K-value, an index for estimating fish freshness and quality

P. T. Lakshmanam*,§ and K. Gopakumar†

*Central Institute of Fisheries Technology, Cochin 682 029, India
†Indian Council of Agricultural Research, Krishi Bhavan, New Delhi 110 001, India

Limitations of using a few chemical compounds, originating from deteriorative changes in fish muscle, for evaluation of fish quality, are focused. Hypoxanthine, a product of nucleotide degradation, can be used as a marker for fish quality in hypoxanthine-forming fish species. *K*-value, representing the ratio between the sum of inosine and hypoxanthine to the sum of all other ATP breakdown products, is a more reliable method for estimation of quality of fresh and preserved fishes and prawns. The storage life in ice ranges between 8 and 15 days for most Indian marine fishes, and between 12 and 15 days for freshwater fishes, prawns and molluscs when evaluated based on *K*-value and sensory methods.

As a healthy alternative to other animal protein, the sea-foods is on the increasing demand all over the world. Fish and fish products are now transported between nations and hence the freshness or quality of these products is becoming more and more important, as it reflects on the price. In 1995, world wide turnover of fish and fish products was 113 million mt valued at US $ 52 billion. In the fish industry, the quality is generally assessed by standard sensory methods for grading and sorting. In the EEC scheme, 4 grades corresponding to different stages of spoilage are recognized. However, commercial fish buyers now demand and depend on more reliable, objective quality criteria. The objectives of this article are (1) to focus the limitations of a few chemical methods and (ii) to highlight the reliability of the nucleotide degradation methods, as indicated by *K*-value in the evaluation of fish quality.

A chemical quality test is expected to accurately reflect the sensory characteristics of the fish and fish products. Based on the post-mortem deteriorative changes in the muscle associated with spoilage bacteria and enzyme reaction, a number of chemical methods have been used to evaluate the quality of fish. Total volatile base nitrogen (TVB-N), trimethyl amine (TMA) and dimethyl amine (DMA) are compounds of such bacterial spoilage and/or enzyme reaction and have been used as an index to evaluate fish quality. However, the usefulness of any of these most commonly used indices is limited. These compounds are produced in the fish muscle at later stages of spoilage and hence may not be useful to detect the spoilage at an early stage[1,2]. TMA is also not applicable to freshwater fish, as they contain very little or no TMAO. Essentially, an acceptable marker compound or index is expected to have the following attributes: the compound is (i) produced at the earliest stage of spoilage, (ii) ubiquitously present in most edible fish species, (iii) present in fishes that are freshly undergoing spoilage, kept in iced storage or frozen for a long or short period, and (iv) compatible with the commonly used sensory score.

§For correspondence

Hypoxanthine as an index of fish quality

As autolytic changes are taking place rapidly in fish muscle before the commencement of microbial spoilage, the nucleotide-based methods have assumed greater importance in assessing the freshness of fish[3–5]. Individual nucleotides and nucleotide ratios have been used to indicate quality in a number of fish species[6–13]. Thus the quantity of hypoxanthine (Hx) is regarded as an index of quality. The pattern of nucleotide breakdown in most fish species is known to proceed as follows: ATP → ADP → AMP → IMP → inosine –> hypoxanthine → xanthine → uric acid. Since Hx is formed as a result of both autolytic and bacterial activities, it has an advantage over TMA assay[4]. After reviewing the chemical indices of fish quality, Martin *et al.*[4] proposed Hx concentration as a useful index of fish quality for several species. Boyd and Wilson[14] found significant linear relationship between Hx concentrations and the days in ice in 210 trawled snapper caught at various times of the year. Fletcher and Hodgson[15] also used Hx levels to assess the shelf life of iced snapper and observed a linear relationship with storage time. The Hx content of seafrozen whole or filleted rock cod showed significant negative correlation with sensory score[16]. Jacober and Rand[5] found a remarkable increase of Hx with chill storage in winter flounder and remarked that Hx can serve as a good index to measure spoilage. They suggested standards of Hx for grading the quality of fish. Based on molecular ratio of inosine : hypoxanthine, Ehira and Uchiyama[17] arbitrarily defined a species as inosine-forming species (5 inosine : 1 hypoxanthine ratio),

hypo-xanthine-forming species (1 inosine : 5 hypoxanthine ratio) or intermediate type species (with a smaller ratio). Of the 100 Japanese fish species studied by them, 35 species alone were hypoxanthine-forming species. Clearly, the freshness of inosine-forming species and intermediate type species cannot be assessed by measuring Hx accumulation. Hence Hx is now considered not as good indicator of quality for all types of fish species. Secondly, the rate of formation of Hx and inosine differs between species[18]. Poor correlation has been observed between Hx concentration and quality change in the iced salmon[19], red fish[20], rough grenadier[21] and lemon sole[22].

K-value as an index of fish quality

A high level of any adenosine-related compounds or inosine monophosphate (IMP) in the muscle imparts sweet, meaty flavour and is regarded as a reliable index of freshness. Post-mortem accumulation of inosine or Hx generally reflects poor quality. The conversion of ATP to IMP is very fast and is usually complete within a day[2,23]. Subsequent accumulation of inosine or Hx is related to both autolytic and/or microbial action[4]. Reviewing the objective chemical indices for fish quality, Saito *et al.*[24] were the first to estimate the freshness of fish muscles from the ratios of the sum of the inosine and Hx to the sum of all other ATP breakdown products. This ratio expressed as a percentage, is called the *K*-value, thus

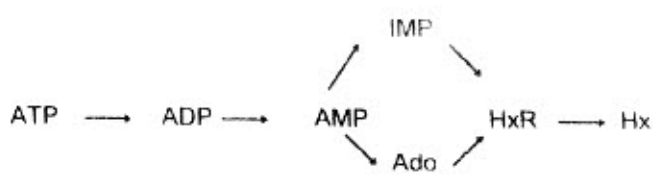
$$K\text{-value } (\%) = \frac{[\mathrm{HxR}] + [\mathrm{Hx}]}{[\mathrm{ATP}] + [\mathrm{ADP}] + [\mathrm{AMP}] + [\mathrm{IMP}] + [\mathrm{HxR}] + [\mathrm{Hx}]} \times 100,$$

where Hx = hypoxanthine, HxR = inosine, ATP = adenosine-5′-triphosphate, ADP = adenosine-5′-diphosphate, AMP = adenosine-5′-monophosphate, IMP = inosine monophosphate. Subsequently, Karube *et al.*[25] deleted the adenosine compounds and proposed Ki as an index of fish quality.

Selecting 104 fish samples drawn from commercial sources, Uchiyama *et al.*[6] made a comparative study on the reliability of 3 indices namely, *K*-value, VB-N (volatile base nitrogen) and TMA-N (trimethyl amine nitrogen); the selected samples were classified into 3 groups: (i) fish immediately after being killed, (ii) good quality tuna and sliced raw fish, and (iii) material of medium quality. Whereas VB-N and TMA-N failed to detect the differences in the freshness among the 3 groups, *K*-value readily distinguished the quality of the three groups, and the results were comparable to those of commercial sensory evaluations. From these results, they concluded that the *K*-value for the prime quality fish is around 20% and can reliably be used as an index of evaluating the real freshness of fish. In a nucleotide degradation study, Ehira and Uchiyama[18] measured IMP and Hx concentrations of muscle in 100 fish species from iced storage until the *K*-value reached 30% or more. Whereas the *K*-value could really and reliably distinguish the freshness in all the investigated fish species, Hx could not be used as an index in inosine-forming species. Comparing nucleotide degradation and sensory analysis in 4 tropical fish species during iced storage, Bremner *et al.*[8] observed that shelf-life and overall acceptability were more related to IMP degradation and *K*-value than bacterial spoilage. Ehira and Maruoka[26] used *K*-value for evaluating the freshness degradation of triploid (3*n*) rainbow trout induced by chromosome manipulation and kept in ice after being killed. Its shelf-life was comparable to its diploid (2*n*) counterpart. Malle and Pezennec[27] employed a rapid test strip method for the determination of *K*-value to assess the freshness of fish, the *K*-value was determined together with TMA-N, TVB-N, the mesophilic count and the freshness index. Correlations of *K*-value with freshness index were very high ($r = 0.93$, 1.98 and 0.98) for salmon, mackerel and whiting, respectively. For whiting (low fat fish), *K*-value and TVB-N were equally good but for the fatty fish, the *K*-value was superior. In an earlier study, Kiesvaara *et al.*[28] could establish that *K*-value served as a freshness indicator for several Finnish freshwater species. Ryder *et al.*[12] and Lakshmanan *et al.*[13] also found that *K*-value is good objective index for the freshness of hoki and rainbow trout, respectively during iced storage. The changes in freshness and flavour components of milk fish (*chanos chanos*) at various storage temperatures, (on ice, 5, 15, 20 and 35°C) indicated that *K*-value was a reliable indicator for milk fish.

K-value as an index of freshness in shellfish

Marine molluscs and crustaceans degrade ATP in a different pathway from that of fishes. High levels of AMP in the post-mortem shell fish muscle have been recorded[24,29]. The major pathway of ATP degradation in these invertebrates proceeds via the adenosine pathway but through two alternate pathways as proposed by Sakaguchi *et al.*[30]



However, these pathways do not interfere with the measurement of *K*-value in molluscs or crustaceans.

Relatively less information is available on the post-mortem nucleotide degradation of shrimps[31,32]. Fatima

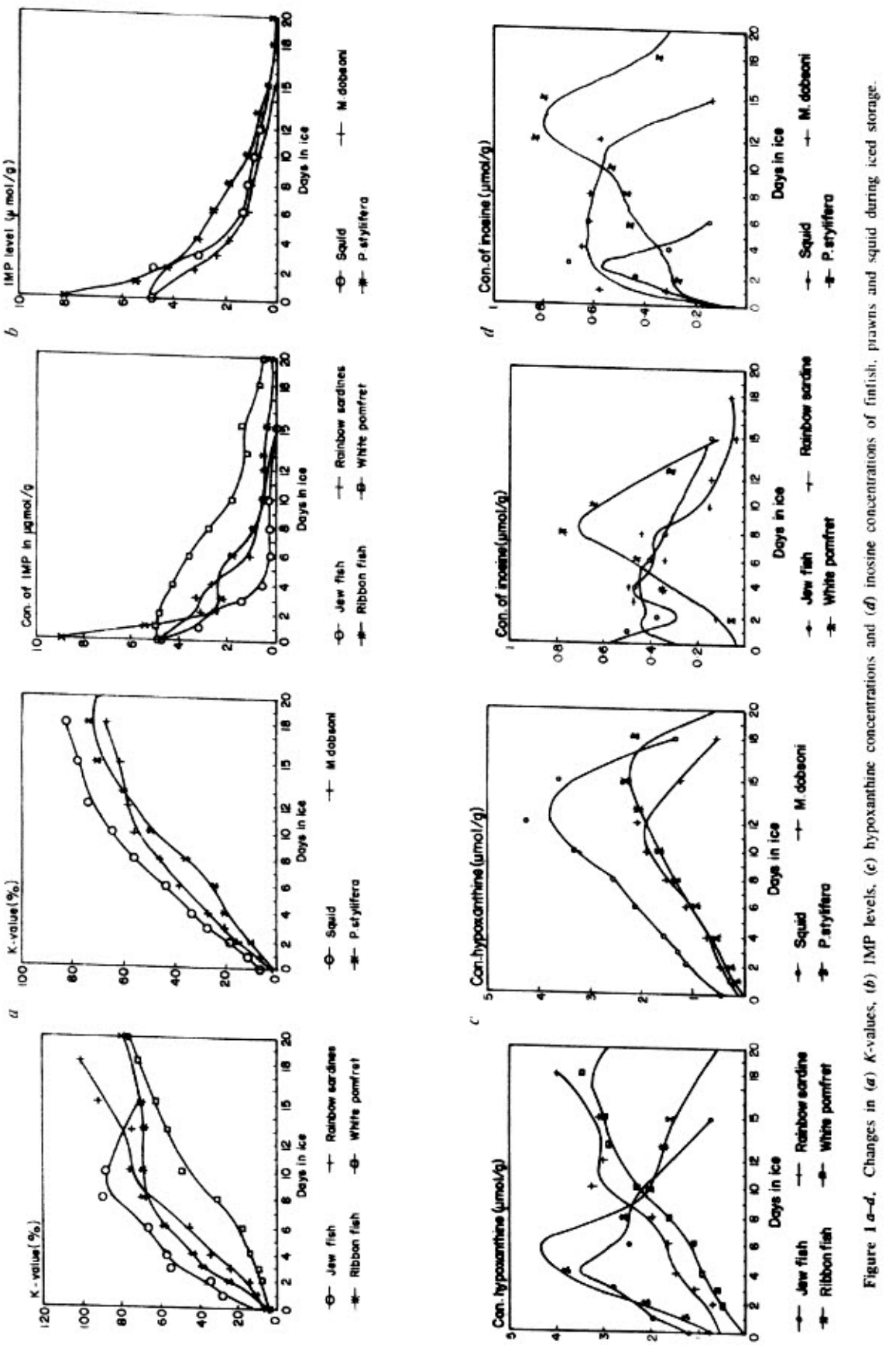


Figure 1 *a–d*. Changes in (*a*) *K*-values, (*b*) IMP levels, (*c*) hypoxanthine concentrations and (*d*) inosine concentrations of finfish, prawns and squid during iced storage.

Table 1. Acceptable level of *K*-value and storage-life in ice for the Indian marine and freshwater fishes, prawns and squid[13,17,34,38–40]

Species	*K*-value at the limit of acceptance	Storage life in ice (day)
Pampus argenteus	61	15
Pseudosciciena sp.	89	6–8
Lepturacanthus savala	69	10
Dussumeria hasseltii	67	8
Rastraliger kanagurta	45	10
Eleutheronema tetradactylum	54	12
Liza corsula	50	8
Etroplus suratensis	50	13
Catla catla	55	12
Labeo rohita	53	15
Metapenaeus dobsoni	58	12
Parapenaeopsis stylifera	60	13
Puneaus monodon	46	14
P. indicus	44	12
Loligo duvaceli	74	12

et al.[32] observed that hypoxanthine accumulates rapidly in the prawn muscle *Penaeus merguensis* during iced storage; the IMP level also gives a measure of freshness in the shrimp. Consequently, there is high correlation between IMP, Hx, *K*-value and sensory assessment[32]. Reilly *et al.*[33] used *K*-value along with microbiological investigations, TMA and TVN to assess the storage stability of the brackish water prawn (*Penaeus monodon*). However, the *K*-value is a more useful index for freshness of this brackish water prawn than TVN and TMA. There is a significant correlation between *K*-value and ice storage life, and the prawn can be stored in ice for 5 days without quality loss. *K*-values for the prawn ranges between 2 and 3%, when fresh, and 35 and 44%, when spoiled. Hence, *K*-value of 20–30% can be used for fresh prawns.

While studying the storage characteristics of two species of prawns, *Penaeus monodon* and *P. indicus* in ice and frozen condition, Lakshmanan *et al.*[34] observed high concentration of AMP and IMP in the muscle. They also recorded good correlation between freshness scores and the levels of IMP, (AMP + IMP) or *K*-value. For prime quality prawns, a *K*-value of around 20% is recommended, a value known to represent the prime quality fishes too.

K-value was found to be a useful index of freshness and quality for the Japanese spiny lobster, *Palunirus japonicus*[35]. *K*-value ranged from 20 to 25% at the stage of initial decomposition and observed after 1,5 and 11 days during storage at 20, 5 and 0°C, respectively. Dingle *et al.*[36] observed that *K*-value increased slowly, but consistently in the muscle of *Homarus americanus* during storage in ice and hence forms a useful indicator of spoilage.

Sagakuchi *et al.*[30] observed high concentration of AMP and IMP in the adductor muscle of the oyster, *Crassostrera gigas*. Suwetja *et al.*[29] too detected appreciable amounts of IMP in the muscle of ark-shell, short-neck clam and cuttlefish. *K*-value increased with storage time for squids held at 26–30°C. Upon rejection on the 11 day, the value was 53%. However, the *K*-value fluctuated widely in samples held at chilled sea water (CSW) in ice and at 5°C, probably due to leaching effect and hence its use as a quality indicator is questionable[37]. Lakshmanan *et al.*[34] also observed an increase of *K*-value with storage in ice in the common squid *Loligo duvaceli*.

Review of research carried out in India

India is a major sea-food exporting country and is realizing huge amount of foreign exchange every year. However, the use of nucleotide degradation or *K*-value as an index of fish quality is assessed only during the recent years by Lakshmanan and his associates[17,34,38–40]. They have studied the nucleotide degradation in a dozen fishes, and prawns and squids. Table 1 gives the *K*-value at the limit of acceptability and storage-life in ice for these species. In general, the storage life for marine fishes lasts between 6 and 15 days; however, it is longer (12–15 days) for a number of freshwater fishes, and marine prawns and squid. Their studies indicate that *K*-value is a good index for quality assessment of iced fishes. The results are illustrated in Figure 1 *a–d*. However, in cooked IQF prawns, nucleotide concentration was very low and *K*-value did not indicate quality.

In spite of the fact that *K*-value has been proposed as the most reliable objective index of quality, no easy method was available for its determination until the high performance liquid chromatography (HPLC) method of Ryder[41] was adopted.

1. Spinelli, J., Ekluno, M. and Miyauchi, D., *J. Food Sci.*, 1964, **29**, 710–714.
2. Jones, N. R., in *The Technology of Fish Utilisation* (ed. Kreuzer, R.), Fishing News Books, London, 1965, pp. 179–183.
3. Amu, L. and Disney, J. G., *Trop. Sci.*, 1973, **15**, 125–140.
4. Martin, R. E., Gray, R. J. H. and Pierson, M. D., *Food Technol.*, 1978, **32**, 188–198.
5. Jacober, L. F. and Rand, A. G., in *Chemistry and Biochemistry of Marine Food Products* (eds Martine, R. E., Flick, G. J., Hebard, C. E. and Ward, D. R.), AVI Publishing Company, 1982, pp. 347–365.
6. Uchiyama, H., Ehira, S., Kobayashi, H. and Shimiza, W., *Bull. Jpn Soc. Sci. Fish.*, 1970, **36**, 177–187.
7. Gill, T. A., Thomson, J. W., Gould, S. and Sherwood, D., *J. Food Sci.*, 1987, **52**, 580–583.
8. Brenner, A., Olley, J., Statham, J. A. and Vail, A. M. A., *J. Food Sci.*, 1988, **53**, 6–11.
9. Greene, D. H., Babbit, J. K. and Reppond, K. D., *J. Food Sci.*, 1990, **55**, 1236–1238.
10. Price, R. J., Melvin, E. F. and Bell, J. W., *J. Food Sci.*, 1991, **56**, 318–321.
11. Hattula, T. and Kiesvaara, M., *J. Sci. Food Agric.*, 1992, **58**, 485–488.
12. Ryder, J. M., Fletcher, G. C., Stec, M. G. and Seelye, R. J., *Int. J. Food Sci. Technol.*, 1993, **28**, 169–180.
13. Lakshmanan, P. T., Brown, R. T. and Ames, G. R., *Trop. Sci.*, 1993, **33**, 75–80.
14. Boyd, N. S. and Wilson, N. D. C., *N. Z. J. Sci.*, 1977, **20**, 139.
15. Fletcher, G. C. and Hodgson, J. A., *J. Food Sci.*, 1988, **53**, 1327–1332.
16. Lakshmanan, P. T., Varma, P. R. G., Iyer, T. S. G. and Gopakumar, K., *Fish. Res.*, 1990, **9**, 1–12.
17. Ehira, S. and Uchiyama, H., in *Seafood Quality Determination* (eds Kramer, D. E. and Liston, J.), Elsevier, Amsterdam, 1986, pp. 185–207.
18. Ehira, S. and Uchiyama, H., *Bull. Tokai. Fish Res. Lab.*, 1973, **75**, 63–73.
19. Murray, J., Jones, N. R. and Burt, J. R., *J. Fish. Res. Bd Canada*, 1966, **23**, 1795–1797.
20. Frazser, D. I., Pitts, D. P. and Dyer, W. J., *J. Fish. Res. Bd Canada*, 1968, **25**, 39–253.
21. Botta, J. R. and Shaw, D. H., *J. Food. Sci.*, 1975, **40**, 1249–1252.
22. Ehira, S. and Uchiyama, H., in *Seafood Quality Determination* (eds Kramer, D. E. and Liston, J.), Elsevier, Amsterdam, 1986, pp. 185–207.
23. Hiltz, D. F., Dyer, W. J., Nowlan, S. and Dingle, J. R., in *Fish Inspection and Quality Control* (ed. Krenzer, R.), Fishing News Books, London, 1972, pp. 191–195.
24. Saito, T., Arai, K. and Matsuyoshi, M., *Bull. Jpn Soc. Sci. Fish.*, 1959, **24**, 749–750.
25. Karube, I., Matsuaka, H., Suzuki, S., Watanabe, E. and Toyama, K., *J. Agric. Food Chem.*, 1984, **32**, 314–319.
26. Ehira, S. and Maruoka., in *Post-Harvest Technology, Preservation and Quality of fish in Southeast Asia* (eds Reilly, *et al.*), International Foundation for Science, 1989, pp. 93–102.
27. Malle, P. and Pezemec, I., *Sci. Ailmentes*, 1992, **12**, 257–269.
28. Kiesvaara, M., Hattula, T. and Karppinen, S., Technical Research Centre of Finland Research Notes, 1990, 1193.
29. Suwetja, I. K., Hori, K., Miyazawa, K. and Ito, K., *Nippon Suissan Gakkaishi*, 1989, **55**, 559–566.
30. Sakaguchi, M., Yamashita, K. and Murata, M., in *Chilling and Freezing of New Fish Products*, International Institute of Refrigeration, Aberdeen, UK, 1990, pp. 141–145.
31. Matsumoto, M. and Yamanaka, H., *Nippon Suissan Gakkaishi*, 1990, **56**, 1145–1149.
32. Fatima, R., Farroqui, B. and Quadri, R. B., *J. Food Sci.*, 1981, **46**, 1125–1127, 1131.
33. Reilly, A., Bernate, M. A. and Dangla, E., *Food Technol.*, 1984, **24**, 1355–1360.
34. Lakshmanan, P. T., Iyer, T. S. G. and Antony, P. D., in *Technological Advancements in Fisheries* (eds Hameed, M. S. and Kurup, B. M.), Cochin University of Science and Technology, 1998, pp. 388–395.
35. Yamanaka, H. and Shimada, R., *Fisheries Sci.*, 1996, **62**, 821–824.
36. Dingle, J. R., Hines, J. A. and Frazer, D. I., *J. Food Sci.*, 1968, **33**, 100–103.
37. Sengupta, W., in *Post Harvest Technology, Preservation and Quality of Fish in Sourtheast Asia* (eds Reilly *et al.*), International Foundation for Sciences, 1989, pp. 131–139.
38. Lakshmanan, P. T., Antony, P. D. and Gopakumar, K., *Food Control*, 1996, **7**, 277–283.
39. Lakshmanan, P. T. and Gopakumar, K., Asia Pacific Fishery Commission, Working Party on Fish Technology and Marketing, FAO fisheries report No. 563, 1996, APFC, Colombo, pp. 55–64.
40. Lakshmanan, P. T., Iyer, T. S. G., and Antony P. D., in *Advances and Priorities in Fisheries Technology*, Society of Fisheries Technologists (India), 1998 (in press).
41. Ryder, J. M., *J. Agric. Food Chem.*, 1985, **33**, 678–680.