CREDIT SEMINAR
ON
QUALITY ISSUES ASSOCIATED WITH CEPHALOPODS

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Quality issues associated with cephalopods

1. Introduction

Cephalopod is a member of the molluscan class, Cephalopoda (Greek: "head-feet"). Cephalopods are exclusively marine animals characterized by bilateral body symmetry, a prominent head, and a set of arms or tentacles (muscular hydrostats) modified from the primitive molluscan foot. The study of cephalopods is a branch of malacology known as "Teuthology" Fishermen sometimes call them "ink fish", referring to their common ability to squirt ink. They became dominant during the Ordovician period, represented by primitive nautiloids.

The class cephalopoda contains two, distantly related, subclasses: Coleoidea, which includes octopuses, squid, cuttlefish and Nautiloidea, represented by *Nautilus* and *Allonautilus*. In the Coleoidea, the molluscan shell has been internalized or is absent, whereas in the Nautiloidea, the external shell remains. So far 800 living species of cephalopods have been identified.

2. Classification

Phylum : Mollusca

Class : Cephalopoda

Subclass : Celeoidea

Orders (Commercially important)

1. **Tathoidea** (squid)
2. **Sepioidea** (cuttlefish)
3. **Octopoda** (octopus)

2.1. Squid

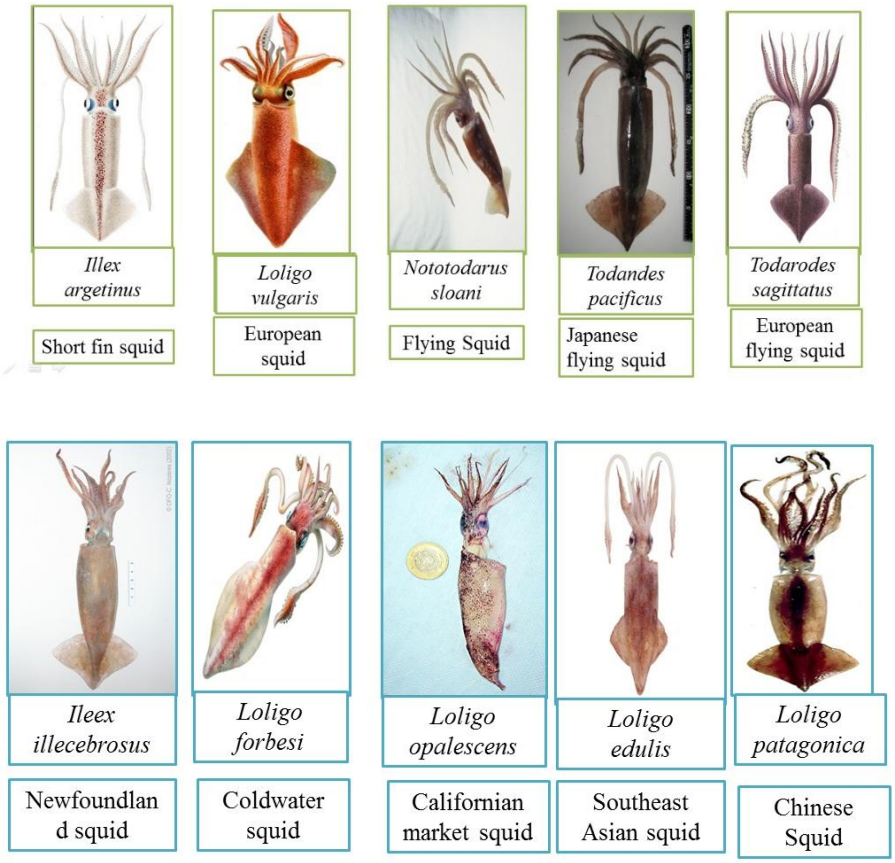
Squids are cephalopods of the order Tathoidea, which comprise around 300 species. Like all other cephalopods, squids have a distinct head, bilateral symmetry, a mantle, and arms. Squids, have eight arms arranged in pairs of which two are usually longer tentacles. They are

strong swimmers and certain species can "fly" for short distances out of the water.They live in the open sea and have the largest brain responsible for vision.There are ten commercially important squid species.

They are

- Newfoundland squid (*Ileex illecebrosus)*
- Coldwater squid (*Loligo forbesi)*
- Californian market squid (*Loligo opalescens)*
- Southeast Asian squid (*Loligo edulis)*
- Chinese squid (*Loligo patagonica)*
- Short fin squid (*Illex argetinus)*
- European squid (*Loligo vulgaris)*
- Flying squid (*Nototo darussloani)*
- Japanese flying squid (*Todandes pacificus)*
- European flying squid (*Todarodes sagi)*
- Indian squid (*Loligo duvauceli*)

Figure 1. Commercially important squid species



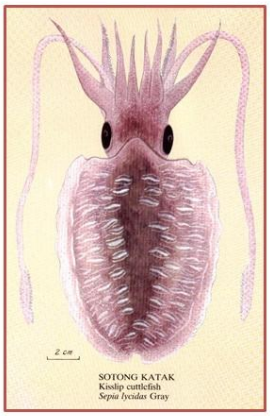
2.2. Cuttlefish

Cuttlefish are marine animals of the order Sepioidea..'Cuttle' refers to their unique internal shell, "the cuttlebone". Cuttlefish eat small mollusks, crabs, shrimp, fish, octopuses, worms, etc. Their predators include dolphins, sharks, fish, seals, seabirds, and other larger cuttlefish. Their life expectancy is about one to two years. Recent studies indicate that cuttlefish are among the most intelligent invertebrates. They swim near the ground and live between plants.They have smaller brain area than squid. There are several commercially important cuttlefish species

They are

- European common cuttlefish (*Sepia officinalis)*
- Golden cuttlefish (*Sepia esculenta)*
- Seiches (*Sepiidae keferstein*)
- Flamboyant Cuttlefish (*Metasepia pfefferi*)
- Paintpot cuttlefish (*Metasepia tullbergi*)

Figure 2. Commercially important cuttlefish species



Sepia officinalis

European common cuttlefish



Sepia esculenta

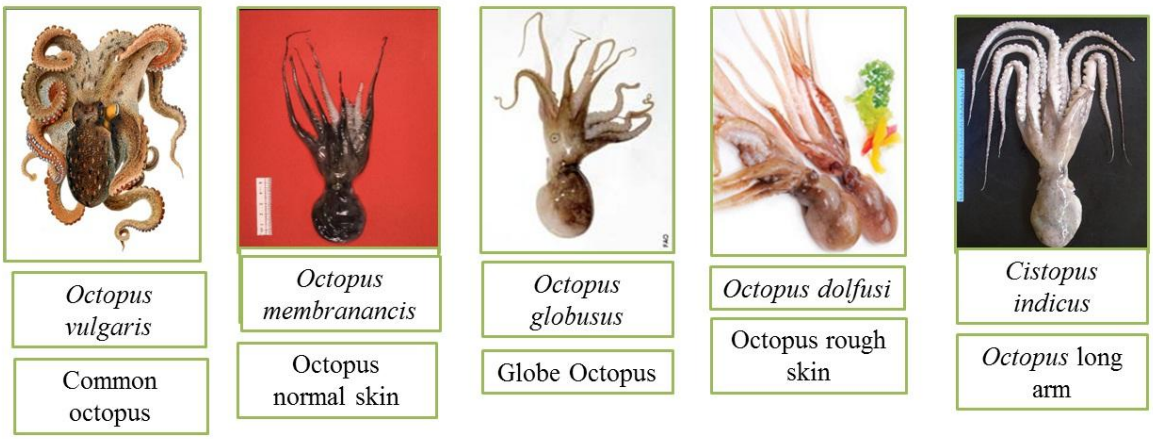
Golden cuttlefish

2.3. Octopus

The octopus is a cephalopod mollusc of the order, Octopoda. They have two eyes and four pairs of arms and, like other cephalopods, they are bilaterally symmetric. They have a hard beak, with its mouth at the center point of the arms. They have no internal or external skeleton allowing them to squeeze through tight places. They are among the most intelligent and behaviorally flexible of all invertebrates. They inhabit many diverse regions of the ocean, including coral reefs, pelagic waters, and the ocean floor. They have numerous strategies for defending themselves against predators, including the expulsion of ink, the use of camouflage and deimatic displays, their ability to jet quickly through the water, and their ability to hide. An octopus trails its eight arms behind it as it swims.They live on the ground and hides most of the time in holes or between rocks.They have a very small brain but have developed sense of touch in tentacles. There are five commercial important octopus species. They are

- Common octopus (*Octopus vulgaris)*
- Octopus normal skin (*Octopus membranancis)*
- Globe octopus (*Octopus globusus)*
- Octopus rough skin (*Octopus dolfusi)*
- Octopus long arm (*Cistopusindicus)*
- Tehuelche octopus (*Octopus tehuelchus*)
- Gloomy octopus (*Octopus tetricus*)
- Whip arm octopus (*Octopus variabilis*)
- Atlantic banded octopus (*Octopus zonatus*)

Figure 3. Commercially important cuttlefish species



3. Cephalopods as food

Spain is the leading country in cephalopod market, followed by Japan and Korea. The other major markets for cephalopods are Italy, Hong-Kong, France, Germany, Greece and Portugal. Cephalopods have received increasing importance as a marine fishery resource because of their high export value in India. They account for 4-5% (1.13 lakh tonnes) of the total Indian marine fish landings. Of the total cephalopod production, 82% is from west coast. In 2004 the total cephalopod production decreased by 3.85%.

The percentage of the edible portion of cephalopods is exceptionally high, between 60 and 80% of total weight, depending on species, size of specimen and sexual maturity, whereas in fish the percentage is only 40-70% (Sikorski and Kolodziejska, 1986).

Octopus is generally popular in Japan and Korea, but less common in South East Asia. In North America and Europe, the people consume cephalopods as frozen breaded rings .In South East Asia and Mediterranean coast they are commonly used in household preparation, as dried squid is part of their daily diet.

Many species of squids are popular as food in cuisines as diverse as Chinese, Greek, Turkish, English, American, Japanese, Portuguese, Italian, Spanish, Korean, Vietnamese and Filipino. In English-speaking countries, squid as food is often marketed using the Italian word "*calamari"*. Squid are found abundantly in certain areas, and provide large catches for fisheries. The body can be stuffed whole, cut into flat pieces, or sliced into rings. The arms, tentacles, and ink are also edible; in fact, the only parts not eaten are the beak and gladius (pen). Squid is a good food source for zinc and manganese, and high in copper, selenium, vitamin B_{12}, and riboflavin

Cuttlefish are caught for food in the Mediterranean, East Asia, the English Channel, and elsewhere. In East Asia, dried, shredded cuttlefish is a popular snack food. Cuttlefish are popular in Italy. For example, in the northeast, they are used in *risotto al nero di seppia* 'risotto with cuttlefish ink', also found in Croatia as 'black risotto'. Spanish cuisine, especially that of the coastal regions, uses cuttlefish and squid ink in a variety of dishes. Breaded and deep-fried cuttlefish is a popular dish in Andalusia. In Portugal, cuttlefish is present in many popular dishes. *Chocos com tinta* 'cuttlefish in black ink', for example, is grilled cuttlefish in a sauce of

its own ink. Cuttlefish is also popular in the region of Setúbal, where it is served as deep-fried strips or in a variant of *feijoada*, with red kidney beans. Black pasta is often made using cuttlefish ink.

Octopus is considered as a delicacy in many countries since it has a sweet, mild flavor. It is a source of easily digestible protein with a high nutritional value, which is necessary for the building of new cells in the human body. In addition to that, it contains a spectrum of important vitamins such as thiamin, riboflavin and microelements. It is an excellent source of iron, selenium and vitamin B12, good source of zinc and omega-3 fatty acids, derived from EPA and DHA.

In the octopus species both tentacles or arms and mantle are edible. It is consumed in different form around the world. Number of products are being prepared from octopus all around the world viz., octopus in red wine, pickled octopus, cooking octopus in Japanese style, barbecued stuffed baby octopus, baby octopus, octopus salad, baby octopus with tomato and black olives, char-grilled baby octopus, octopus pie, octopus cooked in wine, octopus with smoked paprika, fried octopus, braised octopus, Greek casseroled octopus, octopus fritters.

4. Biochemical composition of cephalopods

Cephalopods have high edible parts (80-85%) compared to fish (40-75%), elasmobranches (25%) and crustaceans (40-45%). The biochemical composition of three cephalopods (squid, cuttlefish and octopus) did not show significance differences in respect to moisture, protein, ash, fat and carbohydrate contents. Table 1 shows the biochemical composition of cephalopods

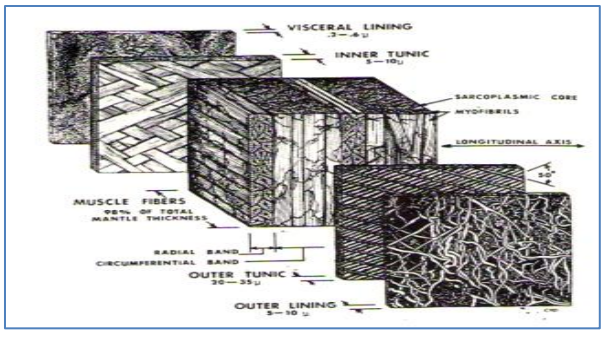
Table 1. Biochemical composition of cephalopods

Species	Moisture	protein	Ash	Fat	Carbohydrate
Squid	81%	16%	2%	1%	1%
Cuttlefish	81%	14%	2%	3%	1%
Octopus	84%	15%	0.5%	1%	0.6%

4. Uniqueness of muscle structure

Cephalopods have unique muscle structure compared to finfish and elasmobranches. The characteristics feature of the squid muscle is their very small size. The squid mantle is composed of five layers of tissue. The middle layer contains the muscle tissue with the muscle fibers grouped in bands that are arranged orthogonally. Circumferential muscle bands comprise fibers running about the entire circumference of the mantle cone. Radial bands comprise fibers that connect two tunics of connective tissue. Regardless of their orientatio0n all the muscle fibers are thin, approximately 3.5µm in diameter (Lluch *et al*., 2001). The elongated cells of myofibrils are surrounding a central core, which contains sarcoplasmic proteins. The muscle fibers are obliquely striated and covered with a thin sarcolemma (Llorca *et al*., 2007). The muscle layer is make up about 98% of the total thickness of the mantle .The muscle layer is covered on each side by layers of connective tissue called outer and inner tunics. These tunics have collagenous fibers. Squid meat has three times more collagen than finfish. (Guthworth *et al*., 1982). The outer side of each tunic is covered by linings. The lining about the outer tunics is known as outer lining. The lining in the inner surface of the mantle thesis is called "visceral lining" which consists of non-fibrous tissue (Otwell and Hamann, 1979). Figure 4 shows the Arrangement of muscle cells in cephalopods.

Figure 4. Arrangement of muscle cells in cephalopods



4.2. Unique muscle protein

The muscle protein of the cephalopods is different in their composition when compared to teleost and elasmobranchs. Squid meat is highly soluble because of the presence of myofibrillarproteins. Sarcoplasmic (water soluble) proteins constitute 55% in squid muscle but in the case of fish, it is only 25%. Myofibrillar (salt soluble) proteins contain myosin, actomyosin,

actin and paramyosin. Actomyosin occupies more than 60% of the total proteins of the squid muscle and it is the dominantmyofibrillar protein. The actomyosin of the squid muscle is extractable with water and hence known as "M-actomyosin". Paramyosin is also present in appreciably high amount in cephalopods, constituting about 14% of myofibrillar protein. Squid myosin is quickly digestible by trypsin than fish.

4.3. Unique NPN compounds

The NPN fraction constitutes 20-25% of the total nitrogen contents in cephalopods and crustaceans while it is low in teleosts (9-18%) and high in elasmobranchs (33-38%). Among the NPN fractions, free aminoacids constitutes 50% and remaining were peptides (15%), TMAO (15%) and octopine (10%). Table 2 shows the compounds present in NPN fractions of different marine organisms

Table 2. NPN fraction of different marine organisms

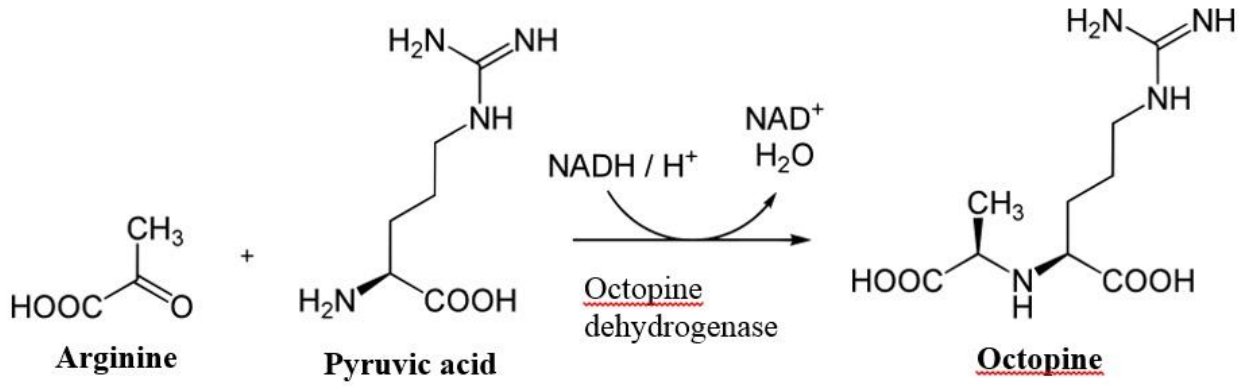
NPN compounds	Teleosts	Elasmobranches	Crustacean	Cephalopods
Free amino acids	25	5	65	**50**
Peptides	5	5	15	**15**
Nucleotides	15	5	5	**5**
Creatine compounds	50	10	0	**0**
TMAO	15	20	5	**15**
Urea	0	55	0	**0**
Betaines	0	0	10	**5**
Ammonia and amides	5	0	0	**0**
Octopine	0	0	0	**10**

Octopine is a derivative of the amino acids viz arginine and alanine. It was the first member of the class of chemical compounds known as opines to be discovered. Octopine gets its name from octopus, which was first isolated in 1927. Octopine is formed by reductive condensation of pyruvic acid and arginine through the action of the NADH-dependent enzyme

octopine dehydrogenase (ODH). The reaction is reversible so that pyruvic acid and arginine can be regenerated.

Glycine, alanine, proline, arginine, serine, threonine and octopine are the major aminoacids present in the cephalopods. Among that, octopine (878mg/100g) and proline (1090 mg/100g) are dominant free aminoacids. Taurine (957mg/100g), betaine (743mg/100g) and homorine (232mg/100g) are the major quartnary ammonium bases present in the cephalopods (Suyama and Kobayashi, 1980). Figure 5 shows the formation pathway of octopine.

Figure 5. Pathway of octopine formation



4.4. Unique lipid profile

Phospholipids are the major lipid component in squid mantle comprising about 97%, of which cholesterol constitutes 4%. Triglycerides, free fatty acids and sterol esters were detected in the mantle in trace amounts only, while in the liver, triglycerides were the major components and the rest were phospholipids, free fatty acids and sterol esters. Table 3 shows fatty acid composition of cephalopods in comparison with fish.

Table 3. Fatty acid composition of cephalopods

FA	Cuttlefish	Octopus	Squid	Fish
C14:0	1.20	1.50	2.10	2.24
C15:0	0.70	0.23	0.50	1.15
C16:0	**20.30**	**15.86**	**22.1**	30.83
C16:1	0.80	0.48	0.10	2.14

C17:0	1.70	1.40	1.00	1.56
C17:1	0.30	0.08	0.00	0.77
C18:0	11	6.59	9.10	0.27
C18:1	4.30	3.63	0.50	38.20
C18:2	0.60	0.43	0.10	0.11
C18:3	0.10	0.01	0.00	0.03
C18:4	0.00	0.01	0.00	0.00
C20:0	0.00	0.01	0.30	0.00
C20:1	3.60	0.00	3.50	0.02
C20:2	0.50	0.26	0.30	0.00
C20:3	0.30	0.00	0.60	0.01
C20:5	7.60	20.08	14.50	1.45
C22:0	0.00	0.01	0.00	0.00
C22:1	0.00	0.00	0.00	0.00
C22:5	3.90	7.50	0.60	1.04
C22:6	**28.30**	**26.32**	**29.30**	8.80
C24:0	0.00	0.77	0.20	0.00

The fatty acid composition of cephalopod examined should that Very high omega 3 fatty acids are present in cephalopods (38-45%) compared to fish. The DHA accounts for more than 26% in cephalopods and the EPA is 2-20%.Yamada and Hayashi (1975). Unlike other marine animals, the poly-enoic acids like EPA (20:5) and DHA (22:6) are the predominant.

4.5. Unique taste / flavor compounds

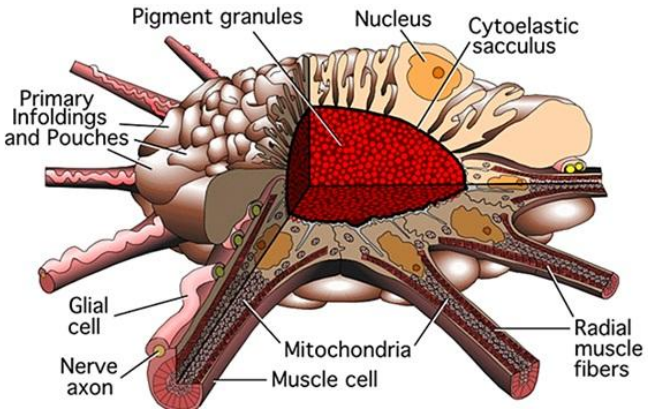
Water soluble free amino acids and other water soluble compounds are responsible for the characteristics flavor and taste of the cephalopod meat. These substances are also used by invading bacteria as a result, ammonia and putritive smell appear and consequently the quality deteriorates. Glycine, proline and alanine are the three free amino acids responsible for superior taste of cephalopod meat. Trimethylamine oxide compounds are responsible for the peculiar taste of squid meat. Proline occurs in sufficiently high concentrations and contributes to the sweetness

of cuttlefish. (Amano and Bito, 1951). Sulfur containing amines are found responsible for the flavor of boiled squid and the inosinic acid gives taste to the dried squid (Yamanishi, *et al*., 1956)

4.6. Unique chromatophores

Chromatophore is a container for the color particles mainly pigments. It consists of a cell with an expandable membrane as a wall. Several muscle fibres are radically attached to the membrane and each fiber is connected with a nerve which links the muscle fibre via the brain with the eyes. They signal to the brain the required color and the brain monitors with high speed via the muscle fibres, the colour change. If the animal is on the light surface, the muscles attached to the cell wall are relaxed, the chromatophores appear small and round or elliptical and the pigment covers only a small area. The skin of the animal thus appears light. When the animal moves onto a dark background, the eyes signal via brain, the shade of the colour. Instantly, the muscle contract and expand the walls of the chromatophores in all directions. Each chromatophores cell can now cover up to 60 times the surface covered by small, contracted cell. The pigment flows to the periphery of the expanded cell covering whole area. The skin now becomes dark. Some cephalopods have up to three differently colored pigments in the store. Black, yellow and red pigments are most frequently found in cephalopods. The differently colored pigments are arranged in different layers of the skin. It is still not fully understood how they are arranged in different layers. The different degree of expansion of the chromatophores is controlled in order to achieve the colour shades required to match the environment. Figure 6 shows internal structure of chromatophore.

Figure 6. Structure of chromatophore



After death, the muscle attached to the chromatophores is no longer controlled by the nervous system. The chromatophores remain expanded and muscle relaxes slowly. The process seems to be concluded with the onset of rigor mortis. Thus, within some hours of death, the skin colour changes from dark to light. (Kreuzer, 1984)

5. Different forms of processing of cephalopods

Cephalopods are processed in various forms such as chilled, frozen, modified atmospheric packaging, dried, smoked, canned and as value added products. Cuttlefish, squid and octopus are processed in the following forms by the processors.

Cuttlefish

1) Frozen: Whole, whole cleaned, fillets, double skinned fillets, pine-cut fillets, shell-cut fillets, tentacles, strips, roe, ink, and wings, dice-cut.

2) Dried: Whole cleaned dried, whole cleaned & smoked, smoked fillets/strips.

3) Prepared/preserved: Seasoned cuttlefish, breaded and battered cuttlefish strips, cuttlefish ball.

Squid

1) Frozen: Whole, whole cleaned, tubes, fillet, slit squid, sliced sushi squid, rings, and blanched tentacles, wings, stuffed.

2) Dried: Whole cleaned dried, shredded, whole cleaned, dried and roasted, smoked fillets, squid meal, and squid oil.

3) Prepared/fermented: Seasoned/fermented, pickled, canned in brine/oil, breaded and battered rings, squid ball.

Octopus

1) Frozen: Whole cleaned, whole cleaned& blanched, whole cleaned and boiled

2) Dried: Whole cleaned and dried

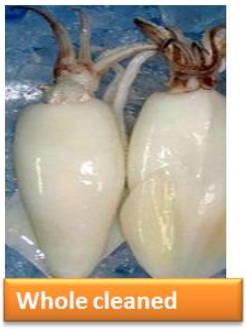
3) Prepared/preserved: Vinegared, seasoned, and smoked

Some of the product prepared using different cephalopods and available in the market are given below

Figure 7. Different products from cephalopods

Squid products


Whole round


Whole cleaned


Fillet


Tentacles


Tube

Cuttlefish products


Whole round


Double finned cuttlefish


Wings


Whole cleaned


Fillets


Tentacles

Octopus products


Baby octopus gutted


Octopus gutted


Baby octopus ink off

Dried and smoked cephalopods



Dried and roasted squid



Dried squid



Dried octopus



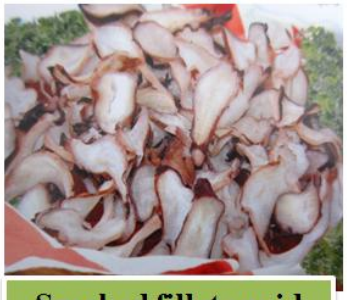
Seasoned smoked cuttlefish



Smoked octopus



Smoked cuttlefish fillet strips



Smoked fillet squid strips



Whole cleaned, smoked cuttlefish

Canned cephalopods



Canned squid



Canned cuttlefish



Canned octopus

Squid byproducts **Octopus byproducts**

















6. Quality Issues associated with cephalopods

The quality issues of cephalopods depend on three important characteristics.They are

- Texture
- Colour
- Flavour

7.1. Texture changes

Cephalopod meat tends to become tough when boiled. The phenomenon responsible for this is structure of tissues, arrangement of fibers. Properties of the various proteins and their thermal behavior. Post mortem quality changes are based on the changes taking place in the proteins and on the properties of new products which occur as result of these alterations**.** The muscle fibers remain in original form at 50^{o}C, disintegration become more than obvious at 60^{o}C and dehydration of myofibrils was evident at 70^{o}C. Thus at temperature higher than 60^{o}C, muscle proteins gradually denature and lose their water binding capacity. This continues until the meat is cooked for some time at 100^{o}C. Heat makes the mantle meat swell and at the same time shrink both lengthwise and widthwise. The maximum swelling of meat occurs at 60^{o}C and the greatest shrinkage within the first five min during cooking at 100^{o}C. These problems are overcome by cooking the cephalopods at high temperature for short duration (3 min) or at low temperature for longer duration (20 min)

7.2. Odor changes

Squid undergo intensive (rapid) spoilage and their shelf life is about 7-8 days in ice storage condition but for fish the shelf life extends up to 12-18 days .This is mainly due to the autolytic activity that degrade proteins and relese more free amino acids. High level of proteolytic activity exists in the muscle due to presence of indigenous proteolytic enzymes (Stanley and Hultin, 1984). The proteolytic activity is 6 times greater than the fish. Spoilage by proteolytic enzymes favours rapid microbial growth.:and subsequent ammonia production, that diffuses from glands to mantle. Ammonia is thus an excellent indicator of freshness of cephalopods. The limit of rejection level of ammonia is 30mg/100g. So, special means of preservation is very much required to preserve the freshness of fresh cephalopods. Gutting can remove liver and pancreas that are source of autolytic enzymes: Quick chilling or freezing of fresh cephalopods and storage at lower temperature are important means to arrest the microbial activity and to reduce undesirable changes in quality.

7.3. Colour changes

Several colour discolorations are formed in cephalopods. They are

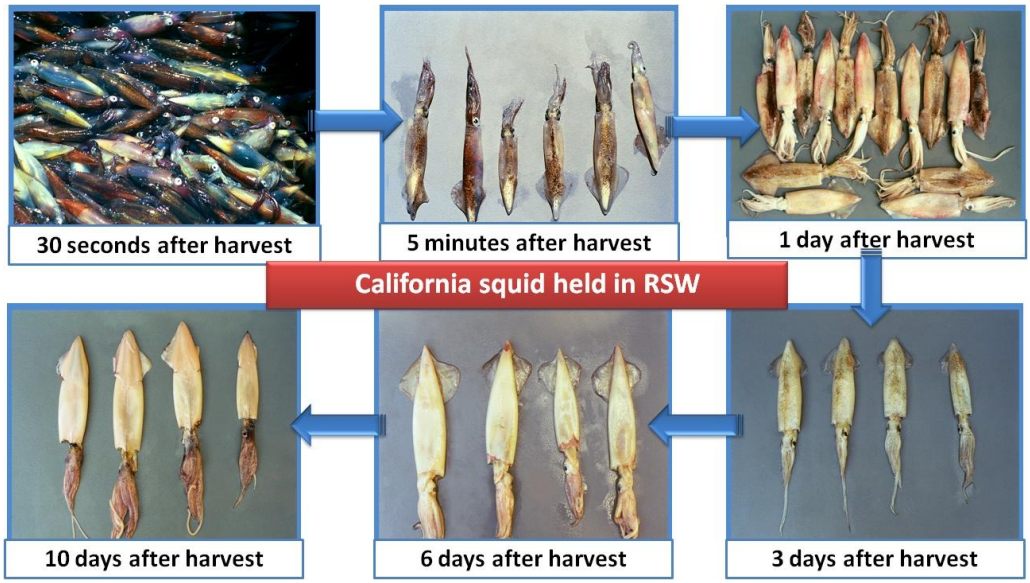
1. Bleaching of original bright colour
2. Pink discoloration in iced squids
3. Black discoloration in frozen squids
4. Black discoloration in dried squids
5. Red discoloration
6. Brown discoloration

7.3.1. Bleaching of original bright colour

Colour changes in cephalopod skin are caused by the pigments particularly by the biological mechanisms causing natural color changes or by the chemical composition of the pigment. Changes in natural color may adversely influence the commercial value of the product. After the death, the biological mechanisms stop working. The colour of the animal when hauled on board remains for some time and then it change irreversibly. Changes in the colour of the fresh skin are regarded as loss of freshness of the raw material. This problem is overcome by

immediate onboard freezing and avoiding ice storage with direct contact with ice. Figure 8 shows the color changing pattern of California squid stored in refrigerated sea water

Figure 8. Color changing pattern of California squid stored in refrigerated sea water



California squid held in refrigerated seawater exhibit the natural color in less than 30 seconds after harvest. When the squid die quickly after harvest the coloration slowly fades within 5 min but the colors do not change. When squid are unloaded from fishing vessels, they are immediately transferred into bins containing ice water. Squid are soaked in ice water for a few hours until they are processed. After 24 hours of harvest, squids show the bleaching effect of the ice water, but retain some of the natural color. Squid held at 4°C (40°F) for 3 days after harvest retain some of their natural color and continue to show some color variation. The top (dorsal) view of the squid appears brownish in color; the ventral view (underside of squid) is reddish in color. When the skin is removed (bottom picture), the squid meat appears nearly white, without discoloration. Squid held at 4°C (40°F) for 6 days after harvest retained some natural color variation. The squid meat still appeared white, although a slight off- color odor was present due to enzymatic breakdown of the squid viscera and muscle. Squid held at 4°C (40°F) for 10 days after harvest retained some natural color variation, but the skin appeared slightly reddish-purple in color. The meat of the squid was mostly white with some red to purple discoloration. The odor of the squid was strongly putrid. Poor quality squid have a dark red or purple discoloration of the meat, and a strong off-odor

7.3.2. Pink discoloration in iced squids

Pink discoloration of whole squid without prior de-skinning was mainly associated with the release of pigments from the chromatophores located in the skin. This change was mediated by the growth of microorganisms. The use of the safe antimicrobial agent together with the sufficient ice could retard pink discoloration and maintain the quality of squid during the extended iced storage. Additionally, de-skinning was also an effective means to prevent pink discoloration. To overcome pink discoloration problems some processing industries add bleaching agents like 3% NaCl with 0.5% H_2O_2 or 1-10 ppm NaOCl solutions. The addition of belching agents, induces lipid / protein changes, enhance protein aggregation, and enhances protein denaturation and leads to loss of quality

7.3.3. Black discoloration

Two types of black discoloration problems are encountered in cephalopods. They are

i. **Black discoloration in cold store**

   This occurs during thawing of squids due to flowing out pigment from the broken chromatophores**.**

ii. **Black discoloration in dried squid**

   When fresh squid are dried in the sun immediately after landing, there appears a black zone near the central line of the back. This blackening of the skin lowers the commercial value of the dried squid product. When the squid is rapidly dried, the muscles attached to the chromatophores remain contracted, thus the chromatophores remain expanded and the pigment covers the surface. It is necessary that the muscles of the chromatophores relax (expand) and the chromatophores cells shrink. For this reason, very fresh squid (before splitting) should be soaked in fresh water for 30 min.

7.3.4. Red discoloration

Red discoloration may occur if the squid is exposed to rain during drying, or if it is kept at a high humidity under warm conditions (Tanikawa, 1971). The cells of the chromatophores break up and the escaping pigment turns red as soon as the squid meat becomes slightly alkaline. When drying outdoors, the squid must be protected against rain and dew. Each day, before sunset, the drying squid must be placed under a cover. It must also be covered before it begins to rain (Tanikawa, 1971).

7.3.5. Brown discoloration

Brown discolouration occurs in dried seasoned squids, when the minced squid meat is processed into seasoned products, and the lipid and fatty acid especially polygenic acids undergo some change. The carbonyl compounds, produced by the joint action of light, oxygen and heat during processing, correspond to the browning reaction that appear during storage of dried and seasoned squid products.

8. Quality testing methods for cephalopods

Quality of cephalopods can be assessed by

a) Sensory evaluation
b) Biochemical tests
c) Microbiology tests
d) Presence of Chemical hazards

8.1. Sensory evaluation

There are two methods by which cephalopods can be sensorically evaluated before processing and subsequent consumption.

8.1.1. EC scheme

In Europe, the most commonly used method for quality assessment in the inspection service and in the fish industries is the EC freshness grading (or EC scheme), which is introduced or the first time in the Council Regulation Number 103/76 (for fish) and 104/76 (for crustaceans) and updated by decision No.2406/96 (for some fish, some crustaceans, mollusk, and cuttlefish). It includes some of the improvements published by Howgate *et al*. (1992). There are three levels in the EC scheme, (E-extra, the highest quality), A (good quality) and B (satisfactory quality). Below level B (sometime called unfit or C).

8.1.2. QIM method

A recent scheme now coming out of it research phase into commercial practice is known as the quality index method (QIM).The QIM is based upon objective evaluation of certain attribute of raw fish (skin eyes, gills, etc.) Using a demerit point scoring system from (0 -3). As

no excessive emphasis is laid on a single attribute, a sample cannot be rejected on the basis of a single criterion.Minor differences in the results of any of the criterion do not unduly influence the total QIM score. QIM methods have been developed for the assessment of squid and cuttlefish (Barbosa and Vaz-Pires, 2004) as well as for octopus. (Vaz-Pires and Seixas, 2006). Table 4 and 5 show the unique QIM method for squid, cuttlefish, and octopus

Table 4. Unique QIM method for squid and cuttlefish (Barbosa and Vaz-Pires, 2004)

Freshness quality parameters	**Description**	**QIM score**
Skin (dorsal side) Appearance/colour	Very bright, well defined pigments of different sizes and colours (brown, purple, rose and dark red)	0
	Iridescent skin bright, becoming discoloured	1
	Rather dull, without shine, purplish in the centra-axis of the body, general orange/pink areas	2
Odour	Seaweedy, (sea) fresh	0
	Slightly seaweedy	1
	Neutral, slightly fishy	2
	Intense, metallic, fishy	3
Mucus	Transparent, watery, shining	0
	Slightly milky, moderate or absent	1
Flesh Texture	Firm, tense, consistent	0
	Soft, less consistent	1
	Flaccid, flabby	2
Eyes Shape/appearance	Convex	0
	Flat	1
	Concave	2
Eyes Ocular tissue	Translucent, watery	0
	Slightly opalescent	1
	Opalescent	2
Mouth region Odour	Seaweed, fresh	0
	Neutral	1
	Slightly fishy	2
	Intense, fishy, acid	3
Mouth region Mucus	Absent or clear transparent	0
	Slightly yellowish	1
	Range of QIM score	0-16

Table 5. Unique QIM method for octopus (Vaz-Pires and Seixas, 2006).

Parameters	Description	QIM score
Skin (Appearance) Colour	Very bright, well-marked colors, white in the clearest parts of the body, skin elastic	0
	Bright, less colored, slightly pink in the clearest parts of the body, skin with low elasticity	1
	Less bright, colorless, orange or brown spots, color somewhat more orange, rose in the clearest parts of the body, shrunken skin	2
Odour	Seaweedy, (sea)fresh	0
	Slightly seaweedy, slightly grassy, neutral	1
	Metallic, grassy, acid, intense	2
Mucus	Transparent, watery	0
	Slightly milky, viscous (sticky), moderate or absent	1
Flesh Texture	Firm, tense	0
	Flaccid, soft	1
Eyes Cornea	Translucent	0
	Slightly opalescent	1
	Opalescent	2
Pupil	Black, shining	0
	Black, dark red, muddy	1
	Dark red, opaque, normally bloodstained	2
Mouth region Colour	White, yellowish	0
	Slightly rose	1
Odour	Seaweed or neutral	0
	Sulpurous, citric, sweet, acid	1
Mucus	Clear	0
	Milky	1
	Yellowish	2
Arms Material in the sucker	As a film all over the sucker	0
	Starting to agglomerate in the centre of the sucker	1
	Completely agglomerated in the centre of the sucker	2

8.2. Biochemical tests

The various biochemical tests examined to assess the quality of cephalopods are

i. Ammonical nitrogen,
ii. α-amino nitrogen
iii. NPN compounds,
iv. TVB-N,
v. Free fatty acid value
vi. Autolytic activity
vii. Agmatine, cadaverine ,putresine

The presence of ammonical off-odour is in accordance with the changes on sensory characteristics of spoiled squid. Hence ammonia, has been shown be an excellent indicator of squid quality according to LeBlanch and Gill (1984). The early onset of agmatine production indicates an autolytic mechanism, but the fact that is produced by decarboxylation of arginine suggests the involvement of bacterial decarboxylase activity. These amines seems to be an excellent freshness index in squid (Yamananaka *et al*, 1987). Cadavarine and putrescine have both been suggested as spoilage indicators of squid (Takagi *et al*., 1971)

8.3. Microbiological tests

- Total psychrophilic bacteria
- Total H_2S producing bacteria,
- Presence of specific spoilage bacteria
    i. Psuedoalteromonas spp and
    ii. Photobacterium phosphoreum

***Psuedoalteromonas* spp**

It is marine bacterial species dominant in whole squids. It is an antagonistic bacterium and produces bacteriostatic and bacteriolytic compounds. It also produces iron binding siderophores. It possesses the enzyme "urease" and hence converts urea into ammonia. This bacterium contribute for the late ammonia production in cephalopods.

Photobacterium phosphoreum

This bacterium is present mainly in the digestive gland and stomach of whole spoiled squids. It converts TMAO into TMA at 10^7cfu/g concentration or above. This bacterium level in

squid at the time of harvest is only up to 10^6cfu/g. It possesses arginine decarboxylase enzyme and hence produces agmatine. Naturally squid possesses photophores (light organs) and forms the substrate for the *Photobacterium sp.* (symbiosis)

8.4. Presence of chemical hazards

Cephalopods accumulate high levels of trace elements, particularly cadmium (Cd) and copper (Cu) in different parts of the body viz., muscle tissue, liver, digestive gland etc. Squids (*Loligo* sp.) and squid products from India and Thailand has high concentrations of toxic heavy metals, especially cadmium and lead (Oehlenschlaeger, 1989). Oehlenschlaeger (1990) has observed low levels of cadmium and lead contents in squid and cuttlefish mantle and suggested to remove the intestines from the mantle immediately after catch to avoid transportation of heavy metals from the intestines to the mantle during freezing. Metals such as Ag, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn are accumulated in the tissues like digestive gland, branchial hearts, gills, digestive tract, kidney, genital tract, muscle, skin, shell of *Eledone cirrhosa* and *Sepia officinalis* (Miramand and Bentley 1992). .

9. Conclusion

Cephalopods Spoils rapidly than fish. Spoilage is mainly by autolysis activity, not microbial activity**.** Ammonical odor and pink discoloration are major quality issues associated with cephalopods. Freeze the cephalopods on board to avoid the discoloration. Avoid stacking of cephalopods to prevent the damage of skin (chromatophores).Icing should be done in non-contact ice (ice slurry) after gutting. De-skinning the cephalopods as early as possible to avoid diffusion of chromatophores in mantle

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