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Effect of salt on the level of histamine in preserved fish (Herring, *Clupea harengus*)

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Abstract

Histamine levels in batches of heavily salted (fish:salt ratio 4:1) herring (*Clupea harengus*) were monitored during ripening at 4°C and 25°C. The batches studied were prepared from both pre-spawning and post-spawning (spent) fish using new and used salt. Salt levels in the flesh, which reached 11 to 14% (wet weight basis) during the ripening period, were found to retard histamine formation. During normal spoilage of ice chilled fish, histamine levels had been reported to exceed 50mg/100g flesh as it approached the limit of edibility whilst, in the heavily salted fish, levels remained below 20mg/100g flesh throughout the ripening periods of 18 months for the 4°C batches and 3 months for the 25°C batches. This was the case when the samples were set up and the salt allowed to penetrate the flesh at 4°C. When, however, the samples were set up and initially stored at ambient (10-15°C) temperature the histamine levels in the flesh rose above 20mg/100g before enough salt had penetrated to inhibit its generation. The gradual rise in levels which, nevertheless, occurred over the ripening periods followed significantly (5% level of significance) different trends, being greater in the batches prepared from pre-spawning than those from spent fish.

Key words : Histamine, Herring, Salt, Preservation

Introduction

The brine salting process has long been considered as an effective method of oily fish preservation inhibiting the oxidation of lipid which would take place under dry salting. This process in its various forms, is practiced in many parts of the world. Although heavily salted fish is now less popular in Western Europe than the other alternatives available throughout the year, certain products such as maatje cured herring remain popular. Maatje herring (lightly salted herring) is gaining popularity in many countries of Europe. Barrel salted herring is still produced in some parts of the United Kingdom, but most is produced in the Scandinavian countries.

In Bangladesh, *Hilsa ilisha*, is brine salted in much the same way as herring in Northern Europe except that the process is carried out at ambient

temperature so that the products ripens to "maturity" in about 2 months compared to the 9-18 months required for the herring product ripened at low temperature. For this reason the two experimental storage / ripening temperatures (4°C and 25°C) were chosen. A large proportion of the reported UK incidence of food poisoning, which have implicated fish, have been scombroid poisonings from the consumption of scombroid and clupeid species, which may have been less-than-fresh. Whilst there is doubt as to whether histamine is the cause of this type of poisoning (Clifford and Walker 1992, Ahmed 1992) its level in the fish flesh is still regarded as a good indicator of the freshness and of the likelihood of their having been subjected to any temperature abuse during handling. For this reason, Council Directive 91/493/EEC (1991) relating to the conditions required in the production and trading of fish product for the EC, specifies a maximum allowable limit (MAL) of 20 mg/100g flesh (with the mean value for 9 samples not to exceed 10mg/100g flesh) (EEC 1990). In view of the increasing attention being paid to biogenic amines by health and regulating authorities the present study was undertaken to study histamine level during the ripening of barrel salted herring. Factors which might influence histamine formation such as catching season, presence or absence of viscera, salt purity and storage temperature were investigated.

Literature on the formation of histamine in foods suggests that the decarboxylation of histidine results in the production of histamine. Early workers believed that histamine was generated due to the activity of the enzyme histidine decarboxylase, although the production rate varied widely under what were regarded as normal, optimum ripening conditions (Kimata 1961, Ferencik 1970, Yoshinaga and Frank 1982). Proteolysis, either autolytic or bacterial, has been suggested on playing a role in the release of free histidine from tissue protein (Ababouch *et al.* 1991). Some species of fish of the scombroid and clupeid families have large amounts (in order of 1% of the fish of wet weight) of free histidine (Hibiki and Simidu 1959, Ababouch *et al.* 1991) normally in their muscle tissue. This serves as a substrate for bacterial histidine decarboxylase. Studies on the effect of salt on histamine formation showed that histamine can form in vacuum-packaged, lightly-salted herring fillets stored at in-store, refrigerated display temperatures, particularly after contamination with psychrophilic *Photobacterium* spp. (Van Spreekens 1986). Mackerel stored at 5°C, even in the presence of 2% salt showed markedly increased histamine content following a prolonged refrigerated display period (Yamanaka *et al.* 1985). The effect of higher salt concentrations on histamine formation in fish is less known. In this paper the formation of histamine in heavily salted herring where the fish to salt ratio is 4:1, is reported.

Materials and methods

Source of fish

Herring (*Clupea harengus*) used in the study was provided from the purse seining vessels of Alexander Buchan Ltd., Peterhead, Scotland. Pre-spawning herring were caught from the nearby Piper field in mid-July and post-spawning (spent) herring from off Scarborough of UK in late September. The herring was kept in RSW tanks at -2°C inside the purse seining vessels but were landed within 24 hours of capture at the Peterhead processing factory. The catch was unloaded by an on-board winch operated scoop net digging the catch out of the RSW tanks and transferring them via a hopper to polypropylene tanks of about 1 m^3 capacity which were fork-lifted to the adjacent processing factory. At this stage the temperature of the fish was 0 to 2°C . In the factory the herring for the investigation were immediately processed according to the scheme selected to examine the effects of salt on histamine level under different condition i.e., before and after spawning, evisceration, salt purity etc.

Source of salt and additives

Pure salt (industrially processed new salt NaCl) and used salt both from the same source was collected from the Anderson's (Fish Merchants) of Peterhead. The used salt had been collected after its use in the dry salting of cod. Potassium sorbate was supplied by Merck Ltd., UK and nisin was supplied by Aplin & Barrett Ltd., UK.

Processing of fish

Both the pre-spawning and spent fish were processed into 4 different groups as follows:

1. Whole fish + new salt (with potassium sorbate and nisin)
2. Whole fish + used salt
3. Gibbed fish + new salt (with potassium sorbate and nisin)
4. Gibbed fish + used salt.

The sample were prepared as described below for maturation (ripening) at 4°C in polypropylene barrels and in polyethylene pouches (Synclair 5-layer pouches Type DBFI; Gee pack, UK). Gutting was carried out to remove gills and viscera in such a way that the pyloric caeca remained in the fish. A ratio of 1 part of salt to 4 parts (by weight) of fish was used in the preparation of all samples. After "rousing", (the hand mixing of fish and salt) the herring were packed into the barrels in the traditional manner. First a layer of fish was placed with belly uppermost and head to tail until the layer was complete. A layer of salt was placed on top and a new layer of fish laid at right angles to the layer beneath. These alternate layers of salt and fish were continued until the barrel was full. An extra two layers of fish and salt were laid on the top of the filled barrel . Within two days of holding at 4°C, the herring inside the barrel had settled down, due to

the pickle formation, and the extra layers had become immersed in the pickle. In a subsequent experiment, this pickle formation stage was allowed to proceed at ambient (10-15°C) temperature before allowing the ripening to proceed at the selected storage temperatures. The barrel was then closed and made airtight with a galvanised steel collar. To suppress bacteria and mould growth, nisin and potassium sorbate had been mixed with the new salt at a dosage level of 20 ppm and 1000 ppm respectively. Each barrel on closing contained 100 kg of fish and 25 kg of salt. In the same way 260 polyethylene pouches for each batch were prepared by vacuum sealing 1 kg fish and 250 g of salt in each pouch. Other conditions were exactly the same as for the barrel-salted fish.

Similarly 8 plastic tubs (4 for prespawning and 4 for spent fish) and 60 polyethylene pouches for the prespawning batch and 60 for the spent batch were prepared for storage at 25°C . Other conditions were exactly same as for the fish stored at 4°C.

All of the experimental materials were transported from Peterhead of Sctoland to Hull, England by insulated vehicle, which took nearly 24 hours. Final storage was performed at Hull until ripening. During the first 2 weeks of storage the barrels were rolled every other day to ensure uniformity of admixture and prevent the fish sticking together.

At 4°C storage, the barrels were opened once in every three months for sampling but the polyethylene pouch stored fish were sampled every month. At 25°C storage, the tubs were opened for sampling at the end of ripening (2-3 months) and the polyethylene pouch stored fish were sampled every month they were judged to have achieved maturity/ripening.

In the subsequent experiment, the herring were allowed to ripen in smaller plastic tubs as well as 60 kg barrels so that sampling could be performed every week, whilst leaving some tubs and barrels unopened for long-term storage. The fish in these containers could, thereby, ripen normally without suffering the effects from air ingress at sampling times.

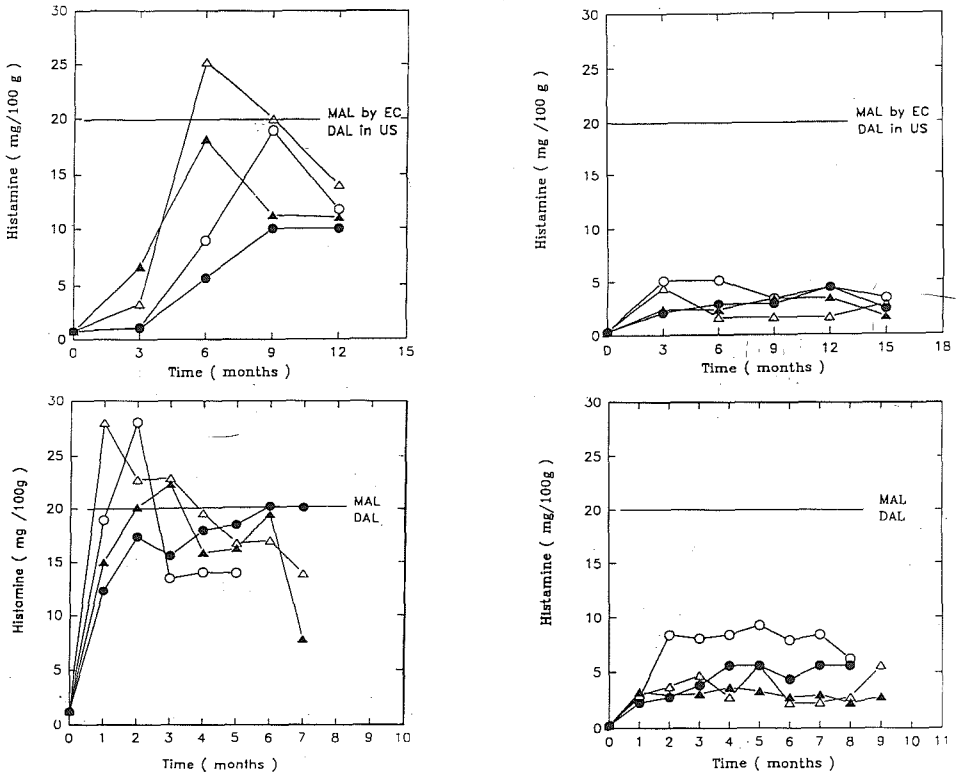
Analysis

The histamine content of the maturing salted herring was determined according to the method of Hardy and Smith (1976) which was based on the principle of colorimetric determination by coupling with a diazonium salt. The moisture content was determined according to the AOAC (1965) method. The salt (NaCl) content was determined by accurately weighing 25 g of fish flesh (homogenised from at least 6 fillets) and blending with 225 ml of cold water. This was filtered and 10 ml of aliquot was titrated against 0.1N $AgNO_3$ using potassium chromate as indicator. The percentage of NaCl in fish flesh was calculated from the relationship 1 ml 0.1N $AgNO_3 \equiv 0.005845$ g NaCl.

Paired t-test were carried out to assess the significance of the experimental results according to Miller and Miller (1988).

Results and discussion

In the herring stored at low temperature (4°C) the histamine levels of the pre-spawning batches increased with storage time up to 6 months for the gibbed fish, and up to 9 months for the whole fish, then gradually decreased as the fish continued to ripen (Fig. 1). Less histamine was produced in fish treated with new salt than in those treated with used salt. There were differences in histamine level among the 4 different groups of herring during barrel salting but all those where ripening had been allowed to proceed at 4°C contained acceptable levels of histamine at the end of ripening/maturation. In the spent batch at the same storage temperature (4°C), histamine levels were found to be considerably lower than those of the fish of prespawning batch (Fig. 2). Very little difference in histamine level were found among the 4 different groups of spent fish during the ripening/maturation period. Similar trends were observed in the fish stored in vacuum-sealed polyethylene pouches (Figs. 3 and 4).



● Whole fish + New Salt (with additives) ○ Whole Fish +Used Salt MAL-Maximum allowable limit by EC
▲ Gibbed fish + New Salt (with additives) △ Gibbed Fish+Used Salt DAL- Defect action level by US FDA

Figs. 1-4. Levels of histamine during the ripening of (1) pre-spawning, (2) spent herring (barrel salted), (3) pre-spawning and (4) spent herring (polyethylene pouch salted).

The batches where ripening had been allowed to proceed at 10-15 °C temperature were found to have unacceptably high histamine levels (ranging between 16 and 77mg/100g flesh) when first sampled, after 30 days storage (Figs. 5 and 6).

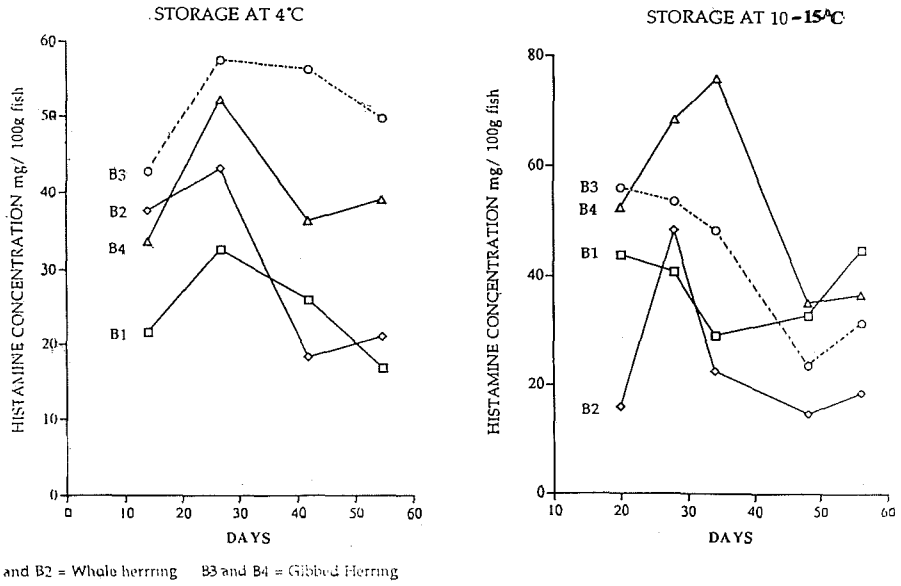


Fig. 5-6. Levels of histamine during the early ripening of pre-spawning herring barrel salted and kept 2 days at ambient temperature.

Ritchie and Mackie (1980) studied the histamine level in ice-stored herring (at 1 °C) where histamine reached 50mg/100g flesh within 17 days of storage.

Farnandez-Salguero and Mackie (1987) observed 53mg/100g in whole fish and 52mg/100g in fillets of herring by the 7th day of storage at 5 °C. In the present study the barrel salted fish were allowed to mature for 12-15 months and those for in the polyethylene pouches for 7-9 months of storage. During this extended period, histamine levels remained below the EC Council Directive MAL and the US Food and Drug Administration Defect Action Level (DAL). High salt concentrations, therefore, appear to effective in retarding histamine formation in herring.

The results of the present study also show histamine levels in stored fish vary with the catching season. They were found to be much lower throughout the ripening period in the spent fish. Variations in the histamine level with season in mackerel have been reported by Smith *et al.* (1980). Such variations were suggested as being due to the differences in the activity of intrinsic enzymes. The pre-spawning fish were caught in July and were still in active feeding condition and, for this reason, the proteolytic enzymes may have been more active than in the fish caught at the end of September, soon after spawning. The effect of gibbing on histamine formation, however, is not clear. In the pre-spawning herring, histamine levels trend in the barrel salted samples were contrary to those salted in the polyethylene pouches. It might have been expected that the presence of viscera would lead to higher histamine levels. Yet, the ambient-prepared batches showed higher histamine levels in the gibbed than in whole herring over start of the ripening period. This would probably be due to the spread of gut contents over the belly cavity in the process. The

contradictory results obtained, however, leaves the question of whether the enzymes from the gut bacteria play a significant role in histidine decarboxylation during 4°C ripening unanswered.

Nevertheless, differences in the levels of histamine in the batches treated with new salt and used salt suggested that histidine is being decarboxylated through the activity of both tissue enzymes and bacterial enzymes. Batches prepared with new salt were treated with antibacterial and antimould agents whilst those prepared with used salt were not. The former were found to contain lower concentrations of histamine than the latter. This differences was found in all groups of experimental fish. In the fish stored with pure salt (with antibacterial and antimould agents) the role of bacterial enzymes in histamine generation would have been expected to be reduced. Histamine formation in these fish therefore would be largely due to the activity of the intrinsic enzymes of fish. The level of histamine in the fish treated with used salt was always higher than the level of fish treated with new salt. It is to be expected that the used salt would be contaminated by halophiles which, over a long period of storage, might remain marginally active and contribute to histamine formation along with the intrinsic enzymes of the fish.

The decline in the histamine level after a certain period of storage, firstly, may be due to some of the histamine formed in the flesh being leached out into the surrounding brine and, secondly, some may enter into another biochemical pathway, for example, taking part in reactions with aldehyde groups of simple sugars or lipid oxidation products.

At higher temperature storage (25°C) the histamine levels in the fish from the plastic tubs at the end of ripening were very similar to those from the barrels kept at low temperature (4 °C). Table 1 shows the results obtained at the end of ripening (2 months) of the 4 different groups of fish from prespawning and spent batches. In the prespawning fish, which were allowed to ripen at 25°C, less histamine was produced in fish treated with new salt (with potassium sorbate + nisin) than in those treated with used salt. Histamine levels were below the specified MAL, although there were differences in histamine level in the 4 different groups of fish. In the spent batch (at 25°C) histamine levels were found to be lower than those of the fish of the prespawning batch (Table 1). The difference in histamine levels among the 4 different groups of spent fish was very small. The same trends were observed in the fish stored in vacuum-sealed polyethylene pouches (Table 2 and 3) although the level went slightly above the specified MAL in some samples of fish from the prespawning batch (Table 2) during the first weeks of the ripening period. Only in the ambient-prepared samples did the histamine levels substantially exceed the MAL. In these samples the bacterial activity associated with spoilage would have proceeded rapidly until sufficient salt had penetrated through the tissues to inhibit the further proliferation.

Table 1. Histamine contents of the fish ripened in plastic tubs at 25°C

Sample	Histamine (mg/100g)	
	Prespawning	Spent
Whole fish + new salt	10.85	3.35
Whole fish + used salt	12.95	3.87
Gibbed fish + new salt	13.32	1.04
Gibbed fish + used salt	17.35	1.75

Table 2. Histamine levels of the pre-spawning fish ripened in polyethylene pouches at 25°C

Storage period (month)	Histamine (mg/100g)			
	Whole fish + new salt	Whole fish + used salt	Gibbed fish + new salt	Gibbed fish + used salt
1	18.36	19.22	21.13	26.34
2	21.60	23.29	17.24	18.23
2 1/2	16.60	17.60	16.84	18.00

Table 3. Histamine levels of the spent fish ripened in the polyethylene pouches at 25 °C

	Histamine (mg/100g)			
Whole fish + new salt	Whole fish + new salt	Whole fish + used salt	Gibbed fish + new salt	Gibbed fish + used salt
1	6.76	8.42	2.89	3.4
2	9.29	10.32	3.83	4.6
3	8.79	10.12	1.80	2.7

The main conclusions of the present study are that:

(i) Low temperature (4 °C) and high salt concentration retard histamine formation in fish flesh.

(ii) If the fish are salted at high (above 15 °C) ambient temperatures, the specified MAL for histamine in the flesh is likely to be exceeded during the first two weeks of the process.

(iii) Even at the high salt concentration, histamine is still produced by the activity of the intrinsic enzymes of fish, but, unless histamine levels in the raw material are high or become high during the period when the salt was

diffusing into tissues, these levels are likely to remain below the specified MAL upto the time of maturity.

(iv) After the salt concentration in the fish flesh has become high enough to inhibit the growth of histidine decarboxylating bacteria, storage temperature has little effect on the histamine formation.

(v) Spent herring is better than the prespawning herring for salt preservation if the lowest histamine levels are considered to be the most desirable attribute but spent fish fails to produce a ripened product with the desired characteristic sensory attributes, compared to those of prespawning fish.

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