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Biogenic Amines in Seafood

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Biogenic amines are aliphatic, alicyclic or hetrocyclic organic bases of low molecular weight formed in foods by the bacterial decarboxylation of free amino acids. Important biogenic amines found in fish are histamine, putrescine, cadaverine, tyramine, spermine and spermidine. Biogenic amines are generally either psychoactive or vascoactive. Their presence in fish usually does not represent any hazard to individuals unless large amounts are ingested or the natural mechanism for catabolism of one of the amines is inhibited. Histamine is mainly formed in certain fish species like mackerel, herring, tuna, sardines and anchovies. At the borderline of organloleptic acceptability, the histamine content of raw mackerel varied from 1-6 mg.100g^{-1} and raw herring from 5-10 mg.100g^{-1}. Incidents of histamine poisoning after eating fish are mainly due to poor quality of the raw material or poor processing. The presence of histamine is considered to be a quality indicator or spoilage indicator, because it occurs in negligible amount in fresh fish and increases upon spoilage. It is heat stable and non-volatile. HPLC, TLC and spectrophotometric methods are used for the determination of biogenic amines especially histamine. Quality criteria with respect to the presence of histamine and other biogenic amines in fishery products are necessary from a toxicological point of view as well as from technological point of view. In this paper, the mechanism of formation of biogenic amines, their significance in seafood, different methods of estimation and permitted levels are presented.

Key words : Biogenic amines, histamine, putrescine, cadaverine, tyramine, spermine spermidine, seafood

Biogenic amines have been defined chemically as aliphatic, alicyclic and hetrocyclic organic bases of low molecular weight. They are formed as consequence of metabolic process in animals, plants and microorganisms. and are formed by bacterial enzymes such as decarboxylases on substrates such as free amino acids. The presence of free monoacids, microorganisms that can decarboxylate the amino acids and favourable conditions for the growth of microorganisms and the production of free carboxylase enzymes, are factors that govern the formation of biogenic amines in certain species of fish like mackerel, herring, tuna, sardines and anchovies. Important biogenic amines in fish are histamine, putrescine, cadaverine, tyramine, spermine and spermidine. Biogenic amines formed in foods such as histamine are important indicators with regard to food contamination and also as chemical indicators of spoilage. Histamine poisoning is historically known as 'scombroid poisoning' because of its frequent association with the consumption of scombroid fish such as mackerel, tuna, bonito etc. Other biogenic amines are also important from the toxicological point of view, as they are identified as potentiators of histamine toxicity.

The presence of biogenic amine is considered as a reliable indicator of quality in fish by Karmas (1981) because they occur in negligible amounts in fresh fish and increase upon storage. They are considered to be the most suitable index of spoilage because of their nonvolatile and heat stable nature. The thermal stability of these amines makes them suitable as quality indices. The index values were developed for salmon, tuna , rockfish, shrimp and lobster tail. The average index values for these seafood products increased in the above order from salmon to lobster tail. Modified atmospheric packing of shrimp delayed microbial growth and TMA and TVBN production. However, production of biogenic amines was enhanced by MAP storage of shrimp (Lopez *et al.*, 2002). Ruiz-Capillas & Moral (2001) reported that amount of cadaverine and agamatine could be used as markers to indicate freshness or otherwise of hake stored in ice.

Good statistics on incidence of histamine poisoning do not exist in any country (Taylor, 1986). For several reasons, instance of histamine poisoning often go unreported. Since the illness is relatively mild and self limited, many patients do not seek medical attention. However, histamine poisoning has worldwide significance as public health problems especially in countries in which fish consumption is high. The countries with most reported incidents of histamine poisoning since 1970 are Japan, USA and Britain (Sours & Smith, 1980). It is second only to ciguatera poisoning in the primary food-borne diseases of chemical etiology in the United States (Taylor, 1987). The fish most commonly included in histamine poisoning are listed in Table 1.

Table 1. Fish species that may be implicated in histamine poisoning

Yellowfin tuna (*Thunnus albacares*)
Blackfin tuna (*T. atlanticus*)
Albacore tuna (*T. alalunga*)
Skipjack tuna (*Euthynnus pelamis*)
Atalantis bonito (*Sarda sarda*)
Atlantic mackerel (*Scomber scombrus*)
Spanish mackerel (*Scoberomorus maculates*)
Bluefish (*Pomatomus altatrix*)
Pacific herring (*Clupaea harengus*)
Horse mackerel (*Trachurus japonicus*)
European anchovy (*Engraulis encrasicohus*)
Pilachad (*Sardina pilachardus*)
Spanish sardine (*Sardinella aurita*)

Formation of biogenic amines

The general reaction taking place in the formation of biogenic amines are as follows:

$$RCH\text{-}NH_2\text{-}COOH \longrightarrow RCH_2NH_2 + CO_2$$

Histidine $\xrightarrow{\text{Histidine decarboxylase}}$ Histamine

Lysine $\xrightarrow{\text{Lysine decarboxylase}}$ Cadaverine

Tyrosine $\xrightarrow{\text{Tyrosine decarboxylase}}$ Tyramine

Ornithine $\xrightarrow{\text{Ornithine decarboxylase}}$ Putrescine→Spermidine→Spermine

Postmortem changes due to autolytic degradation in fish favour rapid proliferation of various spoilage bacteria, including those producing biogenic amines. Bacterial histamine production by the enteric bacteria is greatest in the temperature range of 30°-37°C (Arnold *et al.*, 1980; Behling & Taylor, 1983). The amine forming bacterial population and species are known to vary with storage temperature. *Photobacterium phosphoreum* plays a significant role in the histamine formation in fish stored at or below 10°C. *Pseudomonas* I and II and *Pseudomonas* III (IV-N) are known as typical spoilage bacteria and are the dominant amine forming organisms in fish stored at 5°C, whereas at 30°C, the dominant species were *Vibrio* and *Photobacterium*. A high content of histamine and cadaverine are observed during this stage of spoilage. *Pseudomonas* III/IVNH also produces putrescine at 5°C and *Photobacterium* forms agmatine and cadaverine at 30°C. Halophilic histamine forming bacteria were identified as *Photobacterium phosphoreum* and *P. histaminium*. During the ripening process for salted anchovies *Staphylococcus aurius* and *S. capitis* were found to be the powerful histamine forming bacteria (Rodriguez-Jerez *et al*., 1995).

Proteolytic actions of cathepsins causes degradation of fish proteins to amino acids and these are acted upon by the bacterial decarboxylase enzymes to form nonvolatile amines such as histamine, cadaverine, putrescine, tyramine, spermedine and spermine. These are not uncommon enzymes, they are found in animal tissues, plants, bacteria, yeast and dinoflagellates. Histamine is formed in fish from histidine by decarboxylation by bacterial histidine decarboxylase (EC4.1.1.22) (Taylor, 1986; Brown 1978). Scombroid fishes are commonly involved in histamine poisoning because they possess large amounts of histidine in their muscle tissues (Hibiki & Simidu, 1959; Lukton & Orcolt, 1958).

Biogenic amines occur in negligible amounts in very fresh fish and increase upon storage, which at large is attributed to the decarboxylase activity of bacteria. Bacteria belonging to family Enterobacteriaceae have assumed importance in histamine toxicity, since they are enteric in nature. Non-enteric organisms responsible for this phenomenon are bacteria belonging to the genus *Vibrio*, *Streptococcus*, *Clostridium* and *Lactobacillus*. The most important bacteria belonging to *Enterobacteriaceae* which produces histamine are *Morganella morganii*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Hafnia alvei*.

The bacteria capable of producing putrescine and cadarverine have been identified as *Enterobacter aerogenes*, *Shewanella putrefaciens*, *Escherichia coli* and *Klebsiella pneumoniae*. These organisms posses either one or both of the decarboxylase enzymes, ornithine and lysine decarboxylase. Tyramine is formed by *Streptococcus*, *Lactobacillus* and *Leuconostoc* species possessing the enzyme tyrosine decarboxylase.

Mechanism of amino acid decarboxylases

The decarboxylase reaction can be written either as hydrolytic process producing H_2CO_3 or as a lyase reaction producing CO_2. Owing to the rapid inter conversion of these two substances; it is not an easy matter to establish which is the primary product. Many of these decarboxylases act on nitrogenous substrates and depend on pyridoxal phosphate. Amino acid decarboxylases are highly specific and therefore they have been of great practical use for the estimation of amino acids on micro scale. A few occur in animal tissue and play a part in the metabolism of certain amino acids.

Clinical features of histamine poisoning

Histamine poisoning is intoxication, so the incubation period is rather short, ranging from several minutes to a few hours following ingestion of the contaminated fish. Histamine poisoning is most frequently a rather mild illness presenting with a wide variety of possible symptoms of cutaneous, gastrointestinal, hemodymanic and neurological nature (Taylor *et al.*, 1984; Borysiewicz & Krickler, 1981) (Table 2).

Table 2. Symptoms of histamine poisoning

Cutaneous	**Hemodynamic**
Rash	Hypotension
Urticaria	**Neurological**
Edema	Headache
Localised inflammation	Palpitations
Gastrointestinal	Flushing
Nausea	Tingling
Vomiting	Burning
Diarrhea	Itching
Cramping	

Many of these symptoms of histamine poisoning alone or in various combination can occur with other illness, both food borne and non-food borne. Avoidance of certain types of fish would be an effective means of prevention of histamine poisoning. Although many outbreaks in recent years have been associated with the ingestion of raw fish, elimination of raw fish from the diet would only reduce what is already a relatively modest risk. Care should be taken to ensure that raw fish are fresh and not subjected to spoilage.

Exogenous histamine from dietary sources is not always hazardous. In fact, many foods normally contain small amount of histamine, which can easily be tolerated. The lack of oral histamine toxicity is the result of intestinal detoxication (Tayler *et al.*, 1984). The paradox between the lack of toxicity of pure histamine and apparent toxicity of even small doses histamine in spoiled fish could be explained by the existence of potentiators.

Several potentiaters of histamine are known to occur in spoiled fish. Cadaverine, putrescine, tyramine β-pheoylethylamine are potentiators of histamine toxicity. Other potentiators of histamine toxicity may exist, operating through the inhibition of histamine metabolizing enzymes or other enzymes (Bjeldanes *et al.*, 1978).

Biogenic amines in fishery products

Luten *et al.* (1988) studied the formation of histamine putrescine and cadaverine from raw mackerel and herring during storage at different temperatures. Biogenic amines are generally either psychoactive or vascoactive. Psychoactive amines act as the neutral transmitters in the central nervous system, while the vasoactive amines act as either directly or indirectly as vascular system. Biogenic amine present in fish usually do not represent any hazard to individuals unless large amounts are ingested or the natural mechanism for catabolism of one of the amines are inhibited or generally deficient. Hardy & Smith (1976) reported the formation of histamine in mackerel (*Somber scombrus*) at ambient temperature, ice and frozen storage. The possibility of histamine being the causative agent of disorder from eating spoilt mackerel was also discussed.

Incidence of histamine poisoning after eating fish are mainly due to poor quality of the raw material or poor processing. On basis of a scombroid fish poisoning in Britain, Bartholomew *et al.* (1987) certain guidelines with respect to safety of histamine levels (Table 3).

Table 3. Histamine toxicity levels

Histamine level		Toxicity
< 5 $mg.100g^{-1}$	:	Safe for consumption
5-20 $mg.100g^{-1}$	:	Possibly toxic
20-100 $mg.100g^{-1}$	:	Probably toxic
>100 $mg.100g^{-1}$	:	Toxic and unsafe for consumption

Source: Bartholomew *et al.* (1987)

Wendakoon & Sakaguchi (1992) studied the effect of spices on growth and biogenic amine formation of two strong histamine-forming bacteria, *Enterobacter aerogenes* and *Morganella morganii* in mackerel muscle extract. Clove and cinnamon extracts were found to be the most effective against the bacterial growth and biogenic amine formation in fish. The production of amines as well as histamine decarboxylase activity was inhibited by spices extract. Vijayan *et al.* (1994) studied the formation of histamine in flying fish (*Hirundichthys coramandelensis*) at ambient temperature and ice. Histamine content was within the limits of acceptability. During iced storage, the histamine formation was negligible reaching only 0.22 mg.100g^{-1} in 17 days. Vijayan & Balachandran (1996) studied the formation of histamine in Indian mackerel (*Rastrelliger kangurta)* and tuna *(Euthynnus affinis)* at ambient temperature and in ice. In mackerel the formation of histamine was not significant up to a period of 10 h at ambient temperature (26°C) reaching an average value of only 7.51 mg.100g^{-1} fish and increased significantly thereafter. In iced mackerel, the production of histamine was not significant. Mathew *et al.* (1999) studied the formation of biogenic amines during storage of Atlantic mackerel in ice. No biogenic amine was detected in mackerel during three days in ice storage.

Biogenic amines as quality indicators

The presence of biogenic amines in fish is related to spoilage or quality. All biogenic amines have been proposed as indicators of the quality of fish and fishery products. Meitz & Karmas (1978) proposed a biogenic amine index (BAI) which consists of putrescine, cadaverine, butramine as quality indicators of fresh and processed seafood. There was a correlation between amine formation and decrease in free amino acids (FAA) with the exception of tyramine. Jorgenson *et al.* (2000) reported that biogenic amines are indicators of spoilage rather than causal agents of spoilage off-flavours.

Histamine and other biogenic amine levels vary greatly among species of fish and even within species at times (Rodriguez-Jerez *et al.*, 1995). Luten *et al.* (1992) recorded regulations for the limits of the content of biogenic amines in fish products. Histamine content is not considered as a reliable indicator of decomposition of fish, as the concentration of histidine in scombroid and non-scombroid fish varies. Therefore, cadaverine has been suggested as the most suitable index for both scombroid and no-scombroid fish (Karmas, 1981) Ruiz-Cappilas & Moral (2001) reported that during storage cadaverine levels reached 72.14 mg.kg^{-1} and those of agamatine levels reached 13.47 mg.kg^{-1} for hake. Increase in these amines was remarkable. Results suggested that cadaverine and agamatine could be used as markers to indicate freshness or otherwise of hake stored in ice. In herring and mackerel, similar amounts of histamine accumulated, while cadaverine was formed at higher levels in mackerel, compared to herring (Klausen & Lund, 1986).

Shellfish

In the muscles of squid and cuttlefish, arginine is extremely abundant in the free state and is easily converted into the corresponding nonvolatile amine, agmatine, by the decarboxylation of bacterial enzymes upon storage. So, agmatine could be considered as an index of freshness of squid and cuttlefish. In one study, it was found that agamatine increases before initial decomposition and it is not suitable as an early freshness indicator. But the basic amino acids such as ornithine appear to be appropriate indicator of freshness for squid.

In scallop abductor muscle and *kuruma* prawn, putrescine and ornithine have been reported to be potential freshness indicators whereas agmatine and tryptamine were suggested as indices for ascidian muscle. In crustaceans putrescine can be used as an indicator of decomposition. Huten *et al.* (1992) studied the formation of histamine, putrescine and cadaverine in raw mackerel and herring during storage at different temperatures. Histamine content of raw mackerel varied from 1 to 6 $mg.100g^{-1}$ and of raw herring from 5 to 10 $mg.100g^{-1}$. Hence, for quality control a maximum level of histamine of 5-10 $mg.100g^{-1}$ seems to be adequate for raw mackerel and herring.

Cadaverine is more frequently found in spoiled fish. The amount of cadaverine in spoiled fish varies, but level of 10 to 60 $mg.100g^{-1}$ have been found (Bgeldanes, 1979). Putrescine levels in spoiled fish are usually much lower than levels of cadaverine (Bjeldanes, 1979). Putrescine levels in spoiled fish are usually less than 10 $mg.100g^{-1}$. Ruiz-Capillas & Moral (2001) has reported that biogenic amines can be used as quality indices in muscle of gutted hake stored in ice for 25 days. During storage, cadaverine levels reached 72.14 $mg.kg^{-1}$ and those of agmatine reached 13.47 $mg.kg^{-1}$. Increase in levels of these was observed from 5 days of storage. So they suggested that cadaverine and agmatine could be used as markers to indicate freshness of hake stored in ice.

Fishery products

Toxicity of amins is not only related consumption of fresh fish but also with consumption of fishery products. Amines are heat stable which make them suitable for assessing the quality of raw material used for canned products (Veciana-Nogues *et al.*, 1997; Rogers & Staruskiewicz, 1997). They reported that spermine and spermidine were the only amines determined in all samples but were all lower in canned tuna than in fresh tuna samples.

Thermal processing destroys microorganism but not the amines present and hence amines are useful as chemical indicators for determining the initial quality of processed foods. Toxic amines in canned foods can arise from the use of poor quality raw material or defective handling during processing. The presence of high amounts of amines in canned tuna, indicates that initial raw material had reached a stage of advanced decomposition prior to heat processing (Klausen & Lund, 1986). Histamine at 50 ppm is reported to be the maximum level in canned tuna. A good correlation also exists between the sensory quality and the levels of putrescine and cadaverine (Sims *et al.*, 1992). Biogenic amine content in fish is not expected to increase during the canning process.

Histamine plays a significant role as an index of deterioration of dry salted mackerel, herring and sardines (Suszuki *et al.*, 1994). Histamine may occur during consumption of dry mackerel or herring. Major amines formed were not histamine but putrescine and cadaverine. Histamine was detected after drying for four days but in very low level of 4.31 $mg.g^{-1}$. It is usually formed during handling and salting process, but after drying the unfavourable growth conditions such as the bactericidal effect of salt and reduction of moisture content inhibit histamine forming bacteria and histamine decarboxylase.

During salting and drying process, histamine is increased more in whole fish than gutted fish and in unsalted sun dried fish. During summer, the histamine level in salted products is higher (>100 mg%). This is about double of that in winter. In salted anchovies, the histamine content was found to decrease significantly during the ripening process because along with other nitrogen factors, histamine diffuse into the brine (Veciana-Nogues *et al.*, 1997). Histamine content of about 50 ppm is reported to be safe for consumption.

Semi-preserved/fermented fish produc also, contained biogenic amines, because of the poor quality of material used. Halophilic or halotolerent bacteria were found to be responsible for such high levels of histamine in these products (Rodriguez-Jerez *et al.*, 1995).

Surimi and other fabricated product

Cadaverine seems to be a useful chemical indicator of decomposition in *surimi* and analogue products. Putrescine was found in lower concentration only, because of limited quantities of ornithine present in these products. Regulatory limits for histamine in fish and fishery products has been established in several countries. Regulatory limits in various countries are given in Table 4.

The maximum allowable limits for biogenic amines like putrecine and cadaverine have not been mentioned in any of regulatory agencies. Cadaverine and putrecine could be used as freshness indices for fish and shellfish. Various studies suggested that fish and fishery products containing cadaverine below 15 mg% are considered as possibly safe, between 15–20 mg% as the stage of potential decomposition and over 20 mg% as advanced decomposition.

Methods of detection of histamine and other biogenic amines

The analysis of histamine in the ingested food is critical to the confirmed diagnosis of histamine poisoning. A variety of methods are available for the analysis of histamine and biogenic amine in foods ranging from bioassay to chemical assays, which include flurometric, enzymatic and chromatographic procedures.

Table 4. Permitted levels of limits for histamine in fishery produce

Country	Limit
USFDA	50 mg% (hazard action level) 10 – 20 mg% (defect action level)
EEC	10 mg% (defect action level) 20 mg% maximum allowable limit
Canada	> 10 mg% - indicator of decomposition 10-20% mg% (defect action level)
Germany	20 mg%
Denmark	30 mg%
India	20 mg%
Sweden	20 mg%

Chromatographic and high-pressure liquid chromatography methods are considered to be suitable for determining biogenic amines in seafoods. Histamine can be determined by the method of Hardy & Smith (1976), Suzuki *et al.* (1990) Mathew *et al.* (1999) and Hayman *et al.* (1985) An enzyme based colorimetric method for histamine in fishmeal was described by Albrechet Ruiz *et al.* (2000). This method is a good alternative for the rapid detection of potentially toxic fishmeal.

Regulatory limits for histamine in fish and fishery products has been established in several countries. Maximum permissible limits for other biogenic amines such as putrescine, cadaverine and tyramine were not prescribed by any of the regulatory agencies in US, EU and Japan. Various studies suggest that cadaverine and putrescine could be used as freshness indices for fish and shellfish, respectively. Fish and fishery products containing cadaverine below 15 mg% are considered as good for consumption, 15-20 mg% indicates potential decomposition and over 20 mg% advanced decomposition. Histamine formation varies from species to species and depending on spoilage bacteria and of storage conditions. The use of only histamine as a chemical index of fish decomposition is not always accurate. However, putrescine and cadaverine may act in addition as synergist. Various methods are developed for estimating biogenic amines accurately. Suitability of biogenic amine content as freshness, spoilage indices for fish and fishery products need to be ascertained further.

References

Albrecht-Ruiz M., Clark, L.D. and Alman, P.M. (2000) *J. Aquatic Food Prod. Tech.* **8,** 71

Arnold, S.H., Price, R.J., Brown, W.D. (1980) *Bull. Jpn. Soc. Sci. Fish* **46,** 991

Bartholomew, B.A., Berry, P.R., Rodhouse, J.C. and Gilhouse R. (1987) *Epidim. Inf.* **99**, 775

Behling, A.R. and Taylor, S.L. (1982) *J. Food. Sci.* **47, 1311**

Bjeldanes, L.F., Schtuz, D.E. and Morris, M.M. (1978) *Food Cosmet. Toxicol.* **16**, 157

Borysiewicz, L. and Krickler, D. (1981) *Br. Med. J.* **282**, 1434

Hardy, R. and Smith, J.G.M. (1976) *J. Sci. Fd. Agric.* **27,** 595

Hayman, A.R., Gray, D.O. and Evans, S.V. (1985) *J. Chromatography* **325**, 462

Hibiki, S., and Simidu, W. (1959) *Bull. Jap. Soc. Sci. Fish.* **24,** 916

Jorgensen, L.V., Dalgaard, P. and Huss, H.H. (2000) *J. Agric. Food Chem.* **48**(6), 2448

Karmas, E. (1981) *Lebensm.-wiss. Technol.* **14,** 273

Klausen and Lund (1986) *Lebensmittel-Unters Und Forsch.* **182,** 459

Lopez-Caballero, M.E., Gonclaves, A. and Numes, M.L. (2002) *Eur. Food Res. Tech.* **214,** 192

Lukton, A. and Olcott, H.S. (1958) *Food Res.* **23**, 611

Luten, J.B., Bouquet, Q.B., Seuren, L.A.J., Burggraff, M.M., Reikwel-Booy G., Durand, P., Ettinne, M., Gouyou, G.P., Landrein, A., Ritchie, A., Eclerq, M. and Guinet, R. (1992) *Biogenic Amines in Fishery Products: Standardization Methods within E.C. Quality Assurance in Fish Industry*, (Huss *et al.*, Eds.), Elsevier Science Publications, New York

Mathew, P.T. Ryder, M.J. and Gopakumar, K. (1999) *Fish. Technol.* **36**, 78

Meitz J.L. and Karmas, E. (1977) *J. Food Sci.* **42**, 155

Rawles, D.D, Flick, G.C. and Martin, R.E (1997) *Advanc. Food Nut. Res.* **39**, 329

Rodriguez-Jerez, J.J., Hernandez, L.S., Herrero, M.M. and Mora-Ventura, M.T. (1995) *J. Food Sci.* **59**, 993

Rogers, P.L. and Staruszkiewcz, W. (1997) *J. AOAC Int.* **80**, 591

Ruiz-Cappilas, C. and Moral, A. (2001) *J. Food Sci.* **66**, 1030

Sims, G.G., Farn G., and York, R.K. (1992) *J. Food Sci.* **57**, 1112

Sours, H.E. and Smith, D.G. (1980) *J. Infect. Disease* **142**, 122

Suzuki, K., Kobyashi, K., Noda, J. Suzuki, J. and Takama, K. (1990) *J. Chromat.* **508**, 225

Taylor, S.L. (1987) Scombroid Poisoning in *Human Pathogens and Toxins in the Marine Environment*, (Colwell, R.R., Ed), John Wiley and Sons, New York

Taylor, S.L. Hui, J.Y. and Lyons, D.E. (1984) *Seafood Toxins* (Ragelis, E.P., Ed.), p. 417-430, American Chemical Society, Washington DC

Taylor, S.L. (1986) *CRC Crit. Rev. Toxicol.* **17**, 91

Vijayan, P.K. and Balachandran, K.K (1996) in Proc. Second workshop Scient. Results FORV Sagara Sampada, p. 521-527, Department of Ocean Devlopment, New Delhi

Vijayan P.K., Jose Joseph and Gopakumar K. (1994) *Fish. Technol.* **31**, 142

Veciana-Nogues M.T., Marine-Fond, A. and and Vidal-Carou, A.C. (1997) *J. Agric. Food Chem.* **45**, 4324