CHANGES IN TISSUE PROTEINASE ACTIVITY OF INDIAN MACKEREL UPON CURING IN BRINE AND SALT

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ABSTRACT

Autolytic activity in the muscle of Indian mackerel (*Rastrelliger kanagurta*) cured in saturated brine and dry salt, was monitored at pH 3, 4, 9 and 10 along with weight loss, moisture, salt and α-amino nitrogen, at 3, 6, 12, 24 and 48h of curing. The fish lost 14 to 29% of the initial weight after 48h of curing, in brine and dry salt, respectively. Correspondingly the moisture decreased from 76 to 57% in brine cured and 52% in dry salted fish. Salt content in the muscle increased from 1% to 14.82 and 13.32% in the case of brine cured and dry salted mackerel respectively, with slower uptake of salt in the latter because of the lesser surface area in contact. Tissue proteinases were activated initially in all cases when salting commenced, with higher activation in the case of pH 3 and 4. But subsequently increasing salt concentrations suppressed the autolytic activity. Autolytic proteinases in the mackerel muscle were suppressed between 37 to 78% of their original activity in case of the brine cured fish and between 36 to 74% in the dry salted fish, at the pH assayed. Concentration of α-amino nitrogen in the muscle increased initially in both types of curing at 3 h, but decreased later on. Autolytic activity assayed in the presence of salt containing buffers showed that at 10-20% salt level, (the concentrations prevailing in fish after 48h salting), 36-71% of the original tissue proteinase activity was still intact, which can affect cured product quality.

INTRODUCTION

Curing with salt, drying and smoking are traditional preservation methods of fish, dating from prehistoric times, which are still widely practised in tropical countries. Although successful preservation by salting essentially hinges on lowering of water activity, chemical changes such as lipid oxidation (Khuntia *et al.*, 1994), protein denaturation (Tambo *et al.*, 1992, Raghunath *et al.*, 1995) occurring during salting can also affect the product quality. Proteolytic enzyme activity in fish muscle can lead to rapid deterioration of quality in some fish such as chum salmon (Yamashita and Konagaya, 1991) and squids (Stanley and Hultin, 1984). But their activity is also well known to contribute to the process of fish sauce formation where proteolysis proceeds over long periods, albeit extremely slowly due to the high salt concentrations (Yamashita *et al.*, 1991). In lightly salted products proteinases from salt adapted bacteria contribute to the processing of fermented anchovy paste (Cha and Lee, 1989). However, the activity of fish tissue proteinases under salting conditions where fairly high salt concentrations prevail have not been investigated although their presence has been demonstrated in salted squid preserves (Makinodan *et al.*, 1993). Indian Mackerel (*Rastrelliger kanagurta*), a fish which is widely used for salting in India (Gopakumar and Bhattacharyya, 1992), is well known for its strong tissue proteinase activity (Jose and Raghunath, 1998), and hence was chosen for this study.

MATERIALS AND METHODS

Indian mackerel (*Rastrelliger kanagurta*) procured from the local fish market of Cochin, in post-rigor condition was brought immediately to the laboratory. The fish were beheaded, eviscerated and carefully washed to remove all traces of viscera from the abdominal cavity. Dressed fish were divided into three lots.

For curing in brine, each batch of four pre-weighed fish (Average weight of batch 220g) were immersed in 1.5 vols. of saturated brine (with a small amount excess undissolved NaCl). In case of curing with dry salt, the fish were intimately mixed and covered in 4x their weight of salt. Each batch of fish were taken out of the brine/salt after 3,6,12,24 and 48h, drained/wiped free of brine/salt and weighed again. The weighed fish were cut into small pieces, minced and taken for analysis. Moisture was determined as per AOAC method (AOAC, 1975). Salt was estimated as total chlorides with silver nitrate after digestion with nitric acid (Raghuramulu *et al.*, 1983). Free (α-) amino nitrogen in 10% trichloroacetic acid extract of the muscle was determined by the EBC Ninhydrin method (EBC, 1975). Activity of autolytic proteinases in the muscle homogenate (1:4, muscle: water) was determined by incubating 2ml of the homogenate with 4ml of buffer at 50°C for 1h, reaction terminated with 5ml of 11% TCA and the Folin positive material in the supernatant measured as per Herriott (1955). Activity was expressed as μMoles of Tyrosine released / g muscle / min. Citrate phosphate and Tris -HCl buffers (0.3M) were used at pH 3 and 4 and 9 and 10 respectively. Assays were carried out at pH 3,4, 9 and 10 which are known to contain most of the autolytic activity in mackerel muscle (Jose and Raghunath, 1998). Additionally, autolytic activity of unsalted muscle was also assayed with buffers containing 10, 20 and 30% salt as detailed before.

Commercial solar salt was used for curing purposes and for assaying of autolytic activity in presence of salt containing buffers. All other chemicals used were of AR or equivalent grade.

RESULTS

The loss in weight of mackerel, cured in brine and dry salted conditions over 48h of salting are shown in Tables 1A and 1B. Both the rate and extent of loss were higher in dry than in wet salted (brine cured) samples reaching 30% in dry and 14% in wet salted fish. After 24h, the loss in weight was marginal, indicating establishment of near equilibrium conditions. Concomitantly, the moisture content in the samples decreased quickly in the first 12h, but more slowly later on (Table 2). The dry salted samples reached a lower moisture content than wet salted ones, but difference between the moisture contents was small as the saturated brine contained some undissolved salt to compensate for dilution. Changes in the salt content of the mackerel (Table 2) during curing were similar in both forms of curing, with a marginally higher rate of salt penetration in the first 6h followed by a nearly uniform rate of increase till the end of salting period. Salt penetration was higher in the case of brine cured fish owing to the better contact between fish and curing media.

Tissue proteinase activity (TPA) in mackerel muscle because of the changing composition over time due to moisture egress and salt ingress, have been presented on a moisture and salt free basis (Tables 3 and 4). The TPA in brine cured mackerel ultimately decreased to levels lower than the original, but were initially activated to different extents(Table 3). TPA at pH 3 and 4 were activated to a maximum of nearly threefold after just 3h of salting, and decreased to 72 and 59% respectively after 24h of salting. This was followed by a slight increase to 76 and 78% of the original activity at pH 3 and 4 respectively, at 48h. But the activities remained suppressed below original levels of activity. TPA at pH 9 and 10 increased after 3h of curing as in the acid pH range, albeit to a lesser extent, but decreased steadily later and retained just 50 and 37% of their original activity after 48h curing.

The fate of TPA in the case of dry salted mackerel was largely similar, but extent of activation were much higher than in wet salted mackerel (Table 4). The activation in case of pH 3 and 4 was much higher than in alkaline pH (9 and 10). The activated levels of autolysis decreased steadily with progress of salting, but showed a slight increase of about 1 and 15% at pH 3 and 4 from 24h to 48h, as was earlier observed in case of brine cured mackerel. Decrease in autolytic activity at pH 9 and 10 was quite similar to that at pH 3 4, and retained just 48 and 36% of original activity respectively after 48h of curing.

The effects of autolysis in the tissue should be discernible by the presence of the products viz., free amino acids and peptides. The ninhydrin positive free amino nitrogen (FAN) in the cured fish are shown in Table 5. The concentration of FAN increased initially at 3 h of curing, with higher increase in dry salted fish.

This perhaps is analogous to the increase in autolytic activity (Table 4). Later, in case of wet salted fish, FAN increased marginally at 12h, but this decreased to levels lower than the initial by 48h. The reduction of TPA in the tissue as salt concentration increased, coupled with diffusion of FAN compounds out of the tissue in to the curing brine could be the reason for the decrease in FAN. However, in dry salted mackerel, FAN decreased steadily after 3h, along with decreasing TPA (Table 4), but slightly increased between 24-48h, reaching nearly original levels at 48h. The different behaviour of the dry salted fish is perhaps due to the lower diffusion rates of FAN compounds out of the tissue in the absence of a diffusion facilitating liquid brine.

As the TPA assays were done at low salt concentrations to enable full expression of autolytic activity, the question remains whether the autolytic proteinases were indeed active at the high salt concentrations actually prevailing in the salted fish. This was tested by assay of TPA in unsalted fish muscle, in buffers containing high salt levels. The results are shown in Table 6. Autolysis in the presence of salt was strongly inhibited, the extent of inhibition increasing with salt concentration. Between 10-20% salt level; concentrations which prevailed in the fish after 48h of salting, 36-71% of the original autolytic activity remained at various pH. Thus, at salt concentrations encountered in salted fish, autolytic activity is expressible, even if at a reduced level.

DISCUSSION

High salt concentrations denature muscle proteins like myosin during salting of fish (Iizuka *et al.* 1995), which is attributed to cross-linking (Oka *et al.* 1994). Both *in-vitro* and *in-vivo* digestibility of mackerel subjected to curing and drying are known to be affected (Raghunath *et al.*, 1995) which further affirms the denaturation of muscle proteins. Tissue proteinases may also be affected likewise, but are shown not to be completely inactivated, since activity of cathepsins D, B and L-like proteinases have been demonstrated in 'ika-shiokara', a Japanese salted squid preserve (Makinodan *et al.* 1993). The activation or increase in autolytic activity observed initially in our experiment can perhaps be attributed to denaturation of membrane structures releasing the proteinases bound in tissue lysosomes. Proteinases in the acid and alkaline pH range, however, seem to be at slightly different locations in the cells, as they were activated to different levels and showed slightly different patterns of inactivation. In addition they have also been observed to behave differently when autolytic activity was fractionated into sarcoplasmic and myofibrillar fractions (Jose and Raghunath, 1998). The increasing concentration of salt in the muscle, eventually does suppress autolytic activity, which is in agreement with similar observations in "shottsuru" fish sauce at 5M NaCl concentrations (Yamashita *et al.* 1991). But, as seen from this experiment, salt concentrations in cured mackerel in the absence of drying reach only about 2.5M level on as is basis, and as demonstrated, TPA is only partially suppressed at such salt concentrations. Hence, tissue proteinases can still be active in cured mackerel. Cured fish have a tendency to suffer from physical damage (fragmentation) as observed by Wood *et al.* (1986), but whether tissue autolysis has any role in this, is not known. *Rastrelliger kanagurta* cured even in low strength brine (21%) and dried, is sufficient to produce salted fish with a reasonable shelf life (Poernomo *et al.*, 1992). As autolysis in mackerel is slowed down but not completely stopped by salting, tissue proteolytic activity can affect cured product quality. Further investigations are needed to find out if cured mackerel product quality can be improved by inhibition of autolytic activity using inhibitors other than salt.

REFERENCES

AOAC. 1975. *Official methods of AOAC.* 12th Edn. Association of Official Analytical Chemists. Washington DC, USA.

Cha Y.J. and Lee, E.H. 1989. Studies on the processing of rapid fermented anchovy prepared with low salt concentrations by adapted microorganisms. *Bull.Korean Fish.Soc.* 22(5):363-9.

EBC. 1975. European Brewing Convention Analytica, 3rd edn., Zoeterwoude, The Netherlands. p.61.

Gopakumar, K. and Bhattacharyya, S.K. 1992. Measurement of water activity as an index of optimising salt concentration in brine used for the wet curing of mackerel. *8th session of the IPFC working party on Fish Technol. and Marketing in Yogjakarta, Indonesia.* FAO Rome.1992. No. 470 Suppl. pp257-60.

Herriott, R.M. 1955. In Colowick S.P. and Kaplan N.O. (Eds) *Methods in Enzymol.* Vol.2 Acad. Press. N.Y. pp. 3-7.

Iizuka, S., Mochizuki, Y., Ogawa, H., Mizzzuno, H. and Iso, N. 1995. Physico-chemical properties of salmon during salting process. *Nippon Suisan Gakkaishi* 61(1):71-4.

Leema, Jose and Raghunath, M.R. 1998. Strong binding of tissue proteinase activity by structural proteins of Indian mackerel muscle. Paper no.B4 presented at the Symposium on Advances and Priorities in Fish.Technol. Feb.11-13. Cochin, SOFT(I) and CIFT, India.

Khuntia, B.K., Srikar, L.N., Reddy, G.V.S. and Srinivasa, B.R. 1994. Effect of tissue lipids on the keeping quality of dry salted mackerel and pink perch. *J. Aquat. Food Prod. Technol.* 3(1):77-90.

Makinodan, Y., Nakagawa, T. and Hujita, M. 1993. Effect of cathepsins on textural change during ripening of ika-shiokara. *Bull.Jap.Soc.Sci.Fish.* 59 (9):1625-9.

Oka, H., Hiraoka, Y., Kan, T., Ono, K., and Ninomiya, J. 1994. Change in myofibrillar protein in dried product from anchovy by vacuum frying cooking. *Bull.Soc.Jap.Sci. Fish.* 60(2):253-8.

Poernomo, A., Giyatami, Fawzya, Y.N. and Ariyani, F. 1992. Salting and drying of mackerel (*Rastrelliger kanagurta*) *ASEAN Food J.* 7(3):141-6.

Raghunath, M.R., Ammu, K., Shankar, T.V. and Devadasan, K. 1995. Changes in in-vitro and in-vivo digestibility of mackerel upon curing and drying. *9th IPFC working party on Fish. Technol. and Mktg. In Cochin, India.* FAO, Rome. 1995. No.514, Suppl., pp. 171-7.

Raghuramulu, N., Nair, K.M. and Kalyansundaram, S. 1983. A manual of Laboratory techniques. p 147. National Institute of Nutrition, Hyderabad, India.

Stanley, D.W. and Hultin, H.O. 1984. Proteolytic activity in North Americal Squid and its relation to quality. *Can.Inst.Food Technol.J.* 17(3):163-7.

Tambo, T., Yanmada, N. and Kitada, N. 1992. Change in myofibrillar protein of fish muscle caused by soaking in NaCl solution. *Bull.Jap.Soc.Sci.Fish.* 58(4):677-83.

Wood, C.D., Batty, M. and Rowlands, B. 1986. Studies on the fragmentation of cooked dried fish. Fish processing in Africa. *Proc. FAO Expert Consultation on Fish Technol. Africa.* FAO, Rome. 1985 No.329 suppl. Pp. 369-73.

Yamashita, M., Fujii, T., Konagaya, S. 1991. Proteases in the fish sauce 'shottsuru' mash in fermentation. *Bull.Natl.Res.Inst.Fish.Sci.Japan Chuosuiken Kenpo* No.2 pp. 25-31.

Yamashita, M., Konagaya, S. 1991. Proteolysis of muscle proteins in the extensively softened muscle of chum salmon caught during spawning migration. *Bull.Jap.Soc. Sci.Fish.* 57(11): 2163

Table 1. Weight changes during curing of Mackerel (*Rastrelliger kanagurta).*

Time of Curing, h	Initial Weight, g	Final weight, g	Loss in weight,%
A. Brine Curing			
3	227.48	208.92	8.16
6	220.17	196.88	10.58
12	245.48	215.81	12.09
24	207.13	172.1	16.91
48	213.19	182.48	14.41
B. Dry salting			
3	203.19	184.82	9.04
6	232.56	203.5	12.50
12	222.15	182.13	18.02
24	218.09	163.51	25.03
48	216.41	151.67	29.92

Table 2. Changes in moisture and salt content of mackerel during curing (as is basis).

Time of Curing, h	Moisture, g / 100 g		Salt concentration, g / 100 g	
	Brine cured	Dry salted	Brine cured	Dry salted
0	75.94	75.94	1.04	1.04
3	71.14	70.91	3.69	1.82
6	66.13	69.41	5.61	3.54
12	65.71	62.16	7.94	6.99
24	60.26	60.45	10.95	10.23
48	56.91	52.38	14.82	13.32

Table 3. Autolytic activity in brine cured mackerel muscle during curing on moisture and salt free basis (values in parenthesis indicate activity as % of activity at zero time).

Time of Curing, h	pH 3	pH 4	pH 9	pH 10
	(micromoles Tyrosine / g muscle / minute)			
0	0.1565(100)	0.1130(100)	0.1624(100)	0.1791(100)
3	0.5204(332)	0.3482(308)	0.2763(170)	0.3181(178)
6	0.2518(161)	0.1562(138)	0.1349(83)	0.1541(86)
12	0.2568(164)	0.2409(213)	0.1771(109)	0.2340(131)
24	0.1138(73)	0.0674(60)	0.0904(56)	0.0836(47)
48	0.1189(76)	0.0877(78)	0.0811(50)	0.0668(37)

Table 4. Autolytic activity in dry salted mackerel during curing on moisture and salt free basis (values in parenthesis indicate activity as % of activity at zero time).

Time of curing, h	pH 3	pH 4	pH 9	pH 10
	(micromoles Tyrosine / g muscle / minute)			
0	0.1565(100)	0.1130(100)	0.1624(100)	0.1791(100)
3	0.6807(435)	0.4555(403)	0.3613(222)	0.4160(232)
6	0.3239(207)	0.2001(177)	0.1734(107)	0.1982(111)
12	0.2031(130)	0.1905(169)	0.1400(86)	0.1851(103)
24	0.1119(71)	0.0662(59)	0.0888(55)	0.0823(46)
48	0.1135(72)	0.0838(74)	0.0775(48)	0.0638(36)

Table 5. Changes in alpha amino nitrogen content during curing of mackerel (g/100 g of salt free dry matter).

Time of Curing, h	Brine cured	Dry salted
0	6.53	6.53
3	9.81	15.23
6	6.00	9.16
12	7.95	6.12
24	5.57	5.82
48	2.71	6.23

Table 6. Autolytic activity in presence of salt containing buffers, (μMoles Tyrosine/g muscle/min, as is basis).

Salt concentration (%)	**pH of assay**			
	3.0	4.0	9.0	10.0
Control	0.0382	0.0311	0.0416	0.0402
10.0	0.0174	0.0123	0.0209	0.0297
20.0	0.0000	0.0112	0.0297	0.0223
30.0	0.0032	0.0000	0.0179	0.0061