Chub Mackerel of Thailand (*Rastrelliger neglectus,* Van Kampen): A Short Study of its Chemical Composition, Cold Storage and Canning Properties.

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J. N. Keay[a], P. Rattagool[b] and R. Hardy[a]

Proximate analysis, amino-acid analysis and lipid fatty acid analysis have been performed on Thai chub mackerel. Samples of the fish were stored in the unglazed, glazed and vacuum-packed condition at - 14 and -30°C for 90 day; at intervals various chemical indices of deterioration were determined and taste-panel assessments performed. The data relating to lipid oxidation are considered in particular detail and attention drawn to the lack of agreement between the various indices of degree of oxidation. Samples of the fish were smoked and canned products prepared from these evaluated by a taste panel for possible commercial acceptability.

1. Introduction

In many parts of the world the potential of fisheries as a source of high-grade food has not, for various reasons, been fully exploited. In Thailand the chub mackerel (*Rastrelliger neglectus*, Van Kampen) represents approximately 15% of the fish landings and is the principal species caught. Part of the catch is sold fresh but the greater portion is frozen and cold-stored in order to cover seasonal fluctuations in supply. A recent report[1] indicates that samples of chub mackerel drawn from cold storage in Thailand were of poor eating quality, suggesting a high vulnerability to deterioration, notably the development of oxidative rancidity, and/or inadequate cold storage practice. The work described in this paper was performed in order to provide the basic compositional information for a fuller understanding of the handling, storage and processing properties of the species. Additionally, changes taking place in the fish under several conditions of cold storage were determined by sensory assessment and various chemical tests. Finally, since the chub mackerel is a fatty fish and might, therefore, be expected to yield good canned products which could widen the market for it, the canning properties of the species were briefly evaluated.

2. Experimental

2.1. Fish

A batch of frozen chub mackerel (16 kg) was obtained from Thailand in May 1971. The fish were packed in dry ice for transportation by air and were still in the frozen condition on arrival at the laboratory in Aberdeen where they were immediately placed

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in cold storage at - 30 °C. They were reputed to have been caught some two months previously and held in storage at - 20 °C in Thailand although the initial low indices of lipid oxidation obtained indicate that the actual storage time may have been considerably shorter. An examination of the fish revealed no evidence of bacterial or autolytic spoilage. This is consistent with their having been frozen within 24 h which is the practice in Thailand. Attempts to obtain completely fresh fish at this time were unsuccessful owing to seasonal fluctuations in supply.

2.2 Methods

Proximate analysis, amino-acid analysis and the "zero-time" determinations for the tests used in the storage experiment were performed almost immediately on receipt of the fish. Samples of fish were laid down at - 14 and - 30 °C under three storage conditions namely unglazed, glazed and vacuum-packed in protective pouches. The packaging material used for the vacuum packs was a laminate of PVDC-PVC - coated polypropylene and polythene. The numbers of fish allocated to each storage condition and temperature were sufficient to allow the withdrawal of six fish at the proposed intervals, four fish being used for the chemical tests and two for sensory assessment. The methods used were as follows.

2.2.1 Water content

This was determined on a sample of the minced flesh (10 g) by the azeotropic method of Dean and Stark[2] using toluene as the solvent.

2.2.2 Ash

A sample of the minced flesh (3 g) in a silica crucible was dried overnight at 100 °C and then heated for 2 days at 500 °C after which the crucible and contents were cooled in a desiccator and weighed.

2.2.3 Protein content

This was determined on a sample of the minced flesh by the macro-Kjeldahl method. The result was expressed as percentage crude protein by multiplying the figure obtained for total nitrogen by 6.25. No attempt was made to separate protein from non-protein nitrogen, hence the term "crude protein".

2.2.4 Total lipid content

The lipid was isolated from a sample of the minced flesh (100 g) using the extraction method of Bligh and Dyer.[3] The extract was concentrated on a rotary film evaporator and freed from solvent on a high vacuum line.

2.2.5 Protein-amino-acid analysis

A sample of the minced flesh (10 g) was blended with trichloroacetic acid solution (TCA; 10%; 100 ml) and the mixture centrifuged. The sediment was washed with TCA solution, ethanol and ether, two portions of each being used, and then dried at 105 °C A sample of the dry protein (10 mg) was hydrolysed by heating (3 h) with 6 N-HCI (3 ml) in a sealed, evacuated glass tube. The hydrolysate was then concentrated on a rotary evaporator (bath <40 °C) in order to remove HCI and the residue dissolved in buffer solution (pH 2.2; 10 ml) for analysis by ion-exchange

chromatography on a Locarte Amino Acid Analyzer

2.2.6. Fatty acid analysis

A sample of the total lipid, isolated as described previously, was converted to methyl esters by refluxing (1 h) in methanol containing 5% w/v H_2SO_4 (3 ml); benzene (0.5 ml) was added in order to maintain solution of the esters and a crystal of 2, 6-ditertiarybutyl-4 methyl phenol (BHT) was added to minimise oxidation. The esters were recovered by solvent extraction and the efficiency of conversion checked by thin layer chromatography on Silica gel G plates after which they were purified by column chromatography on silicic acid (Mallinckrodt). Injections of the ester (1 µl; 10% solution in acetone) were applied to a Pye 104 gas chromatograph incorporating a 1.5 m x 3 mm glass column packed with Chromosorb W (80 to 100 mesh) impregnated with 20% DEGS stationary phase. The instrument was operated isothermally at 190 °C with a carrier gas (oxygen-free nitrogen) flow rate of 35 ml/min. A flame ionisation detector was used. Computation of the percentage composition was made by the "peak height x width at half height" formula. Since the BHT present in the solution overlapped on the chromatograms with the 14:0 acid the latter had to be omitted from the calculation of composition.

2.2.7. Peroxide value

This was determined by the method of Banks[4] on lipid extracted with petroleum ether (b.p. 40 to 60 °C) from a sample of minced flesh.

2.2.8. Thiobarbituric acid value

The lipid used for peroxide value determination was also used for this test which was performed according to the method of Dahle, Hill and Holman,[5] as modified by Hardy and Smith.[6] Notable among the modifications were the substitution of acetone for ethanol as solvent in order to effect complete solution of the fish lipid, the use of an incubation period of 45 min at 80 °C and the addition of BHT solution (1% in acetone; 0.5 ml) to prevent further oxidation.

2.2.9. Ultraviolet spectroscopy

For this purpose samples of lipid extracted by the Bligh and Dyer method[3] were used. The extracted lipid was dissolved in spectroscopic grade cyclohexane (BDH) and measurements made on a Unicam SP. 800 spectrometer using 1-cm path-length cuvettes.

2.2.10. Iodine value

This was determined by the Wijs method on samples of the lipid as used for peroxide value determination.

2.2.11. Hypoxanthine determination

This was performed by the xanthine oxidase method of Jones *et al.*[7]

2.2.12. Soluble and insoluble protein determination

Analyses were performed on duplicate samples of the mince from four filleted fish. The soluble and insoluble protein fractions were separated according to the method of Ironside and Love.[8] Nitrogen determinations on the fractions were performed according to the automatic method of

Varley[9] as adapted by Mitcheson and Stowell.[10]

2.2.13 Oxygen absorption

Whole fish were incubated in air at - 14 °C in specially constructed closed vessels incorporating Warburg manometers,[11] one fish (weight approximately 90 g) being contained in each of three such vessels. Absorption of oxygen was followed over a period of two months.

2.2.14. Sensory assessment of cold-stored fish

This was performed by a small panel of judges who were experienced in assessing cold-stored fatty fish particularly for the presence of oxidative rancidity.

2.2.15. Canned, smoked products

Finally, fish were converted to a number of canned, smoked products. The fish were all pickled in sodium chloride solution (75% saturated) for 2 min and smoked in a "Torry" mechanical kiln. They were then canned (225 g oval cans), a processing time of 1 h at 115 °C being used. After a storage period of 2 months the products were evaluated by a small "consumer" taste panel. The variations in treatment and the taste panel reports on each are shown in Table 6.

3. Results and discussion

The proximate composition of the flesh of the chub mackerel (Table 1) is typical of that of many species of fish. It is, of course, to be expected that a full seasonal study would reveal a variation in water and lipid content, with the values for these two components perhaps showing an inverse linear relationship. [12,13] The amino-acid composition of the protein (Table 2) is very similar to that recorded for several European food fish species including herring.[14] When comparison is made of the fatty acid composition of the total lipid of the chub mackerel (Table 3, "zero time") with that of other species[15] the former is seen to be characterised by relatively high 16:1 and 20:5 contents and low 18:1, 20:1 and 22:1 contents

TABLE 1. Proximate analysis of chub mackerel

	composition (%)
Water	72.0
Crude protein	20.7
Lipid	6.5
Ash	1.7

TABLE 2. Amino-acid composition of chub mackerel protein

	g of amino acid/ 100 g[a] protein
Aspartic acid	10.17
Threonine*	4.41
Serine	4.47
Glutamic acid	15.46
Proline	3.30
Glycine	4.20
Alanine	6.23
Cystine/2	2.44
Valine*	5.64
Methionine*	3.73
Isoleucine*	5.29
Leucine*	8.93
Tyrosine	3.69
Phenylalanine*	5.79
Histidine	3.16
Lysine	9.27
Arginine	6.29

[a] Essential amino acids are marked*.

TABLE 3. The percentage fatty acid composition of the total lipid of chub mackerel at "zero time" and after the fish had been in cold-storage for 90 days

		Storage at - 14 °C			Storage at - 30 °C		
Fatty adic	"Zero time" sample	Unglazed	Glazed	Vacuum packed	Unglazed	Glazed	Vacuum packed
16:0	23.2	26.1	21.5	23.8	22.6	23.3	22.9
16:1	14.9	19.5	15.5	15.6	16.5	14.9	17.5
18:0	6.0	6.9	6.2	6.1	5.1	5.0	4.7
18:1	9.2	11.2	8.4	10.0	6.6	8.1	9.1
18:2	2.4	2.7	2.9	2.2	2.8	2.7	2.9
18:4	1.4	1.4	1.2	1.5	1.3	1.5	2.0
20:1	1.9	2.2	2.3	2.3	2.6	2.4	2.5
20:5	14.3	10.2	13.8	12.2	13.1	12.8	14.2
22:1	2.4	2.5	3.0	2.3	2.5	2.8	3.1
22:5	2.2	1.6	1.9	1.7	2.1	2.6	1.8
22:6	10.1	6.8	10.4	9.2	10.5	9.8	10.2
Others	12.5	9.6	13.5	13.7	14.8	14.8	10.2
Total	100.5	100.7	100.6	100.6	100.5	100.7	101.1

In the storage experiments the chemical indices of lipid oxidative change show a qualitatively consistent pattern throughout (Figure 1). Peroxide value (PO), thiobarbituric acid value (TBA) and ultraviolet absorption (u.v.) increase throughout storage at - 14 °C while the iodine value (I) tends to fall. The protective effect of glazing and more prominently vacuum packing is strongly evident. This may be associated not only with the oxygen barrier properties of the glaze and the packaging material but also with the prevention of water loss. Minimal change was observed in the case of - 30 °C 90 storage; exceptionally some variation in I was recorded. It is of practical value to note that glazing or vacuum packing apparently compensates for the deficiencies of storage at - 14 °C. In the taste-panel evaluations for oxidative rancidity only in the case of the unglazed samples stored at - 14 °C were significant levels of rancidity reported at any stage.

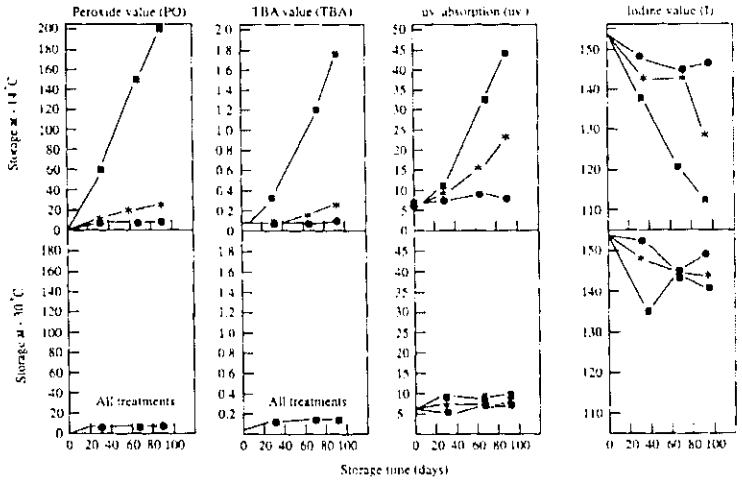

Figure 1 peroxide value (PO) Thiobarbituric value (TBA) Ultraviolet absorption (UV) increase through out storage at -14° C while Iodine Value (I) tends to fall, but Minimum change was observeed in the case of -30° C for 90 days storage.
■ = unglazed, * = glazed, ● = Vacuum packed.

It seems desirable that the chemical data relating to the progress of lipid oxidation should be considered in some detail, particularly that relating to the unglazed fish stored at - 14 °C. If a lipid - autoxidation mechanism is assumed whereby one mol of oxygen absorbed produces one mol of conjugated diene hydroperoxide, [16,17] then the various indices of degree of oxidation shown in Figure I can be equated to the amount of oxygen absorbed per unit weight of lipid as follows.

Iodine value. Only one double bond in a conjugated diene system is measured by the Wijs method, therefore apparent loss of unsaturation provides a measure of conjugation and hence oxidation. If the fall in I = *a*, then:

$$\text{oxygen absorbed} = \frac{a \times 10^4}{2 \times 126.9} \ \mu\text{mol/g lipid.}$$

Peroxide value. Assuming that one µmol of absorbed oxygen produces one µmol of hydroperoxide no conversion of units is necessary to obtain oxygen absorption values.

Ultraviolet absorption. Assuming a molecular extinction coefficient of 29 000 for conjugated diene hydroperoxide[18] and if *C* is the measured $E^{1\%}_{1\,cm}$ value for the lipid then:

$$\text{oxygen absorbed} = \frac{C}{0.29} \ \mu\text{mol/g lipid.}$$

Gas-liquid chromatography. Assuming that the mean molecular weight of the fatty acids which are attacked by oxygen (18:2, 18:4, 20:5, 22:5 and 22:6) is 300, that only one molecule of oxygen reacts with each molecule of unsaturated fatty acid and that d is the total percentage loss of the se acids, then:

$$\text{oxygen absorbed} = \frac{100d}{3} \ \mu\text{mol/g lipid.}$$

Thiobarbituric acid value. Assuming that one µmol of absorbed oxygen produces one µmol of malonaldehyde then no conversion of units is necessary in order to obtain the oxygen absorption value.

Table 4. Conversion of indices of oxidation of the lipids of the - 14 °C/unglazed fish (Figure 1 and Table 3) into theoretical oxygen absorption values

Storage period (days)	I	PO	u.v.	g.l.c.	TBA
0	153; 0; 0	1; 0; 0	6; 0; 0	0.304; 0; 0	0.05; 0: 0
30	138; 15; 591	61; 60; 60	10.5; 4.5; 15.5		
64	120; 33;1300;	142; 141; 141	29 ; 23 ; 79		
90	110; 43;1694	200; 199; 199	43.5; 37.5; 129	0.227;0.077;256	1.7; 1.65; 1.65

The figures shown under each heading at each storage interval are given in the following order: index in original units; change in index with storage time; change in index converted to µmol of oxygen/g of lipid.

The results obtained by performing these conversions are shown in Table 4. While the PO, u.v. and g.l.c. results are of the same order of magnitude they are widely at variance with the I and TBA results as indeed the latter are with each other. It seems that if the former group of results provides a guide as to extent of oxidation then unsaturation has been lost by a mechanism other than that indicated by the simple autoxidation mechanism proposed earlier. Conversely, if unsaturation changes have occurred in accordance with the autoxidation theory then the PO, u.v. and g.l.c. results indicate that the resultant hydroperoxides are redily decomposed and must be more susceptible to oxygen attack than the original lipids. The low TBA values suggest that the hydroperoxides in their decomposition do not produce equivalent quantities of malonaldehyde (or other TBA-reacting substances) or, alternatively, that little peroxide decomposition to such substances has occurred.

Finally, the fish which were incubated in the manometric system showed no significant absorption of oxygen over a period of two months. This phenomenon, along with others described above, has been observed for other species of fish, notably herring and sprats.[11] An explanation may

lie in the fact that dehydration of fish tissue is known to accelerate oxidation and this effect may be avoided in the closed manometric system from which no water loss has occurred.

It must be concluded that the oxidation of lipids in these cold-stored fish is a complex process which cannot be satisfactorily explained in terms of simple autoxidation theory and that the various indices, while empirically valuable in following the course of storage change, must be invoked with caution in assessing the true state of the system.

The hypoxanthine values (Table 5) show an approximately equal increase ralative to the "zero time" sample under all storage conditions up to a period of 64 days. A further increment is observed between 64 and 90 days in the case of the -14 °C fish only. The values are generall low and are equivalent to those observed in Atlantic mackerel *(Scomber scombrus)*[19] and cod *(Gadus callarias)*[20] after approximately 5 days chill storage. The indices obtained for the fish stored at -14 °C are consistent with those obtained for cod[20] and swordfish *(Xiphias gladius)*[21] stored under similar conditions; however, no elevation was obtained for these species when stored at -30 and -26 °C, respectively.

TABLE 5. Hypoxanthine content of chub mackerel held in frozen storage (values are expressed in μmol of hypoxanthine/g of fish)

Treatment	Storage period (days)	-14 °C	-30 °C
Unglazed	30	0.45	0.47
	64	0.43	0.41
	90	0.73	0.49
Glazed	30	0.45	0.45
	64	0.45	0.43
	90	0.65	0.48
Vacuum packed	30	0.36	0.43
	64	0.36	0.40
	90	0.61	0.46

The "zero time" fish sample has a value of 0.17.

The changes in soluble protein content of the stored fish are shown in Figure 2. It is doubtful if, in the case of the fish stored at -30 °C, the recorded differences in protein solubility between the treatments reflect anything other than sampling variation and such variation may also account for apparent anomalies in the data obtained for the -14 °C fish. Multiple fish sampling and replicate determinations would be necessary to clarify this situation. Moreover, it should be remembered that the "zero time" samples had been in cold storage in Thailand for about two months at -20 °C during which time some lowering of soluble protein level would have been sustained. Despite these imperfections a difference in the rate of protein denaturation at -14 and -30 °C is evident and in the case of the -14 °C fish there is some indication that vacuum packing and glazing have a protective effect.

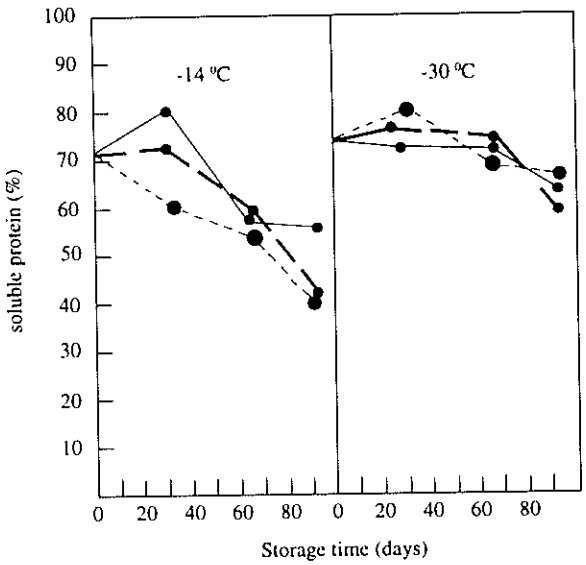

Figure 2. Cold storage of chub mackerel. Measurements of insoluble protein. ....., unglazed; -------, glazed; —— , vacuum packed.

In the brief canning trials which were performed the best products were probably those obtained from fillets smoked for 45 min and from whole fish smoked for 90 min, both being canned in oil (Table 6). Adjustment of the smoking programme to obtain the same deposition of smoke but less drying would almost certainly improve the fillet products and as such they might have a distinct commercial value. It is noteworthy that smoking and canning appear to abolish or at least obscure a slightly unpleasant "metallic" taste which appears to be intrinsic to the species when cooked as wet fish.

TABEL 6. Results of tasting canned chub mackerel

Treatment	Taste panel report
Fillets, smoked for 45 min, canned in maize oil	Good colour and flavour. Slightly tough and "dry"
Fillets, smoked for 90 min, canned in maize oil	Colour too dark. Flavour quite good. Too tough and "dry"
Whole fish (beheaded, eviscerated), smoked for 60 min, canned in maize oil	Good colour. Insufficient smoke flavour Tender but rather "bony"
Whole fish (beheaded, eviscerated), smoked for 90 min, canned in maize oil	Good colour and flavour. Tender but rather "bony"
Fillets, smoked for 45 min, canned in tomato sauce	Overwhelming colour and flavour of tomato sauce. Slightly tough

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