



Seasonal variations in cholesterol content of the Indian squid *Uroteuthis (Photololigo) duvaucelii* (d'Orbigny [in Ferussac and d'Orbigny], 1835)

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ABSTRACT

Among seafood, shellfish and specifically cephalopods are known to contain higher levels of cholesterol. The correlation of dietary cholesterol with heart ailments has led people to become more health conscious and in this scenario the present study was carried out to investigate the monthly variations in Indian squid to aid in better decision making by consumers. Squid samples [*Uroteuthis (Photololigo) duvaucelii*] were collected in quadruplicate on monthly basis for the period of one year (2015-2016) and subjected to analysis for cholesterol, non-saponifiable matter (NSM) and proximate composition. The cholesterol content of squid was highest (279 mg%) and lowest (7.3 mg%) during the months of April and December with respect to total length and size, respectively. A monthly variation in proximate composition of squid with respect to the total length and size was observed. No correlation observed between fat content and cholesterol in squid. Significant difference in cholesterol content was observed monthly in the squid samples. NSM content (0.23%) was negligible and undetectable in the samples.

Keywords: Healthy diet, Heart disease, Proximate composition, Seafood

Introduction

Marine products, mainly shrimps and squids from India have become a major export commodity to European countries in recent years. Consumer awareness about the nutritional quality of fish has increased with reference to cholesterol content, its health issues and other hazardous compounds. Dietary cholesterol levels are directly linked with risks of coronary heart disease (CHD) that has led people to become more health conscious (Sanchez-Muniz *et al.*, 1991; Ammu *et al.*, 1996). As a result, food labelling for displaying the regulatory and safety levels of cholesterol has gained importance. Many of the packaged seafood products need mandatory disclosure of cholesterol content of food items.

Indian squid *Uroteuthis (Photololigo) duvaucelii* is landed in large quantities along the Indian coast mainly by trawling operations. They are processed in frozen conditions in the form of tubes, tentacles and rings and exported to Asian and European countries. Total annual landings of squids are estimated to be 112727 t (CMFRI, 2020). They are regarded as a delicacy amongst the Indian consumers and therefore, a major share of their landings are marketed in fresh condition in the domestic sector. Among seafood, shellfishes in general and cephalopods in particular are known to contain higher levels of cholesterol.

Squids harvested from Indian waters have been reported to contain 188-198 mg% of cholesterol, which is the highest among different seafoods (Mathew *et al.*, 1999). Hence the consumption of squids with regard to the dietary cholesterol pattern has become significant among Indian consumers. Consequently, it was felt necessary to analyse the monthly variations, if any, occurring in cholesterol content of squids with respect to other biochemical parameters which might aid in consumer decision making regarding inclusion of squid in their diets.

Materials and methods

Fresh samples of Indian squid [*U. (P.) duvaucelii*] were procured from Ratnagiri Fishing Harbour on a monthly basis for a period of one year from March 2015 to February 2016. This covered different seasons namely monsoon season from June-September, winter season from October-January and summer season from February-May. They were layered with ice (1:1 fish:ice) and brought to the laboratory in insulated ice boxes. The total length (cm) and weight (g) of samples were recorded and further analysed for cholesterol content, proximate composition as well as non-saponifiable matter. The analyses were carried out from quadruplicate squid samples to enable estimation of variance in the results with respect to length/weight of samples. The mince was

prepared from cleaned squid tube of about 25 g and an aliquot of the same was used and taken for further analysis.

Cholesterol analysis

Cholesterol content of fish was estimated following Zlatkis *et al.* (1953). Extracted lipids were treated with freshly prepared alcoholic potassium hydroxide, glacial acetic acid and ferric chloride-sulphuric acid reagent was added and the colour developed was observed. After 20 min, the absorbance was read at 560 nm in a spectrophotometer. The absorbance readings were plotted and the appropriate cholesterol concentrations were computed.

Total lipids analysis

Total lipid analyses were carried out as per Folch *et al.* (1957); for which 100 g of fish sample was taken and homogenised with chloroform: methanol mixture (2:1, v/v) and filtered. The extract was shaken and equilibrated with 20% distilled water and allowed to stand overnight in a separating funnel. The extracted lipids were concentrated with rotary evaporator and stored in a deep freezer. A small volume was taken in a pre-weighed vial and evaporated to constant weight to determine the percentage of lipid in the samples.

Non-saponifiable matter (NSM)

Estimation of NSM was done as per the standard methods of AOAC (2005). Fat sample (5 g) was taken and 50 ml of alcoholic potassium hydroxide solution was added. The contents were boiled under reflux air condenser until saponification was complete. The condenser was washed with 10 ml of ethyl alcohol. The saponified mixture was transferred in separating funnel, 50 ml of petroleum ether was added and the layers were allowed to separate. The extraction was repeated three times and the combined extract was washed three times with ether. Then the ether solution was evaporated and 2-3 ml of acetone was added while heating on steam. The residue was dissolved in 50 ml of warm ethanol and titrated with 0.02N NaOH.

Crude protein, moisture and ash content were determined as per the standard methods of AOAC (2005). All chemicals used were of analytical grade (HiMedia, Mumbai). Statistical analysis was done using analysis of variance (ANOVA) and correlation coefficients (Zar, 2006).

Results and discussion

Monthly variations in cholesterol content

Monthly variations in cholesterol content of squid samples of varying sizes are represented in Table 1. Highest cholesterol content was observed in the month of April and the lowest in the month of September (Fig. 1). The cholesterol content was observed to reach the highest

value of 279 mg% in sample with a total length (TL) of 42 cm and weighing 105 g in the month of April. The lowest value was observed as 7.3 mg% in sample with a total length (TL) of 22.5 cm weighing 41 g collected in the month of December. Samples could not be procured in the month of June and July owing to closure of fishing activities due to monsoon rains. Significant monthly differences were noted in cholesterol content of samples ($p < 0.05$). However, no correlation existed between the cholesterol and fat contents of the samples (Table 2). Initial analysis for NSM in squid samples were found to be 0.23% which was negligible and in a few samples NSM was not detected. Therefore, further investigation regarding the same was not done.

According to Cox *et al.* (1990) the cholesterol levels below 200 mg dl⁻¹ are classified as “desirable blood cholesterol”; 200 to 239 mg dl⁻¹ as “borderline-high blood cholesterol” and 240 mg dl⁻¹ and above as “high blood cholesterol”. According to Food and Drug Administration (FDA) the recommended daily intake of cholesterol for adults (RDA) should be less than 300 mg per day. With respect to the above results, it is evident that squid possess cholesterol level of 279 mg% which is quite higher than the daily intake recommended by FDA. Consequently, the consumption of squid should be restricted particularly for people with heart ailments.

The variations in the cholesterol content of squid could be attributed to food and feeding habits of squid. Squids are voracious and active carnivores; feeding mainly on fishes and crustaceans (Ayyappan, 2011). Their feeding intensity appears to be penurious in the breeding season. The spawning season in *Loligo duvauceli* [= *U. (P.) duvaucelii*] has been observed throughout the year with peaks during post-monsoon period (September-November). Therefore, higher content of cholesterol in squid can be correlated to the presence of higher levels of cholesterol in shellfish like crustaceans especially shrimps, squid and cuttlefish

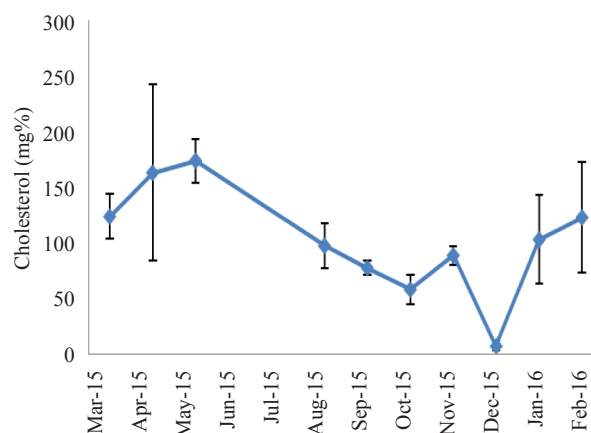


Fig. 1. Seasonal variations in cholesterol content of *U. (P.) duvaucelii*

Table 1. Seasonal variations in cholesterol content and proximate composition of *U. (P.) duvaucelii*

Month and year	Fish sample		Cholesterol (mg%) (mean±SD)	Moisture (g%) (mean±SD)	Protein (g%) (mean±SD)	Fat (g%) (mean±SD)	Ash (g%) (mean±SD)
	Length (cm)	Weight (g)					
March 2015	21.8	68.0	123.75±20.06	83.33±4.21	16.2±0.70	1.26±0.13	1.61±0.28
April	33.0	78.75	163.0±79.30	80.21±2.47	17.48±1.73	1.00±0.1	0.83±0.49
May	27.25	48.50	173.75±19.81	79.39±3.98	17.52±2.86	0.48±0.30	0.52±0.18
June	N A	N A	N A	N A	N A	N A	N A
July	N A	N A	N A	N A	N A	N A	N A
August	30.2	73.2	97.53±20.56	81.48±1.12	15.7±1.49	1.31±0.22	0.26±0.05
September	33.0	94.5	77.7±6.54	81.1±1.21	16.70±1.17	0.3±0.34	0.54±0.23
October	24.0	45.0	58.3±3.32	81.19±.21	16.56±.39	0.48±.45	0.36±.21
November	23.0	33.2	88.93±.40	81.1±.21	14.18±.04	1.1±.20	0.75±.29
December	22.88	33.5	7.52±4.54	81.4±0.96	16.04±2.27	0.95±0.57	0.53±0.34
January 2016	31.5	62.5	103.0±0.0	81.19±.21	17.04±.81	0.6±.61	0.34±.09
February	21.0	30.7	123.00±49.43	78.89±6.01	17.07±1.86	1.33±0.12	0.35±0.14

N A (not analysed).

Table 2. Variations in cholesterol content (mean±SD) in *U. (P.) duvaucelii*, with respect to size

Total length (cm)	Cholesterol (mg%)	Moisture (g%)	Fat (g%)	Month and year
21.82±4.94	123.75±20.05	83.33±4.21	1.26±0.13	March 2015
33±9.83	163±79.39	80.21±2.47	1±0.1	April 2015
27.25±2.75	173.75±19.81	79.39±3.98	0.48±0.30	May 2015
30.25±5.56	97.53±20.56	81.48±1.12	1.31±0.22	August 2015
33±7.16	77.7±6.54	81.1±1.21	0.3±0.34	September 2015
24±6.37	58.3±13.32	81.19±1.21	0.48±0.45	October 2015
23±2.94	88.93±8.40	81.1±1.21	1.1±0.20	November 2015
22.87±3.63	7.52±4.54	81.4±0.96	0.95±0.57	December 2015
31.5±2.45	103±40	81.19±1.21	0.6±0.61	January 2016
21±4.76	123±49.43	78.89±6.01	1.33±0.12	February 2016

on which they feed upon (Mathew *et al.*, 1999). Ozogul *et al.* (2014) had concluded that the variation in cholesterol content in fish meat, shellfish and molluscs varies widely as a result of species, sex, season, water temperature, quantity and quality of food and breeding conditions, and that differences caused by those factors can be very significant. Idler *et al.* (1964) reported variation, during different seasons, in the cholesterol content of the scallop muscle, suggesting a relationship between metabolism and biosynthesis of these sterols. Other squid species also have comparable cholesterol content: *Loligo pealeii* - 243±352 mg% and *Illex illecebrosus* - 108±315 mg% (Krzynowek *et al.*, 1989).

Size dependent variations in cholesterol content

The data on cholesterol content of squid samples with respect to size/weight of squid is detailed in Table 2. The correlation study of cholesterol with regard to the size and cholesterol content indicated a positive correlation from March to November and January respectively, and a negative correlation during December and February (Table 3). Correlation coefficient was determined to study the effect of size and season on the cholesterol

content of squid. Correlation coefficient was determined as per the different lengths obtained in each month with respect to the cholesterol content. Correlation coefficient values ranged between 0.94 to -0.56. As the size of squid increased throughout the period of sampling, cholesterol content showed alternate increasing and decreasing variations. Study conducted by Mathew *et al.* (2005) to evaluate the seasonal variations in cholesterol content in different species of prawns, had noted negative correlation during the months of sampling from March to June.

Seasonal variations in biochemical composition

The evaluation of proximate composition of squid showed monthly variations with regard to the moisture content. Moisture content was highest at 89.57% in squid with a total length of 27 cm and weight 105 g during the month of March. It was lowest at 72.40% in the specimen with a total length of 18 cm and weight 20 g during the month of February (Table 2). With respect to the monthly variation, the highest moisture content was reported in the month of February and the lowest was observed in the month of December. Statistically significant difference was noted in moisture content of

Table 3. Correlation between size and seasons in cholesterol content of squid

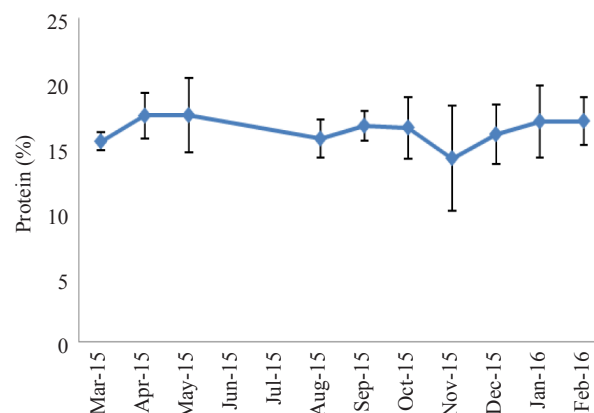
Months	Correlation coefficient
March 2015	0.94
April '15	0.76
May '15	0.97
August '15	0.22
September '15	0.17
October '15	0.58
November '15	0.17
December '15	-0.72
January 2016	0.98
February '16	-0.56

* $p < 0.01$

squid between the seasons ($p < 0.05$). Positive correlation was found between the moisture content and total length of sample; however, negative correlation between fat and moisture seen explicitly in the subsequent months from August to February. Jacquot (1961) in his study reported approximately similar value of moisture content (81.0%) in case of molluscs. Krzynowek *et al.* (1989) also reported similar values for moisture content of *L. pealeii* as 81.6 to 84.1% and for *I. illecebrosus* in the range of 76.1 to 84.7%. Gopakumar (1997) reported similar value of moisture content of squid and cuttlefish at 83.00 and 75.83%.

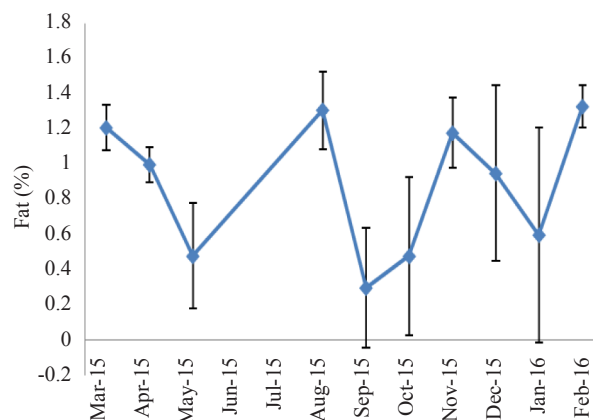
The protein content observed was highest at 19.90% in squid with a total length of 34 cm weighing 70 g in the month of January. While the lowest value was recorded as 10% in a specimen with a TL of 27 cm weighing 41 g in the month of November (Table 2). Variations in protein content were found to be highest in the month of November and the lowest in the month of March as shown in Fig. 2. The two-way ANOVA showed that there was a significant difference in protein content between the months ($p < 0.05$). Jacquot (1961) has reported similar protein content (13%) in squid. Gopakumar (1997) reported protein content of squid and cuttlefish as 14.50 and 18.06% respectively. Krzynowek *et al.* (1989) reported that both species of squid *L. pealeii* and *I. illecebrosus* were high in protein content ranging from 11.5 to 20.5%.

Squid being a non-fatty fish, the fat content was steady throughout the year. Squid with a TL of 38 cm weighing 110 g showed the highest fat content as 1.5% during the month of August. The lowest value of 0.1% was observed in the months of May, September and October in specimens with a total length of 29, 41, 26 and 15 cm respectively and weight of 60.7, 159, 45 and 35 g respectively (Table 2). The highest variation in fat content was recorded in January and the lowest was found in April as shown in Fig. 3. Two-way ANOVA showed that there was a significant difference in fat

Fig. 2 Seasonal variations in protein content in *U. (P.) duvaucelii*

content between the months ($p < 0.05$). No correlation was observed between the cholesterol content and fat. These observations corroborate with the observations made previously by Jacquot (1961). Gopakumar (1997) and Mathew *et al.* (1999) have reported similar values of fat content for *Loligo* spp. as 0.8% and cuttlefish as 0.22%. According to Meiyappan and Mohamed (2003), *U. (P.) duvaucelii* breeds throughout the year while Bal and Rao (1984) observed that their breeding season is prolonged extending from January to June. So, the higher values of fat content obtained during the month of February in the present study may be attributed to their spawning season. The squids (*L. pealeii* and *I. illecebrosus*) were low in fat ranging from about 1 to 2% and averaging about 1.5% as reported by Krzynowek *et al.* (1989).

Ash content was observed to be highest at 2% in squid with a TL of 27 cm weighing 105 g recorded in the month of March. It was observed as minimum at 0.12% in a sample with TL of 26 cm and weighing 45 g recorded in the month of October (Table 2). The highest and the lowest variations in ash content was seen in the month

Fig. 3 Seasonal variations in fat content of *U. (P.) duvaucelii*

of April and August respectively. The results of two way-ANOVA showed that there was a significant difference in ash content between the months ($p < 0.05$). Krzynowek *et al.* (1989) reported ash content in *L. pealeii* and *I. illecebrosus*) ranging from about 0.3 to 2%. Gopakumar (1997) reported the ash content of *Loligo* spp. to be 0.50 and cuttle fish as 1.53%.

This study concluded that the cholesterol content *U.(P.) duvaucelii* varies with respect to season and size. Seafoods are recognised to be hypocholesterolemic with both fish protein and the PUFA of fat having cholesterol lowering effect on blood serum (Mathew *et al.*, 2005). However in shellfishes, the high cholesterol content also should be taken into consideration when we emphasize the effects of PUFA and fish protein on lowering the serum cholesterol. The consumers need to be vigilant with respect to the content of cholesterol in squid and its variations with regard to the season as it would boost the dietary cholesterol levels. The results of this study can be used as aid to design or drive consumer choices with respect to inclusion of squid in their diets.

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