# The Change of Histamine Content in Some Fish-Based Foods During Storage

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## ABSTRACT

*Traditional fish-based food product becomes more interesting because of its active compound which function as health and fitness guard. South Sulawesi as a coastal area has lot of fishery products. Histamine content can be used as quality indicator for fishes and fish products. Some fish-based foods have been analyzed for its histamine content during storage. There were lawa teri, smoked kembung fish, and skipjack burger. These foods represent the different processes for fishery products.*

*Lawa teri is a traditional fish product from South Sulawesi. It is produced by bringing together fish fillet with citrus juice or vinegar, then mixed with fried grated coconut. The storage was carried out at 5°C for 2 weeks in two different packaging, glass and plastic. The results showed that during storage the histamine content increased from 1.009 to 23.023 mg/100g. The storage time showed a significant difference ($p<0.05$) but there was no significantly difference from the type of packaging, and there was no interaction between type of packaging and storage time on histamine content.*

*Smoked kembung fish was analyzed for its histamine content during storage at 5°C for 20 days in plastic packaging with two various conditions, aerobe and vacuum. The results showed that histamine content of raw material decreased after smoking from 7.280 to 7.045 mg/100g then decreased to 5.185 mg/100g after drying the smoked fish. During 20 days storage at low temperature the histamine content increased under aerobe as well as vacuum condition. There was a significant difference of storage time on histamine content and also the condition of packaging ($p<0.05$). The interaction between storage time and condition of packaging showed a significantly difference ($p<0.05$).*

*Skipjack burger was processed through more various steps by using more ingredients such as flour, egg, and spices. Histamine content was analyzed during storage for 4 weeks at 0°C. The histamine content increased from 0.755 to 8.196 mg/100g during 4 weeks storage. There was significantly difference on histamine content during storage.*

## INTRODUCTION

Histamine poisoning, known as scombroid fish poisoning is a world wide problem which occur rather frequently in any country where consumers digest any of fish from the group of *Scombridae* or *Scomberesocidae* (Taylor, 1988). Nowadays, the term scombroid poisoning is seldom used anymore, because histamine poi-

soning can occur due to other food stuffs. However, not all countries with great fish consumers reported the poisoning case. The duration of the illness is also rather short. Symptoms usually subside spontaneously within a few hours. The most commonly encountered symptoms are tingling and burning sensations around the mouth, gastrointestinal complaint, and a rash with itching (Taylor, 1988). The presence of histamine in foodstuffs can be used as an indicator for food hygiene, particularly for fish products.

Histamine (1H-imidazol-4-ethanamine) is a compound of biogenic amine group. Beside it is found naturally in foodstuffs, histamine can occur during processing of foodstuffs through decarboxylation process of free amino acid histidine. High protein foodstuffs, which are decayed by microorganism activity, could lead to high histamine content (Beutling, 1996). However, other types of fish are also commonly involved in scombroid fish poisoning; mahi-mahi, bluefish, jack mackerel, yellowtail, amberjack, herrings, sardines, and anchovies. The halotolerant and halophilic histamine-forming bacteria were isolated from mackerel, tuna, and sardines. Many family of *Staphylococcus, Vibrio*, and *Pseudomonas* have the ability to form histamine by salt concentration of 10 to 15% in test media (Beutling, 1996). Histamine formation in tuna is extremely high at temperature 24 to 30°C and it is low at temperature below 15°C (Taylor, 1988). To avoid histamine formation, it is recommended to store fish at temperature lower than 3°C. The proper handling of raw material can inhibit the formation of histamine.

Fish and other fishery products belong to the perishable commodities. It caused by the high water content, since fish has 80% water content. Besides that, fish body has high protein content and its pH is almost neutral. To prolong the shelf life of fish, its water content must be reduced to a certain amount. The activity of spoiled bacteria can be inactivated by reducing the water content in fish body.

This research aimed to study the change of histamine content in some fish-based foods during storage. There were lawa teri, smoked kembung fish, and skipjack (cakalang) burger. These foods represent the different processes of fishery products. This basic knowledge can give a great contribution on the development of fish processing technology.

Lawa teri was made from fresh anchovy. It was produced by mixing together fish fillet with citrus juice or vinegar and fried grated coconut. Smoked fish was produced by using hot smoking technique. Cakalang burger was made from minced fish which was mixed with wheat flour, eggs, and spices.

## MATERIALS AND METHODS

### Materials and Chemicals

Materials used in this research were anchovy (*Stolephorus sp*) for lawa, kembung perempuan fish (*Rastrelliger neglectus*) for smoked fish and skipjack (*Katsuwonus pelamis*) for burger. Additional materials used were cooking salt, wheat flour, eggs, and spices (garlic, onion, pepper, ginger). All materials used were bought from traditional market in Makassar. Chemicals used in this research were histamine dihydrochloride, trichlor acetic acid (TCA), n-butanol, NaOH, NaCl, n-heptane, HCl, 4-nitrobenzen diazonium tetrafluoroborat, and $Na_2CO_3.H_2O$. All chemicals were bought from chemicals distributor in Makassar.

### Histamine analysis (Mahendradatta and Schwedt, 1998)

*Standard Curve of Histamine*

A 0.207 g histamine di-hydro chloride is dissolved in doubly distilled water and filled to 50 ml. Two standard solution series are made with histamine concentration of 0.5; 1.0; 2.0; 4.0; 6.0; and 8.0 mg/l, and other series of 10.0; 20.0; 30.0; 40.0; and 50.0 mg/l, by dissolving in doubly distilled water. The measurement of absorbance using spectrophotometer is carried out with the method described in ***Analysis of histamine content***.

*Sample preparation*

A 10 g of each sample are homogenized with 25 ml 5% tri chlor acetic acid (TCA), then centrifuged by 4000

rpm for 10 minutes. The supernatant is decanted in 50 ml measured flask. Residue is homogenized once more with 5% of TCA and then centrifuged. The supernatant are combined and filled with 5% of TCA till mark and filtered.

*Sample clean-up*

A 1 ml filtrate is transferred to a centrifuge tube containing 5 ml n-butanol, 0.25 ml NaOH 5M and 0.75 g NaCl. The tube is shaken for 3 minutes and centrifuged for 10 minutes at 4000 rpm. All organic phase is transferred to the second tube containing 2.5 ml NaOH 0.1M saturated-NaCl. After shaken and centrifuged at the same way described previously, 4 ml organic-phase are transferred to the third tube containing 2.5 ml HCl 0.1M and 7.5 ml n-heptane. The solution is shaken and centrifuged. A one ml acidic-phase is ready to analyse.

*Analysis of histamine content.*

1 ml 0,2 % 4-nitrobenzene diazonium tetrafluorborat is added to 1 ml of histamine standard solution. After that 1ml 10 % of $Na_2CO_3.H_2O$ solution will be added. After 5 minutes the absorbance is measured by 470 nm. Histamine analysis procedure was shown at Figure 1.

**Total psychrophilic microbe**

Total psychrophilic microbe was analyzed based on the procedure from Fardiaz (1989).

**Research design**

Research procedures for lawa teri, cakalang burger, and smoked fish are shown in Figure 2, 3, and 4, respectively.

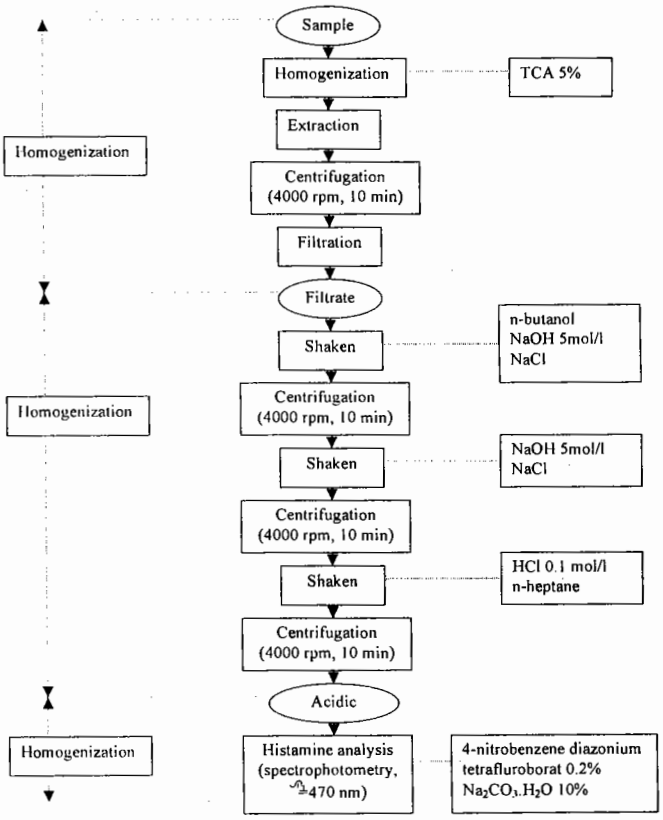


Figure 1. Histamine analysis procedure

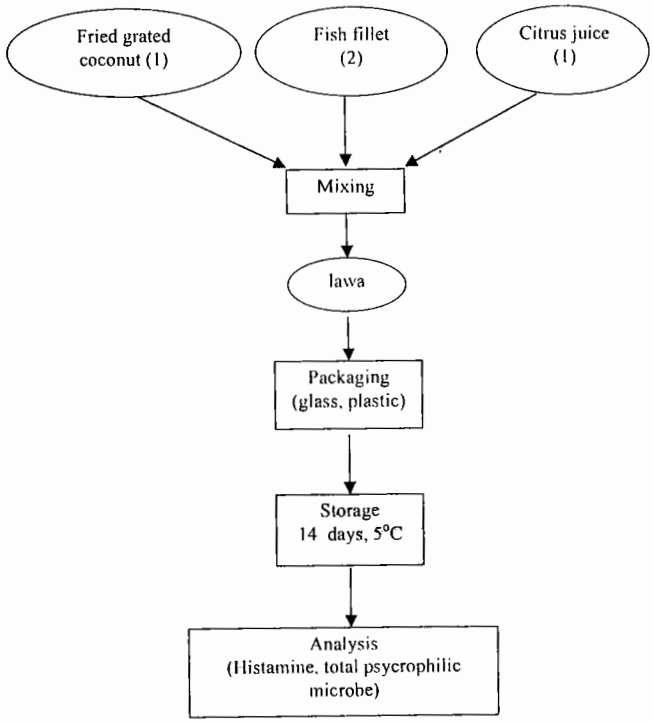


Figure 2. Research procedure for lawa teri

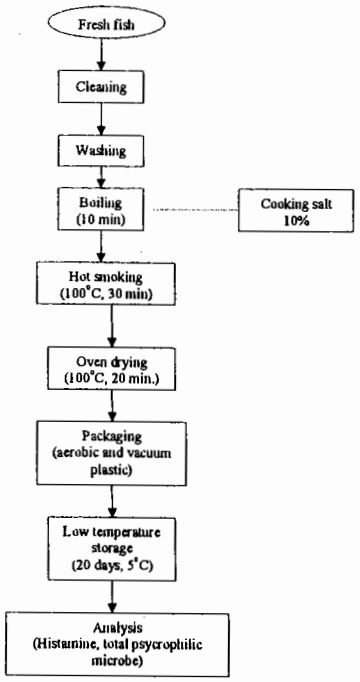


Figure 3. Research procedure for smoked fish

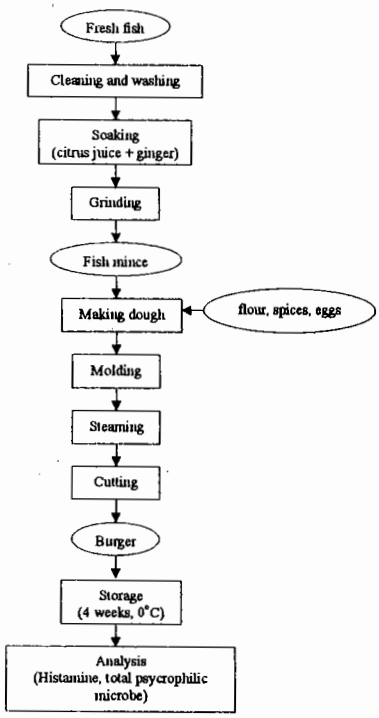


Figure 4. Research procedure for cakalang burger

## RESULT AND DISCUSSION

### Lawa teri (anchovy lawa)

The result was displayed at figure 5. Histamine content in fresh lawa was 1.007g mg/100g. Afrianto and Liviawaty (1989) stated that the freshness of fish played an important role on the histamine formation in end product. After fish is dead, histamine content increased due to the breakdown of free amino acid histidine by proteolitic enzyme. Lawa teri was then stored for 14

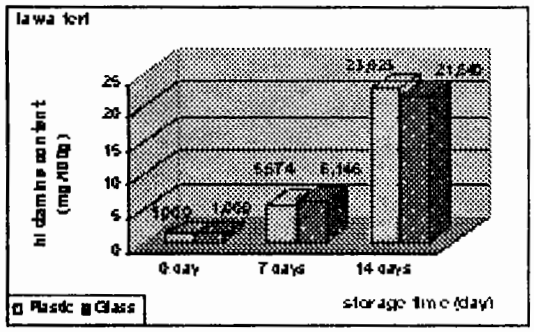


Figure 5. Histamine content in lawa teri during storage

days in two different packaging, i.e. glass and plastic. After 7 days storage the histamine content was increased to 5.574 and 6.146 mg/100g in plastic and glass packaging, respectively. After 14 days storage, the increase of histamine content was great, i.e 23.023 and 21.640 mg/100g. The high content of histamine was due to the addition of fried grated coconut. Coconut meat contains 2.42% of amino acid histidine (Palungkun, 1996). The storage time showed a significantly difference ($p<0.05$) but there was no significantly difference from the type of packaging, and there was no interaction between type of packaging and storage time on histamine content. During storage the free amino acid histidine can be decarboxylated into histamine. The use of packaging aimed to protect the product from contamination, but it could not avoid the product from microbial contamination, especially if the initial contamination has already occurred. Generally, lawa teri can still be consumed

until 14 days storage because the histamine concentration was under the limit concentration of poisoning case 50 mg/100g.

As supporting parameter, total psychrophilic microbes may be used to determine the condition of product. Fishery products can decay due to the microbial growth. The decay of fishery product depends on the rate of microbial growth, especially pathogenic bacteria. The research result showed that there was an increase of total psychrophilic microbes (Figure 6). During storage, there was a decomposition of some nutritive compounds such as protein and lipid which were found in fish and fried grated coconut. These compounds were the suitable media for microbial growth. Storage time showed a significant difference on total psychrophilic microbial ($p<0.05$), but the type of packaging did not.

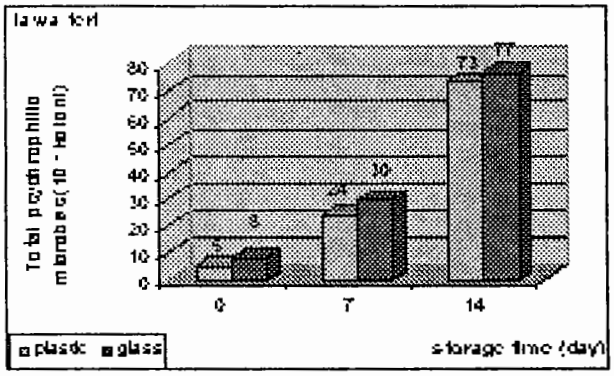


Figure 6. Total psycrophilic microbe of lawa teri during storage

## Smoked kembung fish

The main purpose of smoking process is to cook (to preserve), to dry the fishes, and also to give a specific taste to smoked fish. Smoking process is called also as artificial drying because the air in drying room is a mix between hot air and vapour (Horner, 1997).

Smoking process consists of 4 basic processes, i.e. salting, drying, smoking, and heating. Smoking process causes the decrease of water content, the increase of salt content, and the remains of smoke compound on fish surface. A thin layer of denaturated protein will adhere on fish surface. Drying and salting will reduce the water activity.

The most important role of smoking process is the smoke produced from wood burning. Smoke contains chemical compounds which have the ability to inhibit the bacterial activity which can hydrolyse starch and fat that causes rancidity, as well as bacteria which can destroy protein tissue and causes fish spoiled (Afrianto dan Liviawaty, 1989).

Smoked kembung fish was analyzed for its histamine content during storage for 20 days in plastic packaging with two various conditions, aerobe and vacuum. The result showed that histamine content of raw material increased after boiling from 5.603 to 7.280 mg/100g (Figure 7). Boiling could not inhibit the formation of histamine because this process was carried out only 10 minutes and the decarboxylase enzyme still had the ability to change histidine into histamine. The use of 10% salt in combination with boiling process could not reduce the histamine content of kembung fish yet.

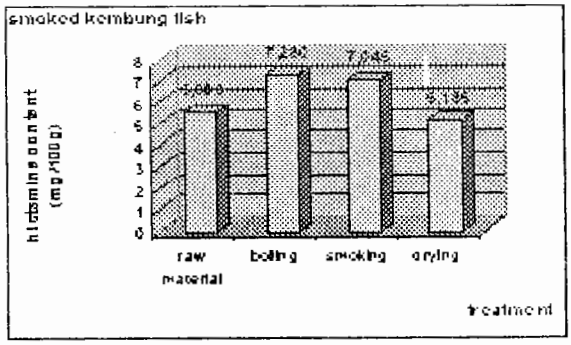


Figure 7. Histamine content in smoked fish during processing

After the smoking process, histamine content decreased from 7.280 to 7.045 mg/100g. Fletchers et al. (1998) indicated that there was evidence that histamine had been formed prior to smoking and that histamine producing bacteria were eliminated during smoking. Hot

smoking process was carried out by putting fish near the heat source, therefore more smoked produced will adhere to fish body. The preservative effect of smoking on fishery products is said to be due to a combination of surface drying, salting, deposition of phenolic antioxidant substances, and deposition of antimicrobial substances such as phenols, formaldehyde, and nitrites (Horner, 1997). Food Safety Advice, Auckland Healthcare (2001) has reported that hot smoking could be used to eliminate *Morganella morganii* and *Hafnia alvei* from seafood products.

Histamine content decreased to 5.185 mg/100g after drying the smoked fish. It was due to the decrease of decarboxylase activity produced by the fish. During 20 days storage at low temperature the histamine content increased under aerobe as well as vacuum condition (Figure 8). Oxygen in packaging can support the growth of microbe and the activity of decarboxylase enzyme. Histamine content in vacuum-packaged smoked fish was lower than in aerobic-packaged because the oxygen was sucked out from packaging. There was a significantly difference of storage time on histamine content and also the condition of packaging ($p<0.05$). The interaction between storage time and condition of packaging showed also a significantly difference ($p<0.05$).

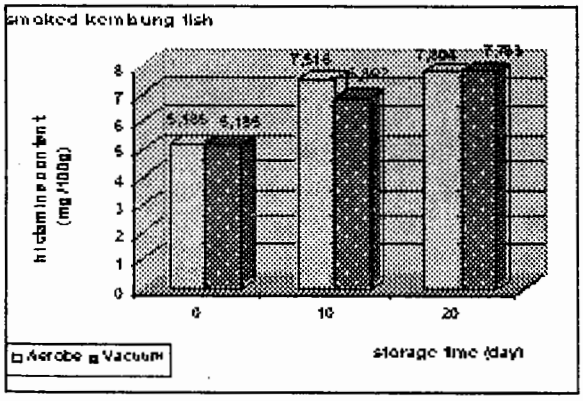


Figure 8. Histamine content in smoked fish during storage

Total psychrophilic microbes of smoked kembung fish ranged between zero (0 day storage in aerobic condition) and $4.6 \times 10^4$ (20 days in aerobic condition) (Figure 9). Cooling the product can not stop the metabolism activity of microbe but only slacks it. The storage time showed a significant difference on total psychrophilic microbial, but the condition of packaging did not show it, though the total psychrophilic microbe in vacuum condition was lower than aerobic condition.

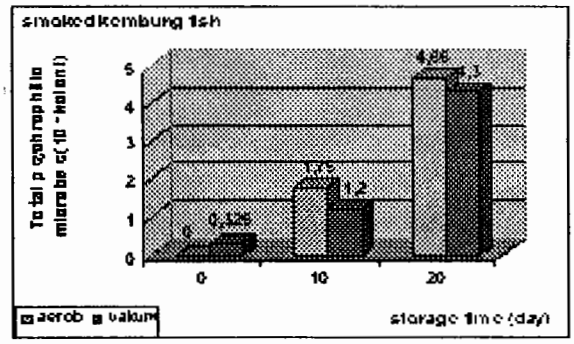


Figure 9. Total psycrophilic microbe of smoked kembung fish during storage

**Cakalang (skipjack) burger**

Skipjack burger was processed through more various steps using more ingredients such as flour, eggs, and spices. The result was shown in Figure 10. Histamine content was analyzed during storage for 4 weeks at 0°C. The histamine content increased from 0.755 to 8.196 mg/100g during 4 weeks storage. The increase was not in consideration due to the microbial activity, because total psycrophilic microbes was not detectable during storage. The other factor beside of the presence of active microbe that can cause the increase of histamine content was the enzyme activity that could change histidine into histamine. Though the enzyme activity was not measured in this research, some researchers stated that histamine can be produced by histidine decarboxylase in a very small amount of bacteria (Yamanaka et al., 1987). Baranowski et al (1985) said that histidine decarboxylase was produced by bacteria

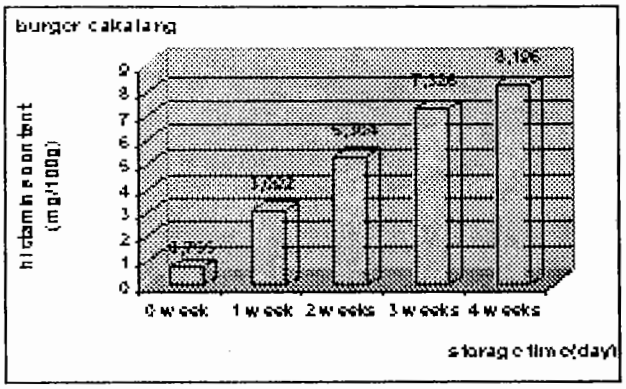


Figure 10. Histamine content in burger cakalang during storage

which its growth has stopped. Such enzyme was able to convert histidine into histamine.

Burger was made by applying procedures which was considered to destroy or inactivate the microoganism such as soaking in citrus juice and ginger, making dough by using spices and eggs, and steaming. Egg contain lysosim which acts as an antimicrobial agent, whereas spices (onion and garlic) contain acrolein as bacteriostatic (Nurwantoro dan Djarijah, 1997). Beside that, ginger showed an antioxidant activity from the group of phenolic i.e. gingerol and diarilheptanoid from non volatile fraction (Jenie dkk., 1992; Prangdimurti dkk., 1999). However in fact, based on the research result, the burger processing could not inhibit the histamine content during storage. Mince of fish meat could increase the histamine content greater than whole fish. Histamine that bound in cell structure of tissue can be released through cell damage (Fujii et al., 1994). Antimicrobial and antioxidant agent in burger could not have ability to inhibit the histamine formation during 4 week storage under given storage condition Statistically, there was a significantly difference on histamine content during storage.

## CONCLUSIONS

From this research, it is concluded that histamine content increases during storage of lawa teri, smoked fish, and burger cakalang. However, histamine concentrations are still below the concentration limit for histamine poisoning.

During processing of smoked fish, it is concluded that histamine content increases after boiling of fish, but then decreases after smoking and drying of fish. Total psychrophilic microbes increases during storage of lawa and smoked fish, but it is not detectable in cakalang burger.

## ACKNOWLEDGEMENT

This work was a part of research financed by CHNIII-Project and "Penelitian Dasar ". Also thanks to Ms. Fifi F. Lebang, Ms. Risna Adriani, and Ms. Rika Mudrikah for their help to conduct the laboratory work.

## REFERENCES

Afrianto, E. dan E. Liviawaty, 1989. "Pengawetan dan Pengolahan Ikan", Penerbit Kanisius, Jakarta.

Baranowsky, J.D., Brust, P.A., and Frank, H.A., 1985. Growth of *Klebsiella pneumoniae* UH-2 and properties of its histidine decarboxylase system in Resting Cells, J. Food Biochem. 9: 349 – 360.

Beutling, D.M., 1996. "Biogene Amine in der Ernaehrung". Springer-Verlag, Berlin-Heidelberg - New York.

Fardiaz, S., 1989. "Analisis Mikrobiologi Pangan". Depdikbud Dirjen Dikti, PAU Pangan dan Gizi, IPB, Bogor.

Fletcher, G.C., Summers, G. and van Veghel, P.W., 1998. Levels of histamine and histamine producing bacteria in smoked fish from New Zealand markets. J. Food Protein, 61(8), 1064 – 1070.

Food Safety Advice. June 2001. "Food safety guide for hot smoked fish", Food Safety Quarterly Report, Auckland Healthcare.

Fujii, T., Kurihara, K. and Okumi, M., 1994. Viability and histidine decarboxylase activity of halophilic histamine-forming bacteria during frozen storage. J. Food Prot. 57: 611 – 613.

Horner, W.F.A., 1997. Preservation of fish by curing (drying, salting, and smoking). In: Hall, G.M., Fish Processing Technology, 2nd ed., Blackie Academic & Professional, London.

Jenie, B.S.L, Undriyani, K, dan Dewanti, R, 1992. Pengaruh konsentrasi jahe dan waktu kontak terhadap aktivitas beberapa mikroba penyebab kerusakan pangan. Buletin Penelitian Ilmu dan Teknologi Pangan 3(2): 1-16.

Mahendradatta, M. and Schwedt, G, 1998. Sample preparation for photometric determination of histamine in foodstuffs compared with capillary electrophoresis. Z. Lebensm. Unters. Forsch. A, 206: 246 – 250.

Nurwantoro dan Djarijah, A.S., 1997. "Mikrobiologi Pangan Hewani-Nabati". Penerbit Kanisius, Jakarta.

Palungkun, R., 1996."Aneka Produk Olahan Kelapa". Penebar Swadaya, Jakarta.

Prangdimurti, E., Zakaria, F.R., Fardiaz, S. dan Sajuthi, D., 1999. Efek perlindungan ekstrak jahe terhadap respon imun mencit yang diberi perlakuan stres oksidatif oleh paraquat. In : Suparmo, D.W. Marseno, dan Sudarmanto (eds.). Prosiding Seminar Nasional Makanan Tradisional, PKMT - PAU Pangan dan Gizi UGM - Kantor Menphor RI.

Taylor, S.L., 1988. Marine toxins of microbial origin. Food Tech., 94 – 98.

Yamanaka, H., Shimakura, K., Shiomi, K., Kikuchi, T., and Okuzumi, M., 1987. Occurrence of allergy-like food poisoning caused by "Mirin"-seasoned meat of torado (*Coryphaena hippurus*). J. Food Hyg. Soc. Jpn. 28: 354 – 358.