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Biochemical Investigations on Antarctic Krill *Euphausia superba*

M.R. Raghunath, T.V. Sankar, A.G. Radhakrishnan, Suseela Mathew, K. Ammu, C.N. Ravishankar, K. Jayan and Leema Jose
Central Institute of Fisheries Technology
Cochin - 682 029, Kerala, India

Antarctic krill (*Euphausia superba* Dana), caught by the First Indian krill expedition was processed on board into whole krill, peeled tail meat and whole krill mince. These were analysed for trace metals, lipids, cholesterol, fatty acids and amino acids. Autolysis at various pH and temperatures as well as thermal coagulation of krill mince suspended in various media were also studied. Trace metals were below toxic levels while no pesticides were detected. Krill tail meat had small amount of lipids and cholesterol (0.81 and 0.033% respectively). The lipids were rich in C18:1, C20:5, C22:6, fatty acids. Autolytic activity at pH 3-4 and 8-10 was predominant in whole krill at 20 and 40°C, but autolysis in tail meat was very low. Nearly 80% of the soluble proteins in krill suspension could be precipitated by rapid heating.

Key words: Antarctic krill, heavy metals, pesticides, lipids, cholesterol, fatty acids, *amino acid, autolytic activity, protein coagulation*

The Antarctic krill (*Euphausia superba* Dana) is quite well known as a valuable source of animal protein, with potentially bulk quantity available for exploitation. Japan and the former USSR have made several investigations and attempts at fishing and commercial utilization of this rich resource (Suzuki, 1981). Several European nations too have made efforts in utilization of krill, notably Norway and Poland (Ellingsen, 1982). However, formidable problems still remain in its utilization because of its tendency to autolyse rapidly at ambient and low temperature and high fluoride concentrations. The first Indian Krill Expedition (Fikex) 1995-96 conducted exploratory fishing for krill, processed samples on board and collected several samples for further analyses. This paper sums up the biochemical investigations conducted on these samples.

Materials and Methods

Antarctic krill (*E. superba*) was caught as detailed elsewhere (Boopendranath *et al.*, 1996). It was immediately frozen as 10 kg blocks at -40°C in an air blast freezer.

Some of the krill was peeled in a prototype roller and frozen similarly. Another portion of the krill was passed through a Baader 694 deboning machine with 5 mm holes and the resultant brown liquid was frozen as before. Krill heads were separated and frozen as well. All these operations were done on board within 1-2 h of the catch and stored at -27°C for 2 months. At the end of the cruise, these frozen samples were transferred to frozen storage at -18°C and the analyses completed within 3 months of being caught.

Heavy and trace metals in whole krill and krill head were determined by atomic absorption spectrometry (Radhakrishnan, 1993a) and whole krill and krill head were analysed for pesticide residues by gas chromatography (Radhakrishnan, 1993b). Krill lipids were extracted from samples with chloroform-methanol mixture as per Folch *et al.* (1957). Cholesterol in the unsaponifiable fraction was estimated by the ferric chloride method (Rudel & Morris, 1973). Methyl esters of fatty acids in the saponifiable fraction were prepared (Metcalfe *et al.*, 1966)

and analysed on a Chrompack CP 9001 gas chromatograph equipped with an Alltech AT225 capillary column (0.53 mm id and 30 m length) and flame ionization detector. Amino acid profile of proteins was analysed after hydrolysing the samples in 6M HCl for 24 h at 110°C in evacuated sealed tubes. Amino acids were separated by ion-exchange chromatography in a Shimdzu high performance liquid chromatograph amino acid analyzer. Tryptophan was determined after alkaline hydrolysis with 5% NaOH (Sastry & Tammuru, 1985).

Autolytic activity was assayed by homogenizing samples with 10 volumes of ice-cold 0.02 M $NaHCO_3$ in a pre-chilled waring blender for 1 min. The homogenate was quickly filtered through a thin layer of absorbent cotton into a pre-chilled beaker and assayed for autolytic activity as outlined by Jose & Raghunath (1998). For thermal coagulation studies, a 1:4 (v/v) homogenate of frozen krill mince and water/0.6M NaCl/ 0.2% citric acid was heated gradually with continuous stirring. Temperature rise was noted and 2 ml samples were drawn at intervals. Samples were kept at 0°C for 30 min and then centrifuged at 37600 x g for 10 min. Protein content in supernates was estimated by the biuret reaction (Gornall *et al.*, 1949).

Machine peeled krill tail meat was used for nutritional studies. As it was not possible to prepare a standard diet with 10% protein for PER studies using cooked tail meat alone owing to its high moisture content, cooked tail meat was used at 50% of the dietary protein, the rest being supplied by Bengal gram (*Cicer areitanium*) flour. The method followed for nutritional evaluation is reported elsewhere (Raghunath *et al.*, 1995).

Results and Discussion

The concentration of various trace metals in whole krill and head are given in Table 1. The concentrations of copper, iron, zinc, chromium were above 10 ppm while those of lead, cadmium, nickel and manganese were less than 1 ppm. Cadmium, copper and iron are present in Antarctic waters (Nolting *et al.* (1991). But, the concentrations of Cd, Pb, Cu and Zn in oceanic waters have been reported to decrease towards Antarctica when proceeding from equatorial waters (Khandekar *et al.*, 1993). Marine crustaceans in Antarctic ocean have been reported to accumulate as much as 13 mg/kg (d.w) of cadmium (Petri & Zauke, 1993). Chinese expedition to Antarctic waters found that *E. superba* does accumulate a number of trace metals such as Pb, Cu, Cd, Zn, Fe and Mn. The maximum was in the case of copper and minimum, in lead (Qun *et al.*, 1990). The higher concentrations of copper, iron and zinc reported in this paper are in agreement with previously reported values, although high concentration of chromium has not been previously encountered. The concentrations of pesticides in krill were all below detectable limits and no traces of any organo-chlorine pesticides were found in any of the samples. But there are reports on the presence of DDT and BHC in Antarctic waters, sediment and organisms (Yao *et al.*, 1992). PCBs were also reported to be present in antarctic waters in the Indian sector (Joiris & Overloop, 1991) The absence of pesticides in Antarctic krill samples analyzed in the present study may be probably due to difference in the areas from which samples were taken. However, it is to be noted that in recent surveys of Antarctic air, decreasing trends of pesticides over the last decade have been observed (Bidleman *et al.*, 1993).

Table 1. Metal concentration (in parts per million) in krill and krill head

Sample	Hg	Cd	Pb	Ni	Cu	Fe	Zn	Mg	Cr	Co	Mn
Whole krill	Nill	0.63	0.41	0.54	18.42	17.02	12.92	8.96	18.8	1.54	0.5
Krill head	Nill	0.58	0.36	0.49	17.36	16.56	11.36	7.84	14.4	0.98	0.32

The total lipid content of 0.81% (Table 2) found in tail meat of krill is lower than lipid concentrations reported in whole krill (Ellingsen, 1982; Pond *et al.*, 1995) but agrees with the values reported by Kinumaki (1980), who also reported seasonal variations in krill lipid content. Anatomical and seasonal differences could be the reason for the lower values reported here. Eighty percent of krill lipids are reported to be phospholipids (Kolakowska, 1988). The proportion of unsaponifiable fraction of lipids was 7.26%, which was comparable with earlier findings (Watanabe *et al.*, 1976). The cholesterol concentration was 101.7 mg per 100 g of whole krill and 33.4 mg/100 g of tail meat. Cholesterol content of whole krill was found to be slightly higher than values reported by these workers.

Table 2. Fat, Nonsaponifiable matter and cholesterol content of whole krill and krill tail meat (g %)

	Fat	NSM	Cholesterol
Whole krill	0.96	0.131	0.1017
Tail meat	0.81	0.509	0.0333

The fatty acid composition of krill lipids has been extensively investigated. The concentration of various fatty acids in the saponifiable fraction of krill lipids is given in Table 3. Thirty five percent of the fatty acids in krill tail meat were saturated, 19% monounsaturated and 36% polyunsaturated. Among the saturated fatty acids, palmitic acid (C16:0) was the dominant fatty acid amounting to 28%. Oleic acid (C18:1 n-9), eicosapentaenoic acid (C20:5 n-3) and docosahexaenoic acid (C22:6 n-3) were the major unsaturated fatty acids contributing 12, 25 and 10% respectively of the total fatty acids. Palmitic acid, eicosapentaenoic acid and docosahexaenoic acid together accounted for about 60% of the total fatty acids. Stearic acid (C18:0) was not present in detectable levels. In case of whole krill, the proportion of the polyunsaturated fatty acid fraction remained similar, but the concentration of C16 and C18 monounsaturated fatty acids was higher. These findings are similar to the fatty acid composition of the phospho lipid fraction of krill reported earlier (Ellingsen, 1982). Krill lipids on the whole have fatty acid compositions similar to fish lipids, except for the near absence of C18:0.

Table 3. Fatty acid composition of whole krill and krill tail meat (% of total fatty acids)

Fatty acid	Whole krill	Krill tail meat
C12 : 0	0.29	nd
C13 : 0	0.19	nd
C14 : 0	10.85	5.65
C14 : 1	0.24	0.34
C15 : 0	0.61	0.33
C16 : 0	21.94	27.65
C16 : 1	7.6	3.26
C16 : 2	0.16	nd
C17 : 0	1.94	0.69
C18 : 1 n-9	21.43	12.6
C18 : 2 n-6	0.34	5.84
C18 : 3 n-6	0.99	0.86
C18 : 4 n-3	0.16	nd
C20 : 1 n-9	1.03	0.28
C20 : 4 n-6	1.32	1.17
C20 : 5 n-3	16.85	24.98
C22 : 1 n-9	nd	0.39
C22 : 5 n-3	0.25	0.26
C22 : 6 n-3	8.1	10.93

nd - not detected in measurable quantities

Table 4. Amino acid composition of krill (grams amino acid per 100 g protein)

Amino acid	*Krill mince*	*Tail meat*	*Whole krill*
Aspartic acid	10.05	12.19	9.30
Threonine	3.88	4.34	4.29
Serine	3.46	4.30	3.34
Glutamic acid	12.74	17.27	12.13
Proline	6.41	4.43	6.35
Glycine	6.28	4.63	5.66
Alanine	6.79	7.13	6.65
Cysteine	1.08	0.95	–
Valine	4.99	5.13	4.90
Methionine	1.63	3.06	0.44
Isoleucine	4.78	5.52	4.99
Leucine	7.80	9.27	8.22
Tyrosine	3.28	4.76	2.80
Phenyl alanine	4.35	5.42	4.39
Histidine	2.84	3.16	2.97
Lysine	6.50	6.08	5.33
Arginine	7.86	7.82	5.68
Tryptophan	0.88	0.88	0.99

The amino acid profile of whole krill, krill tail meat and krill mince are given in Table 4. Protein of the krill tail meat, appeared to be quite balanced in its amino acid composition. All the essential amino acids were present in adequate amounts. Whole krill had a slightly lower concentration of sulphur containing amino acids.

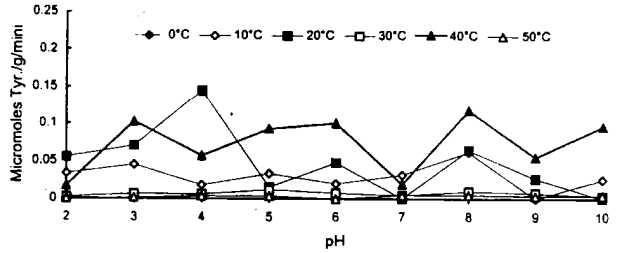

Fig. 1. Autolytic activity of whole krill measured at various temperatures and pH. The following buffers were used; 0.3 M Citrate-phosphate at pH 3, 4, 5, 6 and 7, Tris-HCl 0.3 M at pH 8, 9 & 10, 0.3 M phosphoric acid titrated to pH 2.0 with NaOH. Activity is expressed as µMol tyrosine released/min/g sample.

Autolytic activity in whole krill at different temperatures is presented in Fig. 1. Autolysis was marked at 20 and 40°C and moderate at 10°C. Acid proteinase activity at pH 3-4 and alkaline proteinase activity at pH 8 and 10 were particularly prominent. Autolysis activity at 0°, 10°, 30° & 50°C was less by comparison with 20°C or 40°C. Autolyfic in krill tail meat activity at different temperature and pH is presented in Fig. 2. It was observed that autolytic activity in tail meat was very low at all pH and temperatures. The autolytic activity in krill mince, a product of semi liquid consistency, was quite different (Fig. 3). High activity was observed at pH 8 at 40°C and 50°C,

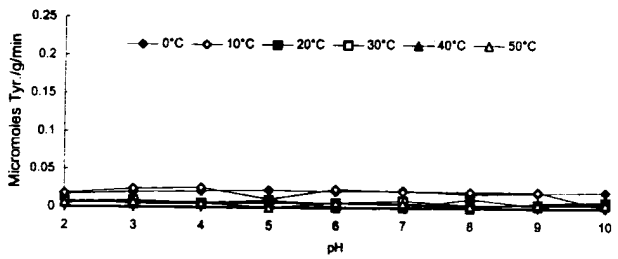

Fig. 2. Autolytic activity of krill tail meat measured at various temperatures and pH. For details of buffers used see figure 1. Activity is expressed as µMol tyrosine released/min/g sample.

while at the lower pH range of 2-4, activity was much lower.

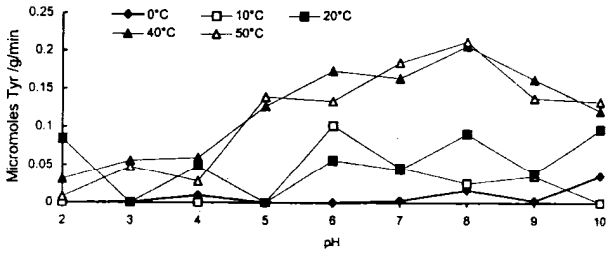

Fig. 3. Autolytic activity of krill mince measured at various temperatures and pH. For details of buffers used see figure 1. Activity is expressed as µMol tyrosine released/min/g sample.

Autolytic activity of krill is a major obstacle in its utilization as it leads to rapid tissue degradation. The whole krill was highly fragile when thawed. Major portion of the proteolytic activity appears to reside in the head or cephalothorax, as the machine peeled tail meat had very little autolytic activity. Similar observations have been reported earlier also (Konagaya, 1980). Thus separation of the cephalothorax portion immediately after catch could help in enhancing the quality of processed products. Autolytic activity in whole krill was observed to be due to acid and alkaline proteinases with some activity in the neutral pH range as well. The multiplicity of proteinases present and their activity even at low temperatures of 10-20°C, has been observed earlier (Seki *et al.*, 1975, 1977; Ellingsen, 1992) and poses a formidable problem in processing of krill. The anomalous pattern of autolytic activity in krill mince could be due to contamination with micro flora from processing equipment, but Ellingsen (1982) reported that krill autolysis proceeded most rapidly at 50 and 60°C, although after 1-3 h of incubation, peptide release ceased due to inactivation of the proteinases. The optimum pH for the formation of perchloric acid soluble peptides were 6 and 8-9. Similar results were obtained from this study also.

Thermal coagulation of krill proteins as a means of protein recovery has been attempted by Kuwano *et al.* (1979, 1980) who

heat denatured minced krill by steam for 30 min. It was seen in this study that nearly 80% of the initially soluble krill proteins can be precipitated by rapidly heating a dilute suspension of krill mince. The initial solubility can be further brought down by using an acidulant such as 0.2% citric acid. However, the solubility of the precipitated proteins has been found to be reduced by 40-70% of the solubility of the original krill proteins (Ellingsen, 1982). The amino acid composition of the insoluble fraction was however not different from the original fractions.

Table 5. Nutritional parameters of krill tail meat

Parameter	Value
Protein efficiency ratio	2.2
Protein digestibility	82.3 %
Nitrogen retained / nitrogen intake	67.2 %
Nitrogen retained / nitrogen absorbed	84.2 %

Note : Cooked krill tail meat was incorporated in diet to provide 50% of total dietary protein. Rest was provided by bengal gram (Cicer arietanium) flour.

The nutritional parameters of krill tail meat are given in Table 5. Krill meat was evidently a good dietary source of protein. There were no ill effects seen in the test animals after 4 weeks of feeding, although the digestibility was slightly low.

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