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Changes in Sulfhydril Groups during Processing of Milkfish, *Chanos chanos*

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The changes in SH group during various processing operations of milkfish such as iced storage, frozen storage, salting (wet and dry salting), drying, steaming, retorting and frying were studied. During iced storage except for the initial few days SH group decreased from 85.10 to 70.8 mmole.g^{-1} dry matters in 21 days. Frozen storage was carried out at two temperatures viz., –35 and –18°C. The decrease in SH group of the fish stored at –35°C was less than that stored at –18°C during 3 months study. During salting the extent of reduction in SH group was more in wet salted fish compared to that of dry salted fish. On further drying of the salted fish SH group decreased continuously. Drying at constant temperature (60°C) for different length of time also reduced the SH group from 76.1 to 16. 8 μ mole.g^{-1} dry matter within 24 h. SH group reduced to a great extent during retorting compared to other heat treatments such as steaming and frying.

Key words: Preprocessing, processing, sulfhydril group, *Chanos chanos*

Preprocessing and processing operations leads to changes in SH group. These changes are of great significance since SH and SS bonds play critical role in the functional properties of the proteinaceous foods. Sulfur compounds posses unique properties that benefit food safety, nutrition and health. Different processing conditions influence the SH groups in different ways and to different extent. During iced storage of fish, actomyosin SH groups are known to decrease throughout the storage period (Buttkus, 1971) except a slight increase in the initial few days (Benjakul *et al.*, 1997). SH groups are known to be lost during frozen storage of fish. The studies of Lim & Haard (1984) on Greenland halibut (*Reinhardtius hippoglossides*) proteins and cod proteins provided evidence for the loss of SH groups in agreement with some early observations by Buttkus (1970) on trout and rabbit myosin. Later studies on the changes in protein denaturation and SH groups have cleared many gaps in the understanding of the role of SH groups and SS bonds. Chen *et al.* (1989) reported that during frozen storage of milkfish

(*Chanos chanos*) myosin at –20°C, total SH of samples decreased significantly but not at –35°C. The above study points out the effect of storage temperature on SH group. It is supported by Jiang *et al.* (1988a) and Jiang *et al.* (1989). They observed that total SH group content at –40°C slightly decreased during the early 2 weeks of storage and no significant changes occurred during further prolonged storage and more disulfides were formed in the sample at –20°C than at –40°C. Jiang *et al.* (1988b) reported that the formation of disulfide bonds as a result of oxidation of SH groups might effect the denaturation of milkfish actomyosin during freezing and frozen storage.

Voskresensky (1965) has reported a reduction in sulfhydril group of salted fish depending on the salt concentration. Salt is also known to induce chemical reactions leading to the formation of cleavage of covalent bonds in proteins, because proteins may be more accessible for such reactions (Sikorski & Ruiter, 1994). The formation of sulfide bond during processing is one of the factors leading to loss of solubility (Mao & Sterling, 1970). Not much work is available on changes in SH group during frying. However, Castrillon *et al.* (1997) have reported significant decrease of SH groups during frying.

The SH content of canned meats, which are sterilized at 110-115°C is reported to be much lower than that of meat pasteurized at 76°C. The difference is attributed to unwinding of protein till 95°C (Opstevedt *et al.*, 1984), which will expose more SH group. Even though unwinding is there, it is immediately followed by oxidation of SH groups to SS bonding formation (Hamm & Hofmann, 1965) and this loss of SH groups is also accompanied by formation of H_2S (Opstevedt *et al.*, 1984). Good amount of work has been done on SH group changes during heat treament (Synowiecki & Shahidi, 1991; Synowieki & Sikorski, 1988; Hamm & Hofmann, 1965). Opstvedt *et al.* (1984) noticed a linear decrease in SH group and increase in disulfide bonds when heating temperature was increased from 20 to 90°C. The decrease in the SH group is attributed to the formation of SS bonds by oxidation. Comparative work on the effect of different kinds of preprocessing and processing on SH group content is not available and this area of research is being recommended as a high priority research area. Here an attempt is made to investigate the extent of changes occurring in sulfhydryl groups during the pre-processing and processing of milkfish.

Materials and Methods

Milkfish (25-30 cm total length; 200-235 g) were collected from brackish water pond of Fisheries Research Station (Kerala Agriculture University), Puthuvypu, Vypeen Island. After washing, the fish was iced properly with crushed ice in the ice-fish ratio 1:2. These samples were used for iced storage and frozen storage. Samples used for cooking, frying, salting and canning were of 23-28 cm total length (100-125 g). Samples used for drying at 60°C were of 37-42cm total length (420-430 g).

For iced storage studies, the fish were iced with flake ice in the ratio of 1:2 (fish: ice) in an insulated box. Re-icing was done everyday to supplement the loss due to melting, after draining the melted ice. The ambient temperature during storage study was about 28±0.5°C. The samples were drawn every 3rd day.

The fish were filleted and the meat was cut into small pieces and was minced well, taking care to reduce heat generation. This ground material was used for all estimations without delay and the rest was kept at –35°C until the experiment was over.

For frozen storage studies, the fish were packed in 100 gauge polythene bags, quick frozen and frozen stored, one group at –18±1°C and the other group at –35±1°C. Sampling was done once in a month.

The fish were de-scaled, beheaded and degutted and were washed thoroughly and these samples were used for steaming, frying and retorting studies. Fish were steam cooked in a retort for 10 min at 15 pounds pressure. Sunflower oil was used for frying the fish. Deep frying was carried out by heating sufficient amount of oil (to immerse the fish) up to 170±5°C and maintained for frying for 10 min. Fish were removed from oil and cooled. Shallow frying was carried out by heating the fish in a pan containing sufficient oil to cover the surface of the pan. The oil was heated to reach a temperature of around 170°C and fish were placed. Each side of the fish was fried for 5 min and was removed from the pan and cooled. Fish was cooled, the meat was hand picked and minced well to a homogenous mass. Ground meat was used for estimating all parameters. The fish were cut to steaks of appropriate size followed by washing, draining and dipping in 10% brine for 20 min. About 100 g of the fish steaks were packed in retortable pouches (500 ml capacity) and about 70 ml of 2% brine was added to each

pouch. The pouches were exhausted by steam injection method, and heat-sealed. The heat processing was done to get a F_0 value of 8. Then the pouches were cooled in the retort itself by maintaining required pressure. After cooling to a temperature of 37°C, the pouches were taken out and washed with potable water. The pouches were cut open and filling medium was drained off. The skin and bones were removed and the sample for analysis was prepared as described above.

The samples kept frozen for 25 days were thawed in running water by keeping in watertight polythene bag and used for salting experiments. Dry salting was carried out by properly mixing sufficient amount of salt and fish, and then kept at room temperature (28±2°C) in a small tank. The samples were drawn for analysis at 3, 6, 9 and 24 h. The fish drawn from dry salting experiment were given a quick wash to remove adhering salt crystals. One half of each sample was used for analysis and the other half for drying. The samples drawn from dry salting were stored at 5°C in sealed polythene bags till the 24 hour sample was drawn. The dry salted fish were split and laid skin side down on perforated aluminium trays and sun dried for 2 days. The skin and bones of the dried fish were removed and ground finely. Another set of fish were immersed in saturated brine for 24 h at 28±5°C. The samples were taken for analysis after 3, 6, 9 and 24 h of brining. The samples were prepared in a similar way as mentioned for dry salting.

For drying, the fish were eviscerated and split open from ventral side and washed, kept skin side down on perforated aluminum tray and placed in an air oven preset at 60°C for 24 h. Sampling was done at 2, 4, 6, 8, 12 and 24 h of drying. Estimation of sulfhydryl groups was done according to Sedlak & Lindsay (1968). Moisture, fat, crude protein and ash were done according to AOAC (1975). Salt soluble nitrogen was done according to Gornell *et al.* (1949).

Results and Discussion

Chanos chanos had 74 to 77.6% of moisture, 19 to 20% of protein, 1.2 to 1.6% of ash and 1.6 to 2.0% of fat. Table 1 shows the changes in SH group during iced storage of *Chanos chanos*. A gradual reduction of SH groups from 85 mmole.g^{-1} dry matter to 76.8 μ mole.g^{-1} dry matter was observed. During a series of studies conducted by Sompongse *et al.* (1996a, 1996b, 1996c, 1996d) on the stability of carp actomyosin during iced storage, they found that the decrease in the SH content was due to the oxidation of

SH groups. They observed two substances larger than myosin heavy chain (MHC) and confirmed that these were polymers formed through SS bonding resulting from the oxidation of SH groups. Benjakul *et al.* (1997) observed that the total SH content of Pacific whiting muscle protein increased slightly after 2 days of storage in ice followed by a gradual and continued decrease up to 8 days. In this study only a gradual decrease was noticed, which could be due to the fact that the first sampling was done only on the fourth day. Fig.1 shows the trend in the percentage reduction of SH group during storage. In general, there is decrease in SH group. There was significant correlation ($p<0.01$) between SSN and SH groups.

Table 1. Changes in SH group (μmole.g^{-1} dry matter) during iced storage of *Chanos chanos*

No. of days	0	4	7	11	14	18	21
SH groups	85.1±6.8	77.6±1.16	83.8±1.16	80.6±8.80	80.2±1.60	78.6±1.57	76.8±1.90

Highly significant changes were observed in SH groups at both the temperatures (Table 2; Fig. 2). Lim & Haard (1984) found that approximately 50% of the SH groups in fresh mince of Greenland halibut was oxidized after long term frozen storage at –10°C. These studies conclude that covalent modification of protein during frozen storage cannot be ruled out as contributing to the phenomenon of protein aggregation in frozen fish. In tilapia hybrid actomyosin (Jiang *et al.*, 1989), an increase in the insoluble fraction, a decrease in the total SH groups and the loss of Ca sensitivity suggested that the oxidation of SH group occurred in actomyosin molecules

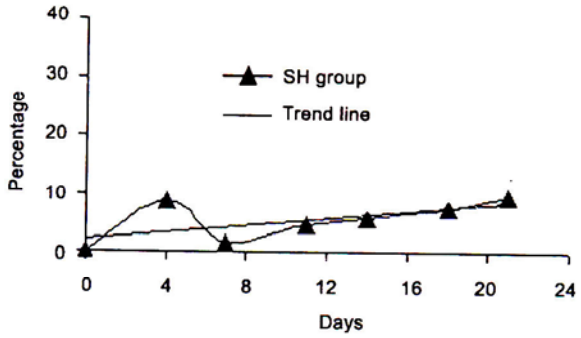

Fig. 1. Percentage reduction of SH group during iced storage of *Chanos chanos*

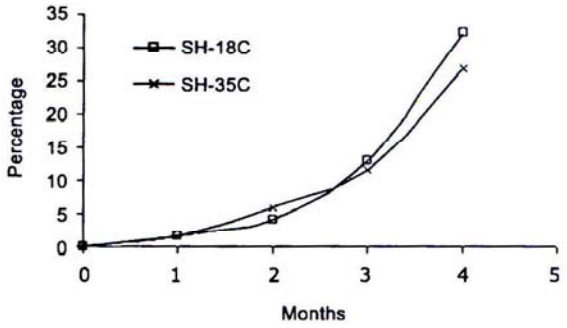

Fig. 2. Percentage reduction in SH group during frozen storage of *Chanos chanos* at -18°C and -35°C

during frozen storage at a much faster rate at –20°C than at –40°C. Myosin aggregates formed in frozen solution were resolubilised only in solvents having the capacity to break both S-S bridges as well as hydrophobic and hydrogen bonds. So the possibility that disulfide bond formation causes insolubilisation cannot be excluded. Iwata & Okada (1971) found that often stable covalent bonds were formed during frozen storage, which prevented solubilization even after cleavage of S-S bridges.

Even though this study did not go into the details of the bonds formed that caused insolubilistion, reduction in solubility and total SH groups was observed during frozen storage at –18°C and –35°C. Percentage reduction in SH was 32.2 at –18°C and 26.9 at –35°C after 4 months storage.

Jiang *et al.* (1988a) reported that during freezing of milkfish actomyosin SH groups decreased at a faster rate at –20°C than at –35°C. They found that during storage disulfides formed at –20°C but not at –35°C (Jiang *et al.*, 1988a; 1988b). But in the case of milkfish myosin, Chen *et al.* (1989) reported that during frozen storage disulfides increased in samples stored at both the temperatures.

Table 2. Changes in SH group (μmole.g^{-1} dry matter) during frozen storage (-18 and -35°C) of *Chanos chanos*

Storage in months	0	1	2	3	4
-18°C	85.10±6.80	83.70±1.67	81.70±2.50	74.10±2.96	57.70±2.54
-35°C	85.1±6.8	83.65±4.00	80.05±0.80	75.20±4.50	62.17±2.50

Table 3. Changes in SH group (μ mole.g^{-1} dry matter) during wet and dry salting and salted and dried *Chanos chanos*

Hours	Wet salted	Dry salted	Salted and dried for 48 h
0	78.5±1.90	78.5±1.90	-
3	68.9±3.40	76.6±1.38	11.63±0.13
6	64.3±1.30	73.9±0.50	12.02±0.39
9	58.8±4.10	68.6±2.96	12.16±0.37
24	58. ±2.30	62.6±2.28	10.89±0.65

There is hardly any work reported on the changes in the SH groups during salting. Dry salting retained 94% of the SH groups until 6 h of salting and reduced only 20% at the end of 24 h salting since protein denaturation during salting is similar to that during frozen storage, the SH groups lost might have formed S-S bonds after getting oxidized and hence indicated lower value.

Only 15% of the SH groups of the salted samples were retained after sun drying for 2 days. This may account for the reduction in the cysteine content. Fig. 3 Shows the percentage reduction in the SH group during wet and dry salting of *Chanos chanos*. Not much change is noticed during last stages of wet salting. The extent of changes in dry salting is less compared to wet salting. Further sun drying of dry salted fish for 2 days suffered a significant loss of SH group (Table 3). Salting may involve cross-linking reactions of myosin heavy chain and formation of ε (γ-glutamyl) lysine by trans glutaminase reaction (Kumazawa, 1993).

Table 4. Changes in SH group (μ mole.g^{-1} dry matter) during steaming, frying (shallow and deep) and retorting of *Chanos chanos*

Treatments	Initial	Steamed	Shallow fried	Deep fried	Retorted
SH groups	77.1±3.86	68.3±0.82	62.9±5.66	60.73±4.86	52.9±4.00

Table 4 gives the changes in SH group during different heat treatments of C*hanos chanos*. Among the heat treatments given SH group reduction was more in the retorted sample followed by the deep and shallow fried samples. In cooking also SH group suffered loss. Extensive work has been done on the effect of heating of fish/ fish actomyosin at different temperatures on

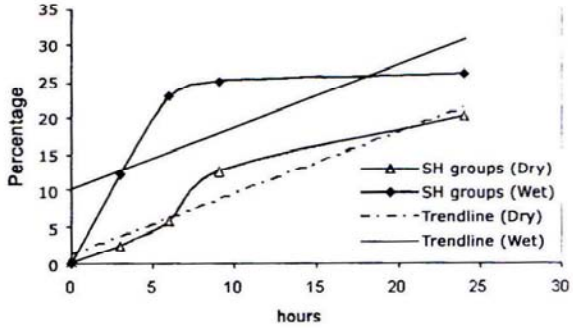

Fig. 3. Percentage reduction in SH group during wet and dry salting of *Chanos chanos*

the changes in SH group, formation of SS bonds and SH, SS inter change. Monetaro & Guillen, (1996) reported that in sardine muscle heated above 60-70°C the bulk of protein-protein interaction took place through the head portion of the myosin molecules by means of either disulfide bonds or hydrophobic bonds which consequently leads to the loss of SH group. In the mantle meat of squid, after heating at 98°C for 45 min, the SH groups reduced by 30%. The correlation coefficients between free SH groups and the solubility was highly significant (Synoweicki & Sikorski, 1988). Hamm & Hofmann (1965) observed that heating meat to 70°C increases the availability of SH groups as a result of denaturation. At temperatures higher than 70°C the SH content decreases, chiefly because of an oxidation to S-S bonds. Itoh *et al.* (1979; 1980) indicated that the sulfhydryl groups of carp actomyosin participated in the gel formation by heating at 40° and 80°C and also suggested that the SH groups were involved in changes to higher molecular weight proteins and in the formation of S-S bonds between protein molecules. Heating resulted in a decrease in the content of sulfhydryl groups of seal meat protein and an increase in the content of disulfide bonds. Increasing the temperature from 20°C to 99°C increased the content of disulfide bonds by a factor of 2.5. However after 40 min of heating at 80°C the content of free SH groups in seal meat was decreased by approximately 50% (Synoweicki & Shahidi, 1991).

Opstvedt *et al.* (1984) found that when ground fillets of frozen Alaska Pollock a low fat fish and pacific mackerel, a high fat fish were heated for 20 minutes at temperature intervals ranging from 40°C to 115°C, a linear decrease in the content of SH group and a concomitant increase in the content of S-S bonds were noticed from 50°C to 115°C. At 95°C the reaction was rapid and had reached equilibrium after 20 min. In myosin prepared from

Sacramento black fish, the amount of freely reacting SH groups decreased slightly during freezing and very markedly upon cooking and dehydration (Mao & Sterling, 1970). In the present study the temperature used in the thermal processing by various methods was much higher than those mentioned in the above studies. Hence a higher decrease in –SH groups was observed. The loss of SH groups during frying is attributed to the oxidative transformation of SS bonds and also to the destruction of cyst(e)ine, which is dropped by 14% followed by the formation of other sulfur containing compounds (Hofmann & Hamm, 1978).

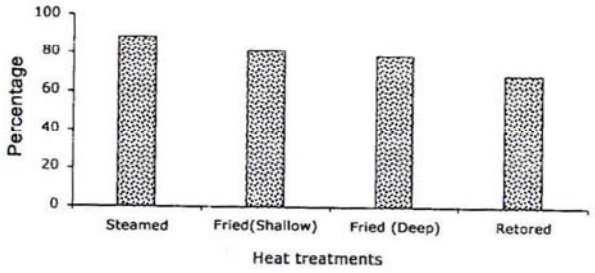

Fig. 4. Percentage reduction of SH group during different heat treatments of *Chanos chanos*

Though the temperature at which retorting was done (121°C) was much lower than that used for deep frying (170°C) SH loss was more since the duration of heating (45 min) was much longer for retorting than for frying (10 min). Percentage retention of the parameters studied during cooking, shallow frying, deep-frying and retorting are given in Fig. 4.

Table 5. Changes in SH group during drying of *Chanos chanos* at 60°C

No. of hours	0	2	4	6	8	12	24
SH group	76.1±1.52	61.3±0.35	53.7±1.14	44.6±0.71	37.6±3.56	20.17±1.0	16.80±1.3

Table 5 gives the changes in SH group during drying of *Chanos chanos* at 60°C. SH content decreases from 76.1 m mole.g^{-1} dry matter to16.80 mmole.g^{-1} dry matter. The SH groups showed a gradual decrease during drying at 60°C retaining only about 23% of the initial value. Raghunath *et al.* (1995) found that the SH groups registered a regular and sharp decrease with drying except at 50°C where initially an increase was observed. The initial increase found in this case may be due to the unfolding of protein chains exposing the buried SH groups, which subsequently become oxidized.

It was reported that temperatures higher than 50°C are required for oxidative transformation of SH groups to S-S bonds (Opstvedt *et al.*, 1984). Loss in cystiene also can cause reduction in SH groups.

In carp actomyosin the total reactive SH content increased slightly from 20°C to 30° C indicating some S-S bonds were reduced to SH. But on increasing the temperature above 30°C, the total reactive SH content decreased considerably, indicating SH groups were oxidized into S-S bonds (Sano *et al.,* 1994). In this study, sun drying of salted fish for two days also reduced the SH content to less than 15% of its initial value though the temperature of drying was only 32-35° C. Salting for 24 h and sun drying for two days seem to reduce the SSN and SH groups to a higher level than that in fish dried at 60°C in an oven for 24 h in spite of the temperature during sun drying being 32 to 35°C.

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