total volatile bases, biogenic amines

Zusammenfassung

Im Handel erhältliche tiefgefrorene Sepien (etikettiert als *Sepia* spp., *Sepiella* spp.), Kalmare (*Illex* spp., *Loligo* spp., *Todarodes* spp.) und Kraken (*Octopus* spp., *Eledone* spp.) wurden analysiert, um einen Überblick über die aktuelle Qualität dieser Produkte auf dem deutschen Markt zu erhalten und um festzustellen, ob die Gehalte an flüchtigem Basen-Stickstoff (TVB-N), Trimethylamin-Stickstoff (TMA-N), Trimethylaminoxid-Stickstoff (TMAO-N) und Ammoniak sowie der pH-Wert geeignete Parameter sind, die sensorische Beurteilung zu bestätigen. Alle Sepien, Kraken und Kalmartuben hatten TVB-N-Gehalte zwischen 1,6 und 16,2 mg/100 g, welche mit der befriedigenden sensorischen Bewertung übereinstimmten. Nur einige Produkte aus ganzen Kalmaren ohne Haut hatten TVB-N-Gehalte bis zu 27,8 mg/100 g Muskelfleisch. Die TMA-N und TMAO-N-Gehalte waren in allen Proben niedrig (≤1,0 und <15 mg/100). Die Messung der pH-Werte und Ammoniakgehalte waren nicht geeignet, eine Verschlechterung der Qualität darzustellen. Das Verhalten aufgetauter Erzeugnisse bei einer Lagerung im Kühlschrank bei 4 °C wurde untersucht, indem die Veränderungen der chemischen und sensorischen Eigenschaften ermittelt wurden. Die Gehalte an Cadaverin, Putrescin und Tyramin nahmen in allen Produkten während der Lagerung unterschiedlich stark zu. Freie Aminosäuren erscheinen als Vorstufen für biogene Amine und aus ernährungsphysiologischen Aspekten beachtenswert. Die Angabe der Cephalopodenart wurde mit einer auf PCR basierenden DNA-Analyse überprüft. Ein DNA-Segment aus 208 Basenpaaren aus dem Cytochrom b-Gen wurde vervielfältigt und sequenziert. Bei einigen Produkten wurde eine falsche Deklaration festgestellt.

Schlüsselwörter: Sepien, Kalmare, Kraken, TVB-N, biogene Amine

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Introduction

Cephalopods such as cuttlefish, squid, and octopus have been fished on an artisanal basis for several thousand years. In more recent times, an increasingly extensive demand for cephalopods by the Japanese led to the development of commercial fishery. It started in the 1960s and rapidly expanded to become global (Garibaldi and Limongelli, 2002). In Germany, the consumer's demand for cephalopod products has considerably increased in recent years. After visiting Mediterranean countries where they experienced such new dishes and food recipes, people want to try them out at home later.

Meanwhile, a variety of cephalopod products can be found in speciality shops and supermarkets. Germany is in particular an importer of semi-processed products mostly from Spanish suppliers. Mainly frozen preparations are sold at retail level, but increasingly they are offered defrosted at fish counters as well.

Nowadays the number of cephalopod species that enter the market has grown significantly. This development is caused by an expansion into new deep water fishing areas and still-growing market demand (Jereb et al., 2010; Kojadinovic et al., 2011). Species identities are hardly ever given on the package labels and often limited to the general designation "spp." which possibly indicates several species of sepia, squid or octopus within one unit.

Previous findings of official German food control laboratories and commercial laboratories showed that the quality of such products was often not satisfactory and that the chemical results were not always consistent with the sensory assessment. Once caught, cephalopod proteins undergo very rapid degradation caused by endogenous and bacterial enzymes. It is known that some of the established quality parameters used to assess freshness or degradation in fish are not always applicable to cephalopods. This includes total volatile basic nitrogen (TVB-N) and pH value (Márquez-Ríos et al., 2007).

The main objective of this study was to obtain an overview of the current quality level of cephalopods on the German market. Another aim of the study was to elucidate whether established quality parameters for fish are also suitable for quality assessment of the various cephalopod subclasses and orders processed for food without further differentiation of the raw material. Thawed cephalopods offered at fresh fish counters should be kept refrigerated post sale. Therefore the remaining shelf life in a refrigerator at 4 °C was also tested. For this purpose chemical and sensory methods were applied. PCR-based DNA-analysis was performed to confirm the labelling (Santaclara et al., 2007).

Material and Methods

Deep frozen products of squid, cuttlefish, and octopus in different packages were bought between October 2009 and March 2010 from local German supermarkets, speciality shops and from the Hamburg whole sale fish market and stored at –25 °C until analysed. Details are given in Table 1. Prior to analysis samples were thawed overnight at 4 °C in a refrigerator. Edible parts of the different product types were used for the chemical analyses. The storage experiment in a refrigerator at 4 °C was carried out with thawed individuals of the same lot. Samples were taken in intervals up to 16 days for chemical and sensory analysis.

DNA analysis

Extraction and quantification of DNA have been described previously (Rehbein and Kreß, 2005). PCR was performed with reagents from SolisBioDyne (Tartu, EST). The assay (volume 25 µL) contained 1.25 units of HotFirePol DNA polymerase I, 2.5 µL of BD buffer, and dNTP mix (50 µM final concentration of each nucleotide), 2.5 mM $MgCl_2$, 0.5 µM of primers, and 25 ng of DNA. The primer pair CEFH and H15149AD was used for amplification of a 208 bp sequence from the mitochondrial cytochrome b gene, according to the protocol of Santaclara et al. (2007).

Amplicons were purified and cycle-sequenced in one direction using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Version 1.1) with the primer CEFH as taken for PCR. DNA sequences were compared with nucleotide sequences from GenBank (National Centre for Biotechnology Information, NCBI) by means of the programme BLAST (Basic Local Alignment Search Tool).

Chemical analyses

Determination of total volatile basic nitrogen (TVB-N), tri- and dimethylamine nitrogen (TMA-N, DMA-N) and trimethylamine oxide nitrogen (TMAO-N)

Thawed samples were homogenised with an Ultra Turrax homogenizer (IKA®-Werke, DEU). A perchloric acid extract was prepared with 20 g homogenised sample and 180 mL 6 % (w/v) perchloric acid using the Ultra Turrax. Kieselgur (Merck, DEU) was added to improve the filtration of the gelatinous mixture. The filtrates were kept at –18 °C until analyses. Aliquots were used for the determination of total volatile bases nitrogen (TVB-N) (Commission of the European Communities, 2005) and free amino acids.

TMA-N, DMA–N and TMAO-N were analysed from the same perchloric acid extract by gas chromatography (HP 6890, Hewlett Packard Company, USA) with a modified method of Oetjen and Karl (1999) using cyclohexane/amyl alcohol (1 : 1, v/v) instead of tert.-butyl methyl ether for extraction.

Determination of ammonia

The ammonia content was measured in an aliquot of the perchloric acid extract. The determination based on the enzymatic method of Da Fonseca-Wollheim et al. (1974).

TABLE 1: *Product overview.*

Type of Product	Product labelling		
	Family	Species	FAO Area
Cuttlefish Whole gutted, skinless, washed and without cuttlebone; tubes, rings, strips	Sepiidae	*Sepia* spp., *Sepia officinalis*, *Sepia pharaonis*, *Sepiella japonica*	FAO 51/57, FAO 71
Octopus Gutted, washed, skin-on	Octopodidae	*Octopus* spp., *Octopus vulgaris*, *Eledone cirrhosa*	FAO 34, FAO 51/57
Squid Whole, gutted, washed; whole, not gutted; tubes without pen, washed and skinless; rings	Loliginidae Ommastrephidae	*Loligo* spp., *Loligo edulis*, *Loligo gahi*, *Loligo opalescens*, *Illex* spp., *Illex argentinus*, *Todarodes* spp., *Todarodes pacificus*	Illex/Loligo: FAO 41 Todarodes: FAO 61

Assay principle: Catalyzed by glutamate dehydrogenase (GLDH) ammonia reacts with α-ketoglutarate and NADH to form glutamate and NAD^+. The amount of oxidized NADH is equivalent to the amount of ammonia in the sample and can be measured photometrically at 366 nm by the resulting decrease in absorbance.

Determination of pH

The pH value was measured in 20 g homogenised sample diluted 1 : 1 with deionised water using a microprocessor pH-meter (WTW 196, Wissenschaftlich-Technische Werkstätten, DEU).

Determination of free amino acids

Free amino acids (including taurine) were determined in the perchloric acid extracts after precolumn derivatization with o-phthaldialdehyde (OPA), according to a modified method of Antoine and coworkers (1999).

High-performance liquid chromatography (HPLC) was carried out by injecting 20 µL of the derivatized sample extract onto the reversed-phase column Nucleodur 100-5 C18 ec. (250 x 4 mm) (Macherey-Nagel, DEU). The separation of the 18 amino acids and the internal standard (2-aminobutyric acid) was established with gradient elution at a flow rate of 0.6 mL/min at 32 °C. The mobile phase A consisted of 15 mM Na_2HPO_4 pH 7.2/methanol/acetonitrile (80 : 18 : 2, v/v/v) and mobile phase B of 15 mM Na_2HPO_4 pH 7.2/methanol/tetrahydrofuran (20 : 76 : 4, v/v/v). Gradient conditions: 100–78 % A from 0 to 16 min; 78–70 % A from 16 to 20 min; 70–60 % A from 20 to 26 min; 60–50 % A from 26 to 28 min; stay at 50 % A for 2 min; 50–40 % A from 30 to 34 min; stay at 40 % A for 10 min; 40–0 % A from 44 to 55 min; stay at 0 % A for 5 min; return to 100 % A in 1 min. The fluorescence detection was performed at an excitation wavelength of 330 nm and an emission wavelength of 450 nm. The calibration curves were linear in the range of 0.1–1 µg/mL for each amino acid.

Determination of biogenic amines

For the determination of biogenic amines aliquots of the perchloric acid extracts were used. Analysis was carried out by means of HPLC on basis of post column derivatization with o-phthaldialdehyde (OPA) and fluorescence detection following the German official method (L 10.00.5; 1999), according to § 64 LFGB with slight modifications.

20 µL of standard solution or perchloric sample extract were injected. Eight biogenic amines and the internal standard (1.7-diaminoheptane) were well resolved on the reversed-phase column Kinetex 2.6 µm C18 100 Å (150 x 4.6 mm) (Phenomenex, USA). The separation was established with gradient elution. Eluent A consisted of 0.1 M sodium acetate and 10 mM sodium octanesulfonate (adjusted to pH 4.5) and eluent B of 0.2 M sodium acetate and 10 mM sodium octanesulfonate (pH 4.5)/acetonitrile (770 mL+230 mL). Gradient conditions: 80–60 % A from 0 to 10 min; 60-35 % A from 10 to 20 min; stay at 35 % A for 5 min; 35–0 % A from 25 to 35 min; stay at 0 % A for 10 min; 0–80 % A from 45 to 50 min. Flow rates of mobile phase and of derivatization reagent were 0.7 mL/min. A 100 cm long and 0.5 mm id peek tubing was used as reaction coil. The analytical column has been installed with the reaction coil in the column oven at 37 °C. The fluorescence detection was performed at an excitation wavelength of 330 nm and an emission wavelength of 446 nm.

Injected standards were in the range of 2–50 µg/mL for each biogenic amine.

Sensory analysis

Sensory analysis was performed for selected raw and cooked products of different quality levels. In order to characterize the criteria with an appropriate and objective terminology including scoring, twelve panellists met in several training sessions. The most relevant attributes were selected for the sensory scheme shown in Table 2.

TABLE 2: *Sensory attributes for different cephalopod products.*

	Appearance		Odour		Taste	Texture
	raw	cooked	raw	cooked	cooked	cooked
Positive criteria	specific bright glossy	specific glossy	fresh seaweed shellfish meat	specific seaweed egg protein sweet	specific sweet cooked mussel	tender elastic smooth rubbery
Negative criteria	bleached discoloured glassy	discoloured sticky	metallic fruity, soapy fishy, chlorine ammoniacal	unpleasant soapy fishy ammoniacal	metallic soapy, fishy bitter ammoniacal	mushy dry tough cartilaginous

Finally seven assessors were chosen and trained in three sessions. The evaluation was conducted by the Karlsruhe evaluation scheme (Paulus et al., 1969). The scores of a 9-point decreasing order scale correspond to different attributes and overall quality. 9 indicates perfect and optimal; 8=typical, without defects; 7=typical, with slight deviations; 6=noticeable deviations; 5=noticeable detractions, slight defects; 4=distinct defects; 3=strong defects; 2=very strong defects; 1=completely changed.

Representative samples of each product were placed in individual boilable film-type pouches and heated in a water bath (96 °C) for 15 min (whole squid, squid tubes, and cuttlefish) or up to 90 min until tender (octopus). After treatment subsamples of 20–30 g were immediately served to the panel in Petri dishes with a lid. Tap water and unsalted crackers were used as palate cleansers.

The sessions were carried out in a sensory laboratory with separate booths. Data were collected and analysed by "FIZZ" sensory software (Biosystems, FRA).

Results and discussion

Species identification

Compared to the extensive literature about species identification of teleost fish, the number of publications dealing with identification of cephalopods is relatively small (Rasmussen and Morrissey, 2009). Three mitochondrial genes, 16S rRNA (Colombo et al., 2002; Chapela et al., 2002), cytochrome oxidase subunit I (Dai et al., 2012) and cytochrome b (Santaclara et al., 2007; Espiñeira et al., 2010) have been applied to authenticate cephalopods.

In order to be able to analyse all kinds of products, a relatively short segment (208 bp) of the cytochrome b gene for cephalopod identification was used. The so-called universal primers CEFH and H15149AD did not react with

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all of the products analysed, but in most cases (22 out of 29 samples) an amplicon of expected size was obtained in an amount being sufficient for sequencing.

The quality of the sequences was not always satisfactory, possibly caused by long stretches of "T"; therefore differentiation between closely related species of the genera Illex and Sepia was not achieved using this short DNA segment (Tab. 3).

Nevertheless, for seven products the cephalopod species were successfully determined. It was also found that six products were mislabelled (Tab. 3).

According to the official German list of trade names (BLE, 2012), the naming of the genus is in case of cephalopods sufficient to fulfil the labelling requirements, e.g. *Illex* spp., *Loligo* spp., *Octopus* spp., *Sepia* spp., *Sepiola* spp., *Ommastrephes* spp.. Only a few species like *Loligo vulgaris* (common European squid, Gemeiner Kalmar), *Loligo pealei* (common American squid, Nordamerikanischer Kalmar), and *Loligo opalescens* (common Pacific squid, Kalifornischer Kalmar) must be labelled by the entire name.

TABLE 4: *pH, total volatile bases (TVB), trimethylamine (TMA), dimethylamine (DMA), trimethylamine oxide (TMAO), and ammonia contents of different cephalopod products (arithmetic mean ± standard deviation; minimum-maximum amount).*

Product (number)	pH	TVB-N (mg/100 g)	Ammonia (mg/100 g)	TMA-N (mg/100 g)	TMAO-N (mg/100 g)	DMA-N (mg/100 g)
Sepiidae (9)	7.0 ± 0.6 (6.6–8.5)	5.5 ± 3.7 (1.7–13.2)	4.0 ± 2.2 (2.2–8.4)	1.0	< 15*	< 0.5*
Loliginidae/ Ommastrephidae whole (7)	7.7 ± 1.0 (6.4–9.0)	11.1 ± 9.4 (2.1–27.8)	6.1 ± 4.3 (1.5–13.0)	< 0.5*	< 15*	< 0.5*
Loliginidae/ Ommastrephidae tubes (9)	8.7 ± 0.7 (7.6–9.7)	6.6 ± 4.5 (1.6–14.9)	4.0 ± 2.0 (1.6–6.4)	< 0.5*	< 15*	< 0.5*
Octopodidae (7)	6.8 ± 0.5 (6.3–7.5)	9.7 ± 4.0 (5.2–16.2)	6.8 ± 3.1 (3.1–7.8)	< 0.5*	< 15*	< 0.5*

*) limit of detection

The product labelled "Seepolype", *Eledona cirros,* consisted of *Octopus vulgaris.* The name *Eledona cirros* is incorrect, presumably the species *Eledone cirrhosa* belonging to the family Octopodidae has been meant. Recently, Espiñeira et al. (2010) analysed 20 commercial cephalopods products from the Spanish market by the same PCR system as used in the present study; the authors reported that 30 % of the products were labelled incorrectly.

TABLE 3: *Results of species identification. Identified species according to GeneBank are given in bold.*

Product labelled as:	Type of Product	Result of BLAST: Accession no., score, Max. ID, species (best match)
"Illex"	Tubes, gutted & skinned	EF423102, 261, 98 %, *Illex argentinus;* E423040, 255, 97 %, *Illex coindetii*
"Illex"	Tubes, gutted & skinned	EF423102, 252, 98 %, *Illex argentinus;* E423040, 235, 96 %, *Illex coindetii*
"Illex"	Tubes, gutted & skinned	EF423102, 263, 98 %, *Illex argentinus;* E423040, 252, 97 %, *Illex coindetii*
"Illex"	Heads	EF423102, 261, 98 %, *Illex argentinus;* E423040, 250, 97 %, *Illex coindetii*
Illex argentinus	Tubes, gutted & skinned	EF423102, 248, 96 %, *Illex argentinus;* E423040, 237, 95 %, *Illex coindetii*
Todarodes pacificus	Tubes, gutted & skinned	EF423142, 276, 100 % ***Todarodes pacificus***
Todarodes spp.	Rings	EF423142, 265, 99 %, ***Todarodes pacificus***
"Loligo"	Whole animal, gutted & skinned	EF423135, 198, 92 %, *Uroteuthis chinensis;* EF423134, 187, 90 %, *Loligo bleekeri**
Loligo gahi	Whole animal	EF423122, 272, 99 %, ***Loligo gahi****
"Baby octopus"	Whole animal, gutted	JN393556, 276, 100 %, ***Octopus aegina****
"Sepia"	Whole animal, gutted & skinned	AB430427, 169, 86 %, *Sepia madokai*
"Sepia"	Whole animal, gutted & skinned	AB430420, 197, 91 %, *Sepia elegans*
"Sepia"	Whole animal, gutted & skinned	AB430420, 215, 95 %, *Sepia elegans*
Sepiella japonica	Whole animal, gutted & skinned	AB675082, 183, 91 %, *Sepiella japonica*
Mislabelled products		
Sepia officinalis	Strips	AB430426, 202, 91 %, *Sepia lycidas;* FJ360586, 172, 88 %, *Sepia aculeata*
Sepia pharaonis	Whole animal, gutted & skinned	AB430426, 268, 99 %, ***Sepia lycidas***
"Sepia"	Whole animal, gutted & skinned	AB675082, 163, 85 %, *Sepiella japonica*
Loligo edulis	Whole animal, gutted	EF423135, 200, 91 %, *Uroteuthis chinensis*
Seepolyp, *Eledona cirros*	Whole animal, gutted	EF423015, 281, 99 %, ***Octopus vulgaris***
"Illex"	Tubes	EF423142, 276, 100 %, ***Todarodes pacificus***

*Species have been reclassified as *Heterololigo bleekeri, Doryteuthis gahi* and *Amphioctopus aegina,* respectively.

Nitrogenous compounds and pH value

The concentrations of the volatile amines present in the different products are shown in Table 4.

Total volatile basic nitrogen (TVB-N) mainly represents the sum of ammonia, TMA-N, DMA-N, and other basic nitrogenous compounds volatile under examination conditions. It is commonly used as criteria for assessing fish quality and known as useful spoilage indicator. The Commission Regulation (EC) No 2074/2005 (Commission of the European Communities, 2005) specifies categories for various fish species with maximum levels fixed between 25 and 35 mg TVB-N/100 g muscle flesh. No limits exist for cephalopods. Ke et al. (1984) suggested values <30 mg/ 100 g for excellent quality of squid held at 2 °C. Values between 30–45 mg TVB-N/100 g and 3–10 mg TMA-N/100 g were estimated as range for acceptable quality. However, TVB-N levels in fresh cephalopods can be much higher than in fresh fish, probably because of its higher autolytic activity (Hurtado and Borderias, 1999) and its intrinsic high levels of ammonium chloride in some species like for the giant squid *Dosidicus gigas* (Clarke et al., 1979; Sánchez-Brambila et al., 2004). TMA-N values ≤1 mg/100 g indicated high freshness (Márquez-Ríos, 2007; Tantasuttikul et al., 2011). Own results for tubes of freshly-caught Atlantic *Todarodes sagittatus* and *Loligo forbesi* were between 4.2 and 10.2 mg TVB-N/100 g and 1.6 and 2.3 mg TMA-N/100 g, respectively (not published). Higher TVB-N contents were reported for example for fresh squid *(L. plei)* with 15.7 mg/100 g (Lapa-Guimarães et al., 2005) whereas Vaz-Pires et al. (2008) analyzed 9.9 mg/100 g in fresh broadtail shortfin squid *(Illex coindetii)* and 7.7 mg/100 g in cuttlefish *(Sepia officinalis)* combined with TMA-N values of 0.1 and 0.3 mg/100 g, respectively.

All cuttlefish, octopus, and squid tubes analysed in this study had low TVB-N contents ranging between 1.6 and 16.2 mg N/100 g, and also very low TMA-N contents. It is known that the processing to skinned products like those of cuttlefish as well as to rings or strips can have a leaching effect on these components. Only within the product type illex/loligo whole skinned individuals approached the range of the proposed TVB-N limit of Ke et al. (1984), but the corresponding TMA-N levels remained low. Such samples had also higher ammonia contents. In various studies ammonia has been shown to be an excellent indicator of squid quality (Leblanc and Gill, 1984; Paarup et al., 2002), but skinning was found to lower the formation of ammonia in squid during ice storage (Sungsri-in et al., 2011).

In this study ammonia did not increase with the basic volatiles to the same extent.

TMAO is an important precursor of formaldehyde and dimethylamine. These substances are responsible for texture alterations in frozen cephalopods. In heated products they can cause adverse effects on human health (Zhu et al., 2012). TMAO-N values were low (< 15 mg/100 g). Only in a few studies TMAO was investigated. Much higher contents were reported in *Sepia officinalis* and several squid species of the Loligo, Illex, and Todarodes genera (80–1683 mg TMAO-N/100 g) (Hebard et al., 1982; Boumpalos and Lougovois, 2005; Lapa-Guimarães et al., 2005; Kani et al., 2008). Own results for tubes of freshly-caught Atlantic *Todarodes sagittatus* and *Loligo forbesi* were between 160 and 230 mg/100 g (not published). Reasons for these large differences are unknown (Ruíz-Capillas et al., 2002; Kani et al., 2007).

The dimethylamine concentration was low in all products.

pH values

The pH values in the mantle tissue of all samples varied between 6.3 and 9.0 and were highest in the squid tubes (Loliginidae/Ommastrephidae). For fresh cephalopods initial pH values between 6.0 and 7.0 are published (Ohashi et al., 1991; Marquiz-Rios et al., 2007). Sotelo and Rehbein (2000) reported that muscle flesh might contain buffering components. An increase in pH is normally associated with spoilage and the formation of volatile bases as evidenced by the increase of TVB, TMA and ammonia. In our study high pH values did not reflect a spoilage process and can be perceived as a result of processing and presumably by the use of water binding additives.

In tubes of *Illex argentinus* with the maximum pH of 9.7 TVB-N and NH_3 values of 1.91 mg/100 g and 1.8 mg/100 g were encountered, respectively. The sensory panel found no reason to devalue this product.

Free amino acids

The total content of free amino acids differed considerably from species to species and between single products of the same species (Tab. 5, Fig. 1). In all cephalopods tested the mean total amounts ranged from 0.2 to 1.0 g/100 g muscular tissue. *Loligo* spp. showed the highest pool of free amino acids. These contents are comparable with the values in many fish species (own results (not published); Haard, 1992).

The high levels of taurine were characteristic for all samples. Taurine is an amino sulfonic acid that is present in free form only in animal tissues, but is never incorporated into proteins. Humans have a limited capability of biosynthesising taurine. It is of importance for many physiological processes. To give an example, taurine is beneficial

TABLE 5: *Mean contents of free amino acids in different cephalopod products (mg/g muscular tissue; arithmetic mean ± standard deviation).*

Product (number)	Cuttlefish		Squid					Octopus
			Loligo		Illex		Todarodes	
	whole (6)	tubes/strips (2/1)	whole (4)	tubes (4)	whole (1)	tubes/rings (7/1)	tubes/rings (1/1)	whole (8)
	mg/g muscular tissue							
Indispensable amino acids								
Histidine	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.05	0.11 ± 0.07	0.02	0.07 ± 0.06	0.15 ± 0.11	0.05 ± 0.02
Isoleucine	0.06 ± 0.02	0.04 ± 0.02	0.12 ± 0.08	0.15 ± 0.08	0.03	0.05 ± 0.06	0.09 ± 0.01	0.09 ± 0.03
Leucine	0.12 ± 0.04	0.07 ± 0.03	0.25 ± 0.17	0.36 ± 0.16	0.05	0.10 ± 0.11	0.15 ± 0.01	0.19 ± 0.06
Lysine	0.15 ± 0.03	0.11 ± 0.04	0.32 ± 0.23	0.36 ± 0.21	0.07	0.12 ± 0.11	0.19 ± 0.04	0.25 ± 0.09
Methionine	0.07 ± 0.02	0.04 ± 0.02	0.15 ± 0.11	0.21 ± 0.10	0.03	0.05 ± 0.06	0.09 ± 0.03	0.09 ± 0.03
Phenylalanine/Tryptophan*	0.07 ± 0.02	0.05 ± 0.01	0.18 ± 0,10	0.25 ± 0.10	0.03	0.06 ± 0.06	0.09 ± 0.01	0.12 ± 0.03
Threonine	0.07 ± 0.02	0.04 ± 0.02	0.13 ± 0.08	0.16 ± 0.08	0.03	0.06 ± 0.06	0.09 ± 0.01	0.10 ± 0.02
Valine	0.08 ± 0.02	0.05 ± 0.02	0.15 ± 0.10	0.20 ± 0.11	0.04	0.07 ± 0.07	0.09 ± 0	0.13 ± 0.03
Conditionally indispensable amino acids								
Arginine	0.58 ± 0.27	0.17 ± 0.15	0.72 ± 0.59	1.00 ± 0.81	0.29	0.29 ± 0.20	0.53 ± 0.13	0.57 ± 0.34
Taurine	1.26 ± 0.52	0.60 ± 0.47	3.89 ± 4.27	4.47 ± 4.68	0.58	0.98 ± 1.35	0.73 ± 0.42	3.31 ± 2.57
Tyrosine	0.07 ± 0.02	0.04 ± 0.01	0.15 ± 0.08	0.20 ± 0.07	0.03	0.05 ± 0.06	0.08 ± 0.02	0.09 ± 0.04
Dispensable amino acids								
Alanine	0.15 ± 0.05	0.08 ± 0.04	0.58 ± 0.46	0.76 ± 0.52	0.14	0.20 ± 0.17	0.36 ± 0.08	0.30 ± 0.14
Asparagine	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	nd	nd	nd	nd	0.04 ± 0.03
Aspartic acid	0.03 ± 0.02	0.03 ± 0.01	0.13 ± 0.10	0.15 ± 0.09	nd	0.03 ± 0.04	0.02 ± 0.02	0.08 ± 0.03
Glutamic acid	0.17 ± 0.04	0.10 ± 0.04	0.36 ± 0.27	0.45 ± 0.27	0.08	0.15 ± 0.14	0.17 ± 0	0.28 ± 0.08
Glycine	0.06 ± 0.02	0.04 ± 0.02	0.40 ± 0.27	0.51 ± 0.20	0.04	0.09 ± 0.10	0.15 ± 0.06	0.08 ± 0.02
Ornithine	0.10 ± 0.03	0.12 ± 0.11	0.28 ± 0.25	0.35 ± 0.29	0.22	0.09 ± 0.06	0.13 ± 0.04	0.16 ± 0.11
Serine	0.10 ± 0.04	0.05 ± 0.02	0.15 ± 0.09	0.19 ± 0.09	0.04	0.08 ± 0.08	0.09 ± 0.02	0.13 ± 0.03
Total mg/g	**3.19 ± 1.06**	**1.68 ± 0.91**	**8.01 ± 7.13**	**9.86 ± 7.45**	**1.72**	**2.53 ± 2.53**	**3.17 ± 0.33**	**6.04 ± 3.03**

nd = < limit of detection (0,01 mg/g); *Phenylalanine and tryptophan could not be separated

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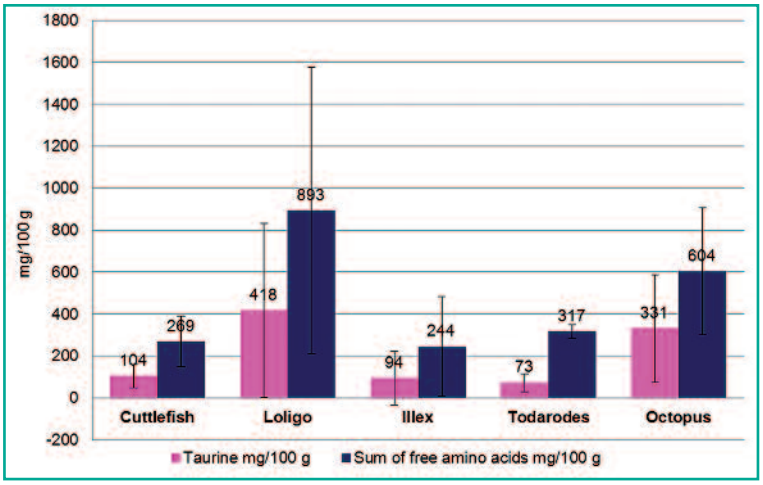

FIGURE 1: *Comparison of the average contents (mg/100 g wet weight; arithmetic mean ± standard deviation) of free amino acids and taurine in various cephalopod products.*

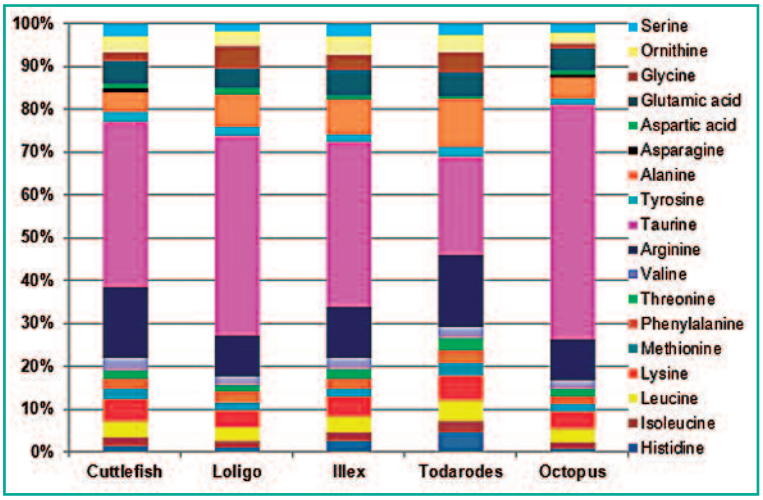

FIGURE 2: *Average percentage composition of free amino acids in different cephalopod products.*

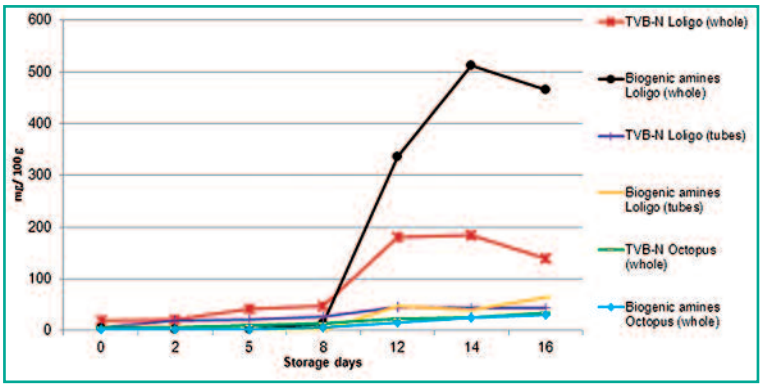

FIGURE 3: *Total volatile basic nitrogen (mg TVB-N /100 g) and biogenic amines (total amounts; mg/100 g) in selected cephalopods during storage in a refrigerator.*

for cardiovascular health, reduces blood cholesterol values, and has antioxidant properties (Undeland, 2009).

Figure 2 shows the average percentage composition of free amino acids which varied depending on the species. Arginine and alanine were also found in relatively large amounts in all samples. This is in accordance with other studies of molluscs (Ruiz-Capillas et al., 2002). The conditionally indispensable amino acid arginine is used for the synthesis of several biological important molecules (Undeland, 2009). It can be easily converted by microorganisms into the biogenic amine agmatine. Alanine contributes to the sweet taste of cephalopods. Because of the small amounts of histidine in all samples, notable amounts of histamine in stored cephalopods are not expected.

In addition, *Loligo* spp. showed higher levels of glutamic acid, glycine, ornithine, leucine, lysine, and phenylalanine/tryptophan compared with the other species.

Biogenic amines

The concentrations of the biogenic amines present in the different cephalopod products are summarized in Table 6. Only in a few cases were agmatine, putrescine, spermine and tyramine detected in small amounts (excluding one whole eviscerated octopus sample). On the other hand cadaverine, histamine, spermidine, and tryptamine could not be detected in any product.

This is in agreement with other investigations (Yamanaka et al., 1987; Paarup et al., 2002; Zhao et al., 2007; Kim et al., 2009) which found only negligible biogenic amine contents in fresh squid samples.

Sensory evaluation

The sensory characteristics were mainly described as fresh, like seaweed, with raw/cooked shellfish meat odour and taste, and as elastic (Tab. 2). The detection of "soapy" and "fishy" components and of a "dry", "tough" and "cartilaginous" texture was associated with a low quality. The majority of samples were rated either good or fair with scores between 7 and 5. All cuttlefish samples belonged to this group which also had really low amounts of volatile bases. In products with TVB-N values >20 mg/100 g undesired attributes as described in Table 2 became evident and did not comply with an acceptable quality level. Values >15 mg/100 g showed first signs of such changes. None of the products was rated under the limit of acceptability (mean score <4). The sensory ratings of the panel members were, however, not consistent and revealed a considerable quality variation of individual samples within a package, resulting in high standard variation which did not permit a meaningful statistical analysis.

Storage of thawed samples in a refrigerator at 4 °C

In preliminary frozen storage experiments with cephalopods (results are not published) whole gutted loligo and octopus showed more potential for quality deterioration compared to cuttlefish tubes and were selected for the storage of thawed products in a refrigerator at 4 °C. The results are presented in Table 7. Most information is docu-

TABLE 6: *Biogenic amines in the tested cephalopod products (mg/kg muscular tissue; minimum–maximum amount.*

Product (number)	Cuttlefish		Squid					Octopus
			Loligo		Illex		Todarodes	
	whole (6)	tubes/strips (2/1)	whole (4)	tubes (4)	whole (1)	tubes/rings (7/1)	tubes/rings (1/1)	whole (8)
	mg/g muscular tissue							
Biogenic Amine								
Agmatine	< 20–24.4	< 20	< 20	< 20–45.4	< 20	< 20	< 20	< 20–122.0
Putrescine	< 20–26.8	< 20–28.2	< 20	< 20	< 20–26.7	< 20	< 20	< 20–25.9
Spermine	< 20	< 20	< 20–35.6	< 20–36.4	< 20	< 20	< 20	< 20–23.4
Tyramine	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20–68.0

Cadaverine, Histamine, Spermidine, Tryptamine: all results <20 mg/kg muscular tissue = limit of determination

TABLE 7: *Evaluation of total volatile basic nitrogen (TVB-N), biogenic amines, and sensory assessment (9-point scale) in selected thawed cephalopod products during storage at 4 °C in a refrigerator (pooled samples of one product with the same lot number).*

Loligo* (whole, ungutted)

Storage days	***0***	***2***	***5***	***8***	***12***	***14***	***16***
Sensory score	8.0	7.2	6.4	4.5	rejected	rejected	rejected
TVB-N (mg/100 g)	18.8	21.2	42.5	46.6	180.2	184.0	140.0
Agmatine (mg/kg)	< 20	< 20	< 20	**67.4**	**1435.0**	**1863.9**	**97.3**
Cadaverine (mg/kg)	< 20	< 20	< 20	**28.9**	**857.2**	**1373.3**	**1645.1**
Histamine (mg/kg)	< 20	< 20	< 20	< 20	< 20	**247.3**	**126.3**
Putrescine (mg/kg)	< 20	< 20	< 20	< 20	**139.4**	**302.4**	**1055.3**
Spermidine (mg/kg)	< 20	< 20	< 20	< 20	**167.3**	**288.3**	**433.5**
Spermine (mg/kg)	**33.6**	**25.8**	**32.2**	**34.6**	**38.4**	**37.6**	**41.5**
Tryptamine (mg/kg)	< 20	< 20	< 20	< 20	< 20	**29.7**	**39.3**
Tyramine (mg/kg)	< 20	< 20	< 20	< 20	**733.8**	**974.0**	**1222.2**
Total (mg/kg)	**33.6**	**25.8**	**32.2**	**130.9**	**3371.1**	**5116.5**	**4660.5**

Loligo* (tubes)

Storage days	***0***	***2***	***5***	***8***	***12***	***14***	***16***
Sensory score	7.2	7.0	6.2	5.5	rejected	rejected	rejected
TVB-N (mg/100 g)	5.9	18.7	21.3	27.5	45.1	43.4	42.8
Agmatine (mg/kg)	**22.5**	**20.7**	< 20	< 20	< 20	< 20	< 20
Cadaverine (mg/kg)	< 20	< 20	< 20	< 20	**24.6**	**25.5**	**37.7**
Putrescine (mg/kg)	< 20	< 20	< 20	< 20	**195.0**	**166.7**	**261.3**
Tyramine (mg/kg)	< 20	< 20	< 20	< 20	**260.9**	**212.2**	**335.9**
Total (mg/kg)	**22.5**	**20.7**	**< 20**	**< 20**	**480.5**	**404.4**	**634.9**

Histamine, spermidine, spermine tryptamine: all results < 20 mg/kg muscular tissue

Octopus* (whole, gutted)

Storage days	***0***	***2***	***5***	***8***	***12***	***14***	***16***
Sensory score	7.6	7.2	6.8	6.3	4.5	4.1	rejected
TVB-N (mg/100 g)	5.5	6.2	10.0	13.4	23.1	24.6	35.0
Cadaverine (mg/kg)	< 20	< 20	< 20	**30.6**	**50.2**	**122.7**	**105.7**
Putrescine (mg/kg)	**27.2**	**22.8**	**26.8**	**27.9**	**54.1**	**74.4**	**131.8**
Tyramine (mg/kg)	< 20	< 20	< 20	< 20	**42.4**	**53.3**	**74.6**
Total (mg/kg)	**27.2**	**22.8**	**26.8**	**58.5**	**146.7**	**250.4**	**312.1**

Agmatine, histamine, spermidine, spermine tryptamine: all results < 20 mg/kg muscular tissue

20 mg/kg = limit of determination of each biogenic amine, * product name

mented for shelf life of cephalopods in ice with maximum limits between 8 and 14 days (Paarup et al., 2002; Vaz-Pires, 2004; Ganesan et al., 2005; Vaz-Pires and Seixas, 2006). Compared to this, storage life at 4 °C was only slightly shorter. After a slow increase up to day eight, the accumulation became very intensive. TVB-N and biogenic amines (Fig. 3) in whole loligo increased much more rapidly than in skinned loligo tubes, indicating that the former underwent the decomposition at a higher rate. Fishy and ammonia attributes contributed to these changes. Again it was found that additional processing steps like skinning and eviscerating can lower the increase rate of undesired components (Lakshmanan et al., 1993). At storage begin the sensory scores for whole loligo were slightly better than for loligo tubes, but the increasing formation of basic volatiles resulted in unpleasant odours and tastes. The end of a commercial acceptable storage life is defined when off-flavours start to develop (Fish Technology Department, 1996). Considering this aspect, the limit of acceptability for whole loligo and tubes was up to five days after defrosting and maintaining the cold chain.

The shelf life of thawed octopuses tended to be slightly longer, combined with a moderate increase of TVB-N and biogenic amines. TVB-N remained below 30 mg/100 g at day of rejection. In order to make a comparison: fresh stored Common octopus *(Octopus vulgaris)* became unacceptable at day eight in ice (Barbosa and Vaz-Pires, 2004).

Some free amino acids result in the rapid formation of biogenic amines during microbial spoilage of food. Free arginine can be ea-

sily decarboxylated by bacterial enzymes to agmatine. Agmatine can be broken down into urea and putrescine which can be further converted in spermidine and spermine. Putrescine can also be formed from ornithine decarboxylation (Vaz-Pires et al., 2008).

Agmatine, cadaverine, putrescine, and tryptamine were frequently detected in stored cephalopods. Besides, spermine, spermidine, and tyramine were analysed in squid and cuttlefish, too (Yamanaka et al., 1987; Paarup et al., 2002; Zhao et al., 2007; Vaz-Pires et al., 2008).

In the opinion of Yamanaka et al. (1987) and Paarup et al. (2002) agmatine is suitable as an index for freshness of common squid. Small amounts could be already detected in fresh squid samples. During storage at 0 °C, 3.5 °C, and 15 °C the concentration of agmatine exceeded 300 mg/kg at the beginning of decomposition and reached 400 mg/kg at the stage of advanced decomposition. On the other hand, twelve-day storage of a sepia sample in a refrigerator lead to an obvious increase of cadaverine, putrescine and tyramine, whereas no histamine and agmatine could be detected (Fiedler, 2011).

According to Resch and Miller (2009), the single contents as well as the sum of cadaverine, putrescine, tyramine, and histamine in cephalopods correlate with the sensory judgment. Values below 60 mg/kg for the sum of cadaverine, putrescine, tyramine, and histamine are suggested for cephalopods of good quality, whereas amounts greater than 100 mg/kg indicate unacceptable quality.

However, agmatine was not suitable as freshness indicator in this study. The concentrations of biogenic amines present in the thawed samples stored in a refrigerator at 4 °C are given in Table 7. Initially their contents were negligible in all samples. After eight to twelve days of storage the contents of all (whole loligo) or at least some biogenic amines increased clearly which was accompanied by adverse sensory effects.

During storage time all biogenic amines in whole ungutted loligo samples increased much more than in loligo tubes or whole gutted octopus. Agmatine, cadaverine, and tyramine were the mostly formed biogenic amines in whole loligo samples. Because the rapid increase of agmatine was limited to whole loligo, this substance seemed not to be suitable as general acceptable freshness indicator.

Biogenic amines other than cadaverine, putrescine, and tyramine were not detected in decomposed loligo tubes and whole octopus.

In accordance with Resch and Miller (2009), the sum of cadaverine, putrescine, tyramine and histamine was below 60 mg/kg in all samples of acceptable quality and greater than 100 mg/kg in products of unacceptable quality.

Conclusion

The study of various cephalopod products showed in general a satisfactory quality that varied within some packages, where a distinctly poorer quality of some individuals could be observed. Mislabelling of species was detected in a number of products, and this topic should be studied further.

Resch and Miller (2009) suggest a limit for TVB-N of 25 mg/100 g for good quality products and >30 mg/100 g for unfit and rejected products. In our study high TVB-N values >20 mg/100 g correlated with poor sensory quality attributes, but not with a fixed limit for rejection. The chemical and sensory degradation processes in whole cephalopod products probably resulted from the presence of the intestinal organs inside the mantel cavity.

TVB-N, TMA-N and TMAO-N were not found to represent reliable and fixed acceptability indicators for frozen cephalopods.

Agmatine is not suitable as freshness indicator for cephalopods. During storage in a refrigerator mainly cadaverine, putrescine, and tyramine increased in all products.

The quality of the cephalopod which reaches the consumer is generally dependent on processing history. Taking this into account, the storage of thawed products should be limited to two-three days.

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