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The influence of salt on rancidity development in salted-dried fish

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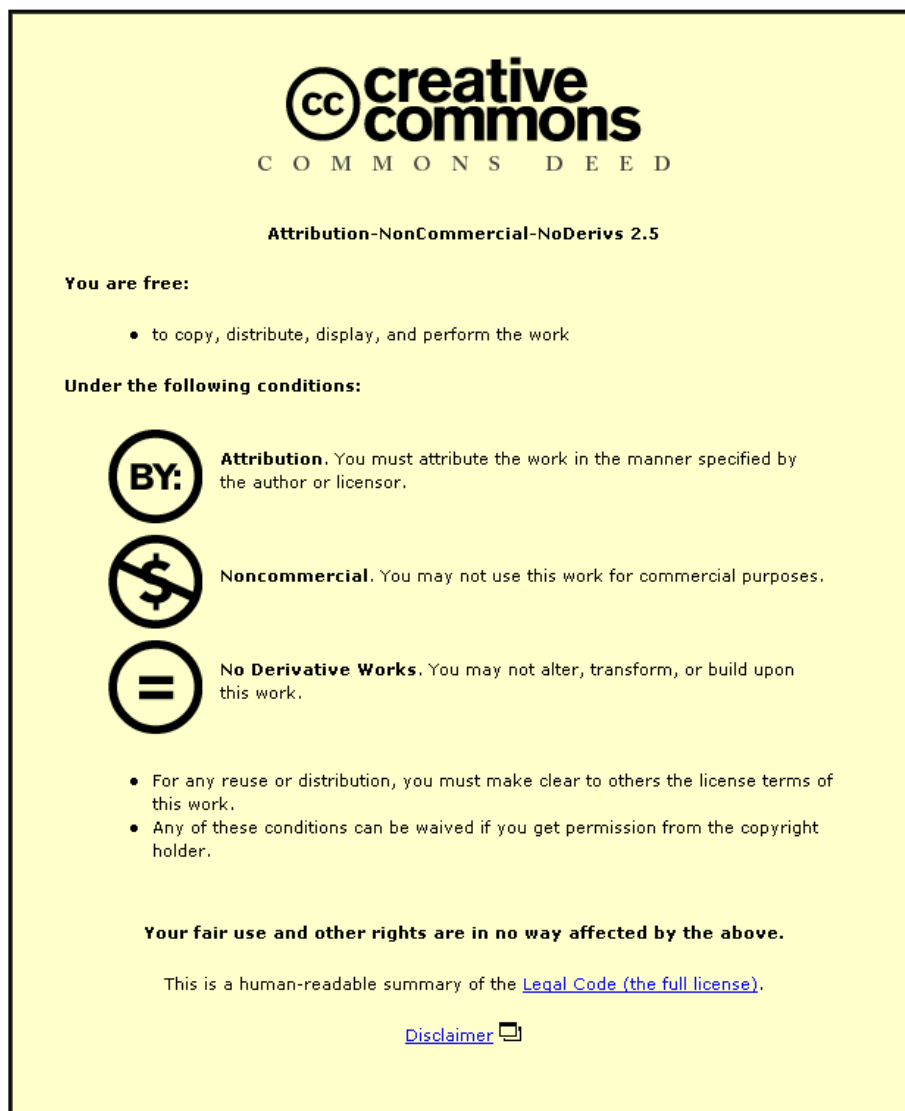
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THE INFLUENCE OF SALT ON RANCIDITY
DEVELOPMENT IN SALTED-DRIED FISH

By

I. N. ESHIET

A Master's thesis
submitted in partial fulfilment of the requirements for the
award of Master of Philosophy of the Loughborough University
of Technology, 1983

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Humberside College of Higher Education,
Grimsby.

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CERTIFICATE OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this thesis, that the original work is my own except as specified in acknowledgements or in footnotes, and that neither the thesis nor the original work contained therein has been submitted to this or any other institution for a higher degree.

DEDICATION

To the memory of my son, Useneno,
the birth of my daughter, Tiemah, and
the love of my wife, Aty -
a lot to miss.

Imeh

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1.

INTRODUCTION

Preservation of fish by salting or drying, or a combination of both, has been used for many centuries in many countries. These methods have developed over the years largely to suit local conditions e.g. availability of salt and climate. In general, rapid drying occurs under low humidity and high temperature conditions, although in practice fish drying is viable provided either the humidity is relatively low or the temperature relatively high. For example, fish drying at low humidities and low temperature is carried out in Canada, Norway, Iceland and the Netherlands, whereas drying of fish at relatively high humidity and high temperature is carried out in many tropical countries. However, dried fish products keep less well in a hot humid tropical climate than in a cool, dry temperate climate (Van Veen, 1953).

World fish production figures show that in 1981, 74.8 million tonnes of fish were caught and of this 10.8 million tonnes (on a landed weight basis) were processed into cured products, i.e. smoked, dried, salted and salted-dried products (FAO, 1983). Of the 4.5 million tonnes of cured fish recorded world wide (on a product weight basis) 3.8 million tonnes were dried and/or salted and the remainder was smoked. This indicates that more than 10% of the world fisheries catch was dried and/or salted in 1981. However, a far higher percentage of the total catch is dried, salted, or salted-dried in many countries, particularly tropical countries; e.g. in India the figure is as high as 50-70% (Pillai *et al.*, 1956), in South East Asia the figure reaches 25 to 30% (Hanson and Esser, 1982) and in Chad and Ghana it can be as high as 70% (FAO, 1981).

These methods of fish preservation assume very considerable importance in Asia and many tropical areas as fish represents a major

source of animal protein (FAO, 1981). In these areas it is known that the total protein content of the diets of millions of people is low, especially when compared with standards commonly accepted in North America and Western Europe (Van Veen, 1953). Even though fish salting and drying is of great importance both nutritionally and economically, the industry is not fully developed and the techniques used are still to a large extent traditional and often wasteful, resulting in losses of about 25% of the 10.8 million tonnes processed into cured fish products. Unfortunately most of these losses occur in Asia, Africa, Mexico and the tropics where protein deficiency is high and where fish would otherwise be a very valuable protein source. Examples of up to 50% loss are known, especially in small scale processing units in S.E. Asia (Hanson and Esser, 1982).

With regard to salted-dried fish, the influence of temperature, humidity, salt purity and the general conditions of hygiene employed are all important in controlling the level of losses. More information specifically concerning the influence of salt on the quality of salted-dried fish, is required - especially in relation to the processes used in S.E. Asia. Such information could be of use in reducing the large losses observed and in improving the nutritional value of that which is consumed.

2.

LITERATURE SURVEY

2.1 FISH PRESERVATION

Fish is a highly perishable commodity and without preservation will become inedible within a few days even in a temperate climate. Chilling will extend storage life up to about 2 weeks but for long term preservation other methods have to be used. These methods can be broadly split into two categories: high technology preservation methods and traditional preservation methods.

High technology methods, which include freezing and canning, are capital intensive and require considerable technical know-how in the operating personnel, hence making them unsuitable in general in third world countries for fish and fish products for internal markets.

The most important traditional preservation methods are smoking, drying, salting and combinations of these methods.

Smoking is of value when suitable wood is available and the catches small and sporadic but it cannot however be easily expanded to cope with the volume of production necessary to serve a large market, especially if product consistency is desired. In many developing countries the taste of smoked fish is not acceptable to the consumer and in these areas drying and salting or a combination of both are the predominant methods of fish preservation.

2.1.1 Drying

Preservation of fish by drying is based on the principle that reduced moisture content and therefore reduced water activity will inhibit microbial growth, enzymic and most chemical reactions and so retard deterioration (See Fig. 1).

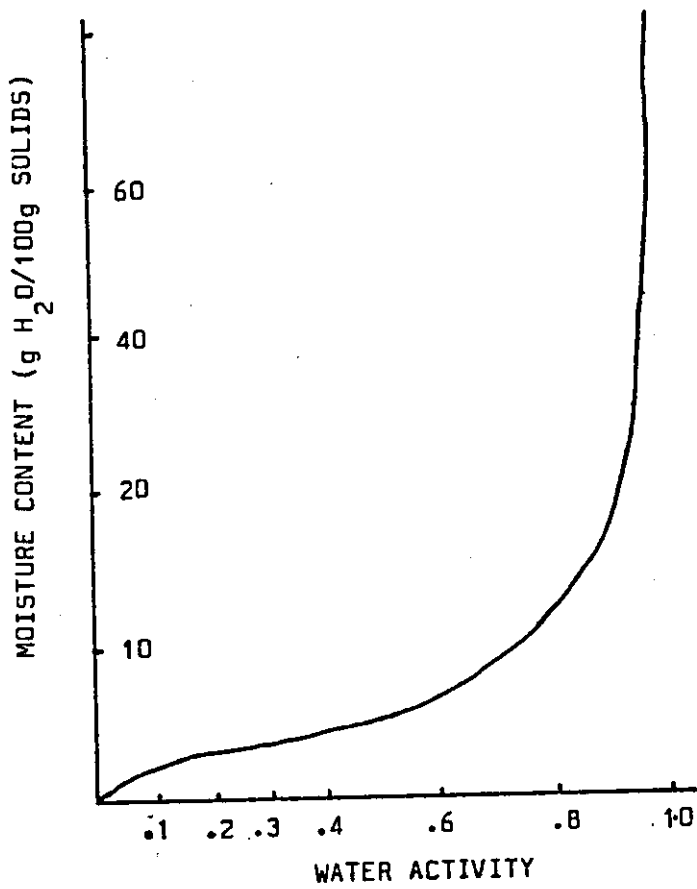


Fig. 1: Water adsorption isotherm, freeze-dried salmon 37°C.

Source: Martinez and Labuza (1968).

Water activity (a_w) is the ratio P/P_0 where P and P_0 are the vapour pressures of the solution and the pure solvent respectively, and under equilibrium conditions, the relative humidity (equilibrium relative humidity, E.R.H.) is equal to $a_w \times 100$. Pure water has an a_w of 1.00 and an E.R.H. of 100%. Solutes e.g. sodium chloride will lower the water activity and for ideal solutions Raoult's law states that the water activity is equal to the mole fraction of solvent, i.e.

$$a_w = P/P_0 = N_2/(N_1 + N_2) \text{ where } N_1 \text{ and } N_2$$

are the number of moles of solute and solvent respectively.

However, the solutes of concern in foods, e.g. salted-dried fish, are not ideal and insoluble components interact with both water and solutes. This results in the water content of food not being directly related to the water activity (see Fig. 1 and Section 2.1.2).

The effectiveness of drying depends on the diffusion rate of moisture from the inner layers of the foodstuff to the periphery. This is ultimately controlled by many factors principally the initial moisture contents and fat content of the fish flesh, the method of drying and the environmental conditions. Only lean and semi-fatty fishes, i.e. those containing about one per cent fat or less can be effectively preserved by this method (Cole and Greenwood-Barton, 1965), because a high fat content will act as a barrier to diffusion and a slow drying rate will result. Details of moisture mobility in fish in relation to relative humidity, wind speed and size of fish have been discussed by Waterman (1976) and Jason (1965).

Sun-drying and wind drying are the two main forms of fish drying.

In sun-drying the fish is laid out to dry under the heat of the sun. This process is enhanced by wind currents and it is mainly used in Asia and some South American countries. Wind drying is used in colder countries, e.g. the Netherlands and Norway, where fish is hung on a line in the wind to dry. Even though wind drying is slower than sun-drying, spoilage is delayed due to the lower ambient temperatures (FAO, 1981). Details of drying techniques vary greatly from place to place and factors like drying times have to be altered to suit the species of fish and the weather (as will be discussed later) (Waterman, 1976).

Fish curers decide, sometimes fairly arbitrarily, when the moisture content of a product is sufficiently reduced and moisture figures up to 65 per cent have been observed by Cole and Greenwood-Barton (1965). A very dry product will be brittle and therefore liable to break-up during packaging and transportation, with subsequent losses, whilst a moist product, i.e. one with a high water activity, will not keep well in storage (see Fig. 2). Economic considerations in terms of selling price and weight of product also determine the extent of dryness allowed.

Although drying is not a dependable means of preservation because of the requirement for suitable weather conditions, it is inexpensive and will therefore continue to be widely used, particularly in developing countries.

2.1.2 Salting

Salting is related to drying in that both processes involve lowering of the water activity of the fish flesh. This occurs on salting in part because of the uptake of salt but also because of loss of water from the fish flesh. Moisture passes from the less concentrated phase of the fish flesh to the more concentrated salt

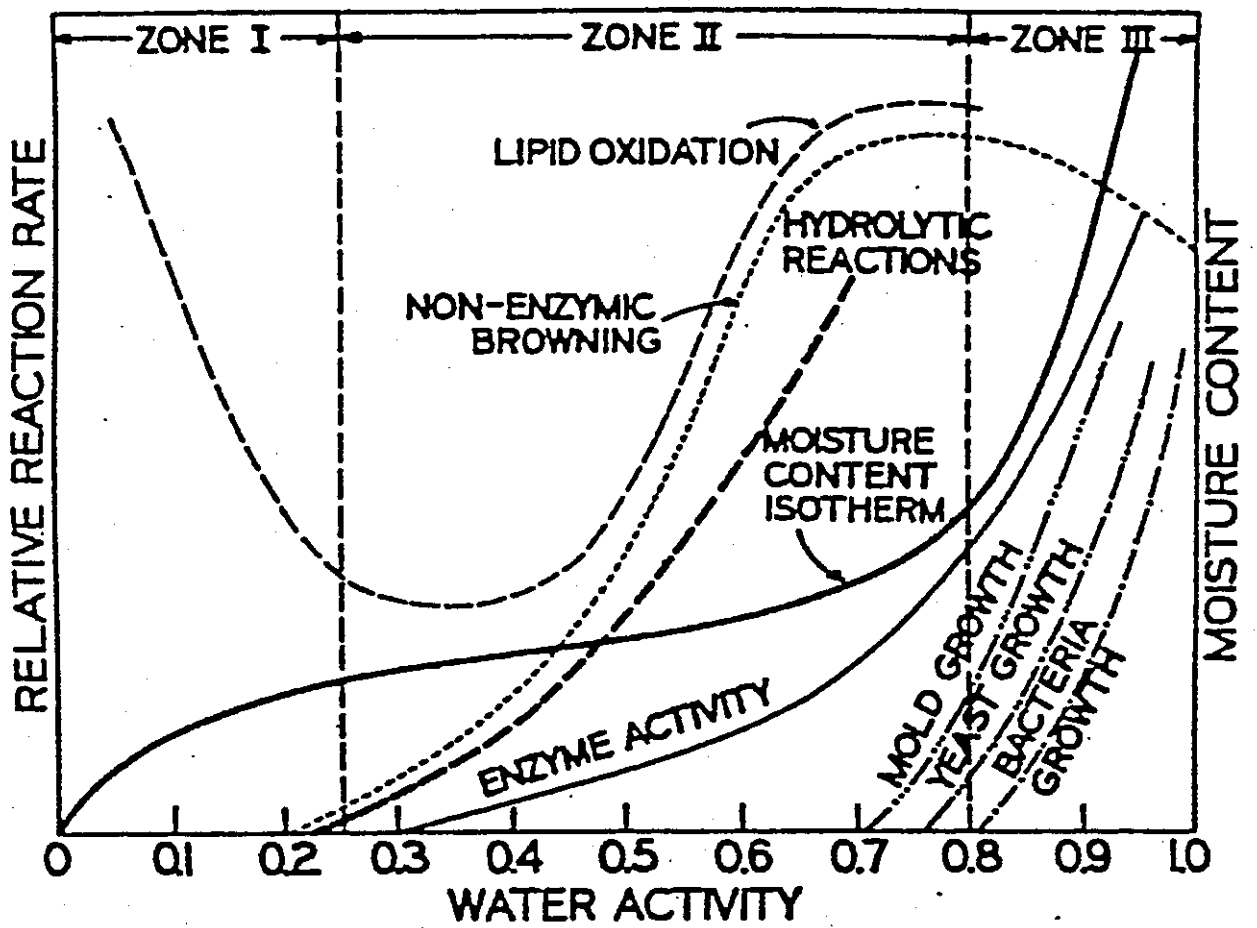


Fig. 2: Stability of food as a function of water activity.

Source: Labuza (1971).

or brine and this process continues until an equilibrium is achieved between the two phases. This lowers the water activity of the fish, with 0.75 being the minimum value obtained without subsequent drying. This is sufficient to inhibit the growth of almost all bacteria and many yeasts and moulds (Heidelbauch and Goldblith, 1975). Lupin et al. (1981) have shown that a linear relationship exists between the molarity (M) of salt in fish and the water activity $(a_w)_{SF}$ i.e. $(a_w)_{SF} = 1.002 - 0.042 M$.

This relationship though approximate (because it assumes the salt to always be in true solution) provides a useful method of estimating water activity of salted fish.

Salting alone is most commonly applied to non-fatty species such as cod. However, in areas where seasonal gluts occur it is common practice to salt all fish initially and follow the process up with drying at a later stage. Salting is also of importance because under adverse weather conditions, i.e. during rainy periods, fish, if heavily salted, can be held in a satisfactory condition for weeks, pending further processing.

Salting is economically feasible only in areas where salt can be obtained cheaply, which is why salting is rare in inland parts of Africa but very common in South America and countries of Asia (FAO, 1981).

2.1.3 Salting and drying

As indicated above, salting of fish is often followed by drying; either by sun-drying or wind-drying. This gives a more stable product due to a further reduction in moisture content and consequently a lowering of the water activity. Moisture contents have been observed to vary between 34% and 60%, salt contents between 6.6%

and 30%, and water activities between 0.69 and 0.92 in salted-dried products from S.E. Asia (Hanson and McGuire, 1982). Poulter et al. (1982) have predicted mould-free storage lives of 4, 10, 38, 100 and 450 days for salted-dried fish with water activities of 0.85, 0.80, 0.75, 0.70, and 0.65 and have produced experimental results to confirm these predictions. This indicates that salted-dried fish should attain a water activity of 0.70 or below to be stored without mould spoilage for three months or more.

Salted-dried fish are common food products in many countries, in particular in S.E. Asia where 30% of the fish catch in Indonesia is converted to salted-dried fish. Also in Angola for example 60% of the population eat salted-dried fish. These products have been described as one of the most highly concentrated protein items on the world food market (Borgstrom, 1962).

In the developed countries freezing has largely replaced salting and drying for locally consumed products. Salted-dried products produced in some developed countries, e.g. the U.K., are specifically for export markets. The method is however still of utmost importance in the less developed world, particularly S.E. Asia, for economic and traditional reasons. Therefore an understanding of the stability of the products and the techniques of processing is necessary if losses are to be prevented.

2.1.4 Methods of cured fish production in S.E. Asia

The main traditional methods of fish preservation in S.E. Asia are drying, salting and salted-drying. These are of considerable importance for the economic, traditional and nutritional reasons described earlier. The techniques involved in these processing methods vary widely, even within a limited area. Details of the

exact chemical, biological and enzymatic changes in the products are limited. Present knowledge of the techniques of processing and the biological and chemical stability of the products so produced have been compiled from empirical findings from local populations (FAO, 1981; Van Veen, 1953; and Cole and Greenwood-Barton, 1965). In these papers some suggestions for improving existing methods have been made.

2.1.4.1 Dried fish

Prior to processing, all fish except the very small ones are split, gutted and scored to expose a large surface area. They are placed on mats, racks or even sandy ground under the sun and wind and left to dry. A 3-10 day drying period has been observed by Waterman (1976), even though this will vary considerably depending on weather conditions.

Suggestions for improving the process include raising the racks and placing them in open grounds to take advantage of the wind current and sunlight. The shelves may be sloped to allow the extracted water to drain. Drying fish should be stock piled inside shades to avoid night dew-condensation. Doe (1977) has suggested the use of a simple tent of polythene sheeting supported on a bamboo frame as this will make the process more hygienic. A trial run in Bangladesh gave improved drying times but problems were encountered with the accurate control of the temperature in the tent and with cracking of the polythene under the heat of the sun. Other forms of solar driers, e.g. brick and glass solar kilns and an insulated solar box, have been tested in Malawi (FAO, 1981). In

all these cases only minimal reduction in drying times were observed; the increased rate of drying being attributed to the increase in the final stages of the drying process. As the process is weather dependent, they were unsatisfactory in the wet season because the lower atmospheric temperatures and air flow rates caused slow drying, leading to early spoilage. The use of electrically powered mechanical drying systems have been developed and used for fish drying. In these systems heated air at about 45°C is drawn over the fish to accelerate drying independent of weather conditions. Aitken et al. (1967) have studied the effect of drying, salting and high temperatures on the nutritive value of dried cod in these accelerated mechanical drying processes and found, surprisingly, no apparent deleterious effects.

However, the process has been found to be expensive and was abandoned in Bangladesh (Shahidullah, 1978) mainly because of fuel costs but also due to the lack of good technical management and the scattered fish landing sites, which created logistical problems.

2.1.4.2 Salted fish

Initial preparation is as for drying and then the fish is salted in three ways:

- i) Kench curing - here the fish is rubbed with salt before being stacked in a pile. The salt penetrates the fish and the extracted moisture is allowed to drain.
- ii) Pickling - in this method salted fish are stacked in watertight containers with the result that the salted fish become immersed in a highly concentrated "pickle" of fluid.

- iii) Brining - here the fish is soaked for a prescribed time in concentrated salt solution.

Pickling and brining are the methods most frequently used in S.E. Asia as these minimise exposure to air and insect pests. In a typical product, Pedah Siam, made with the marine fish Scomber neglectus in Thailand, the salted fish is kept for 12-24 hours before being washed and dried. At times fresh salt is added before the fish is packed in large crates to mature for several weeks or months. The mature product has a pasty consistency and a fresh red colour and its quality can be monitored by pH determinations. A good product should have a pH of 6.0-6.4, weigh about 40-70 g and have a brown colour (Van Veen, 1953). The investigations also reveal that the maturing process must be carried out under strict anaerobic conditions for high quality product. The highly desirable brown colour of Pedah was thought to be due to oxidation products of the fish body gut. The study concluded that besides pH, volatile bases, colour, fat content and flavour, the size, age and outward appearance of the product were important factors in determining the price.

Methods of improving the process include:-

- i) Uniform application of sufficient salt to prevent leaving bare areas, for the growth of spoilage bacteria.
- ii) Keeping treated fish in the shade at low temperatures to inhibit the growth of mesophilic halophiles.
- iii) Using good quality salt of medium crystal size (3mm-5mm) for salting to ensure efficient penetration.

- iv) Storing salt in dry places to avoid the proliferation of red halophiles which can contaminate the fish, and
- v) The use of cement tanks with lids for holding the treated fish (Cole and Greenwood-Burton, 1965).

Observations show that some refinements produce a product that is too bland for the consumer and it has also been noted that even though no one particularly wants a rancid product, people used to eating salted products do expect some degree of rancidity since this contributes to the accepted flavour.

2.1.4.3 Salted-dried fish

Salted fish does not dry at a relative humidity of 75% and above and even at 70% drying is very slow. This renders the salted fish prone to spoilage during storage. The minimum mould-free storage life of salted fish with 40% moisture and 15% salt is reported to be three weeks (FAO, 1981). Therefore to produce a more stable product the salting process is often followed by drying, either under the sun in hot dry climates or by artificial means as already described. Fish salting and drying methods in Indonesia (West Java) have been described by many authors, e.g. (Esser and Warren, 1983) and (Van Veen, 1953). In the processing of Arius sp. in West Java, the fish is beheaded, eviscerated, bled and where possible iced at sea. Otherwise the cleaning operation is done after landing, or in some cases after salting. Salting techniques vary within the locality - some use a pickle cure, others brine and still others a combination of both. Salting takes between 12 hours and 2 days and is carried out in concrete tanks. Usually the pickles or brines are re-used 3 to 4 times with occasional

addition of salt to boost salt concentration for the fresh batch. The salted fish is then washed with either fresh or sea water and laid to dry on wooden bamboo racks raised about 0.6-1 metre above ground - the process taking 2-5 days. After about a day or two the fish may be further split to expose an even larger surface area. There are reports that in some areas of Indonesia fermentation is encouraged in fresh fish before salting in order to give a soft product. Also in some areas salted freshwater fish are treated with roasted and pounded rice before being dried under the sun.

In central Borneo, the Dyaks allow the salted fish to be infested with fly larvae before being put in closed pots for 12 to 24 hours, during which the maggots come to the surface and die, and are removed before the fish is dried under the sun for 3-7 days. The keeping quality of this product is reported to be remarkable (Van Veen, 1953).

The salted-dried fish products are normally dried until the processor decides, sometimes arbitrarily, that drying is complete. Observed values for moisture content of salted-dried fish in S.E. Asia are given in Table 1 (Hanson and McGuire, 1982).

Occasionally dried-salted fish is pressed at intervals to increase moisture loss before being stacked at night in the house or shades. The stacking and pressure piling renders the fish flat on drying and this eases packaging. Salted-dried products may need occasional re-drying as they tend to absorb moisture in long term storage at high humidity conditions.

Table 1: Sample details and analytical data for large salted-dried fish from S.E. Asia

Sample	Country of origin	Common name	Species or genus	Product	Defects	Moisture	Lipid	NaCl	a_w
1.	Thailand	Queenfish	<u>Chorinemus</u> sp.	whole		60	3.4	6.6	0.92
2.	Burma	Yellow croaker	<u>Protonibea</u> sp.	eviscerated		34	2.6	15.8	0.74
3.	Burma	Threadfin	<u>Eleutheronema tetradactylum</u>	fillet		41	1.4	15.6	0.70
4.	"	"	"	fillet		40	3.8	11.0	0.69
5.	Indonesia	Spanish mackerel	<u>Scomberomorus commersonii</u>	split		44	9.7	11.0	0.81
6.	Indonesia	Sea catfish	<u>Arius</u> sp.	beheaded and split	mouldy	36	5.0	11.9	0.73
7.	Indonesia	Tuna	—	eviscerated		43	3.3	13.2	0.74
8.	Indonesia	Sting ray	—	wing		44	1.2	21.4	0.73

Source: Hanson and McGuire (1982).

2.1.5 Production and quality of fish curing salt

Where great financial and technical resources are available salt is mined or extracted from sea water mechanically and purified initially by crystallisation (from the evaporation of a salt solution) and can be further highly purified by an electrolytic process.

Salt has also been produced by heating sea water in open vats to dryness but this is practiced only in the more humid tropical areas where wood fuel is abundant.

In many developing countries, including S.E. Asian countries, the only salt widely and cheaply available to fish curers is solar salt. This is salt made from sea water by evaporation in open ponds. As the evaporation step is crucial, it can only be achieved in areas of intense sunlight and low rainfall. Therefore distribution to other fish curing areas adds to the cost of the otherwise cheap salt.

A typical solar production unit generally consists of a series of ponds dug to present a flow gradient from the top pond to the bottom one (Cole and Greenwood-Barton, 1965). The larger the establishment the more the number of ponds present. Solar salt is produced by letting sea water flow into the top pond where it is allowed to stand until all suspended matter settles out. The clear water is then allowed to flow into the second pond where evaporation is allowed to take place until the specific gravity rises from about 1.025 to 1.196 when iron, calcium and magnesium salts precipitate. The brine is further purified by passing it into other ponds until, at specific gravity of about 1.283, the salt crystallises out and the remaining solution, the "bittern", is drained off. The salt is allowed to dry before being raked, ground to required particle size and bagged. Salt produced in this manner always contains some

impurities, including magnesium and calcium chlorides and sulphate (Shewan, 1951) (see Tables 2, 3, 4 and 5). The calcium and magnesium salts have been found to be desirable in small amounts, i.e. less than 0.5%, as they firm the tissues of the cured fish and prevent some yellow discolorations. Excess of these however can impart a bitter taste and be indirectly involved in spoilage.

The precise size of the salt granule is also an important factor in producing a good quality product by any salting technique (see Table 5). For example, in dry salting, fine particular salt will quickly dissolve and cause the coagulation of surface proteins which in turn will prevent the exit of moisture and penetration of salt into the fish flesh. On the other hand, very coarse salt dissolves too slowly and causes delay in the salt penetration. The best salt for this is therefore a compromise, a mixture of fine particles and coarser particles of approximately 6 mm in diameter or 40-50% of which pass a No. 10 seive. The fine particles will dissolve and penetrate quickly while the large particules will gradually dissolve to maintain a high ionic concentration that is necessary for the removal of moisture from the fish flesh towards the end of the process.

2.2 BIOLOGICAL DETERIORATION OF SALTED-DRIED FISH

Biological deterioration of salted-dried fish can be broadly classified as autolytic, microbial and that caused by various pests, e.g. insects, rodents.

Table 2: The chemical composition of various solar, brine evaporated and rock salts

Type of Salt	NaCl per cent	MgCl ₂ per cent	CaCl ₂ per cent	MgSO ₄ per cent	CaSO ₄ per cent	H ₂ O Insoluble per cent	Moisture per cent
Solar Salt:							
European (15 samples)	89.50-99.50 (95.71)*	0.0-1.19 (0.39)	0.0-0.87 (0.22)	0.0-1.92 (0.56)	0.0-3.97 (0.85)	0.01-1.31 (0.20)	0.10-9.6 (3.5)
Russian (11 samples)	95.60-99.04 (97.74)	0.04-0.33 (0.14)	0.0-0.40 (0.13)	0.0-0.08 (0.01)	0.07-2.48 (1.01)	0.05-1.00 (0.36)	-
N. & S. American (7 samples)	94.98-99.91 (97.30)	0.0-1.28 (0.55)	0.0-0.06 (0.02)	0.0-1.08 (0.28)	0.03-2.21 (1.56)	0.01-0.74 (0.21)	0.16-6.13 (2.23)
Brine Evaporated:							
European and American (9 samples)	97.03-99.96 (98.51)	0.0-0.14 (0.05)	0.0-0.35 (0.17)	0.0-0.46 (0.07)	0.0-1.50 (0.71)	0.01-0.05 (0.04)	0.0-0.84 (0.20)
Rock Salt:							
European and American (9 samples)	94.57-99.52 (97.85)	0.0-0.97 (0.15)	0.0-0.42 (0.09)	0.0-0.14 (0.02)	0.12-2.08 (1.05)	0.12-3.35 (0.83)	0.22-0.75 (0.50)

* The average values are given in brackets. Expressed probably on dry weight basis.

Source: Shewan (1951).

Table 3: Limiting concentrations of calcium, magnesium, sulphate and chloride ions, and insoluble matter in salts used for various processes

Type of Process	Maximal Concentrations in the Dry Salt as per cent				Minimal Chloride Concentration in the Dry Salt as per cent
	Calcium	Magnesium	Sulphate	Insoluble matter	
Pickle Salting	0.50	0.12	1.00	0.30	58.8 - 59.3
Dry Salting	0.50	0.60	1.00	0.20	59.0 - 59.5
Marinated Products	0.10	0.03	0.12	0.05	59.8 - 60.2

Source: Shewan (1951).

Table 4: The Bacterial Counts of various salts
(Expressed as number per gram of salt)

Type of Salt	On Ordinary Media		On Salt-Saturated Media
	At 20°C	At 37°C	At 37°C
Solar Salts - Russian (29 samples)	140-3650 (at 25°C) (Average 760)	-	100,000-200,000 (Average 150,000)
European (7 samples)	20-1100 (Average 650)	60-700 (Average 330)	30-180,000 (Average 65,400)
Brine Evaporated European (5 samples)	10-700 (Average 275)	10-40 (Average 20)	0-1,800 (Average 180)
Rock Salt (1 sample)	10,000 (mainly moulds)	4	0

Source: Shewan (1951).

Table 5: The grain size* and density of some typical fishery salts

Type of Salt		Sieve No.								g/cm ³	
		8	10	12	16	20	30	40	60		70
Solar Salts:											
Sample No. 1		60	47	37	25	19	9	4	1	1	1.136
No. 2		21	12	7	3	2	-	-	-	-	0.88
No. 3		42	31	23	14	10	5	2	1	-	1.12
No. 4		59	39	30	18	11	3	-	-	-	1.104
No. 5		31	20	12	7	5	2	1	-	-	0.912
No. 6		73	56	41	23	15	5	1	-	-	1.056
No. 7		74	53	38	20	15	4	1	-	-	1.056
No. 8		71	58	47	33	26	13	5	1	-	1.088
Brine-Evaporated:											
Sample No. 1)		66	51	37	20	13	5	2	1	-	0.928
No. 2)	Fishery	49	32	22	14	7	3	1	-	-	1.168
No. 3)	salt	79	65	51	31	22	9	4	2	1	1.024
No. 4)		69	52	37	20	13	5	2	1	-	0.944
No. 5	Fine salt	100	100	100	100	99	99	97	58	36	1.264
Rock Salt:											
Sample No. 1		37	18	8	1	-	-	-	-	-	1.056
No. 2		90	85	78	69	56	35	23	15	12	1.312

* Expressed as the percentage passing through British Standard Test Sieves.

Source: Shewan (1951).

2.2.1 Autolytic deterioration

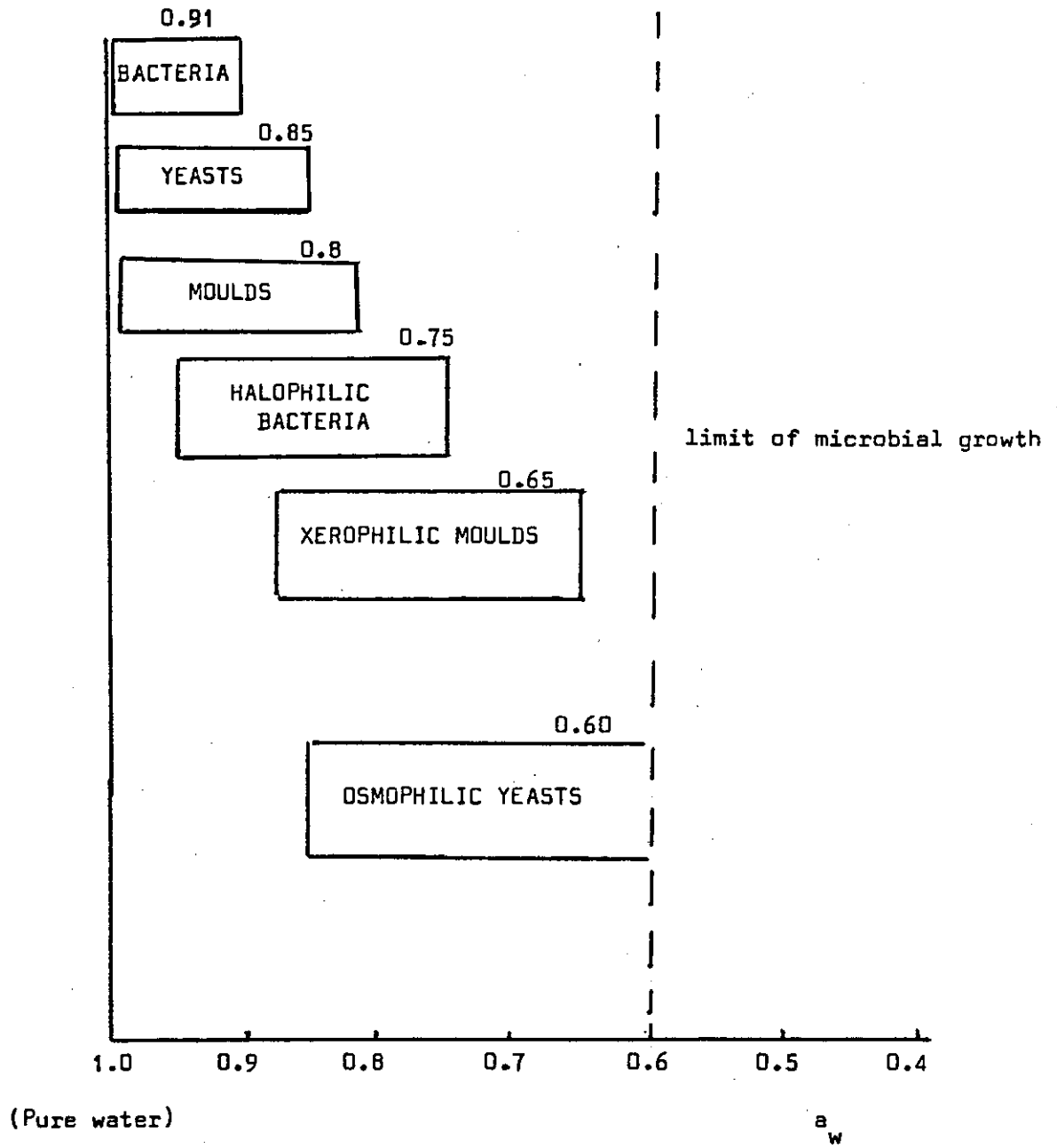
On the death of a fish normal metabolic processes are disrupted and deterioration sets in. Physical tissue damage during handling and processing can increase this effect in which residual body and gut enzymes attack and digest the fish tissue. These enzymes include the proteases, which break down proteins to peptides, lipases which cleave fatty acids from non-polar glycerides (triglyceride etc.), phospholipases and other acyl hydrolases which act on polar lipids.

The extent of damage caused by these enzymes can be minimised by proper handling of the fish, including gutting, bleeding to remove some enzymes, icing to reduce enzyme activity and reduction of the water activity by salting and drying which reduces the activity of the enzymes (see Fig. 2). Most of the autolytic enzymes are inhibited at water activity values below 0.8, but some hydrolases are known to be still active at water activities as low as 0.3-0.2 (Loncin, 1975).

2.2.2 Microbial deterioration

Though live fish flesh is sterile, at death bacteria from the gills, gut and skin start to invade the tissues, which are a source of microbial nutrients, and putrefaction sets in. This process can be slowed down by proper handling procedures, as mentioned above, but can only be stopped by sterilization, e.g. by heating or irradiation, or methods involving adequate reduction in the water activity (see Fig. 3). Generally bacteria do not grow below a water activity of 0.88 but certain halophilic (salt loving) and

Fig. 3: Growth ranges of micro-organisms with respect to water activity



Source: FAO (1981).

haloduric (salt tolerant) ones exist which can grow on fish with water activity as low as 0.75. These include members of the halobacteriaceae, e.g. Halobacterium and Halococcus. The halophiles require a salt concentration of between 5-20% to grow while the haloduric ones can withstand salt concentrations of 15-20%. These conditions have been found to prevail in some salted-dried fish from S.E. Asia (see Table 1) (Hanson and McGuire, 1982). All solar salt contain these bacteria, especially some species of the genera Halobacterium and Halococcus which grow under normal processing conditions to produce undesirable pink colourations leading to severe losses in the salted fish industry. Losses also occur due to their proteolytic activity which softens and breaks the fish flesh besides producing off-flavours and odours. Off-odour compounds include hydrogen sulphide, methyl mercaptan (methanethiol) dimethyl sulphide, esters of acetic, propionic, butyric and hexanoic acids, which are produced from sulphur containing amino acids. Also trimethylamine and ammonia are produced from trimethylamine oxide and urea respectively.

Under tropical conditions some mesophilic bacteria, e.g. Micrococcus, Coryneforms and Bacillus, can grow in unsalted parts of the fish causing proteolysis and tissue degradation - this is more noticeable in dried fish with a hard outer crust and moist inner tissues. Heavily salted, but poorly dried fish, will absorb water at a relative humidity above 76%, to such an extent that it becomes prone to this form of attack.

Some yeasts can grow on salted moist fish at a water activity as low as 0.85 and cause spoilage (see Fig. 3), but this does not appear to cause serious problems in the industry. The main halophilic yeasts include Saccharomyces rouxii, Debaromyces hansenii, Pichia

ohmeri and Hansenula anomala. Spores of these yeasts settle and grow on the fish but Debaryomyces hansenii may also be obtained from the curing salt since it is known to be common in sea water.

Moulds in general can grow under drier conditions than bacteria and yeasts and xerophilic types, e.g. A. halophilicus, A. restrictus, are known to survive in products with a water activity of 0.6. A mould which commonly attacks dried fish is the halotolerant fungi of the genus Wallemia or Geotrichum. These may appear as brown black, fawn or chocolate coloured spots as in Wallemia sebi. Several species of Aspergillus have also been reported to grow on dried fish (FAO, 1981). Moulds do not cause extensive proteolysis like bacteria but they moisten the fish surface and encourage growth of halophilic bacteria which are responsible ultimately for the production of off-flavours and odours.

Besides discolourations, certain toxogenic types of moulds are capable of producing mycotoxins. Aflatoxin by Aspergillus flavus penicillic acid and ochratoxin A by A. ochraceus are the most important in dried foods. However evidence of toxicological problems in man from eating dried fish containing these toxins is lacking (FAO, 1981) probably due to the fact that fish usually forms part of a larger meal, which reduces significantly the concentration of any toxin.

2.2.3 Pest damage

The pests that attack salted-dried fish include common blowfly larvae, beetles, mites and rodents.

In the initial stages of production, cured fish attract the common blowfly (Diptera) which lay eggs that develop into damage

causing larvae. Except in the rare instance (see Section 2.1.4.3) where this is specifically encouraged, it is the voracious feeding habit of the larvae that causes enormous damage to cured fish.

The insecticides Baygon and Startox have been reported to be used by curers in an uncontrolled manner to kill these larvae (Esser and Warren, 1983). Controlled use of insecticides has been tried in several countries (FAO, 1981) with only partial success.

Mites infest the product at the drier stages and also cause considerable damage. Beetles also tend to attack the dried products rather than when the material is still moist and can cause considerable losses during prolonged storage.

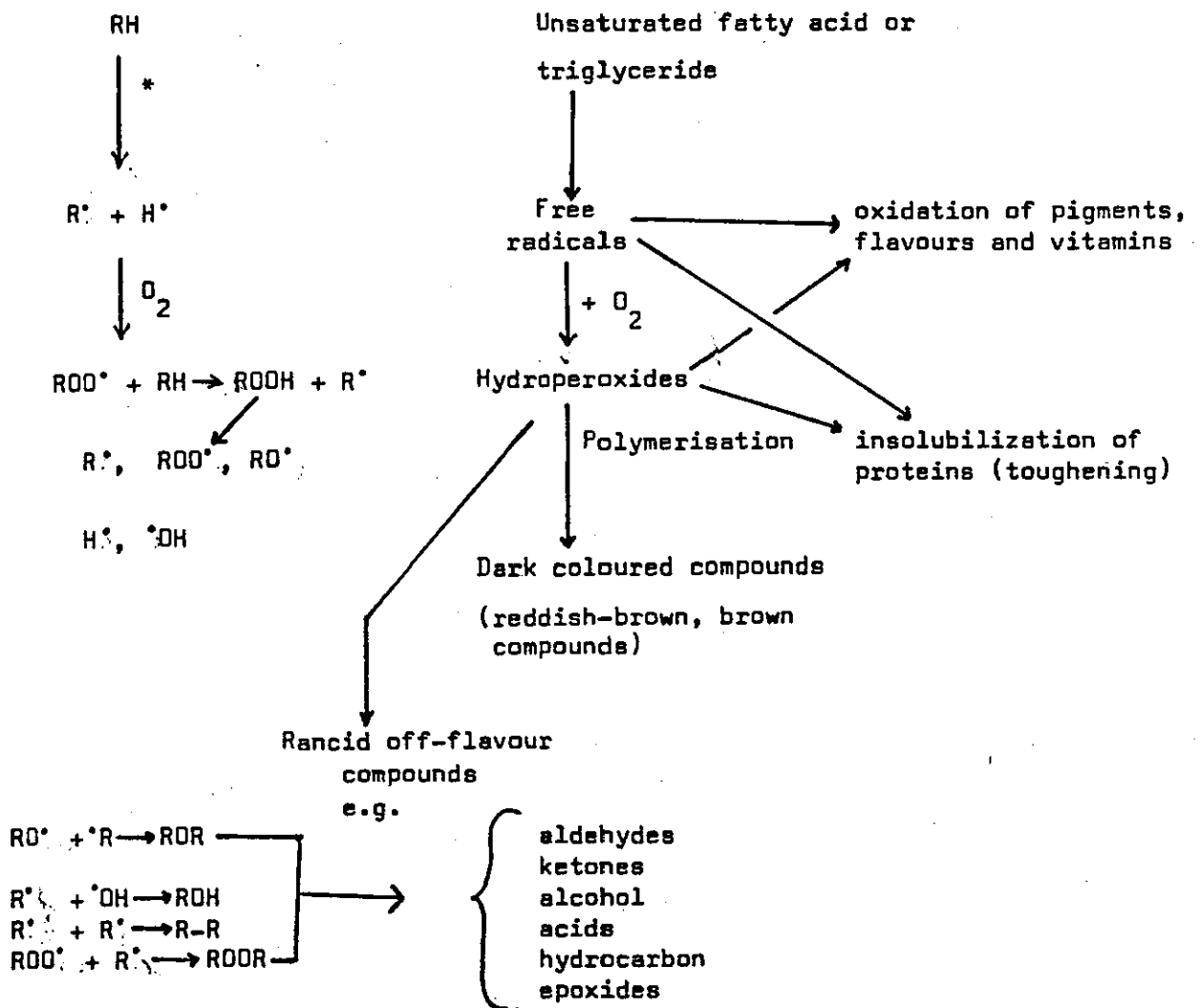
Rodents like rats and mice will eat inadequately protected stored products and can cause severe losses.

2.3 CHEMICAL DETERIORATION OF SALTED-DRIED FISH

Unpreserved fish is mainly subject to biological spoilage, as indicated above, but with preserved fish chemical deterioration becomes significant. This is very evident with fatty fish species in which the unsaturated fatty acids oxidise easily to give rancid flavours and subsequently to a series of other chemical reactions (See Fig. 4) which include protein denaturation, changes in pigments and browning reactions. Besides posing severe limitations to the organoleptic acceptability of stored cured products, these reactions lead to loss of nutritional value (Matsuo, 1961) and may also lead to the formation of toxic by-products (Kaunitz, 1962).

These reactions are affected by the introduction of salt (sodium chloride) and its impurities into the food, as in salted-dried fish during processing (Banks, 1937; Chang and Watts, 1950; Hills and Conochie, 1946; Castell *et al.*, 1965).

Fig. 4: Scheme of rancidity development



Source: Adapted from Labuza (1971).

2.3.1 Rancidity development in salted-dried fish

Rancidity is defined as the chemical deterioration of lipid in foods leading to the development of off-flavours which can render the food unacceptable to the consumer (Labuza, 1971). In salted-dried fish the flavours mainly result from the reaction between oxygen and unsaturated fatty acids (in triglycerides or phospholipids). Pelagic fish species such as Atlantic mackerel (Scomber scombrus) herring (Clupea harengus) and anchovy (Eugraulis spp.) have been shown to contain in their flesh a high percentage of fat (see Table 6). It is well established that percentage fat content is only one of the factors of importance in rancidity development. The degree of unsaturation of the fatty acids and the positioning of the fat in the food matrix are also of great importance.

A scheme representing the general picture of lipid oxidation, development of rancidity and the associated myriad of reactions in food is shown in Fig. 4. Molecular oxygen reacts with the free radicals of the unsaturated fatty acid to form unstable hydroperoxides (Koch, 1962). When these breakdown they form rancid off-flavour compounds which have very low flavour and odour thresholds (Patton, 1964). These reactions are influenced by such factors as temperature, light, presence of natural pro-oxidants and anti-oxidants in the food and general storage conditions.

In dried-salted fish the tissues are porous and therefore permeable to atmospheric oxygen which attack the unsaturated fatty acids. Breakdown products of the hydroperoxides so formed give the off-flavours and can also react with proteins and amino acids to form coloured products and also cause tissue toughening. Lipid peroxides can be toxic (Toyama and Kaneda, 1965) and there is

Table 6: Fatty acid profiles of some fats and oils
(Percentages of total fatty acid content)

FATTY ACID	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	16:1	18:1	20:1	18:2	18:3	20:4	20:5	22:5	22:6
Coconut oil		t	6	6	44	18	11	6		7		2					
Corn oil							13	4		29		54					
Olive oil						t	14	2	2	64		16	2				
Palm oil						1	48	4		38		9					
Butter	3	1	1	3	4	12	29	11	4	25		2					
Beef tallow						3	26	17	6	43		4	t				
Cod flesh						1	20	3	3	12	2	1		5	17	2	30
Mackerel						5	18	3	6	21	6	2	0.4	1	8	1	11
Whole herring (triglyceride)	(also 19% of 22:1)					6	13	1	14	16	14	1	t	t	7	t	3
Whole herring (phospholipid)						2	21	3	5	13	2	1	t	1	12	1	33
Dogfish flesh (triglyceride)						2	17	3	5	16	5	2		3	8	3	22
Dogfish flesh (phospholipid)						6	20	9	3	11	3	1		7	5	2	32
Queen crab (triglyceride)	(also 8% of 22:1)					4	14	2	10	24	12	1	t	2	11	1	9
Queen crab (phospholipid)						3	15	3	6	22	2	1	t	4	31	1	14
Shrimp flesh						3	16	3	6	19	2	2	1	t	22	1	16
American oyster (triglyceride)						4	23	1	5	8	1	t	3		14	1	14
American oyster (phospholipid)						1	5	3	3	5	3	t		t	16	1	20

Source: Ackman and Eaton (1971).

evidence to show that vitamins A and E can be destroyed by these peroxides (Olcott, 1962), which could lead to aggravation of vitamin A and E deficiency. There is also an indication that products of lipid oxidation in food can be carcinogenic (Kummerow, 1962) but there is no conclusive evidence yet. As indicated earlier a slight degree of rancidity is considered desirable and encouraged in some cured products (Waterman, 1976; Van Veen, 1953) however extremely rancid products are unacceptable, which is the condition long term commercial stored products attain under poor storage conditions.

An understanding of the nature and mechanism of the reactions leading to this situation is important if losses in the salted-dried fish industry are to be minimised.

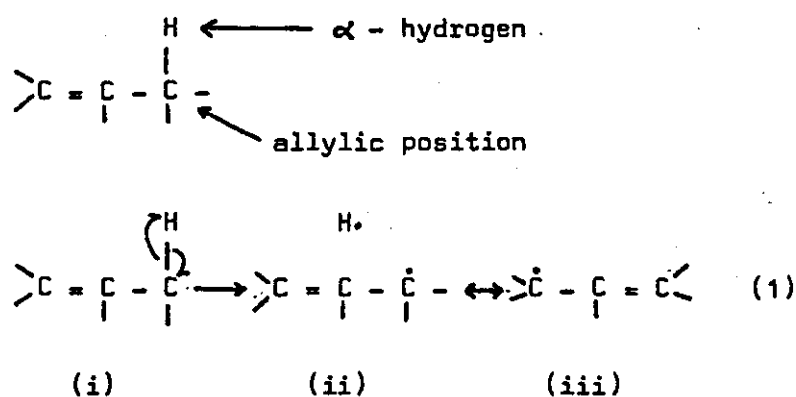
2.3.1.1 Mechanism of rancidity development in pure lipids

The oxidative deterioration of food lipid involves primarily autoxidative reactions (i.e. auto-catalytic oxidation) which are followed by various secondary reactions which may be non-oxidative and may involve pigments, proteins, amines, phenols and aldehydes. In salted-dried fish, the unsaturated lipids (e.g. the oleic, linoleic and polyunsaturated types) are most readily oxidized. Therefore, only the mechanistic aspects of their autoxidation, and how this is affected by other substances will be described here.

Unsaturated lipid oxidation proceeds through a free radical reaction mechanism and this can be conveniently discussed in three stages: (a) free radical formation from unsaturated fatty acid chains: (b) formation of hydroperoxides, and (c) breakdown of hydroperoxides (Labuza, 1971; Dugan, 1976; Lunberg, 1962).

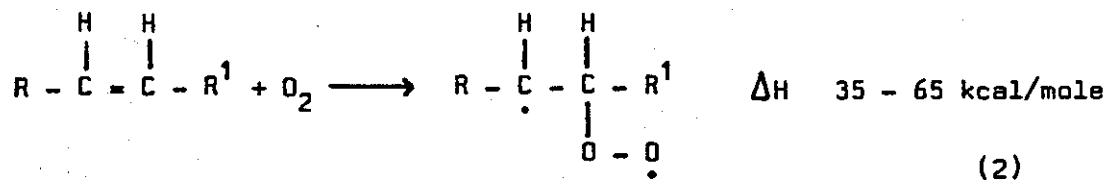
a) Free radical formation

The free radical formation involves the abstraction of a hydrogen atom from the allylic position

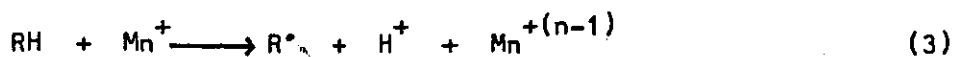


The hydrogen alpha to the double bond is most reactive due to stabilisation of the resulting free radical by electron delocalisation at the double bond(s) as shown above. However, the bond energy approaches 80 kcal/mole and is therefore difficult to achieve directly.

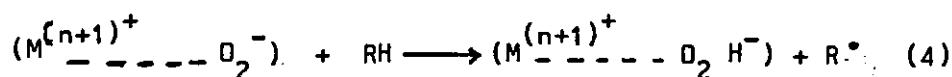
Oxygen can add to the double bonds directly to form a diradical in a process involving lower energy input.



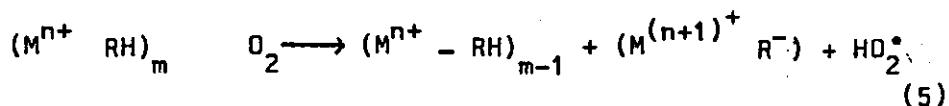
Evidence also exist of a direct metal substrate reaction with the unsaturated fatty acid to produce free radicals.



or an activated metal complex can react with the substrate to form radicals.

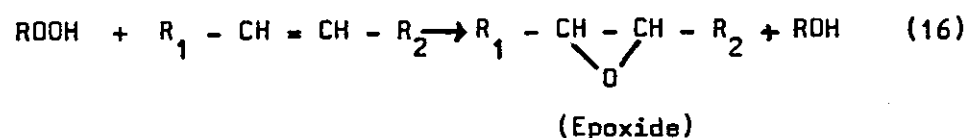
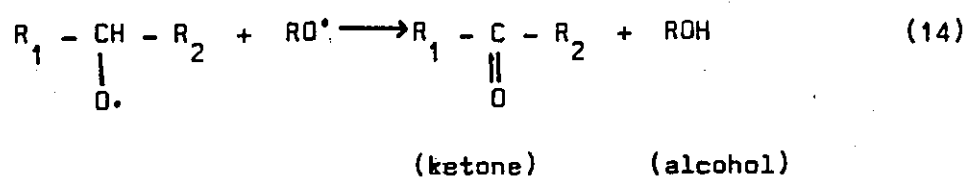
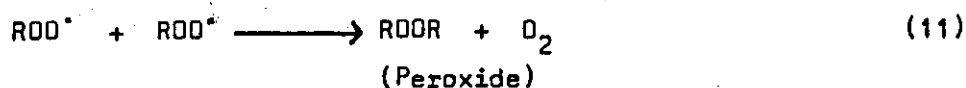
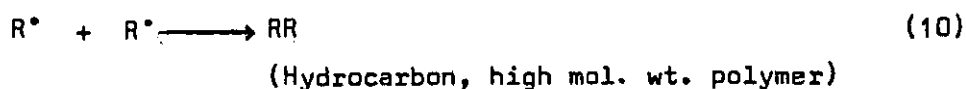


Similarly oxygen activated by copper ion has been found to ease oxidation of benzene to phenol (Anon, 1983). It has also been suggested that the initiation reaction could proceed via an association of the catalyst-substrate complex with oxygen.



These metals are transition metals, copper, iron, cobalt and manganese, that possess two or more valency states, the higher state of which is very reactive making these reactions feasible (Heaton and Uri, 1961).

Also a singlet oxygen molecule (10^3 to 10^4 times more reactive than molecular oxygen) can form by photosensitization in presence of sensitizers like the pigments chlorophyll and haemoglobin and can be interposed between a labile hydrogen to form a hydroperoxide in a concerted reaction.



c) Breakdown of hydroperoxides

Hydroperoxides are known to decompose readily in presence of high energy radiation, e.g. light, heat, transition metals and active enzymes. The decomposition products go to form an additional pool of radicals which propagate the chain reaction without requiring the initial generation of free radicals. The mechanism of decomposition depends on the concentration of the hydroperoxide. When

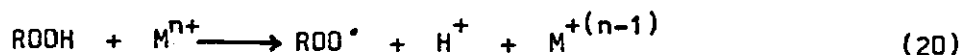
this is low it proceeds by a monomolecular decomposition:



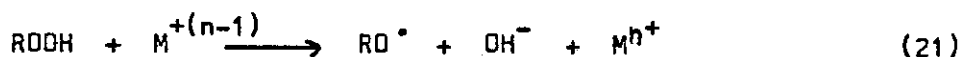
At higher hydroperoxide concentration decomposition is bi-molecular



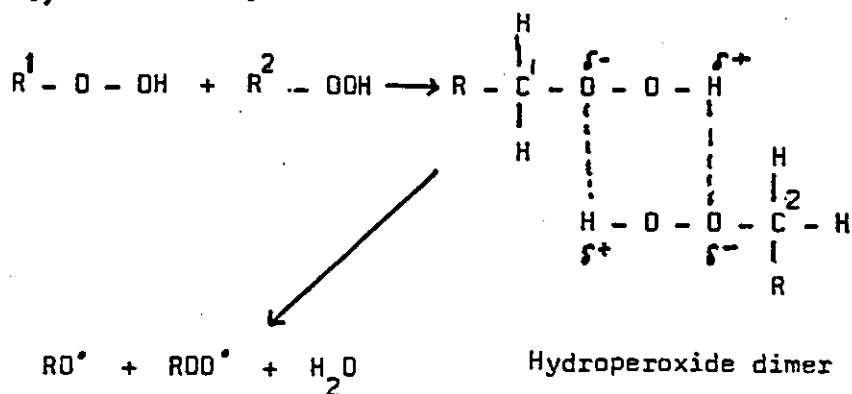
Reaction (17) and (18) can be metal catalysed as follows:-



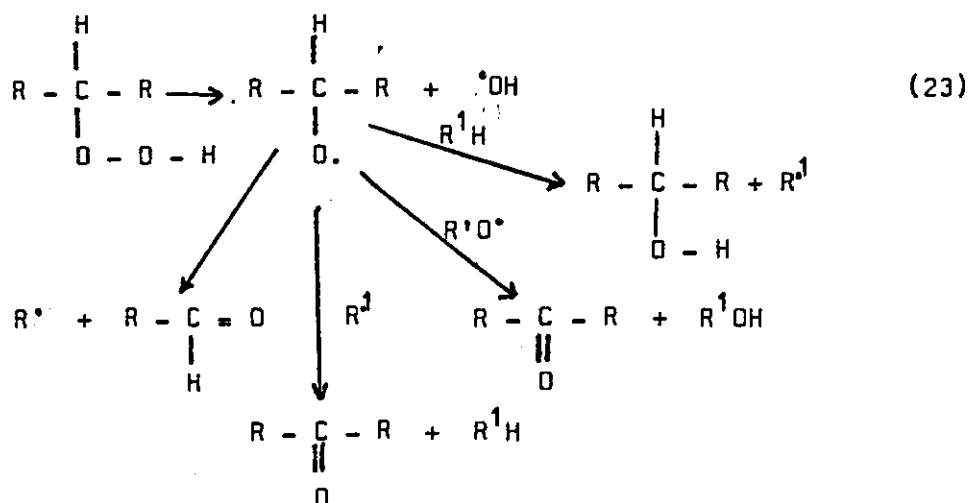
and/or



Also when the hydroperoxide concentration is high or temperature low, there is the tendency for them to associate through hydrogen bonding, most likely as shown:

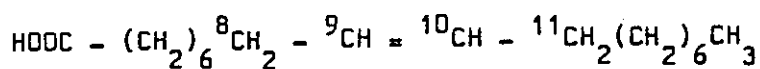


A breakdown of the hydroperoxide is energetically favourable to give products as shown in (23)

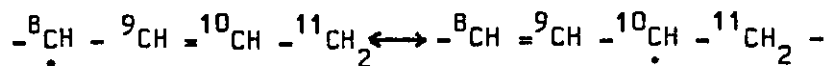


The general scheme is illustrated below by application to typical monoenoic and polyenoic acid components of food lipids.

A. Oxidation of monoenoic acids, e.g. oleic acid (18:1 Δ^9)



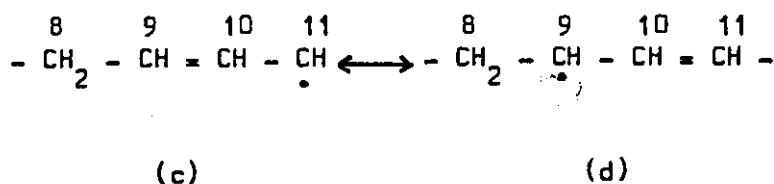
With hydrogen abstraction from the C_8 position, radicals (a) and (b) are possible due to resonance stabilisation.



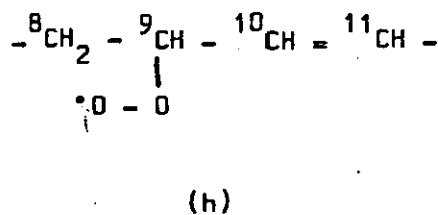
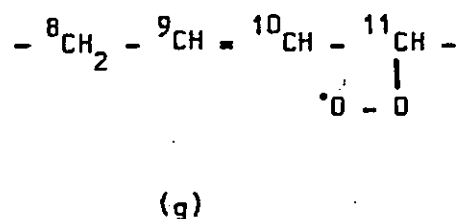
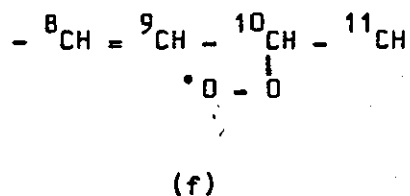
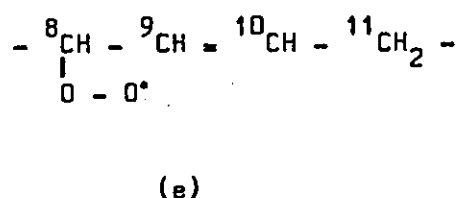
(a)

(b)

Abstraction from the C₁₁ position gives radicals (c) and (d)



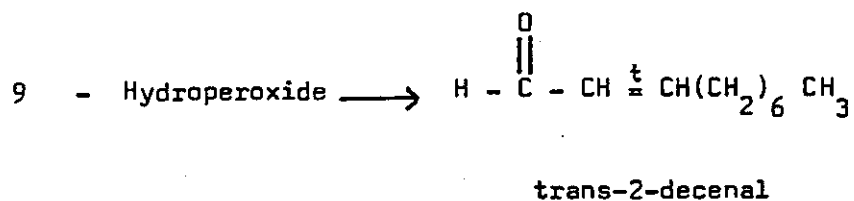
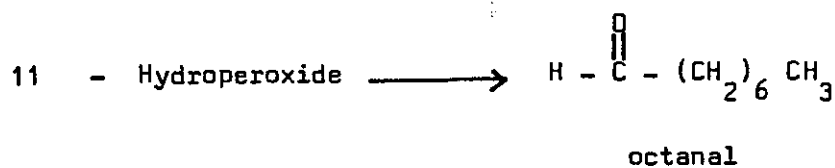
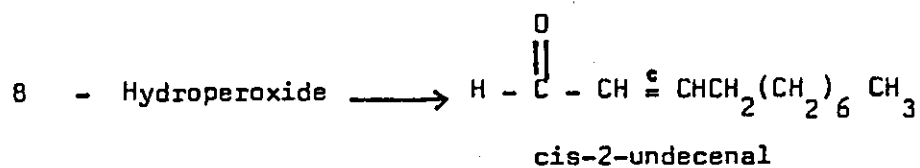
Addition of oxygen forms radicals (e), (f), (g) and (h) as follows:



Each of these radicals is capable of abstracting a H atom from an unoxidized molecule to form four isomeric hydroperoxides.

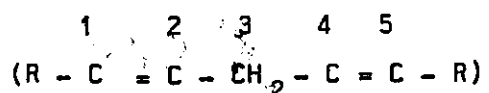
Fig. 5 shows a scheme of the formation of some breakdown products of the hydroperoxides. A shift in location of the double bond during resonance results in a change in the configuration of the bond, usually from cis to trans.

Fig. 5: Some products of autoxidation of oleates

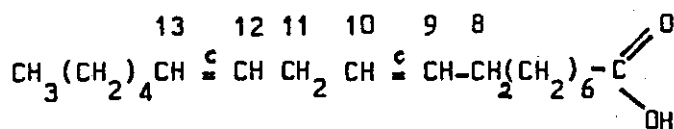


B. Oxidation of polyenoic acids

Polyenes of natural lipids have methylene interrupted 1,4 pentadiene structures

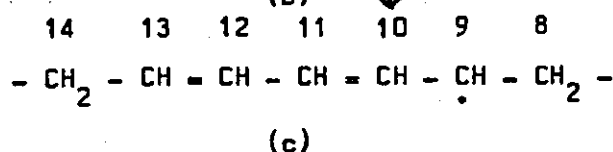
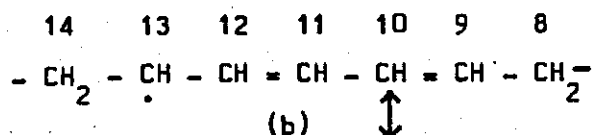
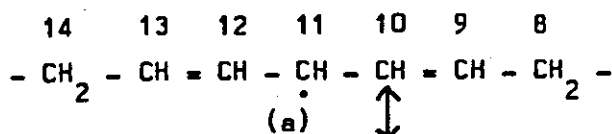


with more reactive allylic positions than the monoenoic acids. The allylic radicals formed are resonance stabilised, i.e. the electrons redistribute to form a more stable conjugated double bond system, giving a radical at either the 1 or 5 carbon of the pentadiene systems (as shown below). Thus the lipid more readily forms the radical necessary for the oxidation to proceed.

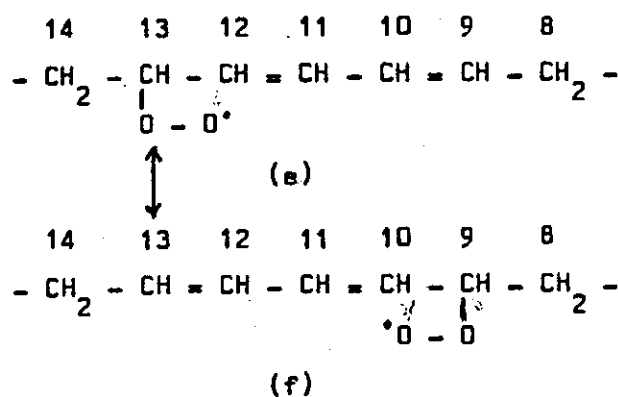
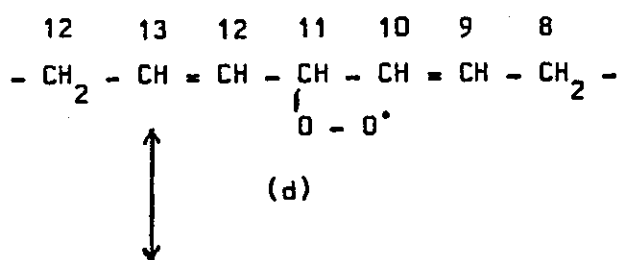


linoleic acid (18:2 Δ9)

If hydrogen is abstracted from C11, three isomeric radicals are possible.



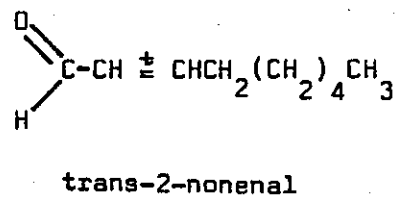
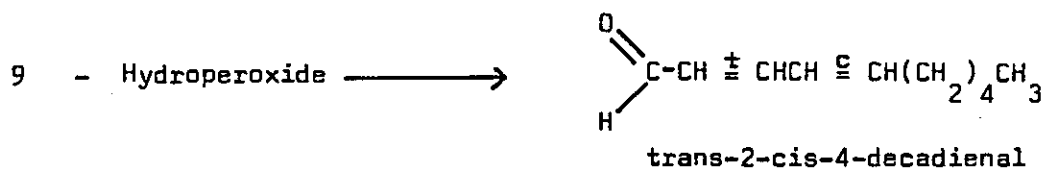
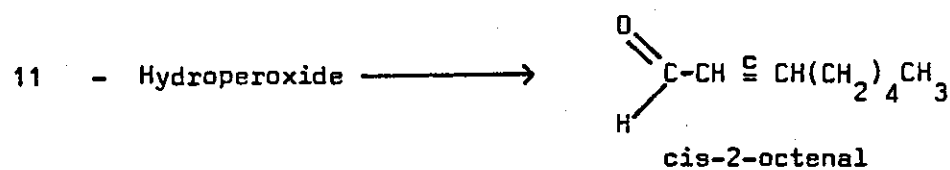
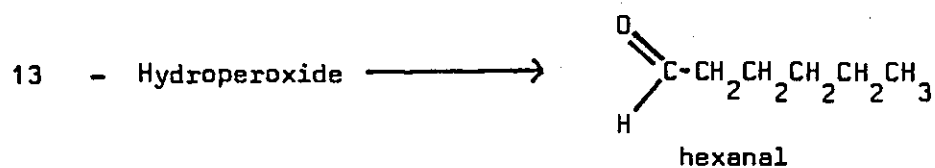
Addition of oxygen forms peroxy radicals d, e and f



From these radicals isomeric hydroperoxides can be formed by abstraction of hydrogen from an unoxidized molecule.

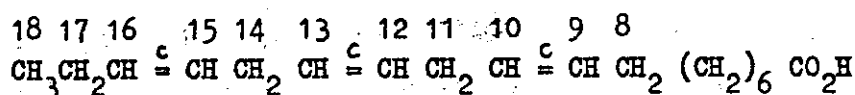
Fig. 6 shows the formation of some of the breakdown products of these hydroperoxides.

Fig. 6: Some products of autoxidation of linoleates

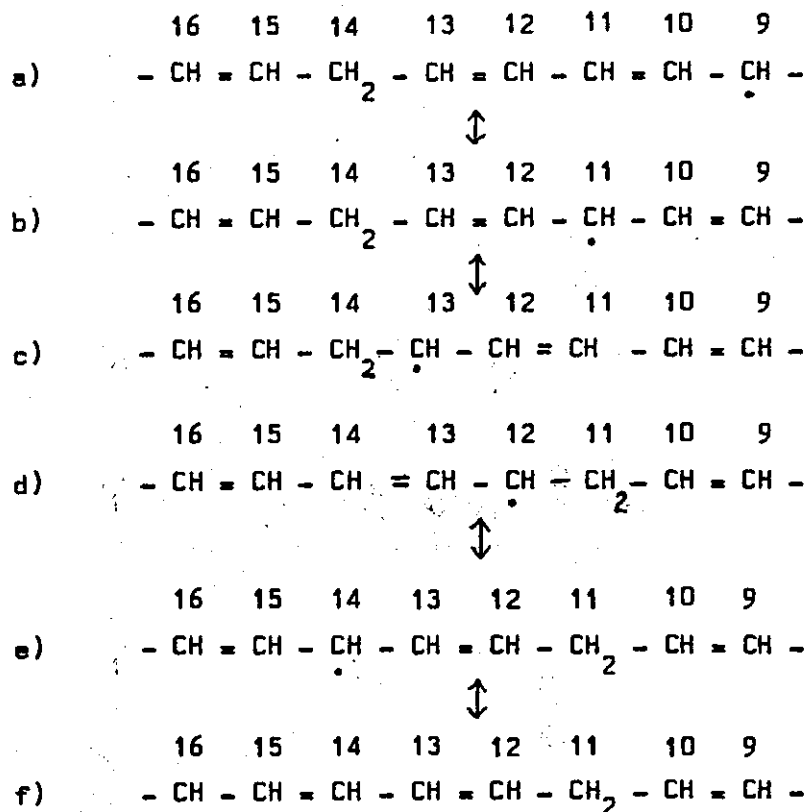


C: Oxidation of other polyenes.

Similar mechanisms occur during the oxidation of highly unsaturated fatty acids, e.g. linolenic (18:3 $\Delta 9$) and arachidonic acids (20:4 $\Delta 5$), as for the dienes. Here a higher rate of reaction is possible through an increased stabilization of the intermediate radical isomers, through electron delocalisation of the conjugated methylene interrupted system.

linolenic acid (18:3 Δ 9)

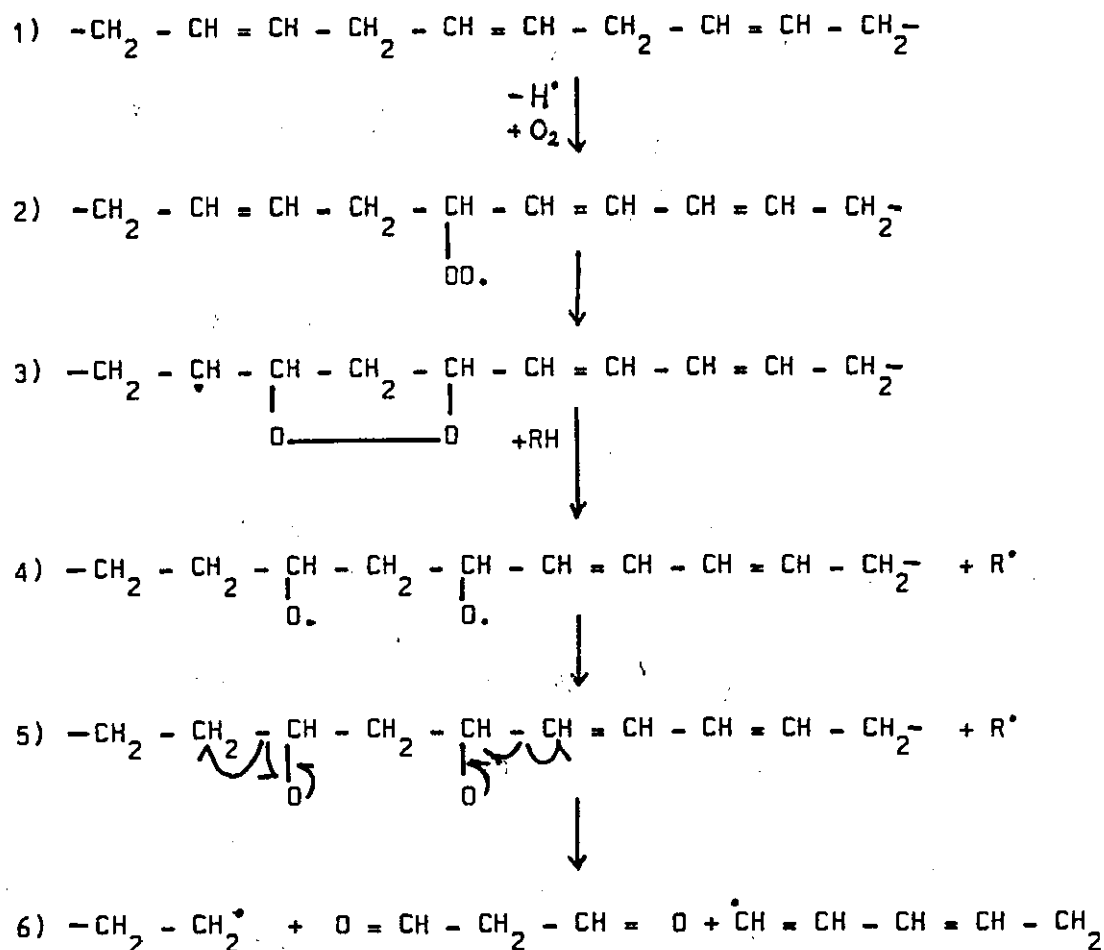
Abstraction of hydrogen atom is possible from C11 and C14; the resonance hybrids which can result are as follows:



Also here isomeric hydroperoxides can be formed by abstracting of H by the radicals. A scheme of their formation and breakdown is shown on Fig. 7.

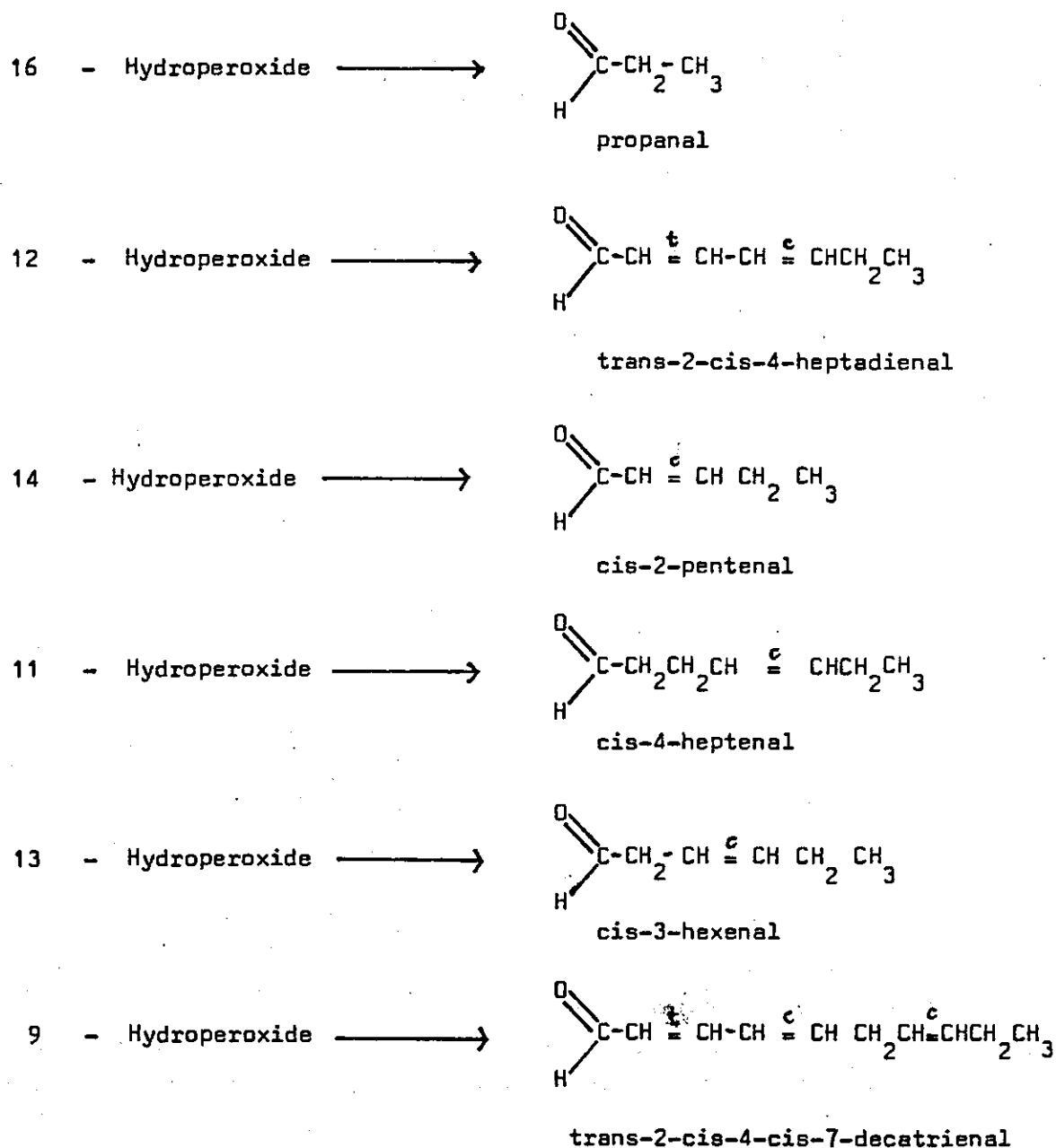
D) Formation of malonaldehyde

A by-product of oxidation of polyenoic acids (with three or more methylene interrupted double bonds) is malonaldehyde (propan-1, 3-dial). A proposed mechanism of its formation is given below.



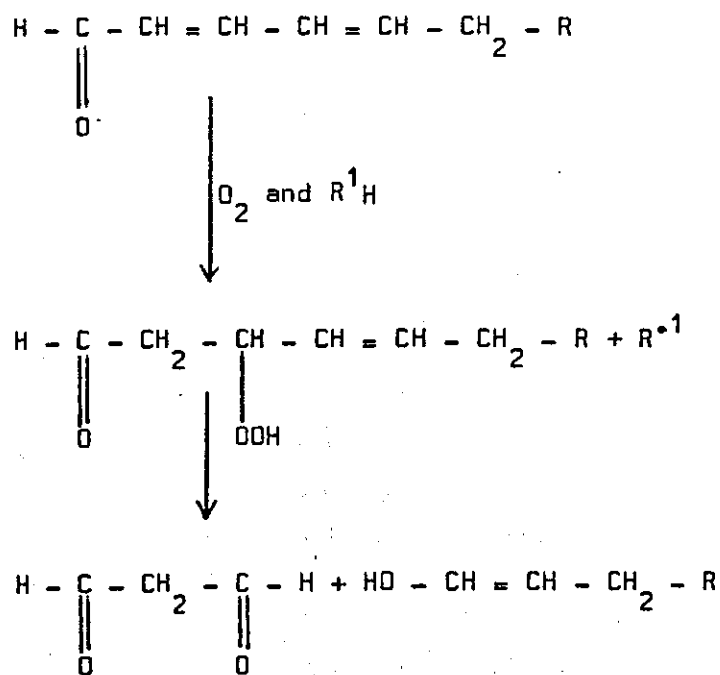
malonaldehyde

Fig. 7: Some products of autoxidation of linolenates



The intermediate product of lipid oxidation, the hydroperoxides, and the by-product of linolenate hydroperoxide breakdown, malonaldehyde can be determined and related to the development of rancidity in methods known as the peroxide value test and the thiobarbituric test respectively (see 2.3.1.2).

Also malonaldehyde measured in oxidizing systems containing linoleate may be derived from further oxidation of 2-enals or 2,4-dienals.



malonaldehyde

2.3.1.2 Methods of measuring rancidity development in salted-dried fish

Measuring the state of rancidity of a lipid-containing food is complicated by the fact that the products of this reaction are unstable and capable of entering into secondary reactions or are volatile (see Section 2.3.1.1). Consequently methods of measurement when made are difficult to relate to sensory flavour scores, with levels being unique to particular foodstuffs.

Presently the analytical techniques used to assay the course of autoxidation in unsaturated lipid and foods containing them include:

a) Peroxide value b) TBA Value c) total non-volatile carbonyls d) total volatile carbonyls, e) gas chromatography method and f) spectroscopic methods.

a) Peroxide value

Peroxides are the main initial products of autoxidation, as discussed above, and are estimated from their ability to liberate iodine from potassium iodide in acetic acid/chloroform mixture. The Peroxide Value of the fat or lipid extract, being a measure of its content of reactive oxygen is given in terms of millimoles of peroxide or milli-equivalents of oxygen per kg of fat. Other, mainly iodometric methods, recommended by the American Society of Oil Chemists are the dichlorophenol/indophenol method, ferric thiocyanate and the stannous chloride method but these are less widely used (Lea, 1962). Peroxide Values have been found by several workers to be useful at the start of the lipid decomposition (Dugan, 1976; Woolfe, 1975). Lea (1962) found it useful in assessing soybean oils but useless for grading herring

meal.

b) The Thiobarbituric Acid (TBA) test, is based on the reaction between products of unsaturated fatty acid (principally linolenic acid) oxidation (e.g. malonaldehyde) and thiobarbituric acid to give a characteristic pink to red colour which absorbs at a wavelength of 530 nm. Several modifications have been made to the original method, and the modification used most frequently involves steam distillation of the food product or lipid (Tarladgis *et al.*, 1960). In experiments to evaluate the method, standard alkanals, alkenals and 2,4-alkadienals were reacted with thiobarbituric acid. It was found that all the aldehydes form a yellow pigment absorbing at 450 nm while only 2,4-dienals, and to a lesser degree the alkenals, produce a red pigment which absorbs at 530 nm. Therefore a measure of both absorption maxima is desirable for the measurement of total aldehydic products of unsaturated lipid autoxidation (Sonntag, 1982). This has however not been the practice because at dilutions normally used in the test, absorptions at 450 nm are small.

The TBA method has found use as a measure of rancidity development in fish samples, but, like Peroxide Values, correlating TBA values with sensory scores is indirect because malonaldehyde does not contribute appreciably to "off-flavours" (Lea, 1962).

c) The estimation of total non-volatile carbonyls, is normally carried out by the colorimetric 2,4-dinitrophenylhydrazine procedure of Henick or the benzidine method of Holm (Lea, 1962). These tests measure the non-volatile non-odorous high molecular weight carbonyls and hence are not directly related to sensory

odour scores. Their importance is recognised however, from the fact that these carbonyls are the precursors of the volatile "off-flavour" compounds. Results for this method do not correlate well with sensory scores.

d) Estimation of total volatile carbonyls. Problems arise in this method because the mono- and dicarbonyls are extremely sensitive to further oxidation and may even re-arrange during the operations used to isolate, concentrate or derivatize them. However, Lea (1962) developed a method involving vacuum distillation which is said to obtain quantitative amounts of propanol. Results for this method too do not correlate well with sensory scores.

e) Gas chromatography, has been employed in detecting volatile carbonyls such as cis-4-heptenal which is an important off-odour compound in cold storage rancidity development in lean fish. The value of this method is limited by the need to identify the main off-odour compounds before gas chromatographic analysis can take place.

f) Spectroscopic method. A spectral method for evaluating the oxidative stability of fats and oils towards autoxidation, has been described by Takasago et al. (1979). The method involves recording the infra-red spectra of a given oil or fat sample and determining the ratio of a varying and a constant absorbance (α).

The varying absorption bands were found to be at 3450 cm^{-1} and 3030 cm^{-1} , which corresponded to the absorption band of -OH stretching frequencies from the formation of hydroperoxides and the absorption band of $\text{CH} = \text{CH}$ bonds respectively, while the constant absorption bands were at 2850 and 1740 cm^{-1} which corresponded to $-\text{CH}_2$ and carbonyl bands respectively.

The ratio (α) is therefore given, for example, by

$$\frac{A_{3450}}{A_{1740}} \text{ and } \frac{A_{1740}}{A_{3030}} \quad \text{where } A \text{ is the peak height.}$$

Other less important methods of rancidity assessment include the refractive indices measurements, UV absorptions to detect conjugated systems, due to isomerism, and other photometric tests (Tsoukalas and Grosch, 1977).

2.3.1.3 Factors affecting the rate of rancidity development in salted-dried fish

An understanding of rancidity development in foods came from reaction kinetic studies in model systems using pure lipids and/or analogues of these compounds (Labuza, 1971). However, results thus obtained are difficult to extend to actual foods because of their complexity and because of the effects of processing and storage conditions. Many factors can affect rancidity development in foods, e.g. the presence of photo-sensitizers like myoglobins and haemoglobins, transition metals, enzymes, the solvent phases present, heat, light, oxygen access and effects of micro-organisms.

In food systems activation energies calculated for C - H bond cleavage and oxygen addition to allylic R - H bond are 4-5 kcal/mole each and 6-14 kcal/mole for both reactions. These figures are appreciably lower than was observed for these reactions in pure systems (see Section 2.3.1.1). In fish a reduction in bond cleavage energies can be envisaged as due to the sensitization of oxygen to the more reactive singlet state by myoglobins and haemoglobin in the presence of ultra-violet light. Ultra-violet light

deactivators, e.g. carbon black are known but are not suitable for food use (Labuza, 1971).

Transition metals, e.g. copper, iron, nickel, cobalt and manganese have been implicated in encouraging the development of rancidity in foods. Most foods naturally contain 1 ppb-500 ppm of these elements and even in carefully purified lipids 0.2 ppm of copper and traces of cobalt and iron have been found (Ingold, 1962).

Rates of chemical reactions are increased by increasing the temperature and lipid oxidation is no exception. High temperatures will facilitate the initiation reactions and also the breakdown of the hydroperoxides (see 2.3.1.1). Chahine and DeMan (1971) have shown that high temperatures give increased rate of oxidation of illuminated corn oil. Their results also show that low temperatures do not necessarily stop the reaction even at -20°C , as metal activators and chromophoric impurities are usually present and can act as pro-oxidants. Salted-dried fish in tropical countries are often stored at temperatures of 30°C or more.

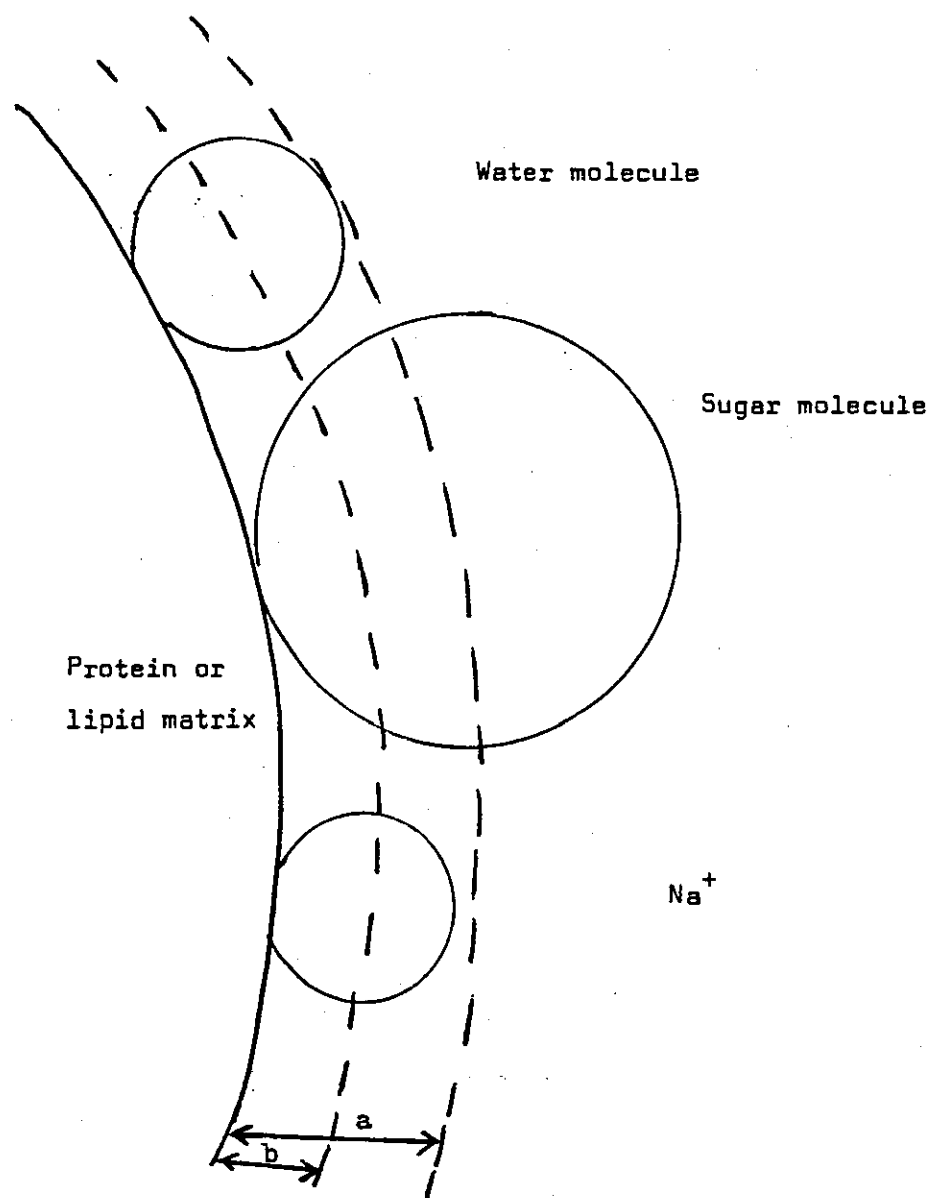
Studies reveal that if the reaction medium is made more polar the catalytic effect of the metal ion is reduced. This has been attributed to the polar solvent solvating the catalyst and thereby altering its redox potential and consequently reducing its affinity for molecular oxygen. Blanchard (1960) showed that metal salts do not catalyze methyl linoleate oxidation in polar solvents like ethanol, ethyl acetate and ethyl caprylate but are very active in non-polar solvents like benzene. The rates in the non-polar solvents can be decreased by adding nitrobenzene, a fairly polar solvent, in molar concentration proportional to that which could be present in the co-ordination shell of the catalyst (Labuza, 1971). This has direct implications in the stability of dehydrated food like salted-dried fish and fish meal, in which at low water activity values greatly increased rates of lipid oxidation occur (Fig. 2, Section 2.1.1).

The water has been thought of as preventing the metal - hydroperoxide complex from forming and also could precipitate the metallic ions as insoluble hydroxides. It has also been suggested (Karel and Young, 1981) that water interferes with normal bimolecular hydroperoxide breakdown by hydrogen bonding with amphi-polar hydroperoxides on the lipid water interface. Therefore, water in dry food systems can act as an inhibitor to lipid oxidation, but in some intermediate moisture foods, increased above the monomolecular layer, acts as a solubilizer and mobilizer for catalyst and substrates thereby increasing catalytic effectiveness (Duckworth, 1981). However, in a system containing chelating agents or antioxidants, solubilization by high water content facilitates the sequestering of metals and therefore causes a lowering of the rate of oxidation (Karel and Young, 1981). Another hypothesis holds that high moisture can cause the swelling of macromolecules, like proteins, to expose more catalytic sites for attachment of the activators and so increase the oxidation rate. On the other hand, it could dilute the catalysts and in effect reduce oxidation rate.

Addition of salt to fish during the curing process will influence the effect of water activity on the rate of oxidative rancidity development. Few studies, however, have been carried out on lipid oxidation in the presence of high salt concentrations and experimental results are conflicting with general conclusions difficult to reach. Banks (1937) reported that salt has no direct effect on lipid oxidation in absence of other food components. But Chang and Watts (1950) found that lard oxidation was accelerated by salt in a simple system of lard and salt. They noted that this effect depended on the amount of moisture present in the system. In contrast an inhibitory effect on the oxidation of an emulsion of methyl linoleate and linoleic acid by sodium chloride has been reported (Mabrouk and Dugan, 1960) and was found to be independent

of pH. As the inhibition increased with concentration of sodium chloride, it was thought that this effect resulted from decreased solubility of oxygen, thus making oxygen availability the limiting factor. Earlier Hills and Conochie (1946) had proposed that sodium chloride catalyzed lipid oxidation occurred from an interaction of the hydroperoxide with chloride ions to form chlorine which can further catalyze the reaction. On the contrary however, Castel *et al.* (1965) have suggested that the active agent is the sodium ion rather than whole salt or the chloride ion, in this mode of oxidation.

Recent experiments with a casein-sodium chloride-water model system conducted by Gal but reported by Duckworth (1981), have revealed that sodium chloride may be present in four different forms - crystalline salt, amorphous salt, salt bound to proteins and dissolved salt - depending on the amount of solvent water and sodium chloride in the system. The presence of solvent water was correlated with a specific water activity, i.e. above this value solvent water existed but below it only non-solvent water was present. Earlier it had been shown that the amount of non-solvent water in a given system varied depending on the solute. This phenomena was considered to be due to steric exclusions. Lately it has been proposed that solution of a particular solute depends on the radii of the ions and the thickness of the film of the surface water and water filled capillaries (see Fig. 8). If the ionic radius of the solute is larger than the water film thickness then the water is non-solvent for the solute. Conversely, if the water film thickness is larger than the ionic radius of the solute, only then is it solvent water. This explains why the water film thickness needed to give solvent water for $\text{Na}^+ \text{Cl}^-$ is less than that for the larger



a - water solvent for Na⁺ but not sugar
b - no water (water not solvent for Na⁺)

Fig. 8: Showing solvent and non-solvent water for Na⁺ and sugar in space around a protein or lipid surface.

Source: Adapted from Duckworth (1981).

sugar molecule. Solvent water for sodium chloride has been determined at a water activity less than 0.3 and this can be related to the water content of any particular food material (Duckworth, 1981). In this theory could lie the explanation of the different observed effects of sodium chloride in presence of other substances and its effect on lipid oxidation, protein denaturation and food spoilage and/or preservation.

The question of salt purity has, however, been raised as traces of transition metals present in the salt could initiate lipid oxidation (see Section 2.3.1.1). This could help to explain some of the conflicting results discussed above.

The species, season, sex cycle, maturity and nutritional status of the fish can influence the course of rancidity development in the preserved fish. It has been generally known that marine fish are more susceptible to rancidity than freshwater fish as the former contains on average larger amount of unsaturated C_{20} and C_{22} fatty acids. However, increase in the percentage content of unsaturated fatty acids especially within a species does not necessarily mean increased susceptibility due to concurrent presence of antioxidants like vitamin E. The differences in the distribution of lipids in the fish also affects oxidative characteristics. Belly and tail muscles contain higher proportions of lipids and are usually more prone to oxidation.

2.3.2 Non-enzymic browning in salted-dried fish

Browning of salted-dried fish has been widely reported in Canada, Iceland and recently in S.E. Asia (Hanson and Esser, 1981). This is considered undesirable by consumers and contributes to losses. Many theories have been postulated to explain the phenomena. For example, Icelandic workers have reported that salt lacking calcium and magnesium was the cause of browning. But in experiments by Shewan (1955), the incidence of browning was not decreased by the addition of calcium and magnesium salts to 99% pure NaCl. Icelandic workers suggested that browning was caused by halophilic bacteria requiring calcium for growth. However, sterilisation of both salt and fish neither prevented nor initiated browning and it was also found that organic matter, living or dead, in the curing salt did not influence the development or the intensity of browning.

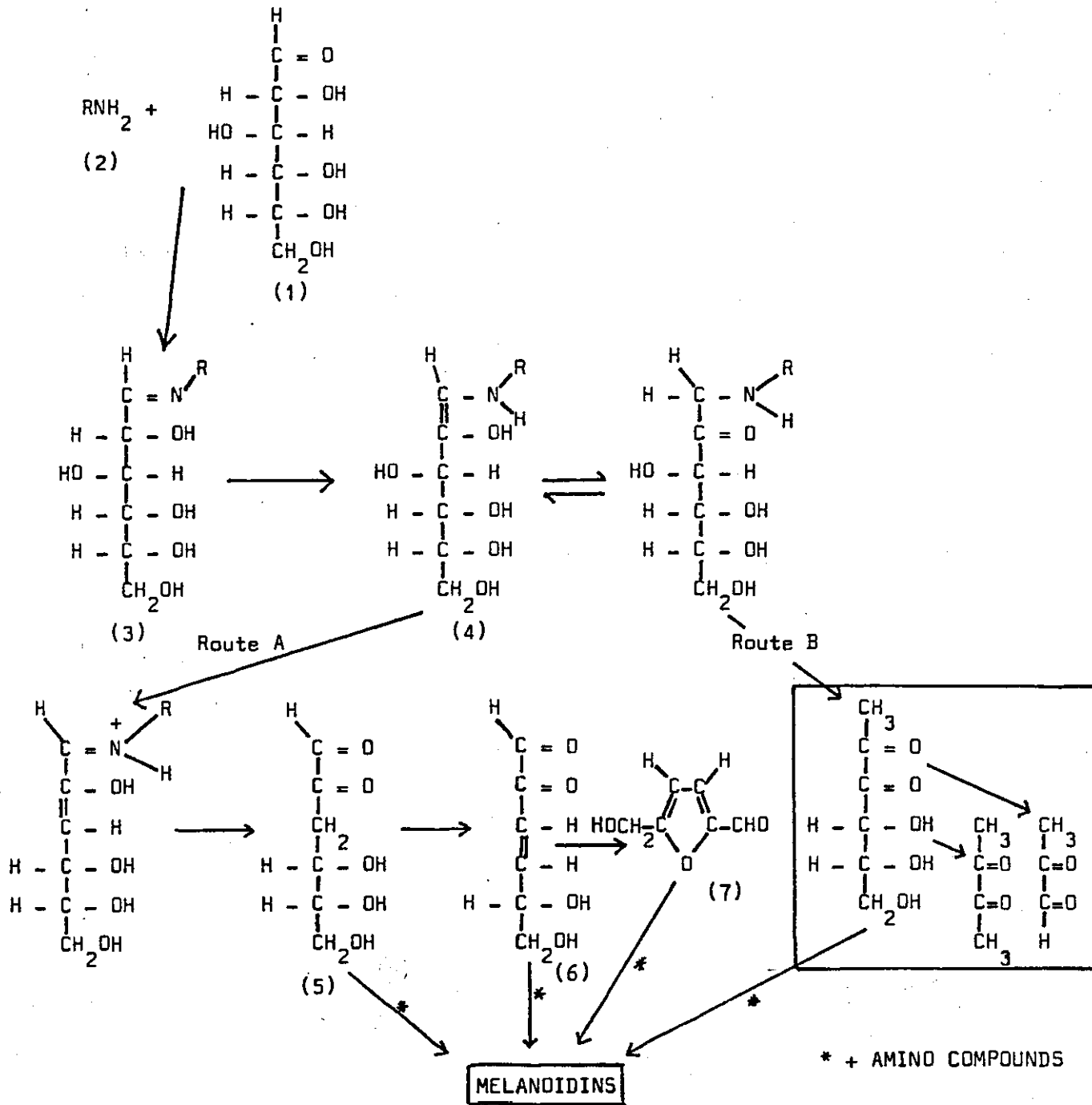
Canadian scientists have reported that salt containing 50 ppm of iron was capable of causing discolouration after 3 months storage. But in Shewan's (1955) experiments ferric and ferrous salts added to fish at levels up to 100 ppm failed to produce the browning reported except after a very lengthy period of storage and at high dosage. Salt in common use in the trade normally contain far less than this amount of iron.

Attention was then turned to reports that susceptibility to browning depended on the area where the fish was caught. Arctic cod was found to brown more quickly and to a greater degree than that from the North Sea. The Arctic cod was found to contain about twice the amount of trimethylamine oxide (TMAO) as the same species from the North Sea and this could relate to browning. Shewan's experiments showed that stale fish, which would have lower TMAO levels due to bacterial decomposition developed browning more slowly than very fresh fish over the same period of time. However, very stale fish containing virtually no TMAO showed browning, although only slowly.

The situation was even more complicated by Shewan's observation that not all curing salt caused browning and that some very pure salts and solar salts did not cause browning at all.

As a result of several experiments involving the use of a more copper sensitive detection method, the dithiocarbamate micro method, and the addition of low levels of copper salts to curing salt, Shewan concluded that copper caused browning. Icelandic scientists also reached the same conclusions. Both groups of workers found that levels of copper present related directly to the amount of browning. The actual mechanism of copper involvement was not elucidated but it was suggested that it could be a Maillard type reaction catalysed by copper but many other factors are now thought to be important in explaining browning development. These factors include temperature, water activity and lipid oxidation products. Most of the studies have however been carried out in model systems (Eichner, 1981; Labuza and Saltmarch, 1981). Only a few studies have been carried out in fish systems (Martinez and Labuza, 1968) and in other related systems (El-Zeany, 1975). There is, however, a general agreement that lipid oxidation products, ketones, aldehydes and other carbonyls (see Section 2.3.1.1), and reducing sugars⁽¹⁾ (free ribose, from breakdown of nucleotopo-myosin nucleic acids is most important in fish) (Tarr, 1954) react with the amino groups of proteins on amino acids⁽²⁾ to form products (see Fig. 9) i.e. a N-substituted glycosylamine.⁽³⁾ This aldosyl derivative undergoes a rapid Amadori rearrangement to ketose amino acids (1,2-enol form)⁽⁴⁾ which can react with one more aldose molecule to give a diketose-amino acid. 3-Deoxyhexosulose⁽⁵⁾ can be released from the diketose-amino acid and another monoketose amino acid which is capable of reacting with another sugar moiety form. Water can be eliminated from the 3-deoxyhexosulose to form unsaturated compounds, reductones⁽⁶⁾ which can be converted into the dehydro reductones. Under acid

Fig. 9: Scheme of some chemical reactions involved in non-enzymic browning (Maillard browning) in foods



Route A is the major route

Route B probably less than 5% of total sugar decomposition

Low pH favours route (A), higher pH favours route (B)

Refer to text for further details

conditions 3-deoxyhexosulose undergoes a ring closure to hydroxymethyl furfural (7). These intermediates (5, 6, 7) can react with amino groups quickly and undergo extensive condensations to form browning pigments of high molecular weight. These are coloured insoluble polymers called melanoidins. This is reported too as Maillard browning.

Carbonyl group blocking reagents (hydroxylamine hydrochloride, phenylhydrazine or sodium metabisulphite (El-Zeany, 1975) are known to retard Maillard browning by making the carbonyl groups less available for reaction with amines. Temperature has also been found to control the rate of development of browning and samples stored in an oxygen free environment produced less colour than those with oxygen at the same temperature (Labuza, 1981). The lack of oxygen could effectively reduce the rate of lipid oxidation and hence the availability of carbonyls for the Maillard reaction (see Fig. 4).

Browning was found to be influenced by the water activity of the system. Martinez and Labuza (1968) found low pigment extraction at low a_w (0.2-0.3) and higher yields at higher a_w (0.75-0.8). The reverse was true of lipid oxidation and it was argued that the water helped to disperse the reacting species more in the medium leading to a more intense reaction.

Thus far there has not been an explanation in the literature as to what role salt (sodium chloride) and copper play in this situation. An observation has, however, been made from kinetic studies that the activation energy of browning was 21 kcal/mole. This would suggest that lipid oxidation is not occurring (see Section 2.3.1.1) and the increased browning observed must therefore be due

to some other degradation reaction involving oxygen and moisture (Labuza, 1981) and possibly sodium chloride and copper where these are present.

There is always the attendant problem of relating the conditions and causative agents of changes in food quality (food deterioration) without considering all degradation reactions which can occur. That is why model systems on analogous materials are difficult to relate to real situations.

2.4 AIMS OF PRESENT WORK

In view of the concern now shown to reduce losses in cured fish due to rancidity development and brown discolourations it is the object of this project:-

- A: To analyse several salt samples from South East Asia and the U.K. to establish accurately the levels of copper, iron, calcium and magnesium present.
- B: To establish whether salt (NaCl) is a pro-oxidant or merely exhibits pro-oxidant activity through its ability to alter the water activity of the fish or because of impurities present in the salt.
- C: To assess the effect of water activity on rancidity development (as measured by PV and TBA methods) and colour development.
- E: To suggest means by which rancidity development in salted-dried fish can be reduced.

3. EXPERIMENTAL

3.1 MATERIALS

Mackerel

Minced flesh of the Atlantic mackerel Scomber Scombrus was used in all the experiments.

Aristar sodium chloride

The ultra pure aristar sodium chloride supplied by BDH Chemicals Ltd., was used as the control with minimum sodium chloride content of 99.9%. Maximum metal content was as follows:-

Copper	-	0.01 ppm
Cobalt	-	0.01 ppm
Iron	-	0.05 ppm
Calcium	-	0.10 ppm
Magnesium	-	0.05 ppm

Analar sodium chloride

Analar sodium chloride also supplied by BDH Chemicals Ltd., was used. Minimum sodium chloride was 99.9% and maximum limits of impurities were as follows:-

Insoluble matter	-	0.003% (30.00 ppm)
Ferrocyanide	-	0.0001% (1.00 ppm)
Arsenic	-	0.0004% (0.4 ppm)
Barium	-	0.001% (10.00 ppm)
Calcium	-	0.002% (20.00 ppm)
Copper	-	0.0002% (2.00 ppm)
Iron	-	0.0003% (3.00 ppm)
Lead	-	0.0002% (2.00 ppm)
Magnesium	-	0.002% (20.00 ppm)
Potassium	-	0.01% (100.00 ppm)

Analar copper chloride

Reagent grade copper chloride supplied by BDH Chemicals Ltd., was used to introduce copper metal to the mince. Minimum content of copper chloride was 98.9%.

Other impurities (maximum levels) were as follows:-

Nickel	-	0.01% (100.00 ppm)
Iron	-	0.01% (100.00 ppm)
Arsenic	-	0.0002% (2.00 ppm)
Barium	-	0.005% (50.00 ppm)
Absorbed oxygen	-	0.01% (100.00 ppm)

South-East Asia salt samples

Samples of salt (sodium chloride) were collected from fish curers in Thailand, Burma, Malaysia, Indonesia and the Philippines. They were kept in airtight polypropylene tubes with cling tapes (Hanson and Esser, 1982).

U.K. salt samples

Salt samples were collected from two fish curers in Grimsby, namely Ernest Cox (Grimsby) Ltd. (Fish Merchants, Curers) and W.M. Kelly Ltd. (Fish Curers). The salts were brands of Imperial Chemical Industries (ICI) PLC. They were treated as above.

3.2 METHODS AND EQUIPMENT

3.2.1 Preparation of mackerel mince

Iced fresh fish was bought from the Grimsby dock approximately 20 hours after catch. The fish was filleted and minced in a mortar with pestle. Homogenisation was performed with Ystral D-7801 homogeniser with polished stainless steel heads, to avoid as much as possible introduction of extraneous metal to the mince.

The mackerel mince was obtained and prepared in this way for every experimental run.

Additives were added on a dry weight basis to mince after freeze-drying except for EDTA which was added before freeze drying.

3.2.2 Freeze drying

1.2 kg portions (approx.) of minced mackerel was spread in two 30 cm x 45 cm polished stainless steel trays of depth 1.5 cm (approx.) and frozen in a blast freeze at -30°C for 12 hours. The trays were then transferred to the pilot and laboratory freeze drier mini-fast model 1700 and dried for 24 hours under a current of nitrogen to a final temperature of 25°C and water activity of about 0.12.

3.2.3 Equilibration of mince to required water activity

The freeze-dried mince was broken up and ground in a mortar with pestle. After the required treatment had been applied (e.g. addition of copper chloride or sodium chloride) 10 g portions (approx.) were placed in water activity dishes, plastic dishes - diameter 2.5 cm and depth 1 cm (approx.). These were then placed on a wire gauze held in a rim greased glass desiccator containing a saturated solution of salt. The desiccator was evacuated to about 10 mm Hg and placed in an oven at 30°C until equilibrated over 24 hours (see Table 7) before opening to air to commence the storage study.

Table 7: Constant humidity solutions

Saturated salt solutions	Water activity at $30^{\circ}\text{C} \pm 0.01$	Wt. of salt to saturate 100 g water at 30°C
Li Cl	0.11	84.50
Mg Cl ₂	0.32	55.00
Mg (NO ₃) ₂	0.52	-
NaCl	0.75	36.30
BaCl ₂	0.84	38.2

Source: Novasina water activity meter, operation manual

Hodgman et al. (1961).

3.3 ANALYTICAL PROCEDURES

3.3.1 Water activity (a_w)

The equilibrium relative humidities, or water activities were measured on the Novasina water activity meter Model EEJA-3. Readings were taken at 30°C after 15 mins equilibration time, when a constant value was reached.

The equipment was calibrated with salt standards provided according to the instructions in the Novasina water activity operation manual.

3.3.2 Moisture

The oven method was used on all samples (Pearson, 1981).

For moisture in salt, about 1 g salt (S_g) was ground and placed in a steel dish with glass rod and accurately weighed (W_g). This was dried in an air oven to constant weight at $105 \pm 1^\circ\text{C}$ for at least 24 hours. Samples were then cooled in a desiccator for 10 mins and reweighed (w). The percentage moisture was determined in the relationship:

$$\frac{W - w}{S} \times 100\%$$

For fish samples about 0.5 g mince was added to the moisture dish containing about 20 g sand and weighed, to this about 5 cm³ of ethanol was added and stirred with the glass rod. The dish was then placed over a water bath at 80°C and the ethanol allowed to evaporate (~ 20 mins). The sample was then oven dried as above and the percentage moisture determined in the same way.

3.3.3 Total lipid

The total lipid content of fish mince was determined by a modified method of Bligh and Dyer (1959). The sample (about 10 g wet mince or 1 g dried mince) was accurately weighed into a 250 cm³ volumetric flask. Water was added to the sample (10 cm³ if wet, 15 cm³ if dry) and stirred. Then 20 cm³ of chloroform (containing 0.01% BHT) and 40 cm³ of methanol were added and the mixture was homogenised for 2 minutes whilst being cooled in ice.

A further 20 cm³ of the chloroform was added to the flask and further homogenised for half a minute. 20 cm³ of water was then added and further homogenised for half a minute.

The homogenate was transferred to a 100 cm³ glass centrifuge tube, capped and balanced with another before centrifuging at 2000 rpm for 20 minutes.

The aqueous top layer was removed by suction and the chloroform layer transferred to a quick fit test tube to known weight and evaporated off using a rotary evaporator with warming in a water bath at 50°C. The lipid was then dried to constant weight using a rotary oil pump (minimum time 15 mins).

3.3.4 Peroxide value

The peroxide value of the lipid extract was determined by a modified Lea method (Bligh and Dyer, 1959).

0.5-1 g (W) of the lipid was accurately weighed in a quick fit test tube. To this was added 20 cm³ of chloroform (containing 0.01% BHT) with shaking to dissolve the lipid. The mixture was then transferred to a 250 cm³ volumetric flask and 50 cm³ of acetic acid: chloroform (60:40) added while gently rotating the flask to facilitate mixing.

1 cm³ of saturated potassium iodide solution was added and mixed, and kept in the dark for 30 minutes. 100 cm³ distilled water was then added and the liberated iodine was titrated with 0.01 M sodium thiosulphate solution using 1% starch solution as indicator.

A blank run was carried out without the lipid.

The peroxide value (PV) was calculated as follows:-

$$PV = \frac{(S-B) \times 0.01 \times 1000}{W} \text{ meq O}_2 \text{ kg}^{-1} \text{ lipid}$$

where S = sample titration in cm³
 B = blank titration in cm³
 W = weight of lipid in g.

This experiment was always carried out under subdued lighting conditions.

3.3.5 Thiobarbituric acid value

This was performed using a modified method of Sinnhuber and Yu (Tarladgis et al., 1960).

The calibration curve

1,1,3,3-Tetraethoxy propane (TEP) and thiobarbituric acid reagent (TBA) were used as standards. The TEP solution was made by dissolving 0.3056 g of TEP in 1000 cm³ of water and then 20 cm³ of this was diluted to 1000 cm³ to give a concentration of 0.02778 μmoles/dm³.

The TBA reagent was made by dissolving 0.2883 g TBA in 100 cm³ 99% acetic acid and kept in a darkened bottle.

Standard solutions were then prepared as follows:-

5	cm ³	of distilled water (blank)	
4	" "	" "	and 1 cm ³ of TEP solution
3	" "	" "	2 " " " "
2	" "	" "	3 " " " "
1	" "	" "	4 " " " "
5	TEP solution		

These solutions contain the equivalent of 0, 0.4, 0.8, 1.2, 1.6 and 2.0 $\mu\text{g}/\text{cm}^3$ of malonaldehyde.

To each tube 5 cm³ of TBA solution was added and heated in a boiling water bath for 35 mins. The tubes were then cooled under the tap for 10 mins and the absorbance read at 532 nm using 1 cm³ cuvetts on a SP 825 Series 2 spectrophotometer.

The absorbance was then plotted against concentration of malonaldehyde ($\mu\text{g}/\text{cm}^3$).

Sample determination

Between 5 g and 10 g of fish mince was weighed into a 150 round bottom flask and blended with 50 cm³ of distilled water for 2 mins when a further 46.5 cm³ of water was added and again homogenised. To this 5 drops of an antioxidant mixture (0.3 g butylated hydroxy anisole (BHA) in 5.4 g of propylene glycol with 0.3 g of butylated hydroxy toluene (BHT) in 4 g of 40) and 1 cm³ of 0.2% ethylene diamine tetra acetic acid disodium salt solution (EDTA).

2.5 cm³ of 4 M hydrochloric acid was added to adjust the pH to 1.5.

The flask was then wrapped with glass wool and covered with aluminium foil before being placed on a heating mantle and connected for downward distillation under nitrogen.

The flask was heated (under a current of nitrogen) at a rate such that 50 cm³ of distillate can be collected over 20 ± 2.0 mins from the commencement of boiling.

To 5 cm³ of the distillate (or suitable dilutions of it) in a ground glass stoppered test tube, 5 cm³ of TBA reagent was added and treated as in the calibration curve.

Absorbances were measured on the SP 825 spectrophotometer and related to the concentration from the calibration curve.

The thiobarbituric acid value (TBA) was calculated from the following equation:

$$\text{TBA value (or number)} = \frac{500 \times T}{70} \text{ mg of MA kg}^{-1} \text{ of flesh}$$

where T is malonaldehyde (MA) concentration in µg/cm³ (obtained from the calibration curves) for a 10.0 g sample of the fish mince.

This equation assumes 70% recovery in the distillation step.

Corrections were made for moisture and salt content of the mince and the TBA value expressed in terms of moisture and salt free fish matter.

3.3.6 Colour

The colour of the mince was determined by recording the absorption of an acetic acid extract of the mince.

0.5-1.5 g of mince was added to 50 cm³ 100% glacial acetic acid in a 150 cm³ beaker, and stirred for 15 mins using a magnetic stirrer.

The extract was filtered with Whatman (G.P.) filter paper and the absorbance of the extract measured at 400 nm on the SP 825 Series 2 spectrophotometer.

The colour was expressed as absorbance per gram of dry matter per cm^3 of acetic acid ($\text{Abs g}^{-1} \text{cm}^{-3}$) i.e. corrections were made for moisture and salt content of the mince.

3.3.7 Chloride ion

a) Salt sample

20 cm^3 of a 1% solution of the sample was titrated with 0.05M silver nitrate using potassium dichromate as indicator. The concentration of sodium chloride calculated from the fact that 1.00 cm^3 of 0.1 M AgNO_3 is equivalent to 0.005845 g sodium chloride and expressed both as percentage wet weight and dry weight.

b) Salt content of fish mince

About 5 g of mince was ashed in a muffle furnace at 570°C for 12 hours. The residue was then weighed and washed with a small volume of distilled water into 250 cm^3 flask. This was filtered twice to obtain a clear filtrate which was made up to 100 cm^3 with distilled water. A 20 cm^3 aliquot of this filtrate was titrated with silver nitrate as above and the results were expressed on a percentage wet and dry weight basis.

3.3.8 Metal ions

Metal ion content of salt samples and fish mince were determined by atomic absorption spectroscopy (A.A.) using the SP 9 Pye Unicam A.A. with SP 9 computer and furnace programmer. All

adjustments made were according to the instructions in the operation manual of the equipment.

Iron, magnesium and calcium were determined with nitrous oxide/acetylene flame and the limits of detection were 0.06, 0.003 and 0.007 ppm respectively. Copper was analysed electrothermally with the carbon furnace and with the aid of the furnace programmer and the computer, the following parameters were adjusted for maximum sensitivity: integration time, precision number, drying temperature and time, ashing and atomisation temperature.

Calibration curve

Stock solutions were prepared for the metal analysis as follows:-

Calcium ($1000 \text{ mg litre}^{-1}$) - 2.7693 g of calcium chloride was dissolved in 100 cm^3 of deionized water and diluted to 1 litre in a volumetric flask with deionized water.

Magnesium ($1000 \text{ mg litre}^{-1}$) - 1.0000 g of magnesium metal was dissolved in 50 cm^3 of 5M hydrochloric acid and diluted to 1 litre in a volumetric flask with deionized water.

Iron ($1000 \text{ mg litre}^{-1}$) - 1.0000 g of iron wire (polished) was dissolved in 20 cm^3 of 5M hydrochloric acid and 5 cm^3 of nitric acid (S.G. 1.42) then diluted to 1 litre in a volumetric flask with deionized water.

Copper ($1000 \text{ mg litre}^{-1}$) - 1.0000 g of copper metal (polished) was dissolved in 50 cm^3 of 5 M nitric acid and diluted to 1 litre in a volumetric flask with deionized water.

These stock solutions were all stored in a polythene bottle with film sealed caps and used within 2 months of preparation.

Duplicates of these stock solutions were adjusted to contain 99.50% of sodium chloride and 0.5% of the metal ion to simulate the nature of the salt sample whose metal content was to be determined. Calibration curves were then drawn for the absorptions in presence and absence of salt. The straight line section of the graph, i.e. where no interference was observed, was chosen for interpreting the results by using a regression equation generated on a computer (see Figures 10, 11 and 12) except for magnesium where readings were taken directly from the graph (see Fig. 13).

3.4 EXPERIMENTAL PROCEDURES

3.4.1 Analysis of salt samples

For the S.E. Asia and U.K. samples a range of concentrations of 0.125 to 0.50% was used for metal ion analysis (see Section 3.3.8). Metals determined included, calcium, magnesium, iron and copper. The chloride ion determination was also carried out to assess the purity of the salt (see Section 3.3.7).

The percentage moisture was determined (see Section 3.3.2). A visual inspection was also carried out to detect organic matter.

3.4.2 Analysis of mackerel mince

About 10 g of mince mackerel was charred in a crucible and ashed in a laboratory furnace. To this 100 cm³ of distilled water was added and properly stirred. The mixture was then filtered twice with Whatman filter paper.

Then the filtrate was analysed for copper as in Section 3.3.8 and for chloride as in Section 3.3.7.

Fig. 10: Calibration curve for calcium determination

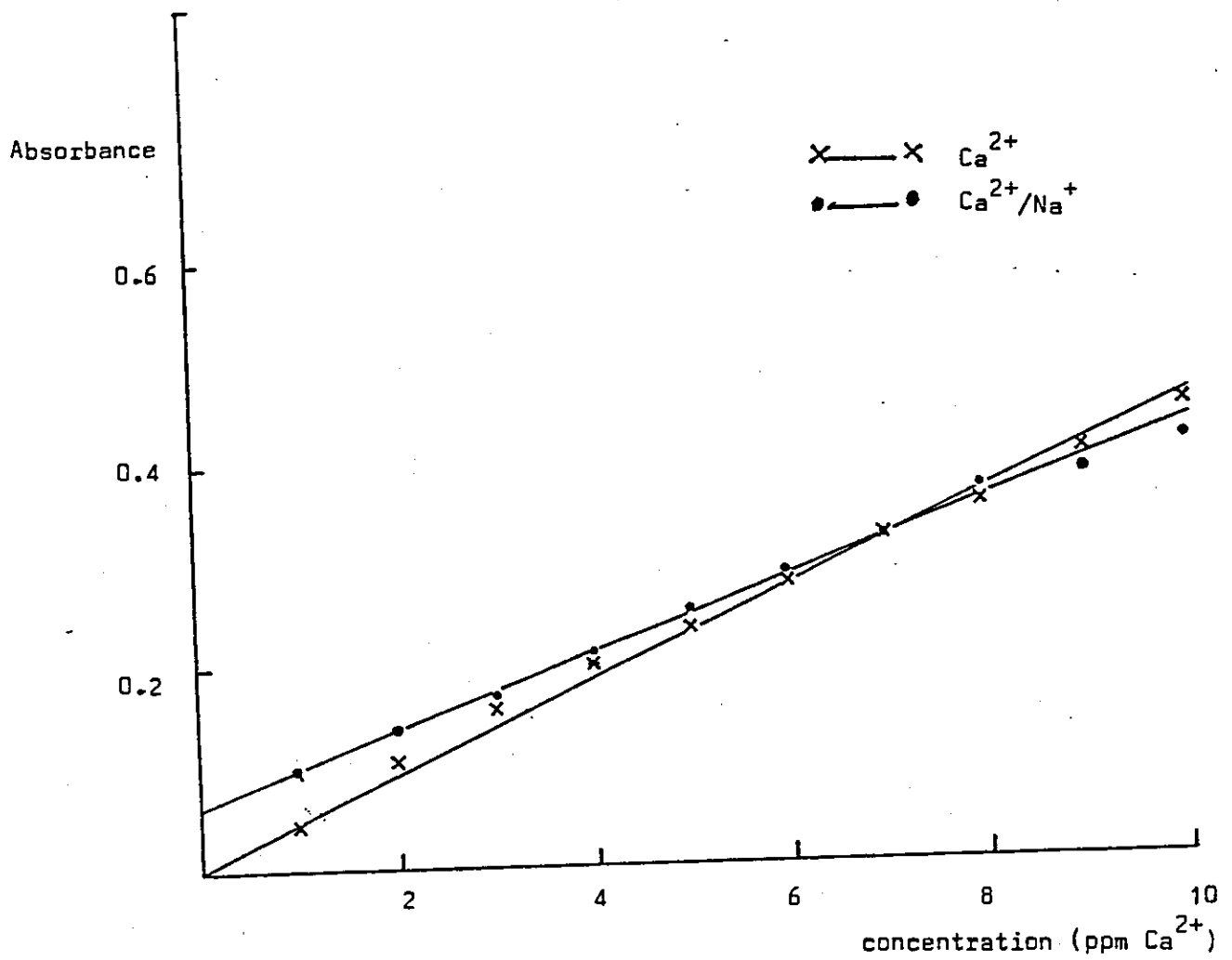


Fig. 11: Calibration curve for copper determination

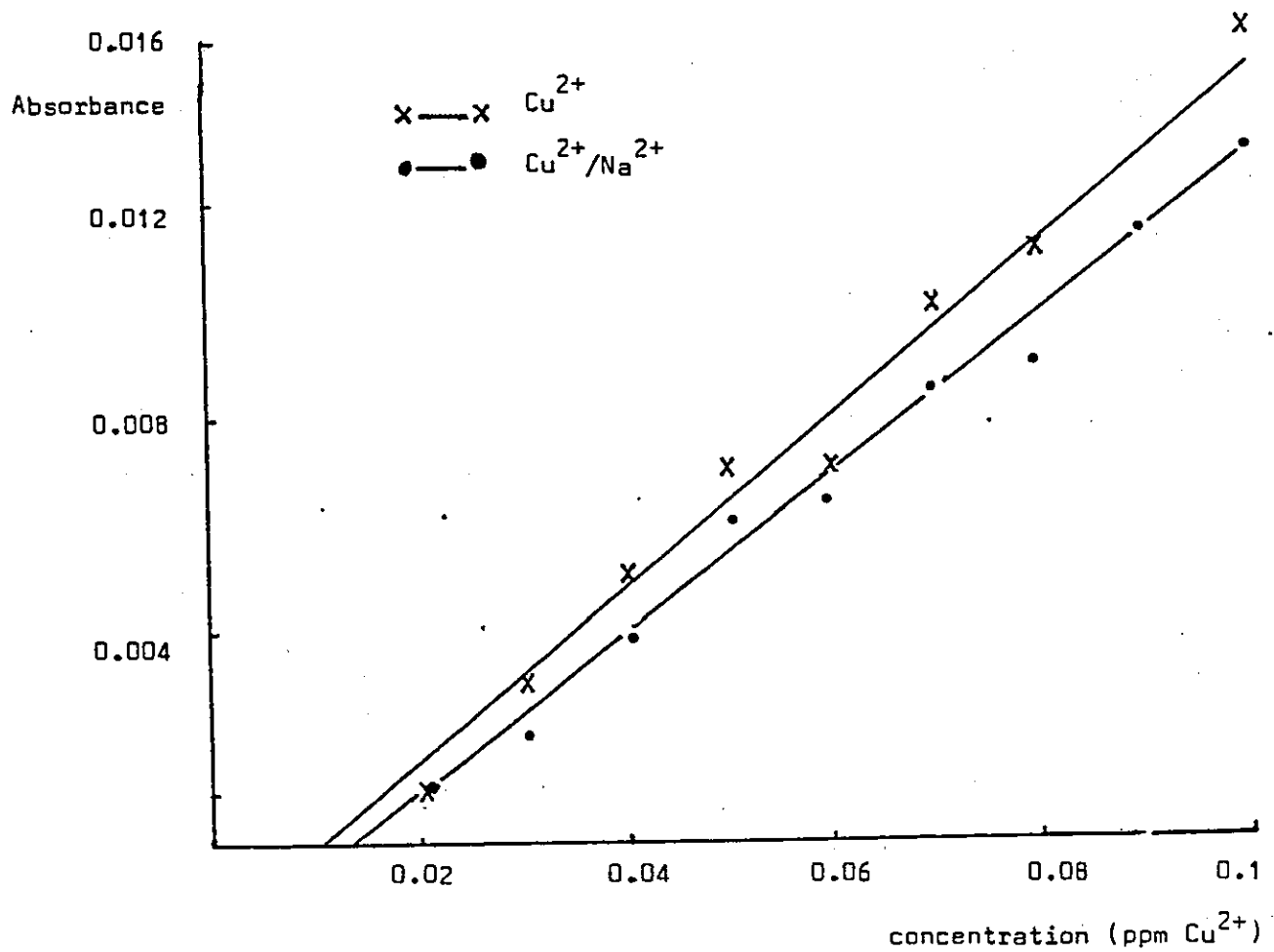


Fig. 12: Calibration curve for iron determination

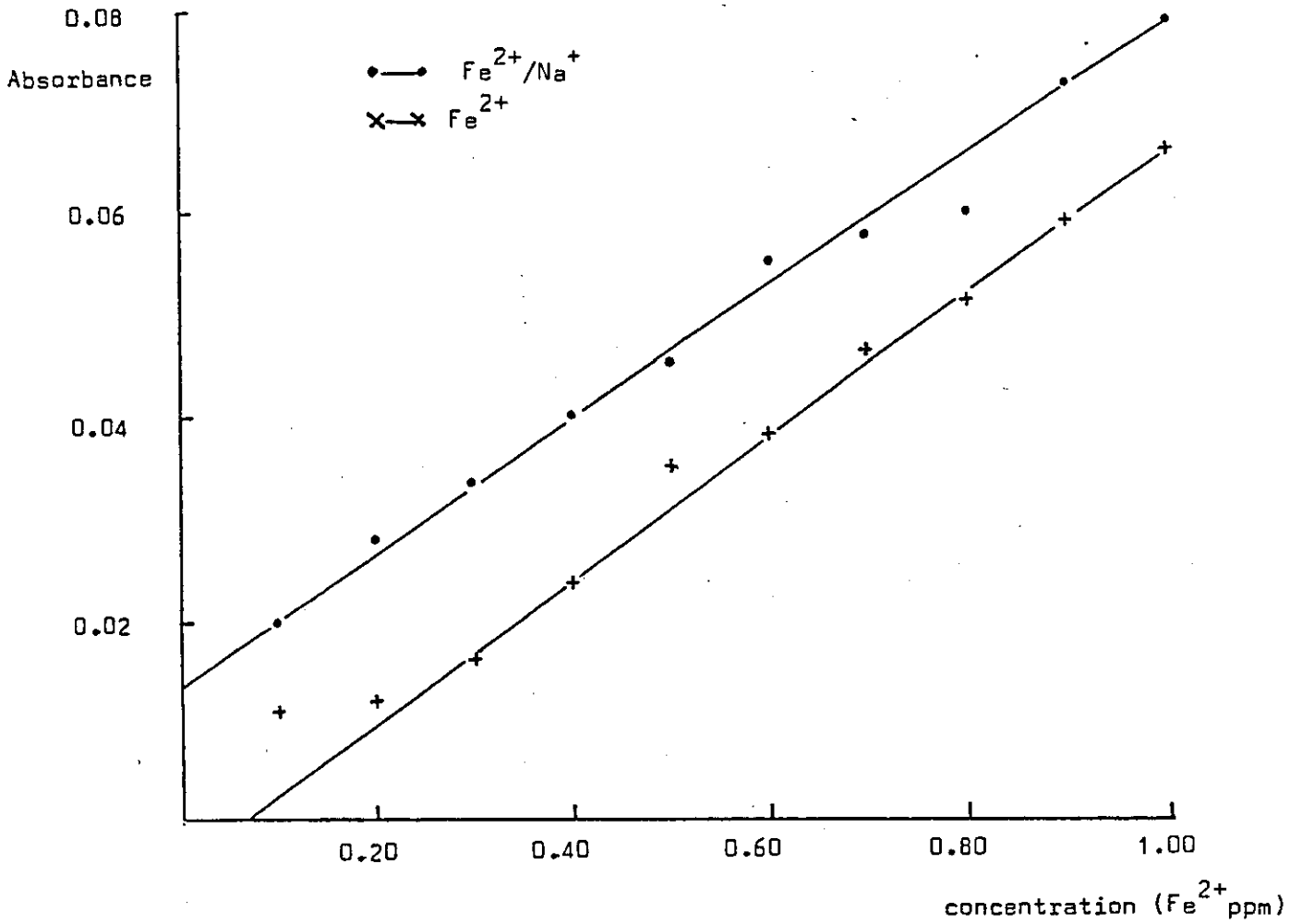
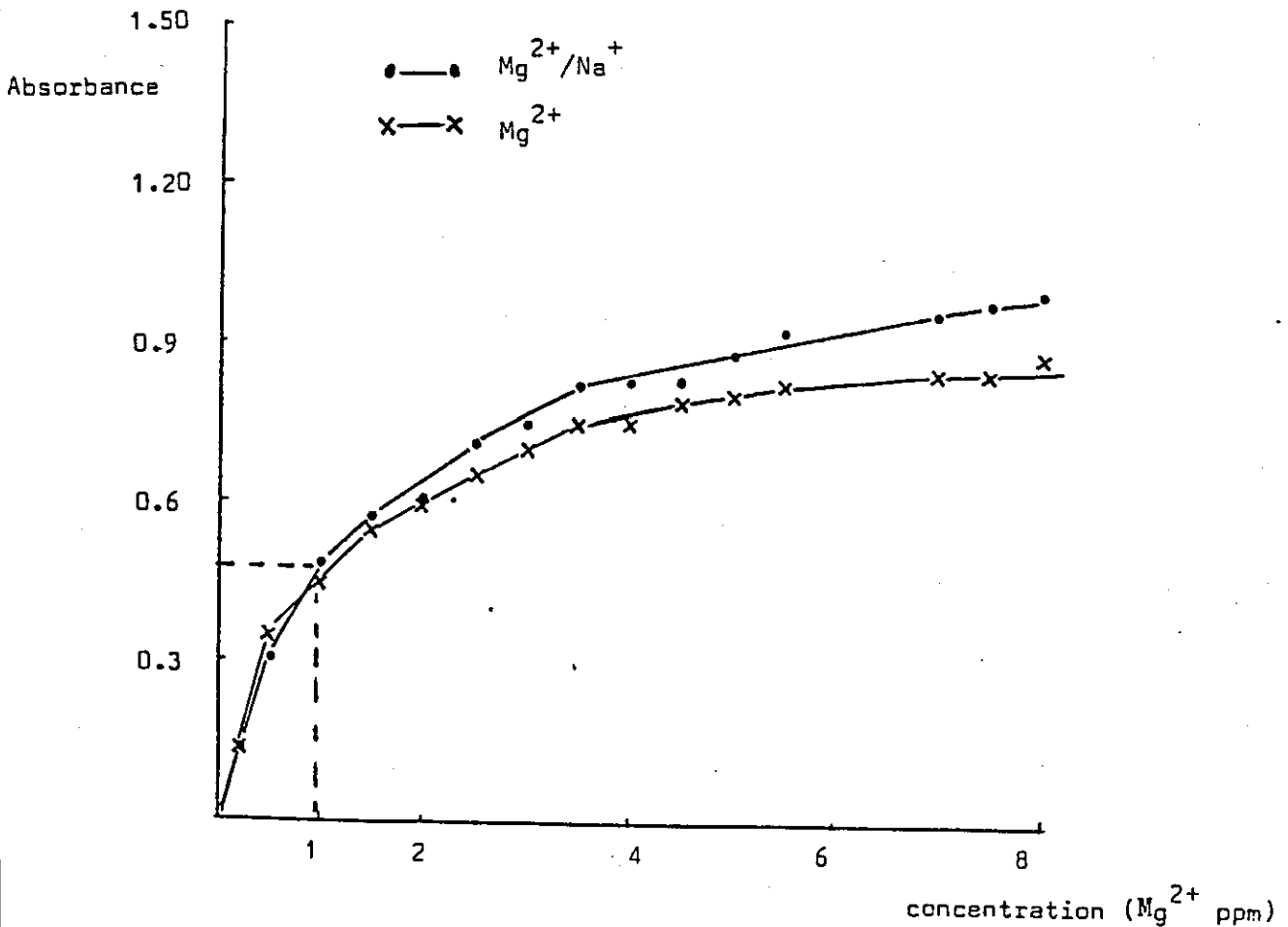


Fig. 13: Calibration curve for magnesium determination



3.4.3 Simulation of S. E. Asian fish salting and drying process

Mackerel mince was prepared as in 3.2.1 and 200 g portions of it were placed in 500 cm³ beakers and given the following treatment:-

- i) mince + 56.63 g Aristar sodium chloride
- ii) mince + 56.63 g Analar sodium chloride +
10 ppm Analar copper chloride
- iii) mince + 10 ppm Analar copper chloride
- iv) mince alone

The sodium chloride was added to saturate the 78% (approx.) moisture content of the mince (based on 36.3 g NaCl saturating 100 g H₂O at 30°C) (Hodgman *et al.*, (1961)).

Copper chloride was added as solution (0.964 cm³ of 0.5366 g/cm³ of CuCl₂.2H₂O) to adjust Cu²⁺ level to 10 ppm. Each beaker was then thoroughly stirred with a glass rod.

They were prepared in duplicate and placed in an air oven at 30°C. The percentage moisture content, peroxide value (PV), water activity (a_w) and colour were determined over 14 days.

3.4.4 Storage at constant water activity in the absence of high NaCl levels

Mince was prepared as in 3.2.1, freeze-dried as in 3.2.2, and then portioned into 3 sets and treated as follows:-

- i) mince + 10 ppm Aristar sodium chloride
- ii) mince + 10 ppm Analar copper chloride
- iii) mince alone.

Samples were thoroughly mixed and portioned in duplicate into water activity dishes and equilibrated as in 3.2.3 to attain water activities of 0.14, 0.32, 0.51 and 0.84 (± 0.01) respectively.

Solid copper chloride was added to avoid complications with equilibration.

PV and colour were determined at each of the four water activities and in addition TBA values were determined for $a_w = 0.14$ over a 12 day period.

3.4.5 Storage at constant water activity in the presence of low levels of copper

Mackerel mince was prepared as in 3.2.1 and freeze-dried as in 3.2.2. The mince was treated as follows:-

- i) mince + 0.2% EDTA (added before freeze drying)
- ii) mince + 0.5 ppm Analar copper chloride
- iii) mince + 1.5 ppm Analar copper chloride
- iv) mince + 3.0 ppm Analar copper chloride
- v) mince alone.

These samples were portioned in duplicate into water activity dishes and equilibrated as in 3.2.3 to water activity of 0.53 (± 0.01).

PV, colour, TBA and a_w were monitored for up to 12 days.

3.4.6 Storage at constant water activity in presence of high levels of NaCl

Mackerel mince was again prepared as in 3.2.1, freeze dried as in 3.2.2 and treated as shown below:-

- i) mince + 25% Aristar sodium chloride
- ii) mince + 25% South East Asia salt sample
- iii) mince + 25% Aristar sodium chloride + 0.5 ppm Cu
- iv) mince + 50% Aristar sodium chloride + 0.5 ppm Cu
- v) mince + 50% Aristar sodium chloride
- vi) mince + 50% South East Asia salt sample
- vii) mince alone.

NOTE: The additives were added on a dry weight basis
calculated for each water activity.

Mixing, portioning and equilibration were carried out as in 3.2.3 and all samples with 25% salt were equilibrated at a_w of 0.84. Those with 50% salt were equilibrated at a_w of 0.51, 0.32, 0.14 (± 0.01). The control was equilibrated at each of the four water activities.

PV, colour, T.B.A. and a_w were monitored for up to 14 days.

4.

RESULTS AND DISCUSSION

4.1 ANALYSIS OF SALT SAMPLES

The results of the analysis of the salt samples are given in Table 10.

Their moisture contents ranged from 0.2% to 12.8% with most of the S.E. Asian samples having higher values (mean = 4.73%) than the U.K. samples (mean = 0.65%).

The total chloride content, calculated as sodium chloride ranged from 99.4% (dry wt.) in the U.K. samples to 94.0% (dry wt.) in one of the S.E. Asian samples.

The Ca^{2+} and Mg^{2+} levels in the S.E. Asian samples was between 0.03-0.26% and 0.04-1.10% (dry wt.) respectively, whereas the U.K. samples had values of 0.001% for Ca^{2+} and 0.0001% and 0.0002% for Mg^{2+} . Fe^{2+} levels in both S.E. Asian and U.K. salt samples were relatively low, i.e. about 0.002% (dry wt.) in S.E. Asian samples and about 0.0003% in U.K. samples.

Atomic absorption analysis using an electrothermal furnace and micro computer control could detect copper accurately down to 0.02 ppm but levels observed in the salt samples were lower than this value.

The salt samples were found to contain negligible amounts of organic matter and upon dissolving in water, no appreciable amount of insoluble matter was observed.

Table 10: SALT ANALYSIS

Samples	Code	Moisture %	Chloride % dry wt.	Chlorine % wet wt.	Calcium % dry wt.	Magnesium % dry wt.	Iron % dry wt.
	S ₁	9.5	95.2	86.2	0.09	0.78	0.0009
	S ₂	9.8	96.9	87.4	0.12	0.60	0.008
	S ₃	5.6	98.5	93.0	0.09	0.47	0.0014
	S ₄	6.4	98.8	98.4	0.03	0.29	0.0005
South	S ₅	5.2	98.5	93.4	0.13	0.24	0.0011
East	S ₆	0.3	98.1	97.8	0.10	0.18	0.0005
Asian	S ₇	0.5	97.5	97.0	0.08	0.06	0.0005
Samples	S ₈	6.4	97.2	96.8	0.05	0.04	0.0002
	S ₉	3.6	99.9	96.3	0.09	0.24	0.0021
	S ₁₀	7.6	99.2	91.7	0.10	0.30	0.0022
	S ₁₁	6.2	98.2	92.1	0.12	0.18	0.0005
	S ₁₂	3.7	97.6	94.0	0.13	0.17	0.0035
	S ₁₃	5.1	97.7	92.7	0.08	0.44	0.0019
	S ₁₄	1.9	97.7	95.8	0.13	0.19	0.0010
	S ₁₅	12.8	94.4	82.3	0.17	1.10	0.0001
	S ₁₆	7.3	94.0	87.1	0.26	0.84	0.0015
	S ₁₇	0.5	99.2	98.7	0.06	0.06	0.0019
<u>Mean values</u>		4.73	97.56	92.92	0.11	0.36	0.0016
UK	ex Kelly	1.1	99.4	98.3	0.001	0.0002	0.0004
samples	ex Cox	0.2	99.4	99.2	0.001	0.0001	0.0003
<u>Mean values</u>		0.65	99.4	98.75	0.001	0.00015	0.00035

4.2 ANALYSIS OF MACKEREL MINCE

This analysis was necessary in order to assess the levels of potential lipid oxidation catalysts present naturally in the fish mince, specifically the levels of copper, iron and sodium chloride.

The average values obtained were 0.73 ppm (SD = 0.01) for copper, 1.88 ppm (SD = 0.31) for iron and 0.35% (SD = 0.05) for sodium chloride (all wet weight basis). These can be compared with the average values for fish flesh of 2 ppm for copper, 15.5 ppm for iron and 0.2% for chlorine (equivalent to about 0.3% for sodium chloride), however, there is wide variation in results obtained, e.g. copper in fish flesh has been found to vary between 0.01 and 37 ppm (Murray and Burt, —).

4.3 SIMULATION OF S.E. ASIAN FISH SALTING AND DRYING PROCESS

Mackerel was salted according to the procedure used in S.E. Asia (the detailed procedure was as employed in W. Java) and the changes in moisture content, water activity, peroxide values and colour were determined.

4.3.1 Moisture changes

The dehydration curves obtained on drying the mince in an oven at 30°C showed a faster initial drying rate for samples with sodium chloride than those without, as shown in Fig. 14. The control sample had the slowest initial drying rate and the highest final moisture content.

4.3.2 Water activity changes

The water activity measurements (see Fig. 15), showed that the samples without sodium chloride (initial $a_w = 1.00$) had a short

Fig. 14: Moisture Content vs Time on drying mackerel mince

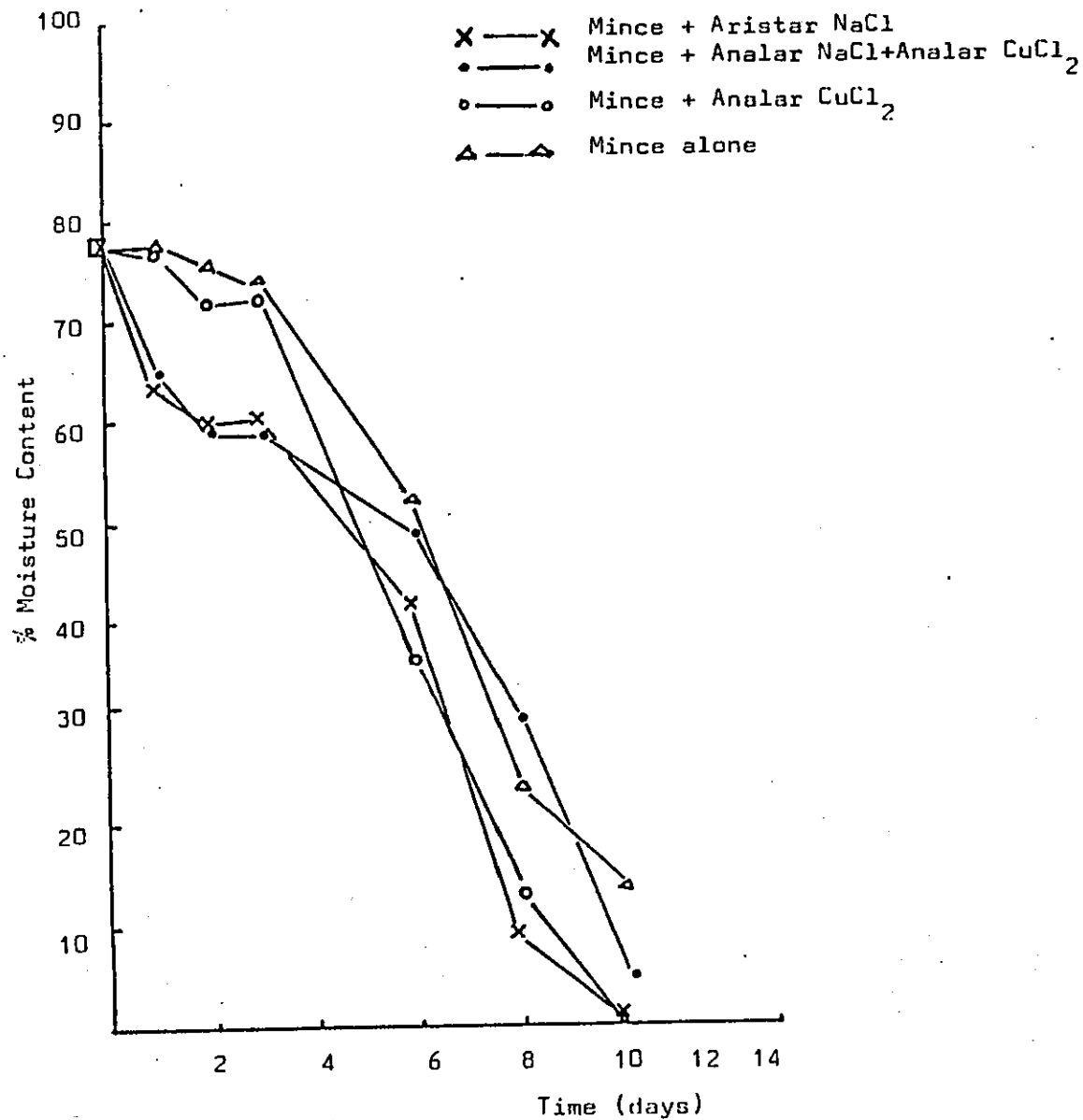
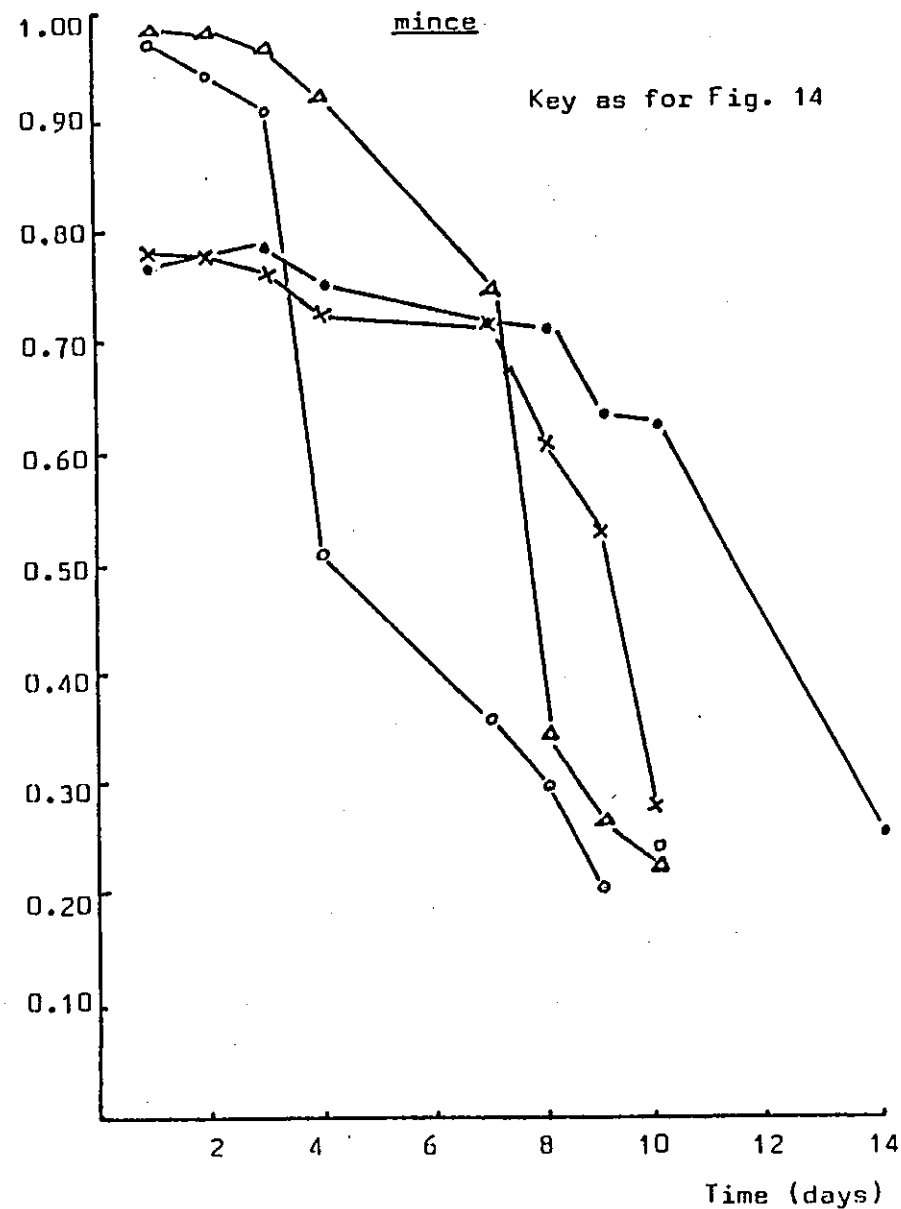


Fig. 15: Water Activity (a_w) vs Time on drying mackerel mince



induction period before the water activity dropped sharply from 0.95 between the 3rd and 5th day to about 0.24. The samples containing sodium chloride (initial a_w reduced from 1.00 to <0.80) showed a steady decline to 0.75 on the 3rd day, remaining constant till the 6th day (corresponding to the saturation zone) before dropping to about 0.26.

4.3.3 Peroxide value changes

Due to the very fast rate of lipid oxidation in the mince containing salt the maximum value of the peroxide value (PV) attained was difficult to determine accurately. As such this model system was repeated four times. The peroxide value results for two of these runs are given in Figs. 16 and 17.

The PV of samples containing NaCl rose sharply to attain maxima between the 4th and 6th day. Thereafter PV values declined steadily till the 9th and 10th day. Samples without NaCl rose to lower maxima between the 4th and 6th day after which the PV's declined to low values.

Therefore, sodium chloride in this system appears to be acting as a pro-oxidant, catalysing the formation of hydroperoxides from the fatty acids (see Section 2.3.1.1) as evidenced by the raising of the PV from an initial $10 \text{ meq O}_2 \text{ kg}^{-1}$ lipid to a maximum of $173 \text{ meq O}_2 \text{ kg}^{-1}$ after 4 days and $74.4 \text{ meq O}_2 \text{ kg}^{-1}$ of lipid after 6 days in models 3 and 4 respectively. These values when compared with those of the control samples (mince alone) which were 49.7 and $29.5 \text{ meq O}_2 \text{ kg}^{-1}$ lipid in models 3 and 4 respectively, are quite high.

The sample containing both sodium chloride (36.30% of flesh moisture) and 10 ppm Cu^{2+} showed a higher maximum of PV than the sample containing sodium chloride alone. Values reached were

Fig. 16: Peroxide Value (PV) vs Time
on drying mackerel mince (Run 3)

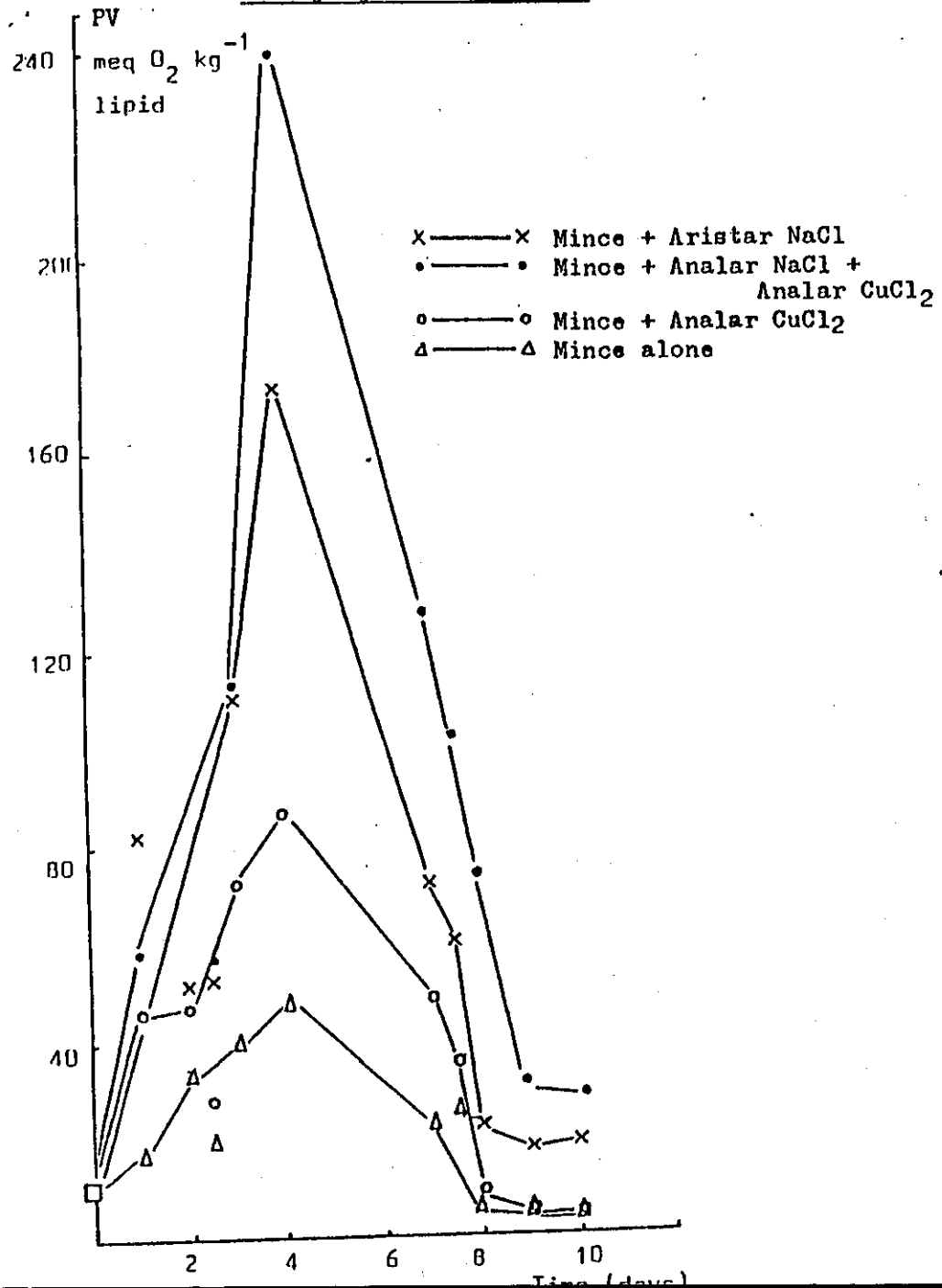
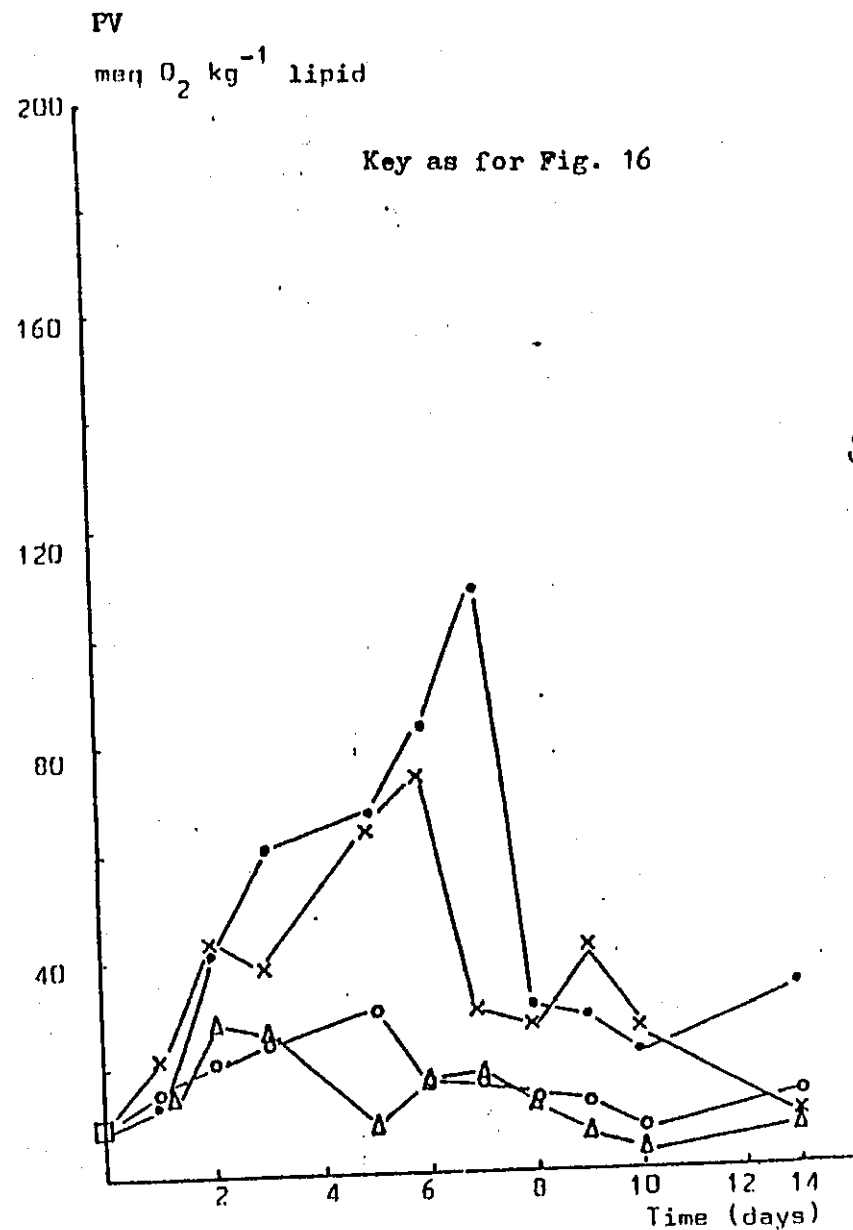


Fig. 17: Peroxide Value (PV) vs Time
on drying mackerel mince (Run 4)



242 (after 4 days) and 110 (after 6 days) meq O_2 kg^{-1} lipid in runs 3 and 4 respectively.

This would indicate a possible additive or synergistic pro-oxidant effect of copper and sodium chloride on the hydroperoxide formation from the fatty acids.

The sample containing 10 ppm copper alone did not show a comparable rise in the PV's. The maxima reached were 86.4 and 30.1 meq O_2 kg^{-1} lipid in models 3 and 4 respectively, suggesting therefore that copper could be catalysing the breakdown of hydroperoxides at a faster rate than it catalyses their formation, in absence of sodium chloride. Evidence that it does catalyse hydroperoxide formation is borne out by the fact that PV's of the control sample (mince alone) though following the same pattern as those with copper alone, attained lower values (max. 49.7 and 29.5 meq O_2 kg^{-1}).

4.3.4 Colour production

The colour produced in this model system was not water soluble but soluble in organic solvents only. After a series of trials with various solvents, it was found that acetic acid and ether extracted more colour than the other solvents, such as acetone and toluene. However, ether being very volatile, tended to give erroneous results after only a short while at room temperature. Acetic acid was therefore used.

Due to the pigment's insolubility in water, colour extraction in acetic acid of highly moist mackerel mince might be expected to be less efficient than for drier minces. As such in some instances the absorbance of the extracted colour did not relate directly to colour visually observed. Colour pictures of this model system are included in the Appendix, Plates 1, 2 and 3.

The colour production for two of the runs are given in Figs.

18 and 19. The mince containing copper (10 ppm) alone attained the highest value of 58.5 and 60.5 $\text{Abs g}^{-1} \text{cm}^3$ (dry fish matter) in models 3 and 4 respectively. These values were followed by those of samples with salt (36.3% of fish moisture) and copper (10 ppm) maximum values observed for these were 26.6 and 39.0 $\text{Abs g}^{-1} \text{cm}^3$ (dry fish matter) in models 3 and 4 respectively. Occasionally within the duration of these experiments a maximum value was followed by a drop in the amount of pigment extracted. However, this drop was not visually noticeable.

On a dry fish matter basis the sample with salt and copper did not produce as much colour as the sample with copper alone.

The sample with NaCl alone, on the other hand, showed only small changes in colour throughout the drying period, not significantly different from the control (mince alone). Maxima obtained in model 3 were 12.3 and 21.6, and in model 4 were 7.2 and 16.0 $\text{Abs g}^{-1} \text{cm}^3$, for samples with sodium chloride alone and the controls respectively.

This indicates therefore that copper, at 10 ppm, very actively catalyses the formation of colour in the fish mince, while sodium chloride, at 36.3%, has only a slight catalytic effect (see Appendix plates 1, 2 and 3).

4.4 STORAGE AT CONSTANT WATER ACTIVITY IN THE ABSENCE OF HIGH LEVELS OF NaCl

Samples were freeze-dried before equilibration to fixed water activities by absorption. Changes in moisture content and water activity during freeze drying are given in Fig. 20.

Freeze-dried mince samples were equilibrated in small plastic water activity dishes instead of watch glasses with large surface areas, for the convenience that the water activity could then be

Fig. 18: Colour vs Time on drying mackerel mince (Run 3)

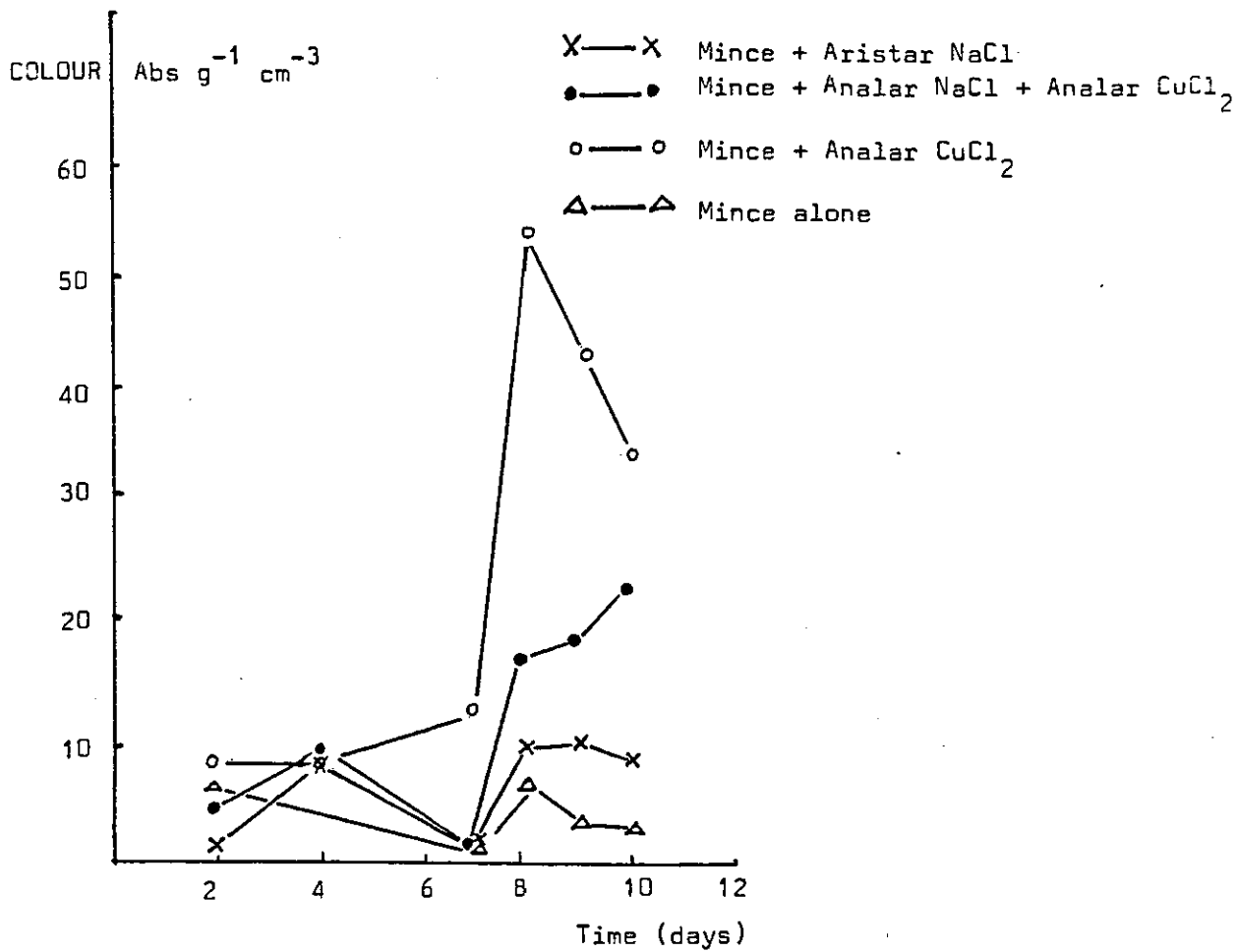


Fig. 19: Colour vs Time on drying mackerel mince (Run 4)

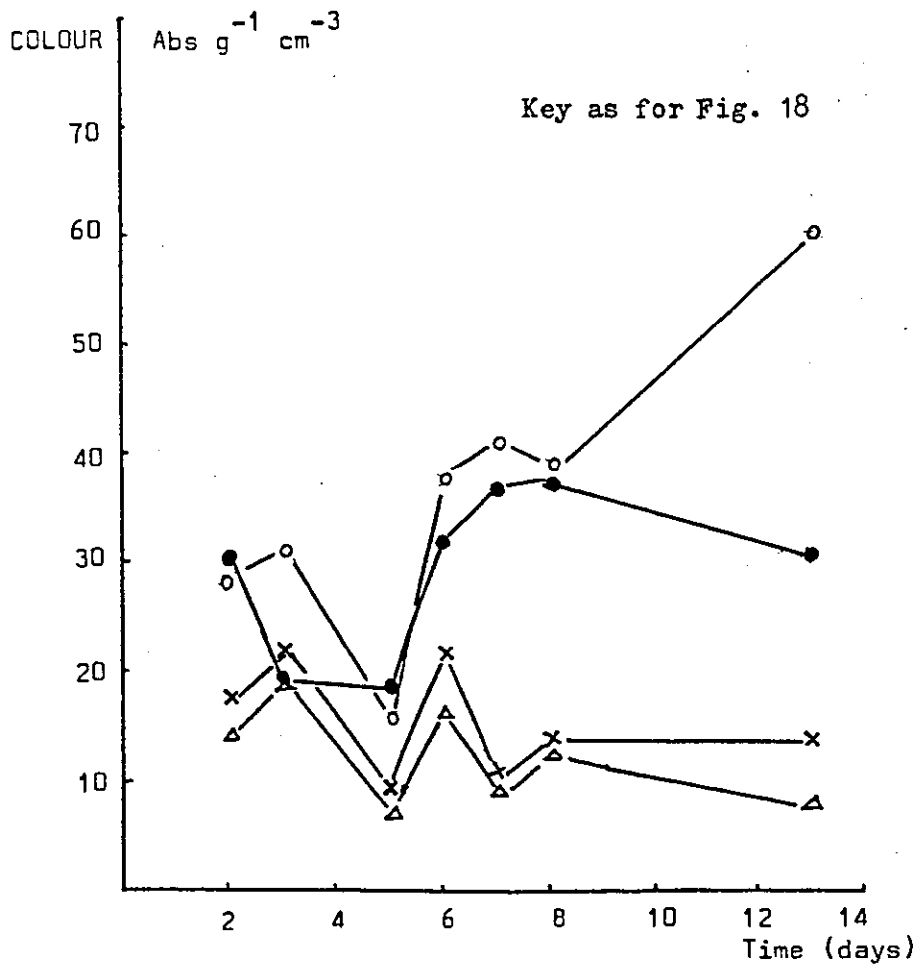


Fig. 20: Desorption isotherm of mince during freeze drying

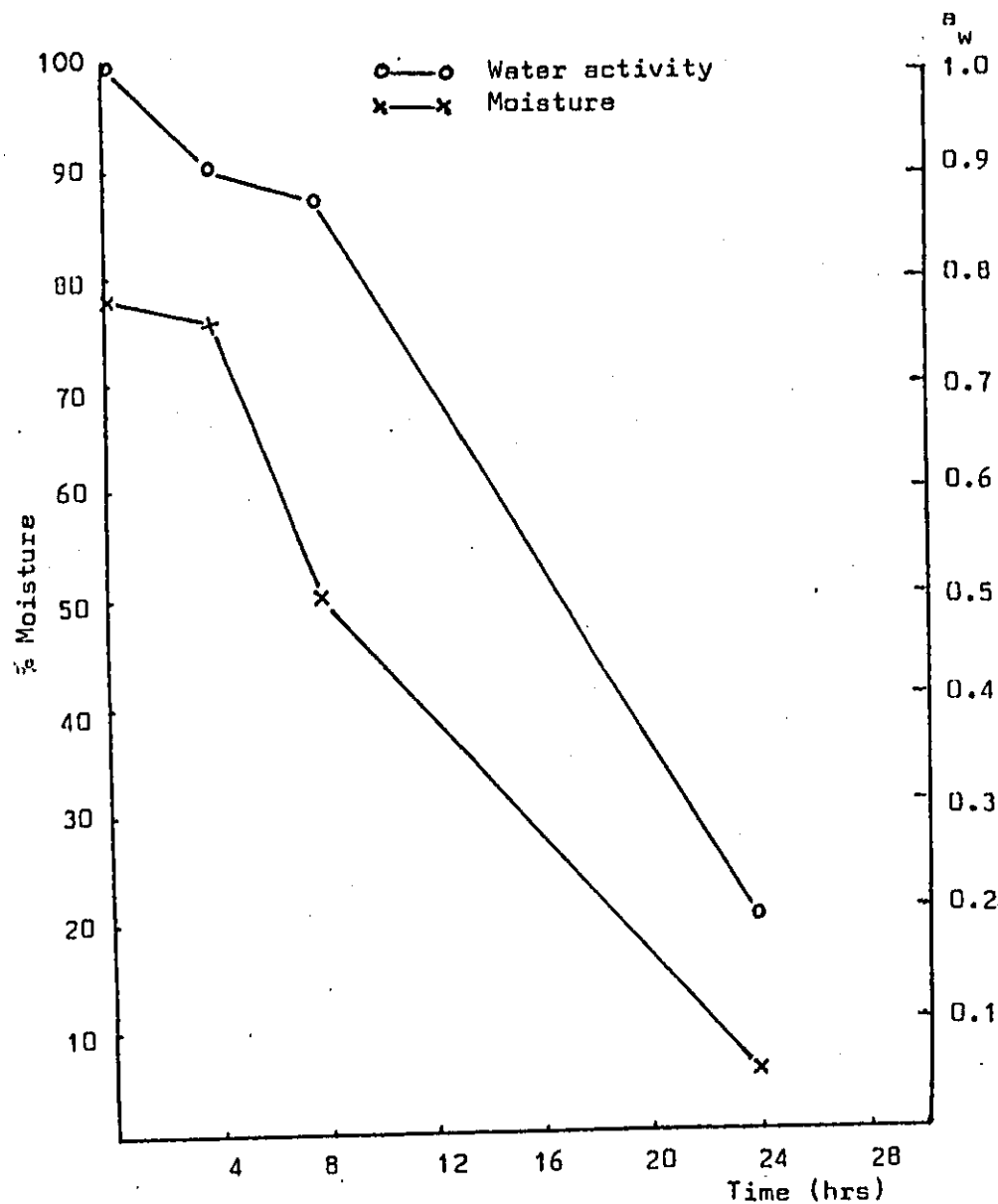
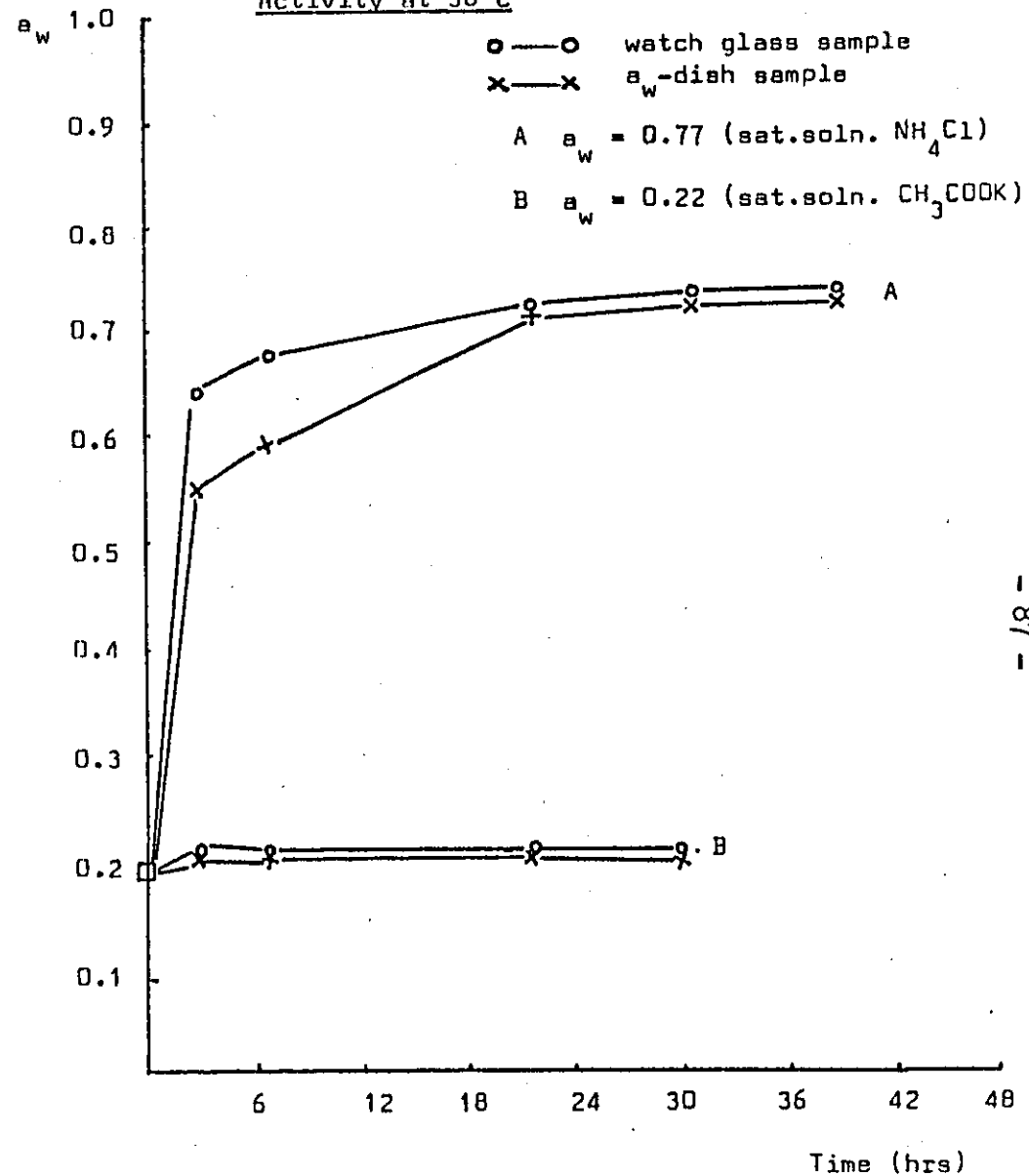


Fig. 21: Equilibration of dried mince to constant water activity at 30°C



determined directly by transferring the dishes to the water activity meter. Fig. 21 shows that the rates of equilibration (by absorption) of the samples spread thinly in a water glass and in a water activity dish are very similar and reach a steady state after about 20 hours. The desiccators were evacuated to enhance the equilibration rate and to remove as far as possible atmospheric oxygen and so prevent rancidity development during the equilibration period. However, high initial PV's (ranging from 50 to 380 meq O_2 kg^{-1} lipid) were observed after equilibration compared to the initial PV of freeze dried mince of 19.6 meq O_2 kg^{-1} lipid. This is considered to be due to the fact that air may not have been completely removed at a vacuum pressure of about 10 mm Hg and that only a small amount of oxygen is required to initiate the rancidity, possibly that residual in the interstitial tissues of the dry mince. This development was observed both in the control and test samples, so it could not have been due to the added sodium chloride and/or copper. A similar rise was not observed in the colour development.

4.4.1 Peroxide value changes

Upon exposure of the equilibrated samples to air the peroxide values in general increased. For the samples of mince in the absence of added salts (Fig. 22), at $a_w = 0.14$ the peroxide value rose to a maximum of 848 meq O_2 kg^{-1} after 4 days, at $a_w = 0.84$ the peroxide value rose to a maximum of 340 meq O_2 kg^{-1} after 5 days. Secondary maxima occurred at 9-10 days. At $a_w = 0.53$ and $a_w = 0.32$, the peroxide values changed erratically, but kept below 300 meq O_2 kg^{-1} .

Fig. 22: Peroxide Value (PV) vs Time
of mackerel mince in the absence
of added salts

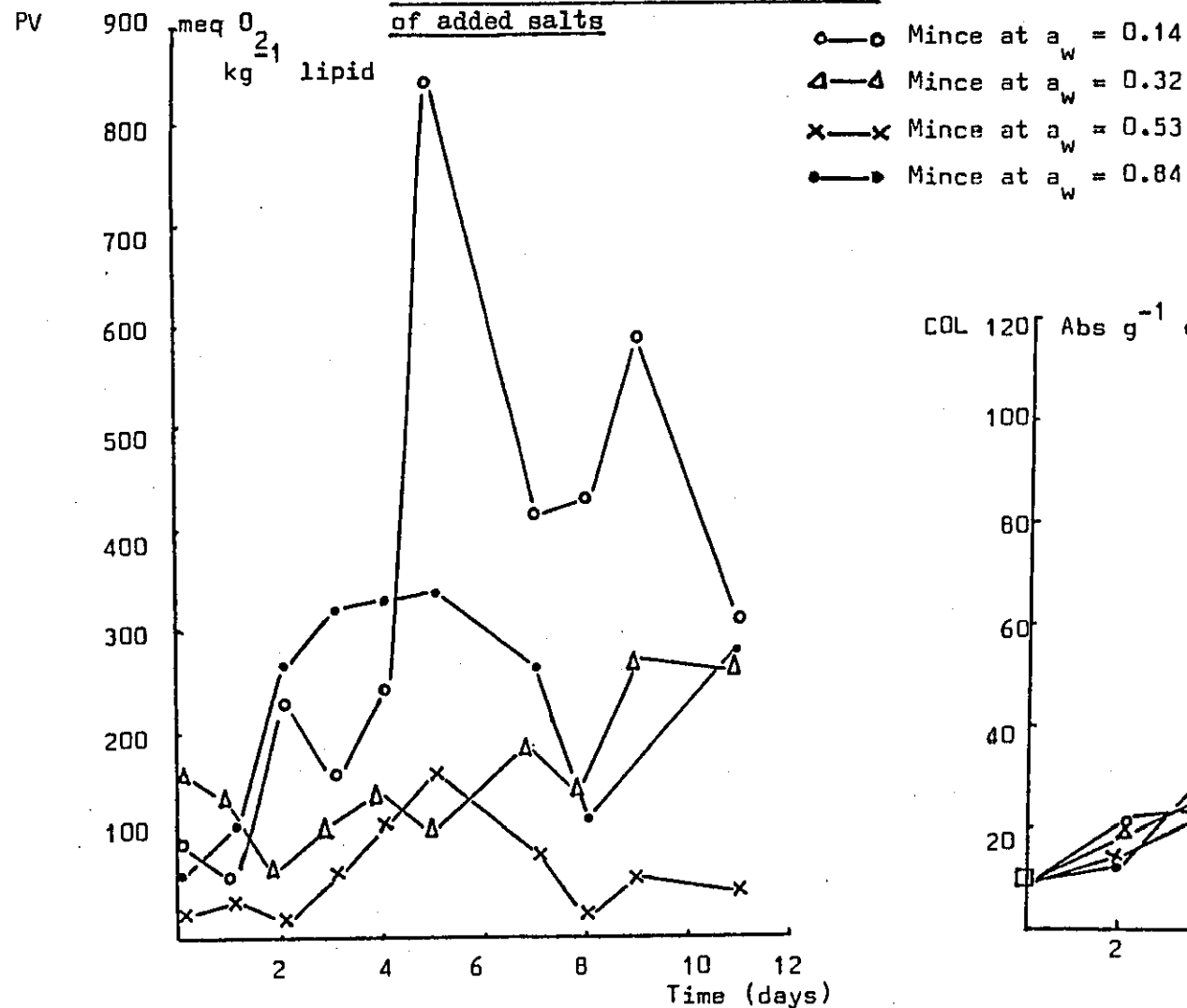
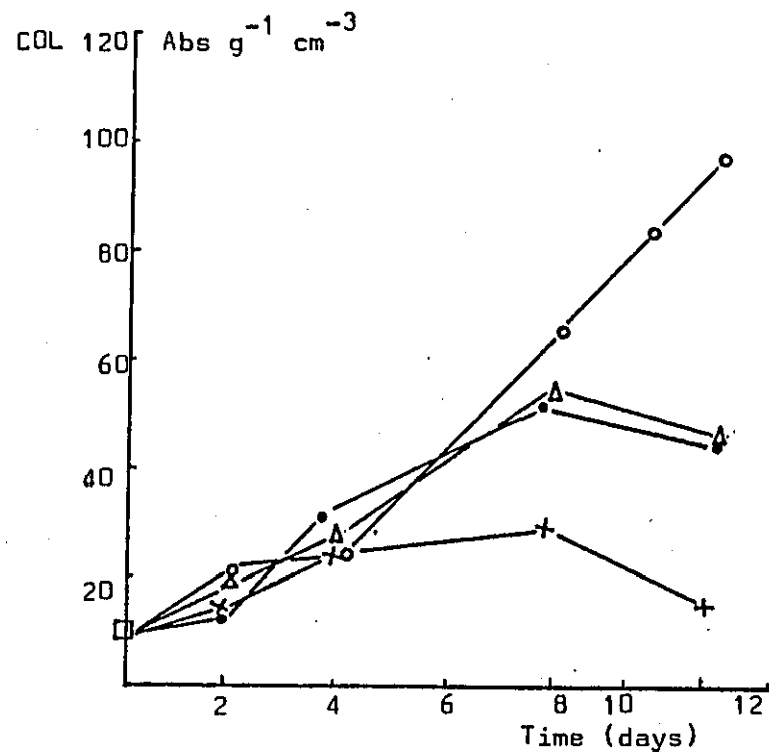


Fig. 23: Colour (COL) vs Time
of mackerel mince in the absence of added
salts



The PV of samples containing sodium chloride (10 ppm) followed a similar pattern to those of the control (see Fig. 24). PV of samples at $a_w = 0.14$ and 0.84 reached 600 (after 5 days) and 415 meq O_2 kg^{-1} lipid (after 4 days) respectively, with secondary maxima after 9 days. Samples at $a_w = 0.32$ and 0.53 had lower peroxide values than for the other samples. The $a_w = 0.32$ sample had a maximum of 270 meq O_2 kg^{-1} after 4 days, with a secondary maximum after 9 days.

The samples containing copper (10 ppm) showed very short induction periods (see Fig. 26), attaining for all the samples values above 300 meq O_2 kg^{-1} lipid on the 2nd day with the maximum values occurring either on the 2nd or 4th days. The maximum of the sample at $a_w = 0.14$ was again the highest at 734 meq O_2 kg^{-1} (after the 4th day). The rest were very similar reaching their maxima at approximately 400 meq O_2 kg^{-1} lipid between the 2nd and 4th days. The rise was followed by a drop with minor peaks occurring between the 8th and 11th days.

Fig. 28 shows the relationship between the maximum peroxide values attained and a_w . It shows a high rate of lipid oxidation at low water activity, decreasing as the water activity increases to a minimum between $a_w = 0.32$ and 0.53 . From here the rate of lipid oxidation increases as the a_w increases to 0.84 , except for samples with copper which, after the initial fall in rate of lipid oxidation with a_w from 0.14 to 0.32 , showed no appreciable rise but had higher values than the control and sample with 10 ppm sodium chloride within the a_w range of 0.32 to 0.84 .

Fig. 24: Peroxide Value (PV) vs Time
of mackerel mince in the presence of 10 ppm of NaCl

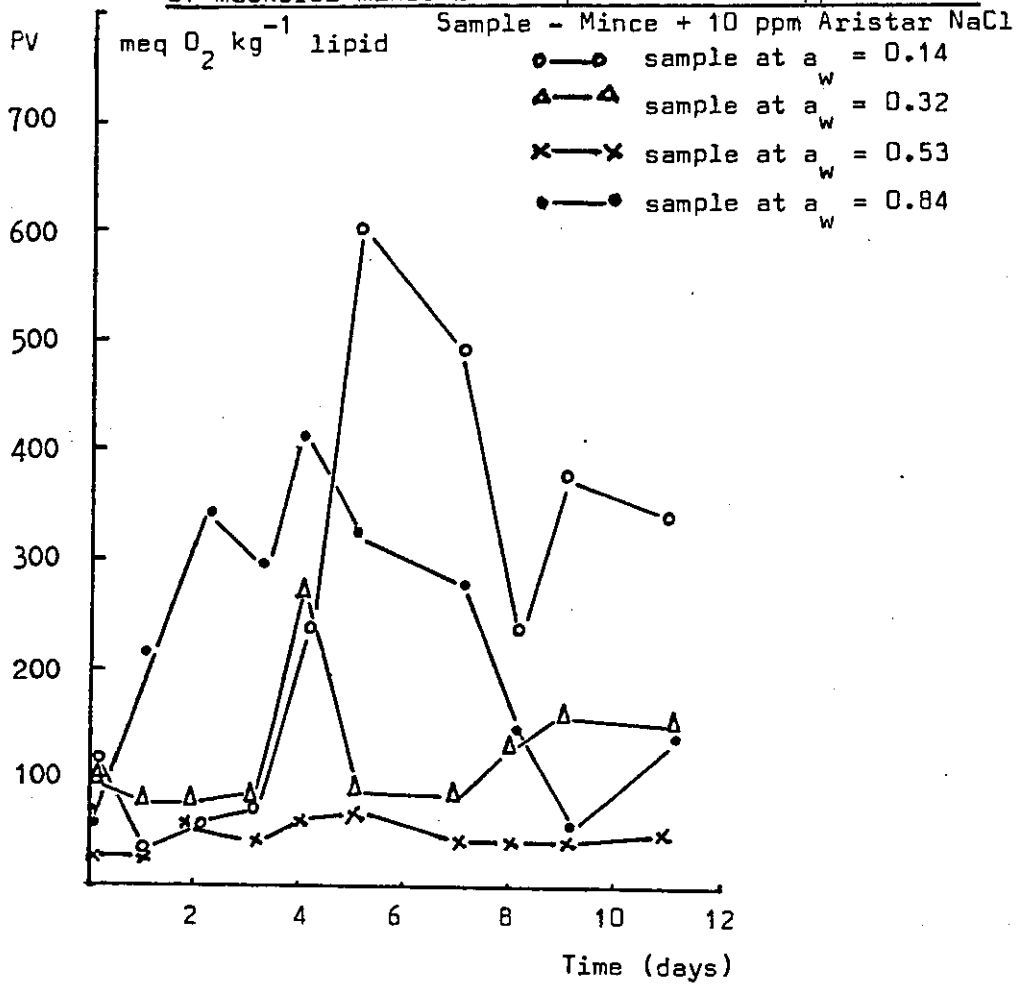


Fig. 25: Colour (COL) vs Time
of mackerel mince in the presence of 10 ppm of NaCl

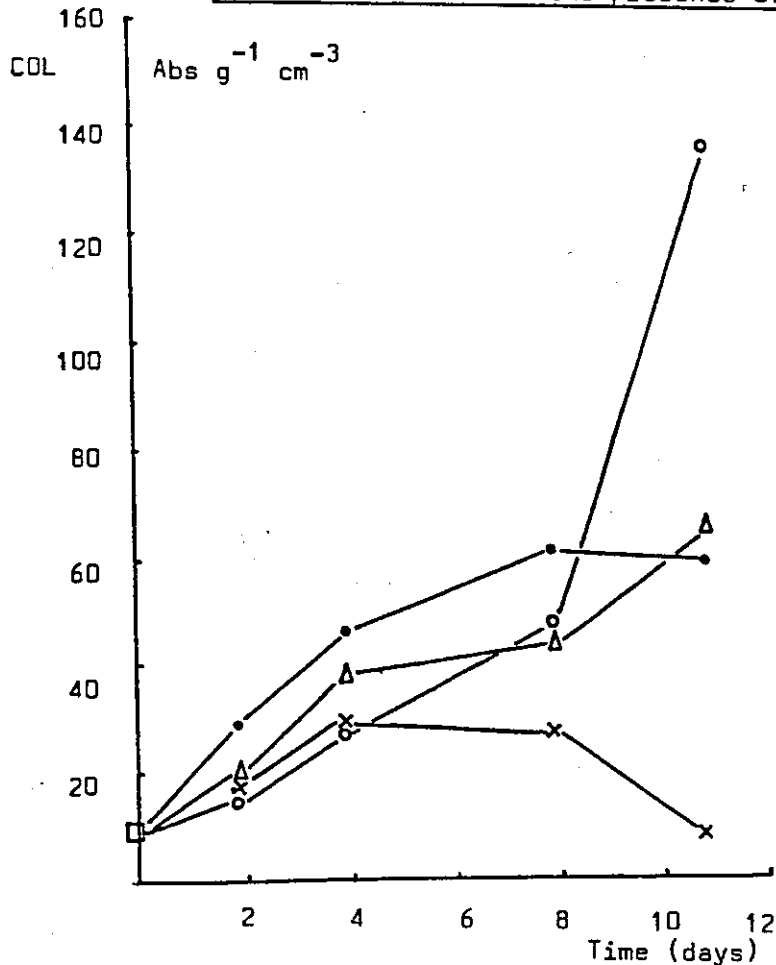


Fig. 26: Peroxide Value (PV) vs Time of mackerel mince in the presence of 10 ppm of copper

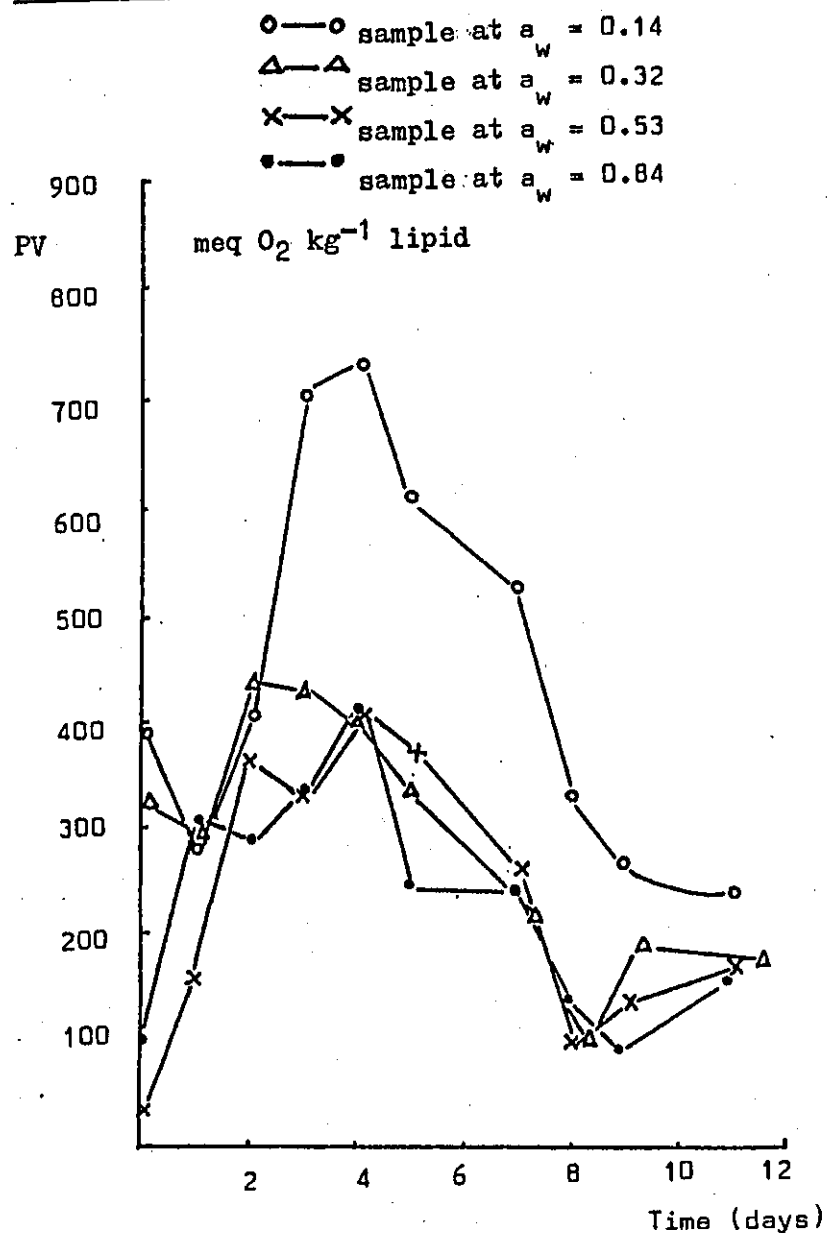


Fig. 27: Colour (COL) vs Time of mackerel mince in the presence of 10 ppm of copper

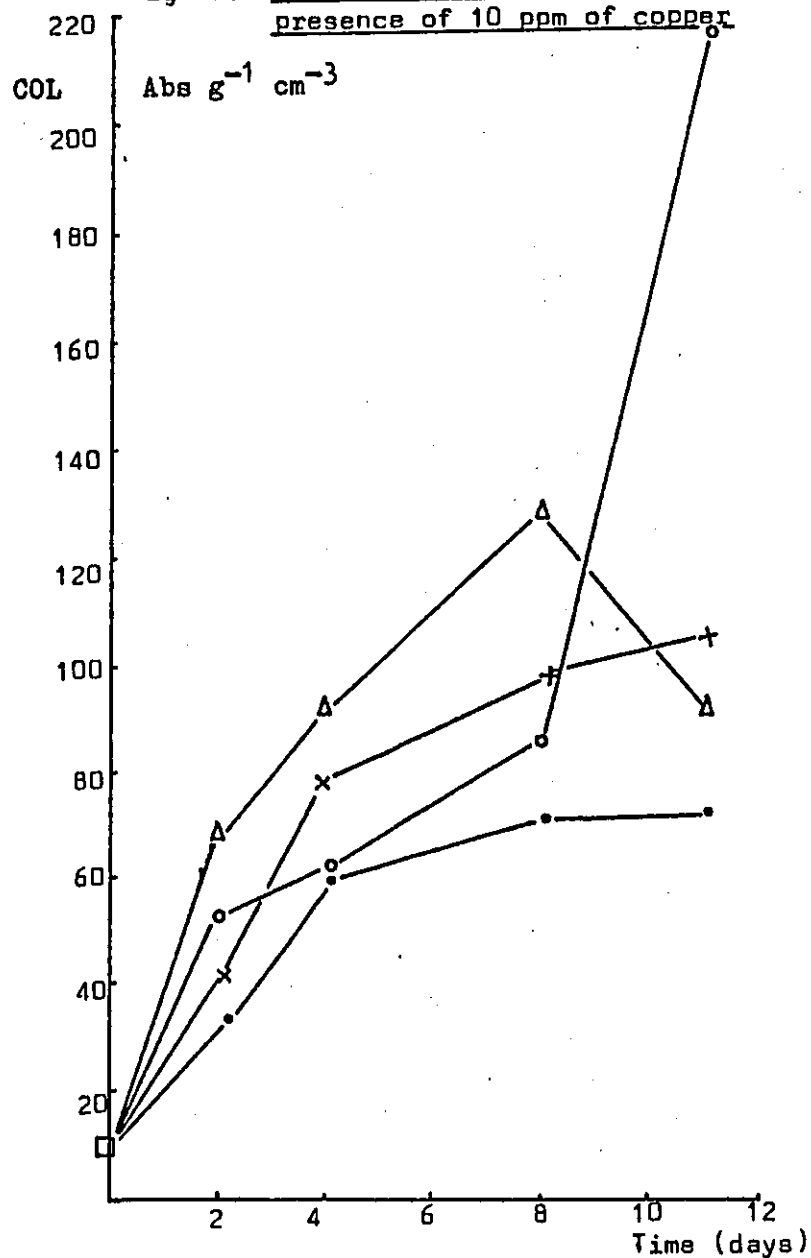


Fig. 28: Maximum Peroxide Value (max. PV)
vs Water activity

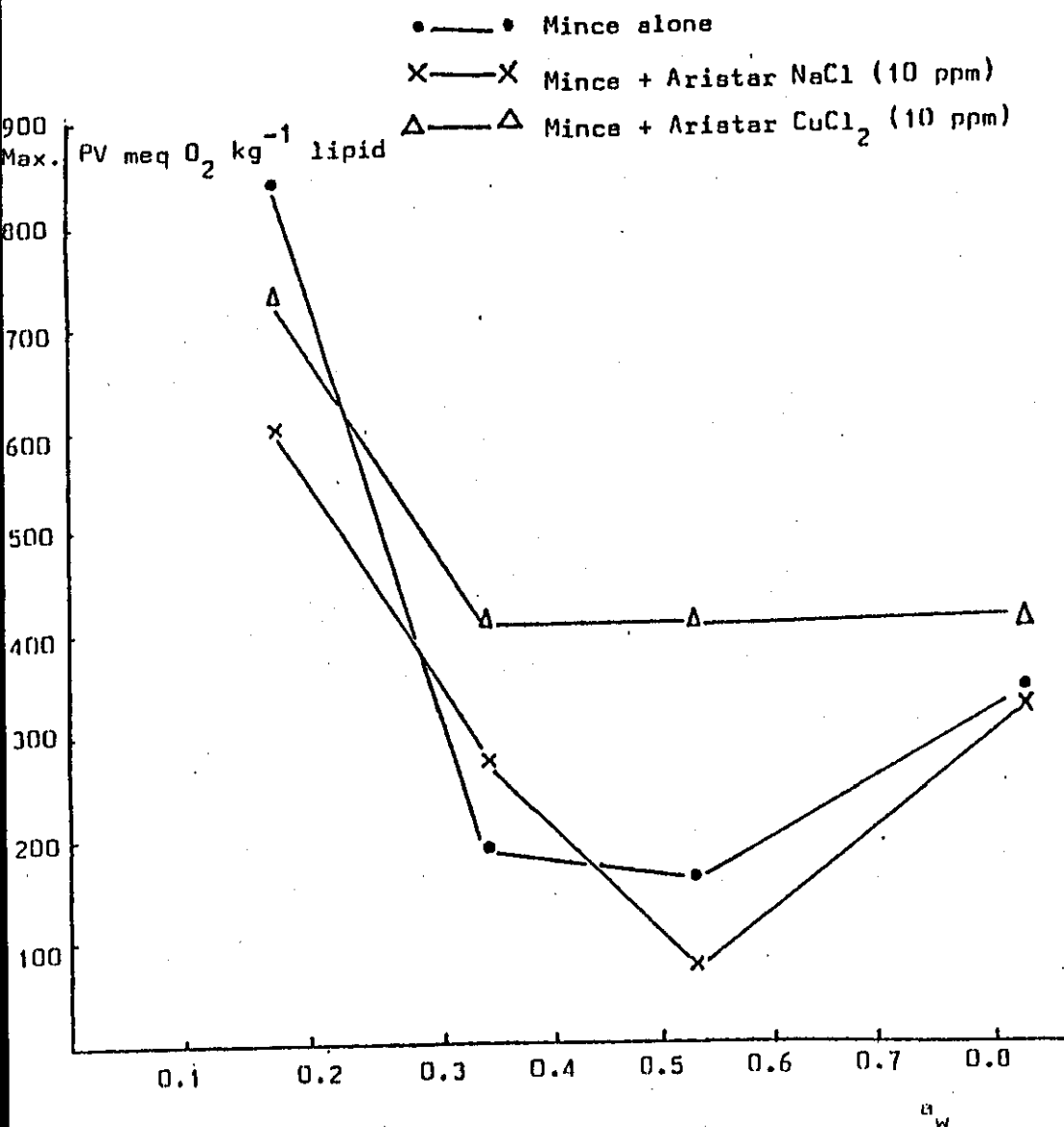
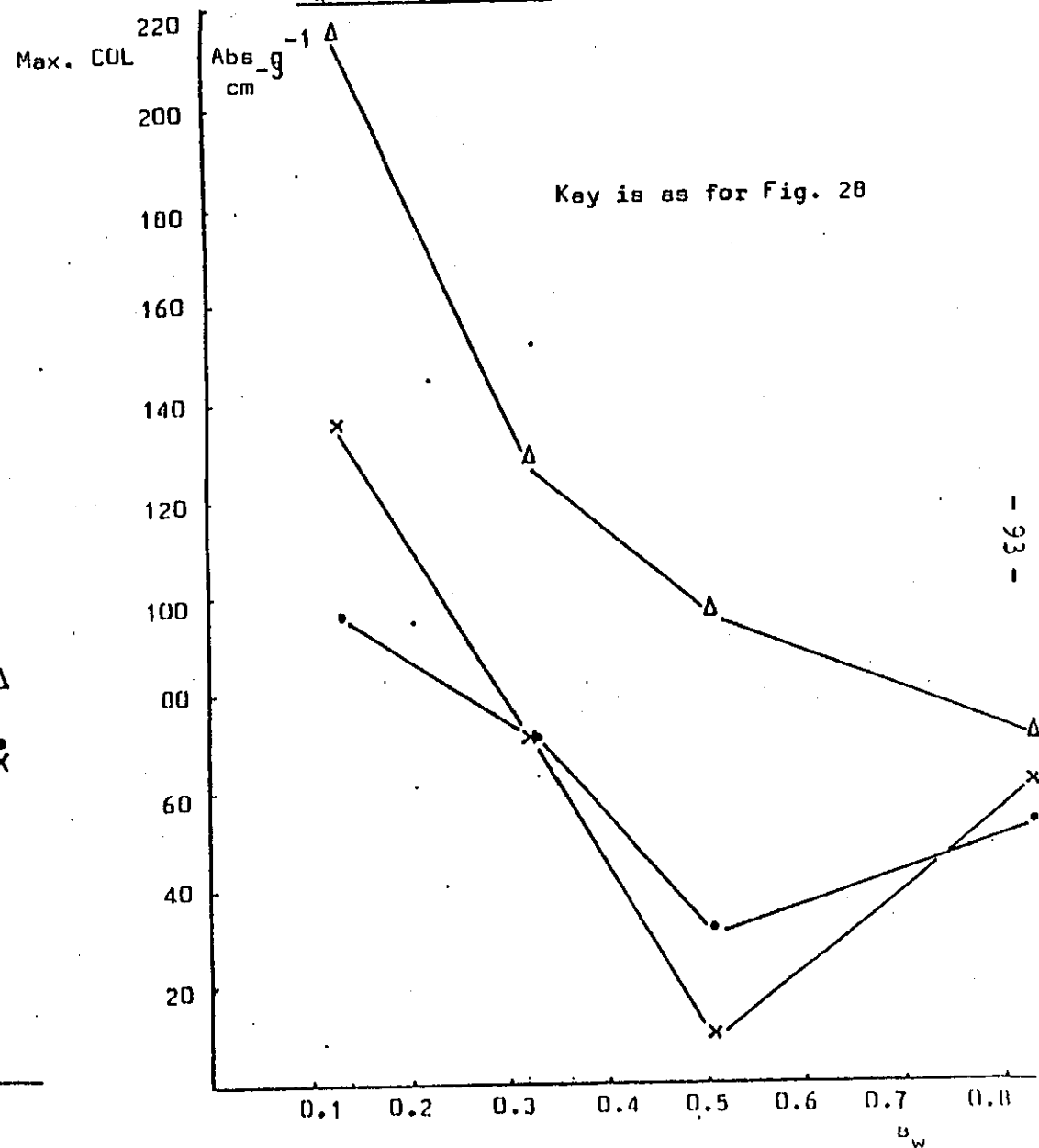


Fig. 29: Maximum Colour Values (max. COL)
vs Water Activity



These results are similar to those of Labuza (1971). Unfortunately insufficient data were obtained to assess if a drop occurred in lipid oxidation rate after $a_w = 0.85$, as observed by Labuza in model systems of methyl linoleate (Labuza, 1971). Troller and Christain (1978) did not observe this occurrence in foodstuffs.

4.4.2 Colour production

The extracted colour changes for mince in the absence of added salts are given in Fig. 23. The samples showed initial low values of $10 \text{ Abs g}^{-1} \text{ cm}^3$ (dry matter) and rose consistently to higher values over the 11 day period of study. The largest value of $96.3 \text{ Abs g}^{-1} \text{ cm}^{-3}$ (dry matter) was observed at $a_w = 0.14$ and the lowest value was given by the sample at $a_w = 0.53$ (maximum value $31.0 \text{ Abs g}^{-1} \text{ cm}^{-3}$). Samples at a_w 's of 0.32 and 0.84 did not show any appreciable difference, and they reached maximum values of 54.5 and $52.9 \text{ Abs g}^{-1} \text{ cm}^{-3}$ (dry matter) respectively. The absorbance of the extracted colour from these latter samples and those at $a_w = 0.53$ showed a decline after the 9th day of the study.

In the samples containing sodium chloride (10 ppm) the colour extracted showed very similar trends to the samples described above, (see Fig. 25) with samples at $a_w = 0.14$ reaching the highest value ($136 \text{ Abs g cm}^{-3}$ dry fish matter) with those at $a_w = 0.32$ and 0.84 remaining again similar, reaching maximum values of 66.7 and $59.4 \text{ Abs g}^{-1} \text{ cm}^3$ (dry matter) respectively. The samples at $a_w = 0.53$ as for the control, had the lowest value (max. $29.0 \text{ Abs g}^{-1} \text{ cm}^{-3}$ dry matter).

Samples containing copper (10 ppm) showed generally higher values at all water activities (see Fig. 27). The highest value ($216 \text{ Abs g}^{-1} \text{ cm}^{-3}$ dry matter) was once again observed for sample at $a_w = 0.14$

but the lowest value (maximum $72.9 \text{ Abs g}^{-1} \text{ cm}^{-3}$ dry matter) was this time at $a_w = 0.84$. The samples at $a_w = 0.32$ and 0.53 had values within these two extremes. Their maximum values were 129 and $96.7 \text{ Abs g}^{-1} \text{ cm}^{-3}$ respectively. A drop from the maximum value was observed in the sample at $a_w = 0.32$ alone (see Fig. 27). It is not clear why the values for extracted colour should have passed through a maximum in some cases. This never occurred with samples at $a_w = 0.14$, possibly indicating that a secondary reaction requires a higher a_w . It should be noted that the intensity of colour as visually observed for the sample containing 10 ppm copper was greater at $a_w = 0.84$ than at $a_w = 0.14$, even though the extractable colour would suggest otherwise i.e. it was more intense for the 0.14 than the 0.84 sample. This was probably due to the difficulties of extraction in presence of high moisture content, as indicated earlier, or due to extensive polymerisation of the colour compounds leading to changes in structure and possibly therefore to reduced solubility in acetic acid. The mince plus sodium chloride (10 ppm) and mince alone had similar actual colour intensities visually and related closely to the absorbance of the extracted colour over the a_w range studied.

The relationship of the maximum values of absorbances of colour extracts for these samples in relation to water activities is shown in Fig. 29. For the mince alone and mince in the presence of 10 ppm NaCl there is a minimum at about 0.5 whereas for the mince in the presence of 10 ppm of Cu decreasing water activity leads to increasing maximum extracted colour.

4.5 STORAGE AT CONSTANT WATER ACTIVITY IN THE PRESENCE OF LOW LEVELS OF COPPER

Mackerel mince was stored at a constant water activity of about 0.5.

This water activity value was chosen because lipid oxidation was relatively slow at that value. The actual water activity obtained on equilibration was 0.53.

The results are discussed with reference to Fig. 30, 31 and 32 where changes in peroxide value, thiobarbituric acid value and extracted colour are presented.

4.5.1 Peroxide value changes

The peroxide values of the mince containing 1.5 ppm and 3.0 ppm copper did not show any induction period but rose to maximum values of 352 and 330 meq O_2 kg⁻¹ lipid after one day. This was followed by a decline to steady values of about 120 meq O_2 kg⁻¹ lipid between the 3rd and 8th day. Minor peaks (max. value 104 and 183 meq O_2 kg⁻¹ lipid) were observed for these two samples on the 9th day, after which the PV dropped to a steady value of about 100 meq O_2 kg⁻¹ lipid. The peroxide value of the mince containing 0.5 ppm Cu^{2+} showed a long induction period reaching a maximum of 180 meq O_2 kg⁻¹ on the 9th day. The minces containing 0.2% EDTA and the control sample (i.e. mince alone) showed no significant difference in their peroxide values and did not attain any prominent peak but had maximum values of 110 meq O_2 kg⁻¹ in presence of 0.2% EDTA on the 7th day and 120 meq O_2 kg⁻¹ for mince alone on the 10th day.

The significance of this is that copper at 1.5 ppm and 3.0 ppm had a strong catalytic effect on the production of peroxides, while copper at 0.5 ppm had only a small effect in the period studied. The addition of 0.2% EDTA to chelate metal ions intrinsically present in the mince did not show any significant difference when compared with the control sample, thus indicating that the copper (0.7 ppm) present in the mince is possibly in a form not available for catalysis of lipid oxidation.

Fig. 31: Thiobarbituric acid (TBA) vs Time
for mackerel mince stored at $a_w = 0.53$

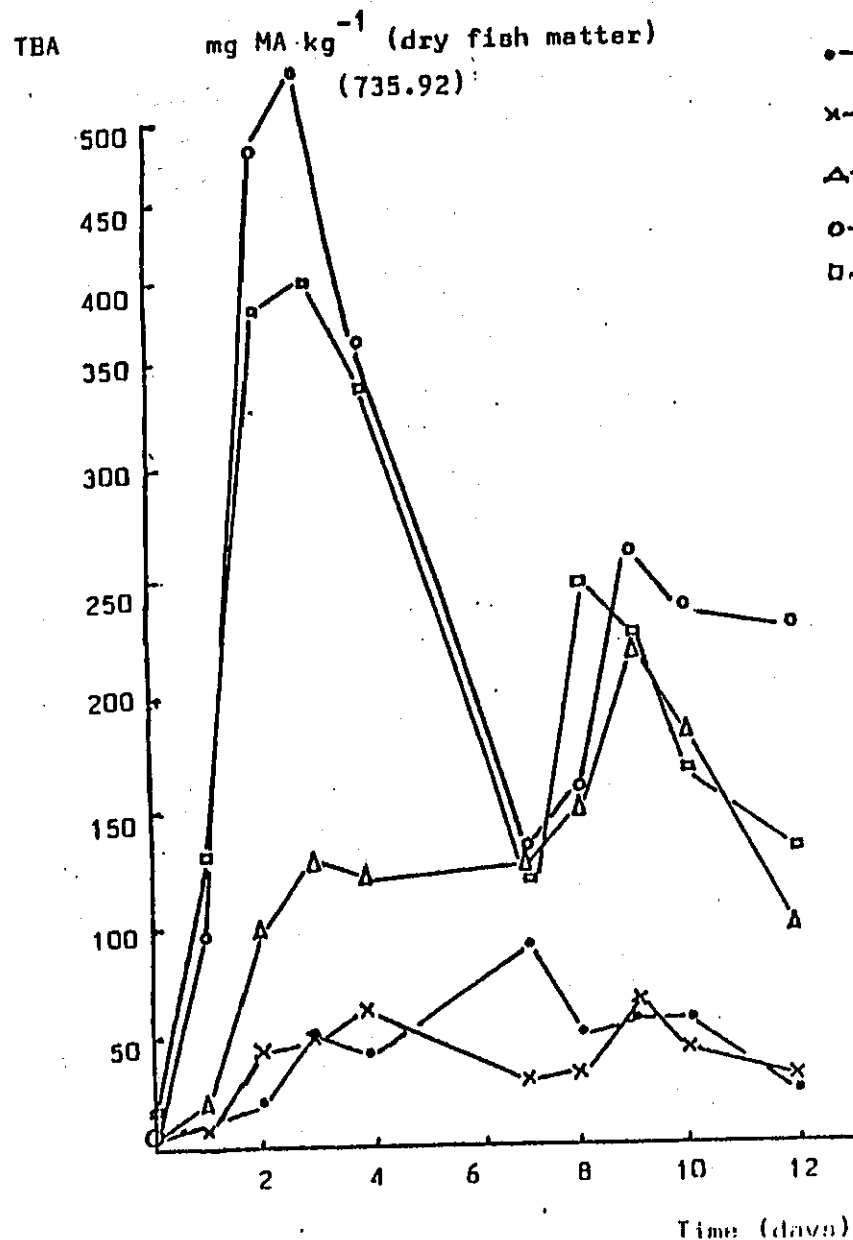


Fig. 30: Peroxide Value (PV) vs Time
for mackerel mince stored at $a_w = 0.53$

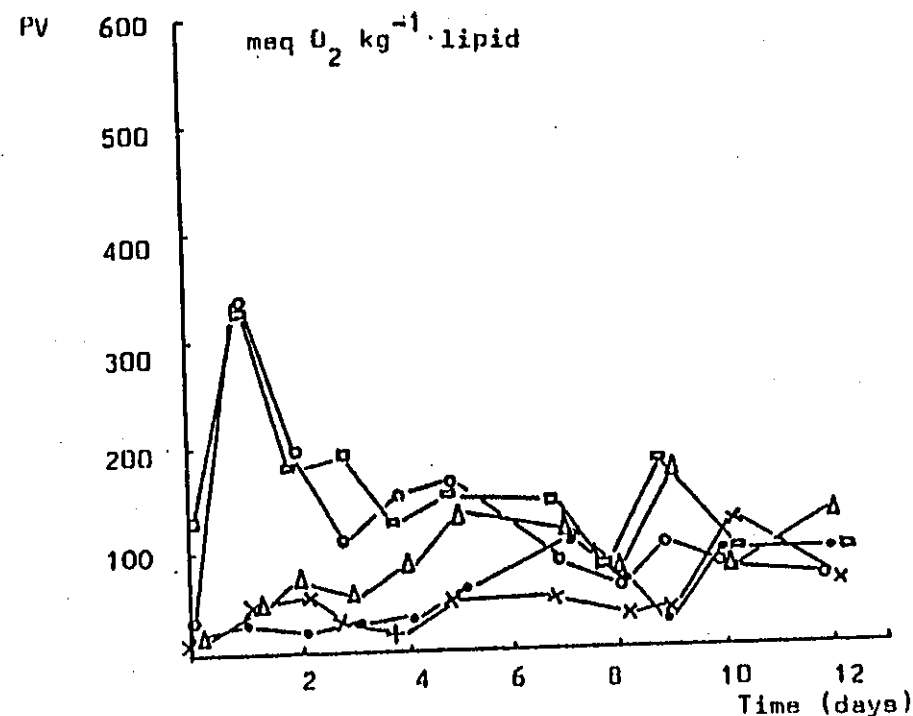
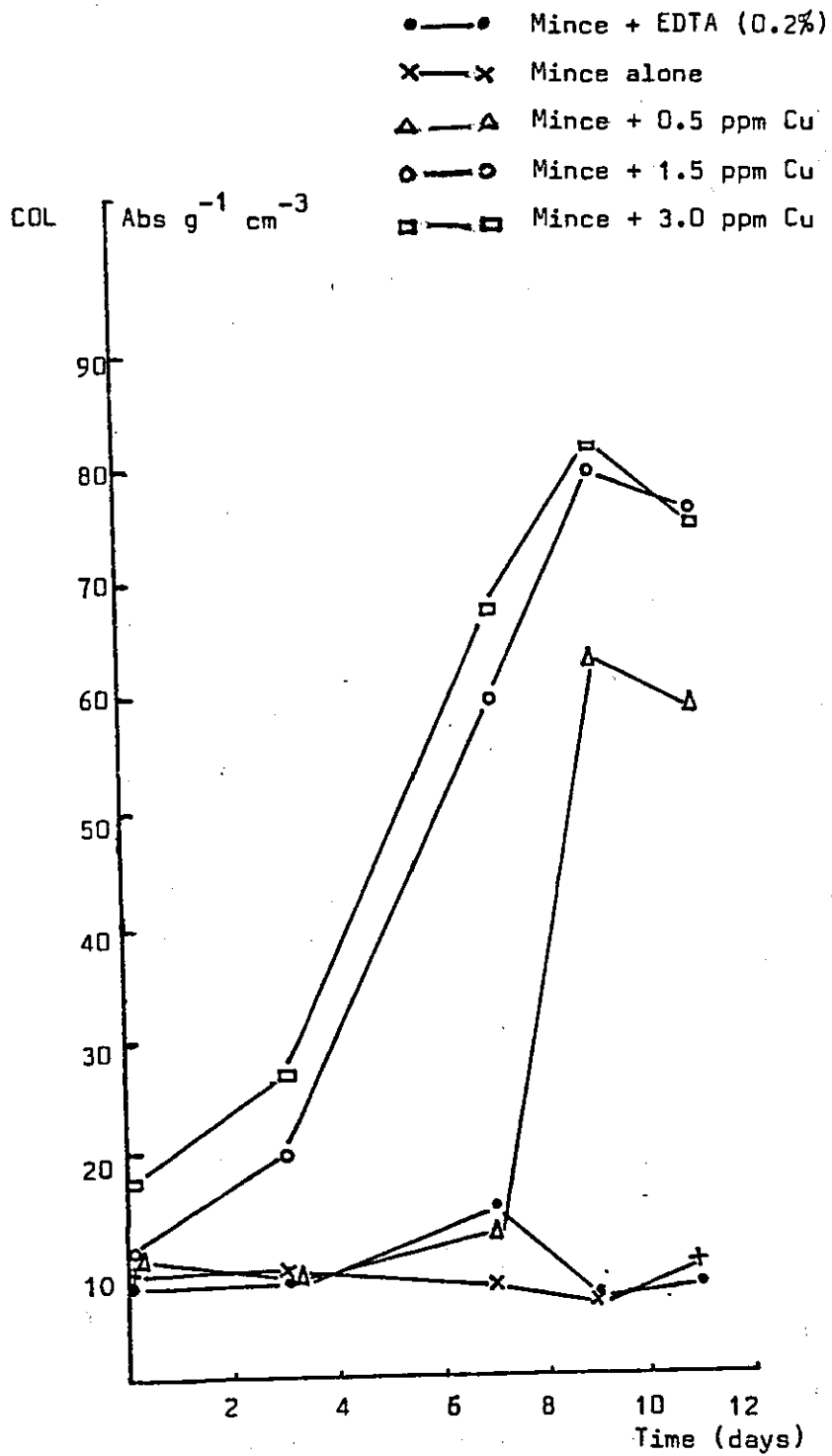


Fig. 32: Colour (COL) Vs Time for mackerel mince stored at $a_w = 0.53$



4.5.2 Thiobarbituric acid value changes

The trends observed above for PV values were also observed for the TBA values (see Fig. 31). Samples containing 1.5 ppm and 3.0 ppm copper rose dramatically from an initial 5.5 and 14.2 mg MA kg⁻¹ of dry flesh respectively to maximum values of 737 and 408 mg MA kg⁻¹ dry flesh respectively by day 3. This rise was followed by an equally sharp drop to minimum values of 144 and 126 mg MA kg⁻¹ dry flesh for mince with 1.5 ppm Cu and 3.0 ppm Cu respectively, with both showing a minor peak on the 9th day. The mince with 0.5 ppm Cu showed an extended induction period as did the control and the sample containing 0.2% EDTA. It reached a minor maximum on the 3rd day at 135 mg MA kg⁻¹ dry flesh and remained around this level until the 9th day when it reached a major peak at 232 mg MA kg⁻¹ dry flesh before dropping to a minimum of 104 mg MA kg⁻¹ dry flesh on the 12th day.

After the much extended induction periods showed by the control sample and that with 0.2% EDTA, they reached maximum peaks at 65.8 (on day 4) and 97.3 (on day 7) mg MA kg⁻¹ dry flesh respectively. A minor peak for both samples was observed on the 9th day as for the copper containing samples.

This indicates therefore that copper is not only catalysing the formation of hydroperoxides from fatty acids, but also catalyses their breakdown to form malonaldehyde, (especially those formed from the linolenic type of fatty acid). The effect is significant even at the 0.5 ppm level in contrast to the insignificant effect on the peroxide value at this level.

4.5.3 Colour production

The colour development in samples with 1.5 and 3.0 ppm was rapid (see Fig. 32), rising from an original 18.0 Abs g⁻¹ cm⁻³ to

84.3 and 86.8 Abs $\text{g}^{-1} \text{cm}^3$ on the 9th day respectively. A slight drop in extracted colour was observed between the 9th and 11th day. The extracted colour in samples containing 0.5 ppm Cu was low and similar to that observed for the control and mince plus 0.2% EDTA, until the 7th day when it increased sharply to a maximum of 67.1 Abs $\text{g}^{-1} \text{cm}^3$ on the 9th day.

The sample with 0.2% EDTA and mince did not show any appreciable rise in extracted colour throughout the run, maximum values reached were 15.2 and 10.4 Abs $\text{g}^{-1} \text{cm}^{-3}$ dry flesh respectively.

It can be inferred therefore, from the above findings, that copper at 1.5 ppm and above actively catalyse the development of extractable colour whilst at 0.5 ppm Cu, this activity is delayed and became obvious only after 8 days. The natural copper content of the mince, at about 0.7 ppm (see Section 4.2), was possibly unavailable to catalyse colour production and there was no significant observable colour development for the mince alone and that containing 0.2% EDTA.

The extracted colour in this set of experiments corresponded well with the visually observed colour.

4.6 STORAGE AT CONSTANT WATER ACTIVITY IN THE PRESENCE OF HIGH LEVELS OF NaCl

Changes in peroxide value, thiobarbituric acid value and extractable colour were determined for mackerel mince stored at four different water activities: 0.14, 0.32, 0.51 and 0.84. The NaCl contents were 25% for the a_w 0.84 samples and 50% for the others.

4.6.1 Peroxide value changes

The results are given in Figs. 33, 34, 35 and 36. The peroxide value of the control sample (i.e. mince alone) at $a_w = 0.14$ was high

Fig. 33: Peroxide Value (PV) vs Time
for mackerel mince stored at $a_w = 0.14$

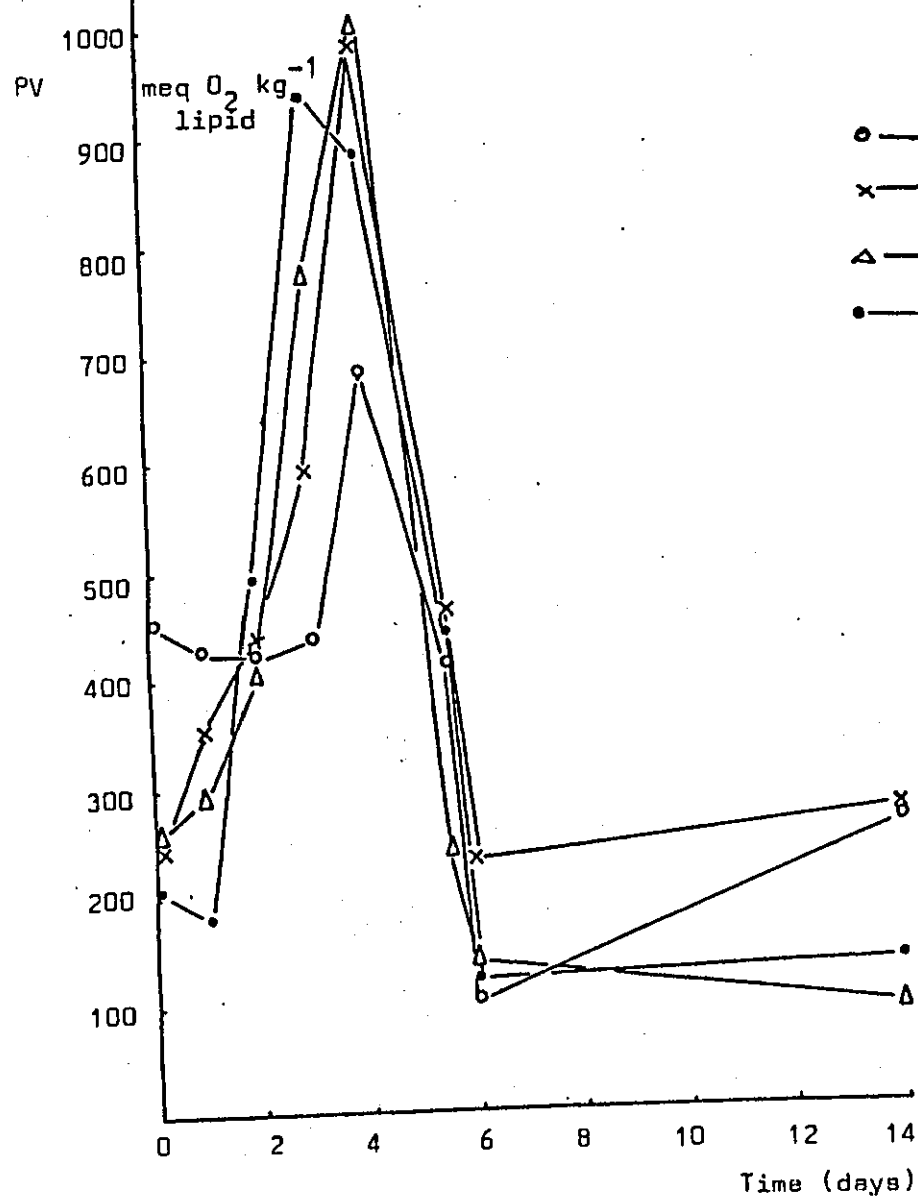


Fig. 34: Peroxide Value (PV) vs Time
for mackerel mince stored at $a_w = 0.32$

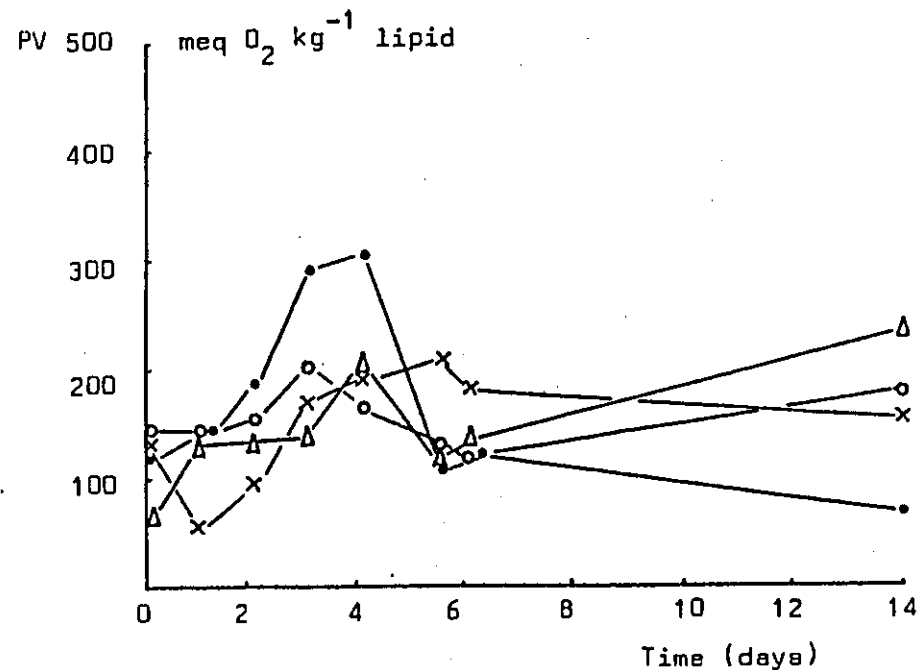


Fig. 35: Peroxide Value (PV) vs Time
for mackerel mince stored at $a_w = 0.51$

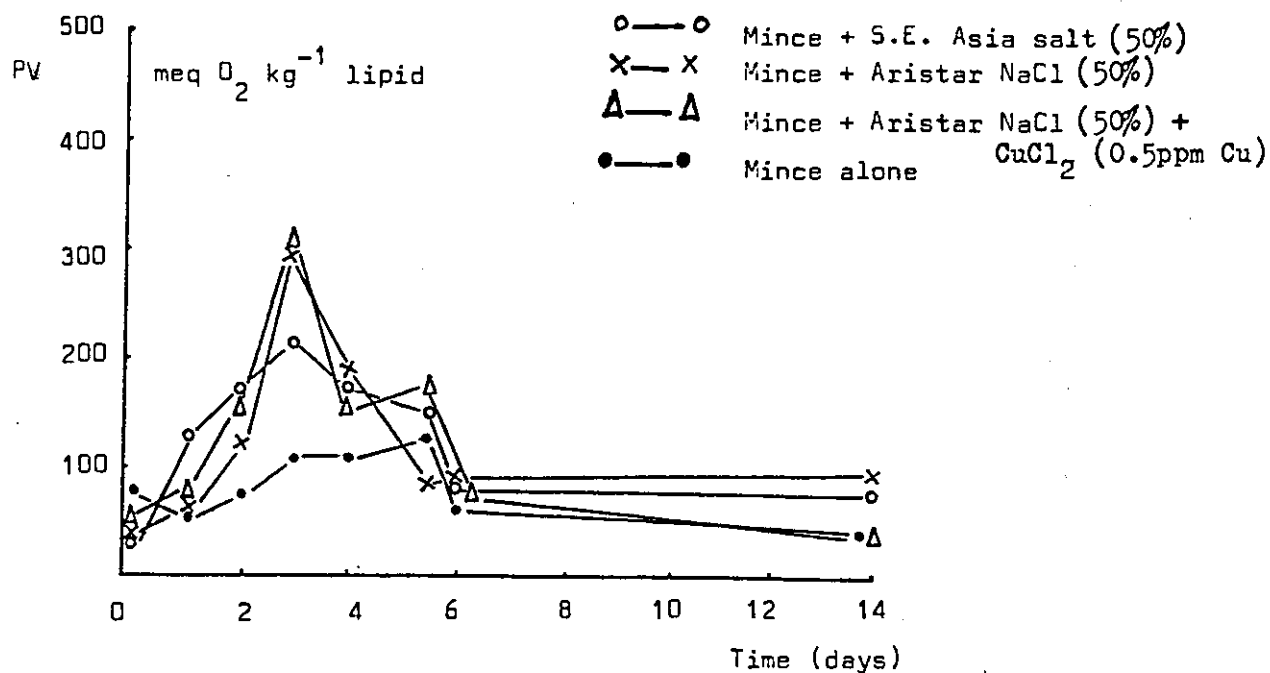
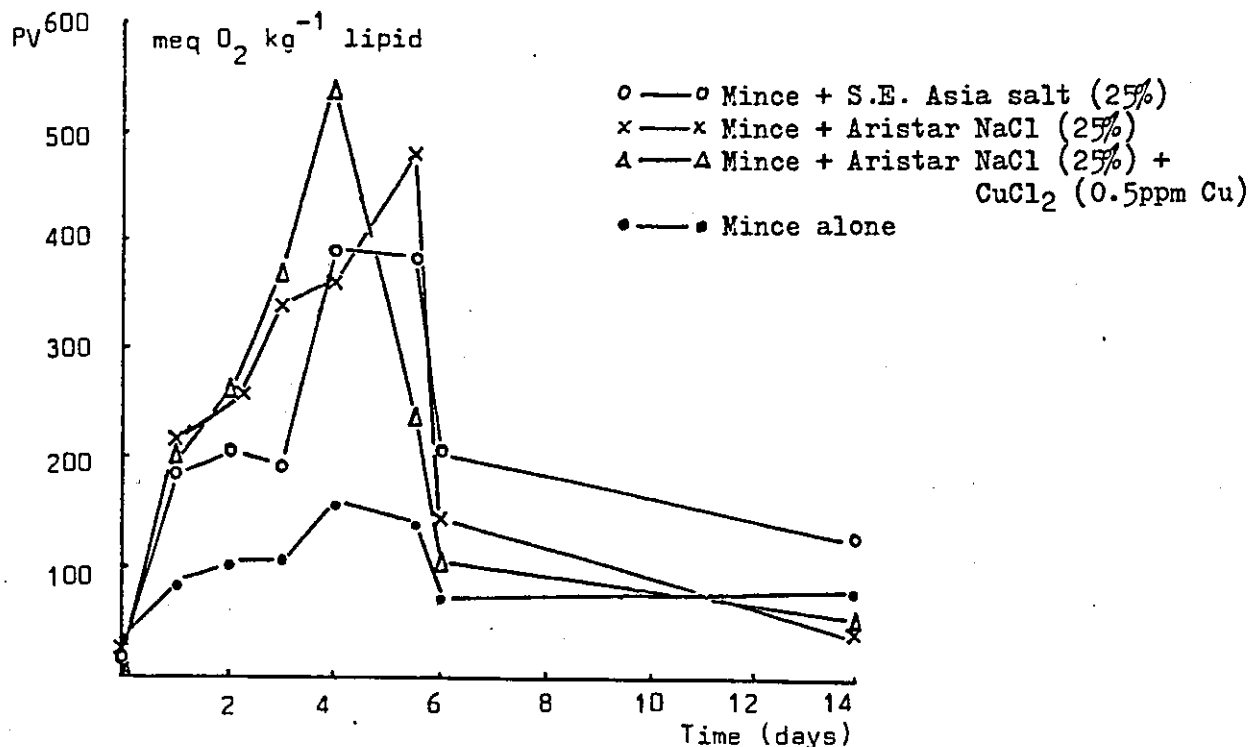


Fig. 36: Peroxide Value (PV) vs Time
for mackerel mince stored at $a_w = 0.84$



showing a short induction period, as was observed in the absence of salt (see Section 4.5.1), reaching a maximum value of $940 \text{ meq O}_2 \text{ kg}^{-1}$ lipid on the 3rd day. The PV dropped equally steeply to a minimum value of $121 \text{ meq O}_2 \text{ kg}^{-1}$ on the 14th day. At $a_w = 0.32$ much lower values were observed for this control sample than at $a_w = 0.14$. It also showed a more extended induction period reaching a maximum of $306 \text{ meq O}_2 \text{ kg}^{-1}$ on the 4th day. Also for this sample much reduced values were observed at $a_w = 0.51$ and only a broad peak of maximum value $129 \text{ meq O}_2 \text{ kg}^{-1}$ lipid was attained between the 3rd and the 6th day. From here values reduced to a minimum of $46 \text{ meq O}_2 \text{ kg}^{-1}$ lipid on the 14th day.

The $a_w = 0.84$ values for the control sample were very similar to those at $a_w = 0.51$ in that they showed long induction periods and broad peaks with maximum value of $158 \text{ meq O}_2 \text{ kg}^{-1}$ lipid on the 4th day, before reducing to a minimum of $75.95 \text{ meq O}_2 \text{ kg}^{-1}$ on the 14th day.

The PV of the mince with Aristar sodium chloride (50% dry wt.), and that which also contained 0.5 ppm Cu, at $a_w = 0.14$ showed a very short induction period, rising sharply to a very prominent maximum peak at about $1000 \text{ meq O}_2 \text{ kg}^{-1}$ lipid on the 4th day. This value is only slightly greater than the maximum observed for the control samples at the same a_w indicating that the sodium chloride is not affecting oxidation rates to a great extent. The values dropped equally steeply to a minimum (between $100\text{--}250 \text{ meq O}_2 \text{ kg}^{-1}$ lipid) on the 6th day, where they remained up to the 14th day.

At $a_w = 0.32$, generally reduced peroxide values were observed for samples with Aristar sodium chloride (50% dry wt.) and with 0.5 ppm Cu. They showed induction periods of about 2 days and reached broad peaks of about $200 \text{ meq O}_2 \text{ kg}^{-1}$ lipid between the 3rd and 4th day.

For these samples at $a_w = 0.51$, the trends of PVs were similar to those observed at $a_w = 0.32$, in that they showed extended induction periods and reached maximum values, with sharp peaks, around $300 \text{ meq O}_2 \text{ kg}^{-1} \text{ lipid}$ on the 3rd day. This is very low when compared to values observed at $a_w = 0.14$, but higher than the maximum value reached by the control sample ($132 \text{ meq O}_2 \text{ kg}^{-1} \text{ lipid}$) at this a_w . A different picture is shown by these samples at $a_w = 0.84$, where a much increased catalytic activity is observed than at $a_w = 0.51$. The induction period for the samples were shorter, rising to a maximum of $544 \text{ meq O}_2 \text{ kg}^{-1} \text{ lipid}$ on the 4th day for the sample with both Aristar sodium chloride (25% dry wt.) and 0.5 ppm copper (dry wt.) and to $481 \text{ meq O}_2 \text{ kg}^{-1} \text{ lipid}$ for that with Aristar sodium chloride (25%) alone, after the 5th day. The slightly higher maximum PV of the sample with Aristar sodium chloride (25% dry wt.) and 0.5 ppm Cu could be attributed to the effect of the copper but the difference is rather small to be definite. At higher copper levels this difference might be more obvious.

The sample with South East Asia salt (50% dry wt.) at $a_w = 0.14$, like the control sample, showed an extended induction period and also rose to a high maximum value of $682 \text{ meq O}_2 \text{ kg}^{-1}$ after 4 days. The much extended induction period and the low maximum PV, relative to the rest at this a_w , is probably related to the more granular nature of this salt rather than to any impurities, since copper added at 0.5 ppm to the Aristar sodium chloride (a level higher than in S.E. Asia salt) has no apparent effect. Also observed for this sample at $a_w = 0.32$ as at $a_w = 0.14$ is an extended induction period, but the maximum PV ($200 \text{ meq O}_2 \text{ kg}^{-1} \text{ lipid}$) is lower than was observed at $a_w = 0.14$. This level is almost maintained ($217 \text{ meq O}_2 \text{ kg}^{-1}$

lipid) for this sample at $a_w = 0.51$, showing on the whole a similar trend. But at $a_w = 0.84$ a shorter induction is observed and the maximum PV reached on the 4th day is almost double, at about 400 meq $O_2 \text{ kg}^{-1}$ lipid.

Fig. 37 shows the relationship between maximum peroxide values reached and a_w . The trends suggest a high rate of lipid oxidation at low a_w , decreasing as the a_w increases to minimum values between $a_w = 0.32$ and 0.53 , as was found for this system in absence of high amounts of salt. However, the influence of salt at $a_w = 0.84$ is evident from the significantly higher PVs in these samples than in the control sample at the same a_w . Copper (0.5 ppm) overall shows some catalytic property at $a_w = 0.84$ but its effect is still minor.

4.6.2 Thiobarbituric acid value changes

The TBA changes are given in Figs. 38, 39, 40 and 41.

The TBA changes at $a_w = 0.14$ of the control sample (i.e. mince alone) showed a slight induction period and rose to a maximum of 756 mg MA kg^{-1} dry matter on the 4th day. Thereafter it reduced to a minimum of about 232 mg MA kg^{-1} dry matter on the 14th day. A reduced activity was observed for this sample at $a_w = 0.32$. The maximum value reached here on the 2nd day was 389 mg MA kg^{-1} dry matter and the minimum value (208 mg MA kg^{-1} dry matter) was similar to that at $a_w = 0.14$. However, at $a_w = 0.51$ a much reduced activity is observed for the mince alone, with an early maximum of 112 mg MA kg^{-1} dry matter on the 2nd day followed by a decline to values as low as 27 mg MA kg^{-1} dry matter. A very similar trend is observed for this sample at $a_w = 0.84$ in that the maximum TBA (262 mg MA kg^{-1} dry matter) which occurred on the 2nd day is lower than that observed at $a_w = 0.14$ and is followed by a decline to values

Fig. 37: Maximum Peroxide Value (max. PV) vs water activity
for mackerel mince stored at constant water activities
and high NaCl levels

- Mince + S.E. Asia salt
- x—x Mince + Aristar NaCl
- Δ—Δ Mince + Aristar NaCl + CuCl₂
- Mince alone

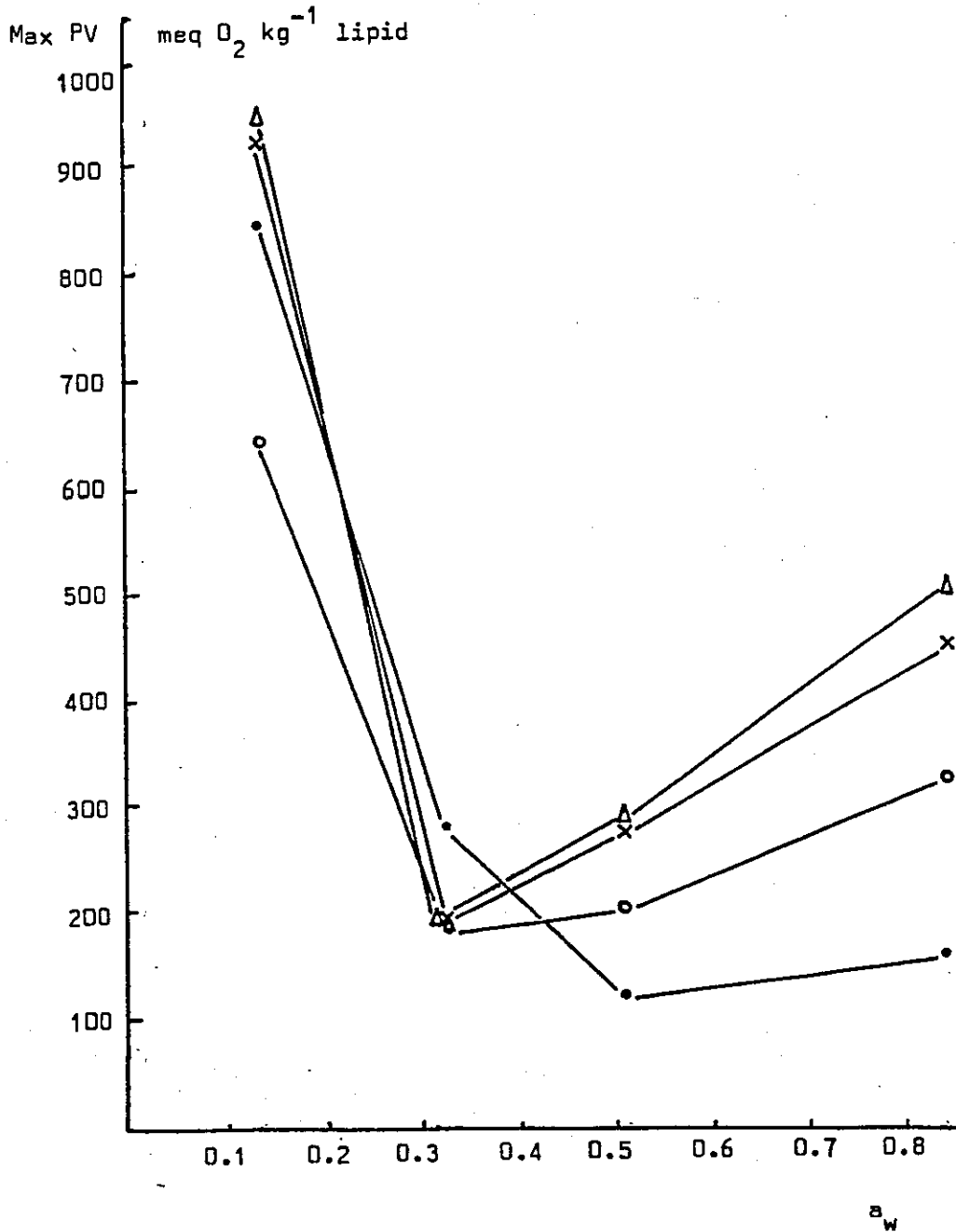


Fig. 38: Thiobarbituric acid value (TBA) vs Time
for mackerel mince stored at $a_w = 0.14$.

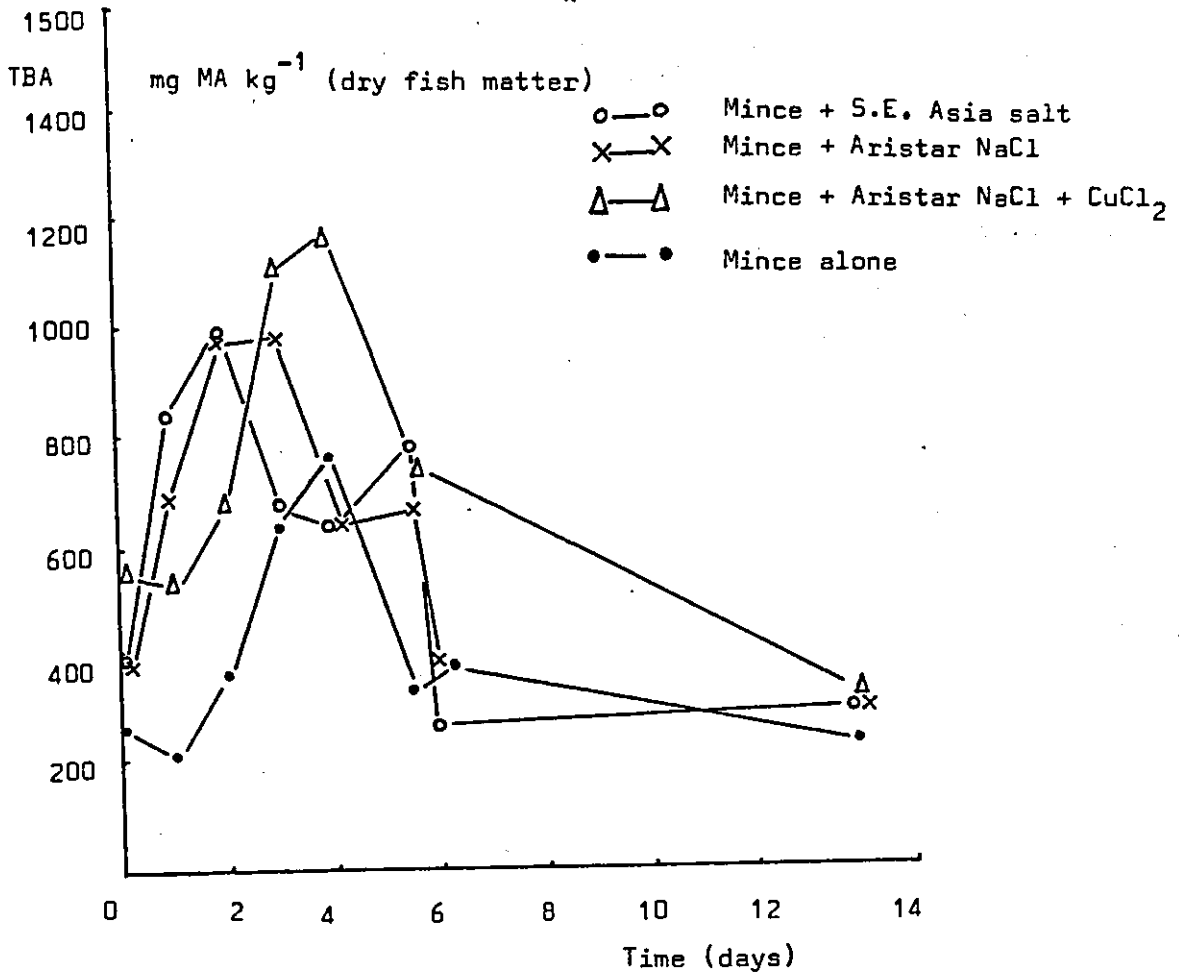


Fig. 39: Thiobarbituric acid value (TBA) vs Time
for mackerel mince stored at $a_w = 0.32$

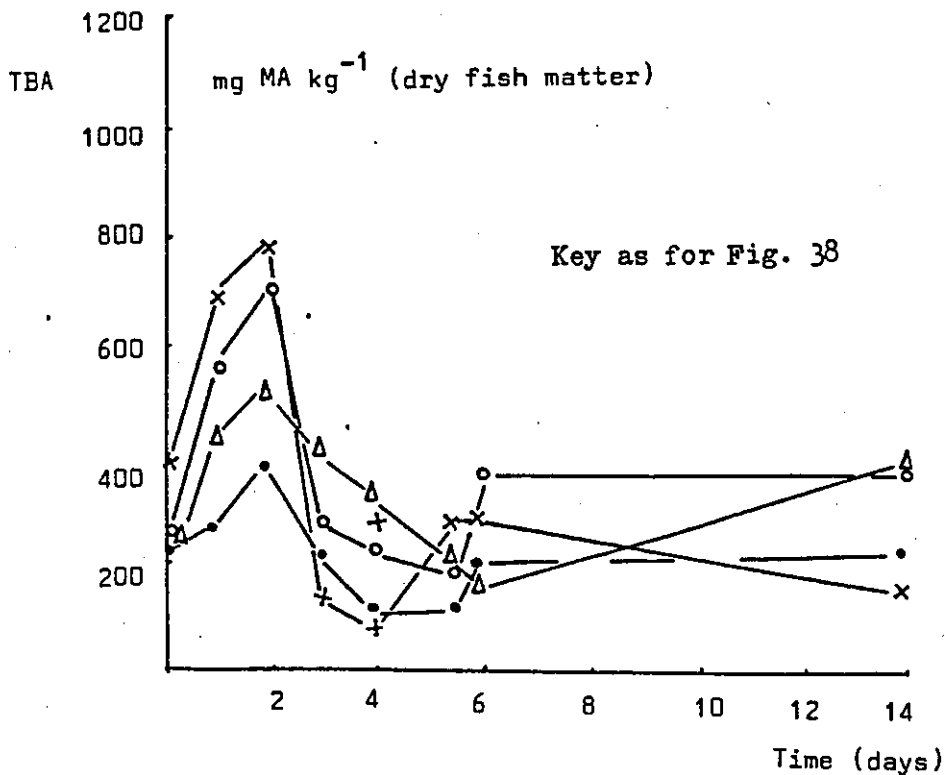


Fig. 40: Thiobarbituric acid value (TBA) vs Time
for mackerel mince stored at $a_w = 0.51$

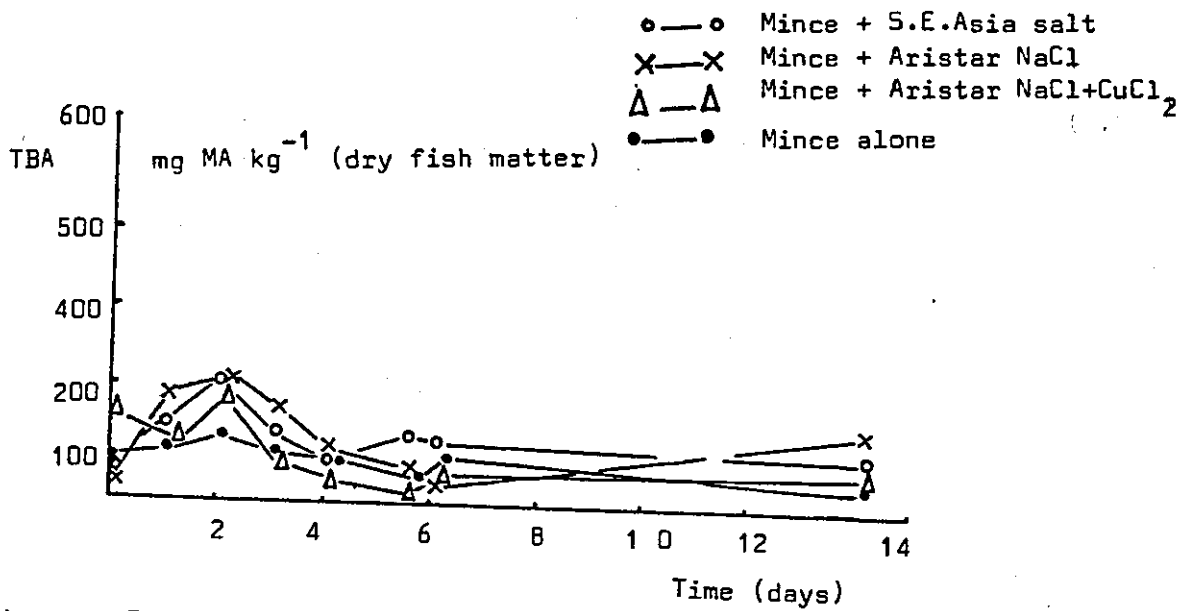
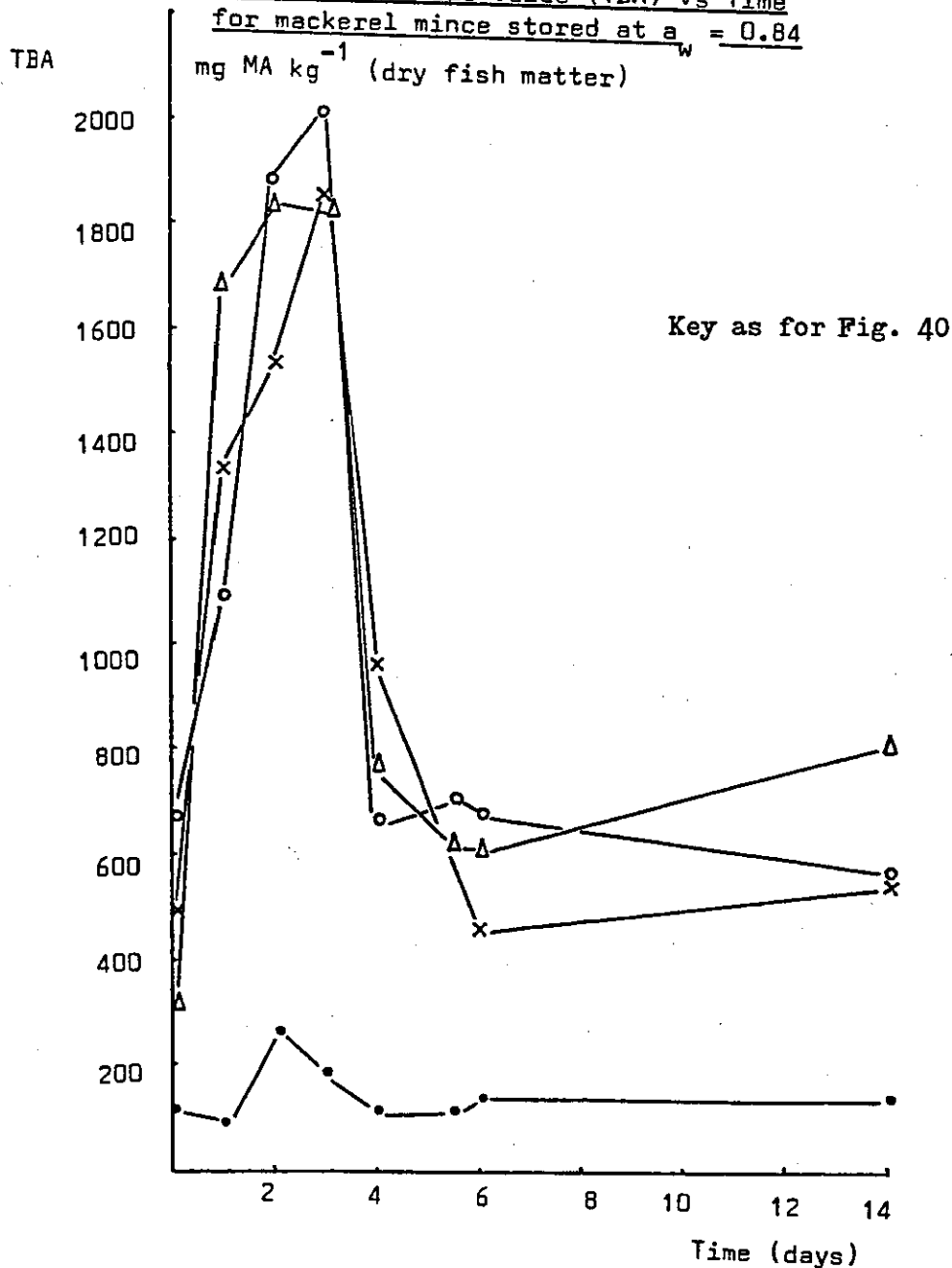


Fig. 41: Thiobarbituric acid value (TBA) vs Time
for mackerel mince stored at $a_w = 0.84$



similar to those at $a_w = 0.51$.

The sample with Aristar sodium chloride (50%) at $a_w = 0.14$ did not show any induction period but rose sharply to maximum value of about 1000 mg MA kg⁻¹ dry fish matter on the 2nd day and then reduced to a minimum (297 mg MA kg⁻¹ dry fish matter) on the 14th day. A similar trend but reduced activity was observed at $a_w = 0.32$ as the TBA values showed no induction period, instead it rose to a maximum of 798 mg MA kg⁻¹ dry fish matter on the 2nd day. A minimum (75 mg MA kg⁻¹ dry fish matter) for this sample was observed on the 4th day but the sample shows a minor maximum on the 6th day before dropping finally to a low value of 160 mg MA kg⁻¹ dry fish matter. As was found for the control sample there was generally a much reduced activity at $a_w = 0.51$. Maximum value was only 209 mg MA kg⁻¹ on the 2nd day and fell gradually to a minimum TBA value of 129 mg MA kg⁻¹ on the 14th day. A dramatic rise is observed for this sample at $a_w = 0.84$. TBA values showed no induction period at all but rose to a maximum (1517 mg MA kg⁻¹ dry fish matter) on the 2nd day and also dropped sharply to a minimum (450 mg MA kg⁻¹ dry fish matter) on the 6th day. The values were observed to change only slightly up to the 14th day (~ 540 mg MA kg⁻¹ dry fish matter).

The sample containing Aristar sodium chloride (50% dry wt.) and 0.5 ppm Cu (dry wt.) showed generally a very similar trend as that which had Aristar sodium chloride alone. At $a_w = 0.14$ however, a short induction period up to the 2nd day and a slightly higher maximum value (1074 mg MA kg⁻¹ dry fish matter) were observed on the 4th day. This was followed by a sharp drop to a minimum value of about 300 mg MA kg⁻¹ dry fish matter on the 14th day. A more reduced activity was observed for this sample at $a_w = 0.32$, the maximum attaining just 500 mg MA kg⁻¹ dry fish matter on the 2nd day

and followed by a drop to a minimum value ($150 \text{ mg MA kg}^{-1}$ dry fish matter) on the 6th day. A secondary minor peak occurred between the 6th and the 14th day. TBA values for this sample at $a_w = 0.51$ were low as for the rest of the samples and reached a maximum of only $200 \text{ mg MA kg}^{-1}$ dry fish matter on the 2nd day. Thereafter, it dropped to very low values (10 mg MA kg^{-1} dry fish matter) before the 6th day and showed a minor peak (50 mg MA kg^{-1} dry fish matter) between the 6th and 14th day. Like the sample with Aristar sodium chloride alone (50% dry wt.), this sample (with 0.5 ppm Cu) at $a_w = 0.84$ showed no induction period but rose sharply to a high maximum value ($1820 \text{ mg MA kg}^{-1}$ dry fish matter) on the 2nd day. This was also followed by a sharp drop to a minimum (about $600 \text{ mg MA kg}^{-1}$ dry fish matter) on the 5th and 6th day, from where it peaked again between the 6th and 14th day.

The mackerel mince sample containing 50% dry wt. of South East Asia salt at $a_w = 0.14$, like the rest of the samples with salt, showed a marked deviation from the control sample. After 2 days it reached a higher maximum TBA value ($997 \text{ mg MA kg}^{-1}$ dry fish matter), from where it proceeded to decline till the 4th day ($630 \text{ mg MA kg}^{-1}$ dry fish matter) peaking again before the 6th day ($777 \text{ mg MA kg}^{-1}$ dry fish matter) and finally declining to about $250 \text{ mg MA kg}^{-1}$ dry fish matter on the 14th day. At $a_w = 0.32$ the TBA values for this sample resembled very closely those of the sample with Aristar sodium chloride. A maximum value of $713 \text{ mg MA kg}^{-1}$ dry fish matter occurred on the 2nd day. Further lower values were observed for the minimum TBA value ($787 \text{ mg MA kg}^{-1}$ dry fish matter) before the 6th day and thereafter levelling off at $370 \text{ mg MA kg}^{-1}$ dry fish matter up to the 14th day. Again at $a_w = 0.51$ for this sample, as with the rest, a much reduced activity was observed. A low maximum TBA value of about

200 mg MA kg⁻¹ dry fish matter was reached followed by a drop to minimum values levelling off at about 120 mg MA kg⁻¹ dry fish matter. As with the other salt containing samples, TBA values of this sample (mince with S.E. Asia salt 50% dry wt.) are very high. Without any induction period it attained a maximum of 2012 mg MA kg⁻¹ dry fish matter on the 3rd day, followed by a fall on the 4th to values around 600 mg MA kg⁻¹ dry fish matter till the 14th day.

Fig. 42 shows the relationship between maximum TBA values and a_w . This graph reveals a high level of malonaldehyde production from lipid oxidation at $a_w = 0.14$ and at $a_w = 0.84$. The least activity being observed at $a_w = 0.51$ followed by that at $a_w = 0.32$.

Also, in the a_w range 0.14 to 0.51 the maximum values were very similar, occurring at a level slightly above that of the control sample, but at $a_w = 0.84$ the samples with additives attain very high maximum values, compared to the control sample.

As for the changes in PVs, the changes in TBA values when compared at fixed times for the overall range of a_w 's studied showed similar trends.

4.6.3 Colour production

The colour production data are plotted in Figs. 43, 44, 45 and 46.

In this model system the development of colour followed a similar trend to that found for mince in the absence of large amounts of salt, in that higher values of extractable colour were observed at the extremes of a_w 's (i.e. $a_w = 0.14$ and 0.84) with minimum values being observed in the middle range ($a_w = 0.51$). However, unlike the previous systems, in the copper containing sample the absorbances here at $a_w = 0.84$ were much higher than those at

Fig. 42: Maximum Thiobarbituric acid value (max. TBA)
vs water activity

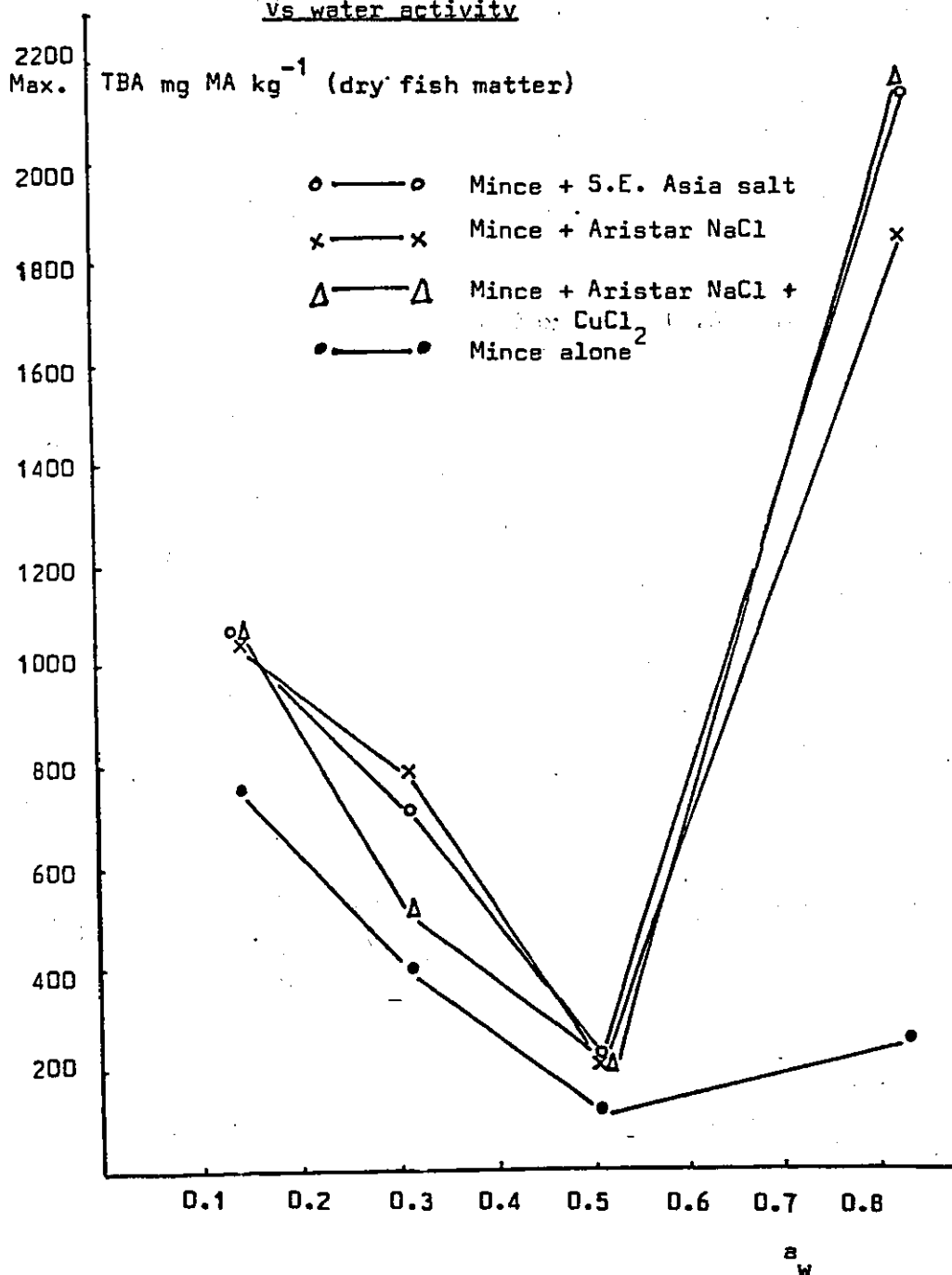


Fig. 43: Colour (COL) vs Time for mackerel mince stored at $a_w = 0.14$

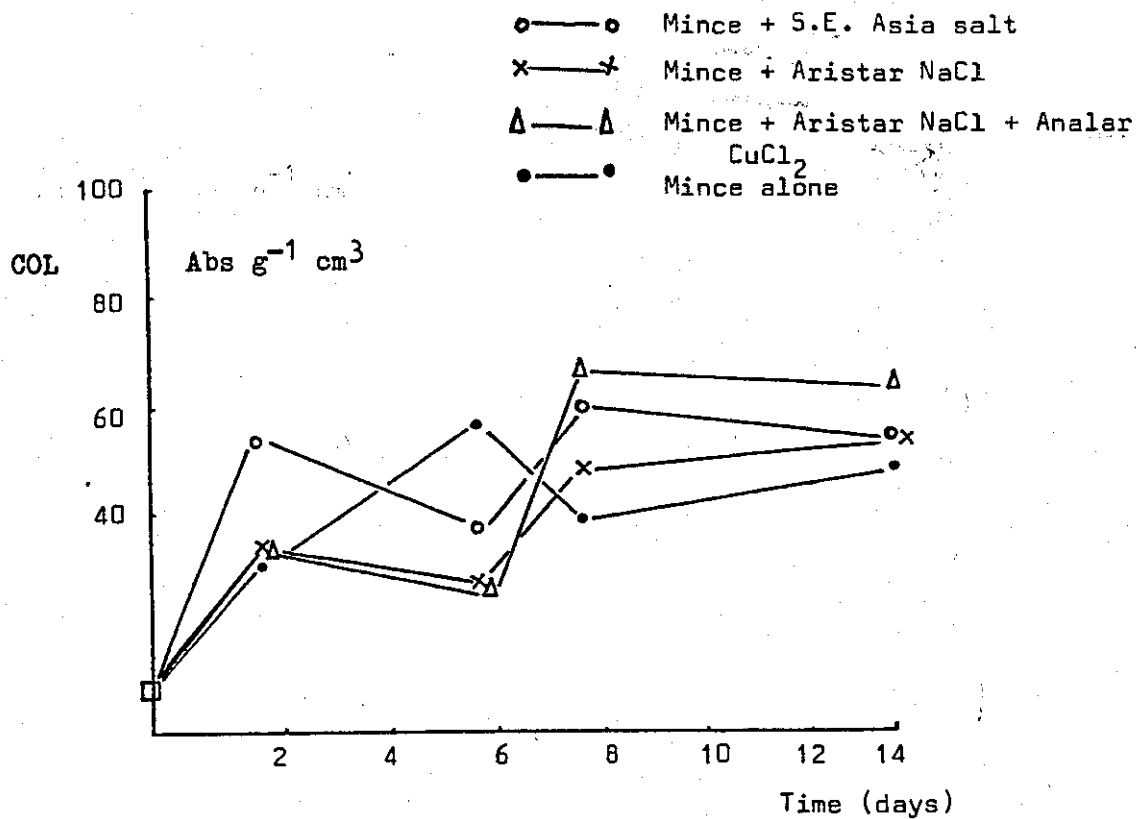


Fig. 44: Colour (COL) vs Time for mackerel mince stored at $a_w = 0.32$

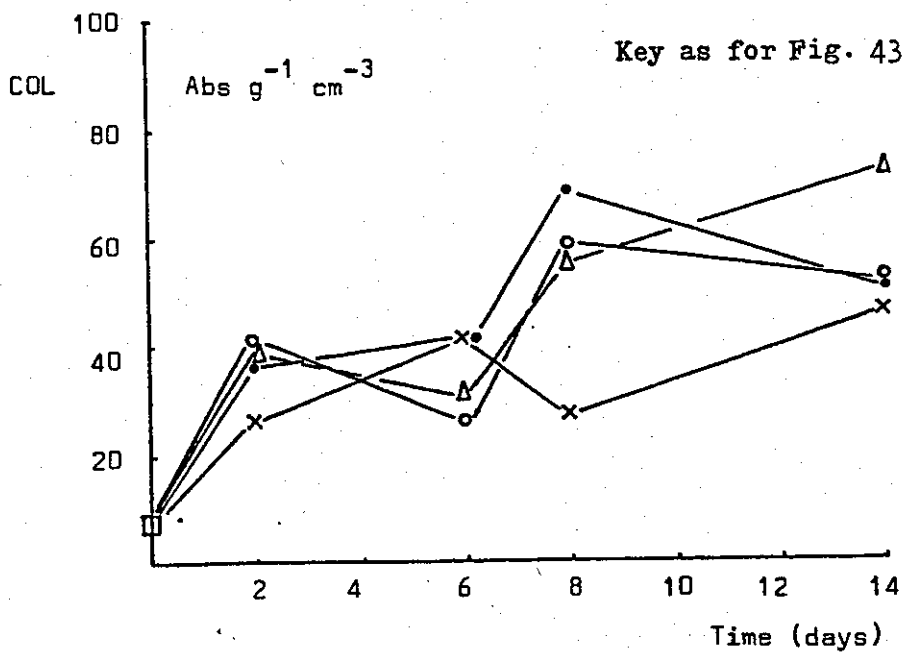


Fig. 45: Colour (COL) vs Time for mackerel mince stored at $a_w = 0.51$

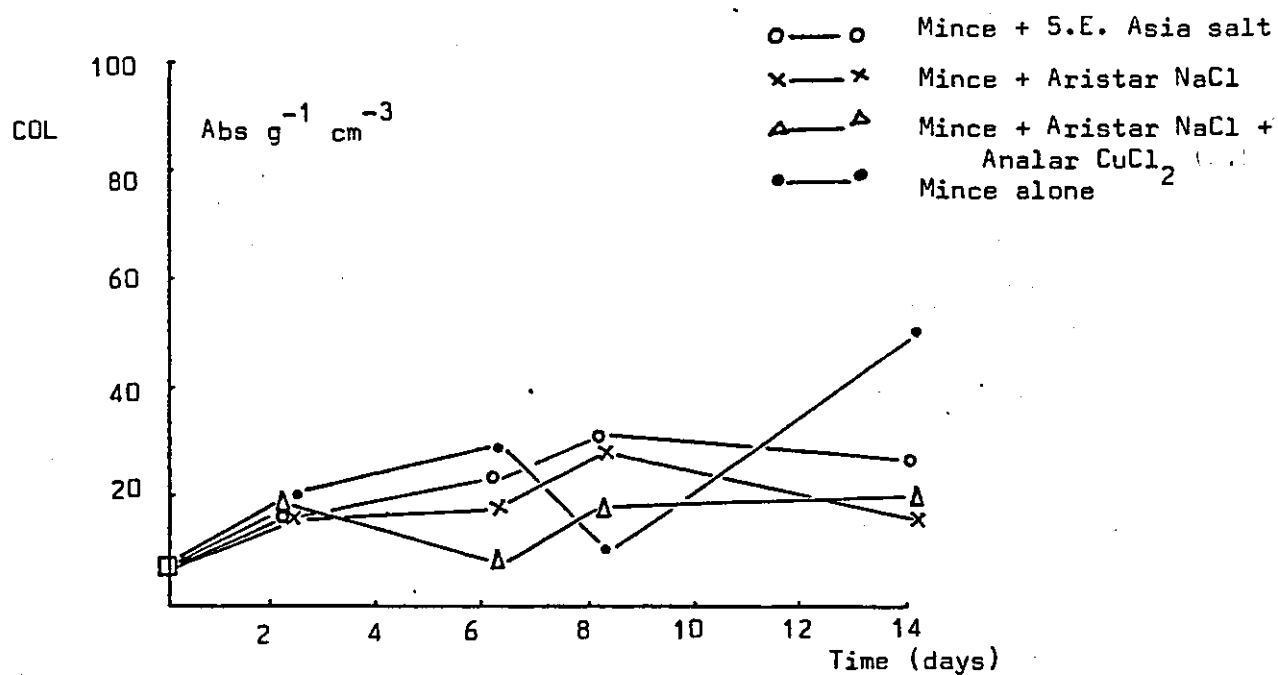
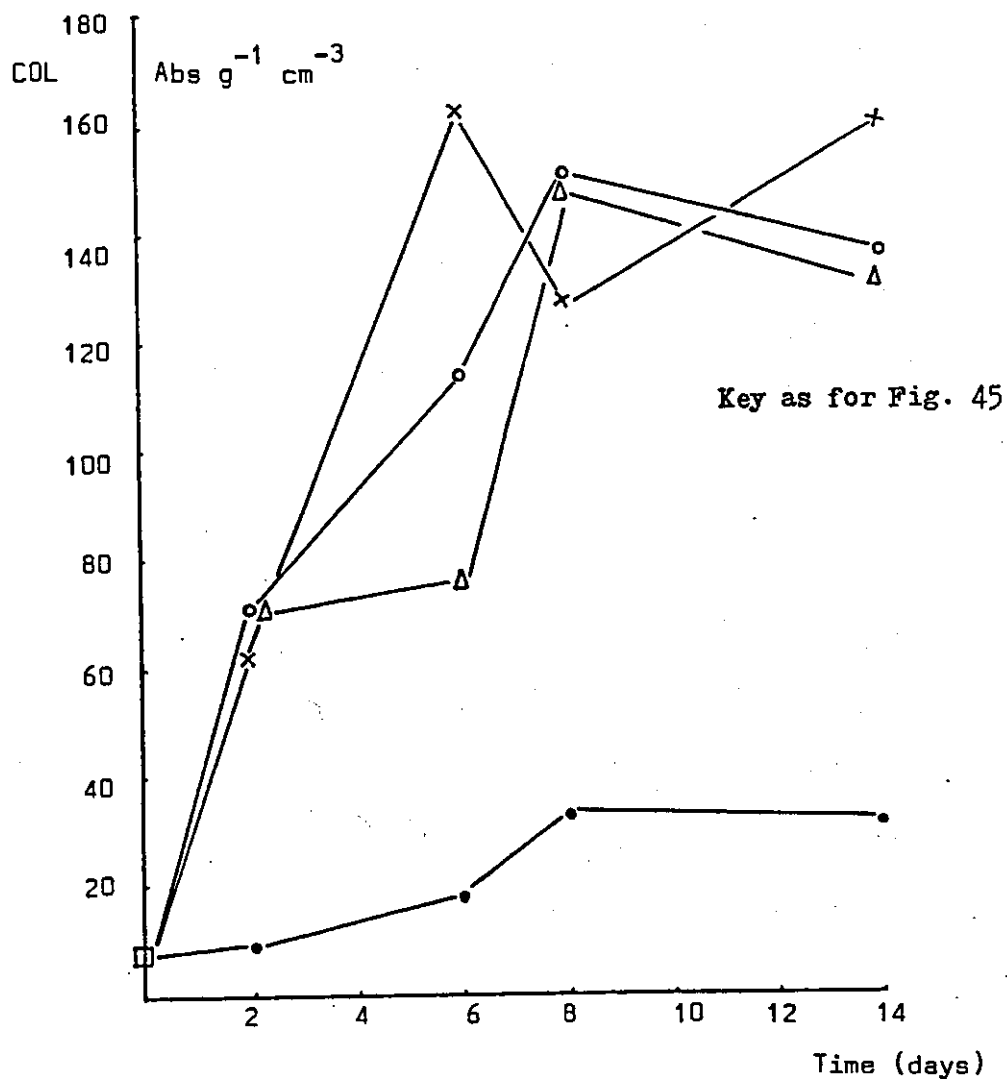


Fig. 46: Colour (COL) vs Time for mackerel mince stored at $a_w = 0.84$



$a_w = 0.14$ for the extracted colour and this was reflected visually.

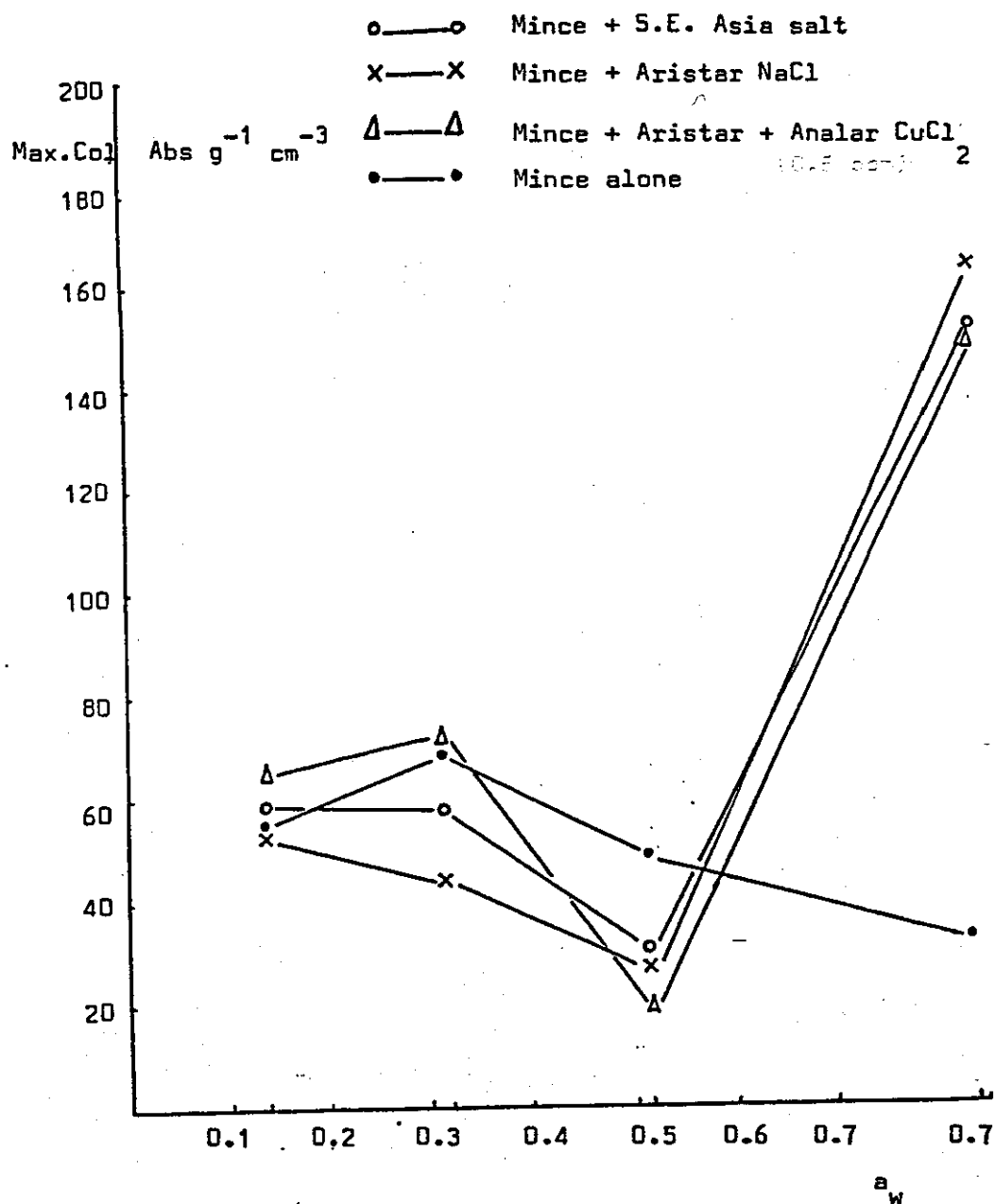
At $a_w = 0.14$ in presence of salt all the samples showed close absorption values (45 to 65 $\text{abs g}^{-1} \text{cm}^{-3}$ dry fish matter) with the mince with copper (0.5 ppm Cu^{2+}) being slightly higher. Maximum values were recorded on the last day (14th) of the run.

A similar observation was made for all the samples at $a_w = 0.32$, where the samples with additives did not vary much from the control sample. However, the sample containing 0.5 ppm Cu^{2+} now showed a wider gap in absorbance at about $72 \text{ Abs g}^{-1} \text{cm}^{-3}$ dry fish matter than the rest which remained around $50 \text{ Abs g}^{-1} \text{cm}^{-3}$ dry fish matter on the 14th day.

At $a_w = 0.51$ a much reduced colour production is observed generally, hardly exceeding $20 \text{ Abs g}^{-1} \text{cm}^{-3}$ dry fish matter for all samples. However, at $a_w = 0.84$ a dramatic increase in absorbances are observed for all samples except the control which remained slightly above $30 \text{ Abs g}^{-1} \text{cm}^{-3}$. Highest absorbances for samples with S.E. Asia salt (25% dry wt.) and Aristar sodium chloride (25% dry wt.) were observed on the 8th day at about $150 \text{ Abs g}^{-1} \text{cm}^{-3}$ dry fish matter. The sample with Aristar sodium chloride alone (25% dry wt.) had a maximum absorbance ($162 \text{ Abs g}^{-1} \text{cm}^{-3}$ dry fish matter) on the 6th day.

A general graph relating the maximum colour values to the water activities is given in Fig. 47. When this graph is compared with the equivalent graph for the study of changes in the absence of high levels of sodium chloride (Fig. 29, page 93), the catalytic effect of sodium chloride at high a_w 's can be clearly seen. (See also Plate 4 in the Appendix.)

Fig. 47: Maximum Colour Values (max. CDL.) vs water activity



4.7 GENERAL DISCUSSION

Minced mackerel is a highly heterogeneous system and so precautions were taken to minimise the likely variation in results. Emphasis was laid on vigorous uniform mixing (without introducing extraneous metal ions) and careful sampling. Thorough mixing of additives appeared effective as indicated by the fact that colour production was uniform as visually observed (see Colour Plates 1, 2, 3, 4 in the Appendix).

Malonaldehyde is known to be mutagenic and carcinogenic but the level of significance to human health of any of the concentrations reported here is not known (Shamberger *et al.*, 1977) due to the variability in the level of occurrence in various foods. A sensory test was therefore not conducted in this study due to the unwholesomeness of the food material resulting from the additives used and the conditions applied.

In this investigation TBA values obtained on storage were much higher than those observed in other studies reported in the literature. PV and TBA values found in this study ranged from 6.0-1000 meq O_2 kg^{-1} lipid and 1.5-2200 mg MA kg^{-1} dry fish matter respectively. Literature values are similar for PVs (Koizumi *et al.*, 1981; Labuza, 1971, 1981) but observed TBA values in natural foods range from 0-39 mg MA kg^{-1} (Gray *et al.*, 1981; Shamberger *et al.*, 1977). However, these literature results were obtained in food systems that were not dehydrated and did not relate closely to the mackerel mince as used in this investigation, i.e. a freeze-dried system containing highly unsaturated lipids.

The conventionally accepted mechanism of lipid oxidation when related to measurements of PV and TBA (see Section 2.3.1.1) would suggest that the PV will reach its maximum value before the TBA because the breakdown products of hydroperoxides (malonaldehyde) are

measured by the TBA test. However, observations in this study indicate otherwise. Instead of the PV showing the expected steady rise and fall as hydroperoxides form and breakup (as predicted from reaction kinetics, Labuza, 1971), a major peak followed by one or more minor peaks were observed over the period studied. The TBA also deviates from the expected steady rise in malonaldehyde production to a maximum concentration, and instead a rise and fall in values were observed. Similar observations have been made in some reported studies (Lanier *et al.*, 1981; Labuza, 1981). This evidently reflects the heterogeneity of the sample and the complexity of the myriad of reactions possible in such a system. Explanations which can be offered for these occurrences are that in a very highly oxidizing system of this nature, the higher unsaturated fatty acids (the linolenic and more unsaturated types) are preferentially oxidised to give the first major peak of hydroperoxides and these then break down to give malonaldehyde measured as the thiobarbituric acid value. The less unsaturated ones i.e. the di-unsaturated forms such as linoleic acid and the monounsaturated forms such as oleic acid) are more slowly oxidised to produce subsequent peaks in PV and also TBA as formed by the alternative route described previously (see Section 2.3.1.1). Because the main route to malonaldehyde production is oxidation of linolenic type fatty acids and in the mackerel system the percentage content of this type is low (see Table 6) and is thus rapidly exhausted, the TBA values reach a peak before the PV.

Changes in the PV and TBA values as they relate to colour production are discussed later (see Section 4.7.4).

4.7.1 Effect of sodium chloride on lipid oxidation

In the S.E. Asia process simulation, samples containing salt were observed to have high PVs compared to the control samples, (see Figs 16 and 17). The salt here may be acting through its ability to retain water by a solvation process (Sherman, 1961), giving a more polar environment and thus stabilizing the newly formed hydroperoxides through hydrogen bonding. This water binding property is reflected in the maintenance of the a_w at the saturation level of $a_w = 0.75$ for a major part of the run (see desorption isotherm Fig. 15). Further experiments in Section 4.4 and 4.6 reveal that at this water activity, peroxide values are high and compare fairly closely to the values found even in the control sample at the same water activity (see Figs 35, 36 and 37). Thus it appears that sodium chloride is acting here merely as humectant, which at saturation level can keep the water activity at 0.75.

At a fixed water activity (see Section 4.4) sodium chloride at 10 ppm did not seem to affect the rate of lipid oxidation at any of the a_w 's studied since the rate of change of PV were similar to that of the control sample.

However, sodium chloride and S.E. Asia salt (50% or 25% dry wt. of mince) at $a_w = 0.51$ and 0.84 (see Section 4.6, Figs 35 and 36) are seen to catalyse lipid oxidation as measured by PV and TBA. This suggests therefore, that at these high concentrations, the presence of a high concentration of "solvent water", which would aid ion mobility, enables the sodium chloride to catalyse the formation and also the breakdown of hydroperoxides. As was observed by Labuza (1971), at this high level "solvent water" access to reaction sites is greater. The effect will, therefore, overwhelm the hydration of the ions at this high "solvent water" level which would otherwise lead to a reduced catalytic effect.

4.7.2 Effect of copper chloride on lipid oxidation

In the S.E. Asia process 10 ppm of copper chloride was observed to catalyse lipid oxidation, as measured by PV, to a lesser extent than sodium chloride (33.6% of mince moisture) but slightly more than the control. This effect could be explained by the fact that copper binds very readily to sulfhydryl groups of proteins (Winge *et al.*, 1975) causing denaturation. This could then lead to the exudation of moisture from the tissues causing a sharp reduction in moisture content through a faster dehydration rate and therefore lower the a_w of the mince to about $a_w = 0.51$ in 4 days (see desorption isotherm Fig. 15). At this a_w , as has been shown (see Section 4.4 and 4.6), lipid oxidation is very slow indeed. Another view is that copper at this level is very actively causing the breakdown of any hydroperoxides formed to malonaldehyde. At a fixed $a_w = 0.53$ (see Section 4.5) copper at 3.0 ppm and 1.5 ppm showed a marked difference in TBA value compared to the control sample (see Figs 30 and 31) while copper at 0.5 ppm did not show much catalytic effect over the same period.

However, in presence of sodium chloride, as in the S.E. Asia process, copper (10 ppm) is seen to have a synergistic pro-oxidant effect, as measured by PV, by boosting the lipid oxidation effect of the sodium chloride (see Figs 16 and 17). An explanation for this comes from the fact that copper alone (10 ppm), as indicated earlier, will react with proteins and lead to a lowering of the a_w . But in presence of salt (saturation level) moisture is retained and this offsets the effect of copper. The resulting ease of mobility of the Cu^{2+} leads to higher catalytic effect on hydroperoxide production and at this high a_w level the hydroperoxides are stabilized through hydrogen bonding.

From Fig. 28 it can be seen that at low a_w (i.e. $a_w = 0.14$) and in the absence of large amounts of salt, copper (10 ppm) has very little effect on lipid oxidation (as measured by PV). Also at 0.5 ppm in presence of salt (Fig. 33) its catalytic effect is not apparent either, at this a_w . However, at the higher a_w 's and in absence of large amounts of salt, the catalytic effect of 10 ppm copper becomes very apparent. The effect of copper at 0.5 ppm in presence of salt is also obvious but minor. This can again be attributed to the presence of high "solvent water" which aids the mobility, accessibility and reactivity of the ions at reaction sites. While at low a_w (i.e. $a_w = 0.14$) lipid oxidation is due mainly to direct contact with oxygen rather than through a copper or sodium chloride catalysed reaction (see Section 2.3.1.1). This is so because at this a_w the fish matrix is dry and porous, allowing direct diffusion of oxygen to oxidize the lipids. The additives at this stage are more localized and their potency as catalysts of lipid oxidation is not so apparent. Hydroperoxides formed at this low a_w will be more stable - hence the high PVs and the relatively low TBA values (Figs 37 and 42) as compared to PV and TBA values at $a_w = 0.84$ in presence of additives. This suggests therefore that at high a_w the additives besides catalysing the formation of hydroperoxides are also involved in their subsequent breakdown.

4.7.3 Effect of sodium chloride and copper chloride on colour production

Sodium chloride at 36.3% of mince moisture did not seem to produce as much extractable or visual colour as the samples containing solely copper (10 ppm). Copper chloride, however, at 10 ppm produced a lot more colour which was also visually perceptible (see Figs 18 and 19).

At fixed water activities sodium chloride at 10 ppm did not show any marked difference in colour producing capability than was found in the control through the a_w range 0.14 to 0.84 while copper alone, also at 10 ppm, showed a marked colour producing effect as visually observed (see Fig. 29).

As already described in Section 2.3.2,1, colour production in these model systems involves non-enzymic (i.e. Maillard browning) and are as a result of the reaction between by-products of lipid oxidation (short chain carbonyls) and the amino groups of proteins and amino acids. Sodium chloride does not have a similar colour producing effect as copper chloride in the system studied. Copper ions are known to denature proteins to expose more reaction sites and to catalyse lipid oxidation. Results of experiments in Section 4.5 show that the lowest level of activity of copper in rapid colour production is 1.5 ppm (see Fig. 32). The role of oxygen access to mince and rancidity development is discussed in Section 4.7.4. From these considerations the difference in effectiveness of copper chloride in producing more colour than sodium chloride can be deduced from the ionic and atomic radii of the reacting species, according to Guert (Duckworth, 1981) (see Fig. 8 Section 2.3.1.3).

Oxygen (atomic radii 0.074 nm) is smaller than sodium (ionic radii 0.098 nm) and therefore will be in more intimate contact with the protein and lipid at the protein/lipid water interface and help form the hydrogen bonds. Copper ion (ionic radii 0.072 nm) is smaller than both oxygen and sodium ions (Mahan, 1972) and therefore will be more reactive with the proteins and lipids leading to protein denaturation and breakdown of the hydroperoxides by preventing hydrogen bonding. This gives a pool of short chain carbonyls and several amino groups. Furthermore, in the transition metal series, copper has the smallest ionic radii and has been found by Castel *et al.* (1965) to be the most reactive amongst them in lipid oxidation.

These additives at low water activities (e.g. $a_w = 0.14$) in presence or absence of salt do not seem to play a very significant part in the colour production as absorbance of the control sample is also high at this low water activity range. Therefore, colour production here could probably be due to the direct oxidation of lipids by oxygen through the porous fish matrix to provide the reacting carbonyls (see Fig. 47). Copper at 10 ppm shows a catalytic effect at this low water activity but the extent is not very remarkable. The presence of large amount of salt appears to have subdued colour production at this low a_w (see Fig. 47). This could be related to problems of diffusibility of oxygen and contact with the fish mince lipids. At high a_w ($a_w = 0.84$) copper alone at 10 ppm or 25% sodium chloride with added copper (0.5 ppm) are shown to have high catalytic effects on colour production. This can again be attributed to the high "solvent water" which aids reacting species dispersion and accessibility to reaction sites, and hence the very high TBA values that are recorded at this a_w .

The drop in extracted colour for mince with 10 ppm copper alone, at $a_w = 0.84$ especially, and the occasional drop observed for other samples were not noticed visually and could be due to structural changes in the pigments to give less extractable coloured compounds (melanoidins) (see Colour Plate 4 in the Appendix).

4.7.4 Effect of water activity on rancidity development

High PV and TBA values have been observed at $a_w = 0.14$ both in absence and presence of sodium chloride alone and copper chloride alone and could be related to the ease of access of oxygen to the mince tissue. These high values at this a_w are reflected in the relatively high extracted colour observed at this a_w , as breakdown

products are channelled into Maillard browning reactions. So the reduced PV, TBA and colour observed as water is added to the system to increase the water activity ($a_w = 0.32-0.51$) reveals that water is acting here as an anti-oxidant. This effect has been thought of as water dislodging the oxygen from the tissues. Also it has been seen as the solvation of metal ions in the system rendering them incapable of catalyzing the development of rancidity. But the results of experiments in Section 4.5 with 0.2% EDTA added to mince show that the intrinsic metals of the fish mince is not in a form or quantity for lipid oxidation catalysis, as the PV and TBA values of this sample did not differ very much from that of the control sample (see Figs 30 and 31). So only added metal additives can be solvated in this way, and any excess would be expected to lead to rancidity development. This effect has been shown in the results of the experiments reported here (see for example Fig. 31). It is of interest to note that the colour observed throughout the a_w range varied directly with the TBA values.

The lowest values of PV, TBA and colour were observed at $a_w = 0.51$. But as the a_w increased past this range water becomes slightly pro-oxidant i.e. PV and TBA values of control samples were increased. This effect is, however, greatly amplified by the presence of copper and sodium chloride at 25% dry weight of mince (see Figs 37 and 42). This is contrary to the reduced catalytic effect that might be expected from increased hydration of metal ions and stability of hydroperoxides through hydrogen bonding. Instead, the higher TBA values at $a_w = 0.84$ than at $a_w = 0.14$ and the lower PV at $a_w = 0.84$ than at $a_w = 0.14$ in the presence of the additives indicates positively the active breakdown of the hydroperoxides, due to the presence of the additives at $a_w = 0.84$. Hence the mobility of the reacting species and contact with reaction sites seem to be the more important considerations.

The previously reported reduced catalytic effect at high water activities (i.e. greater than 0.84) due to dilution aspects was not investigated.

5.

CONCLUSIONS

The results of the experiments reported in this study allow the following conclusions to be reached.

- A: Salt samples collected from S.E. Asia contained higher levels of calcium, magnesium and iron than samples collected in the U.K. However, all the samples had copper levels below 0.02 ppm.
- B: The intrinsic metal content of mackerel mince does not appear to significantly affect the rate of lipid oxidation.
- C: Pure sodium chloride at levels used in fish processing significantly catalyses lipid oxidation of fatty fish at water activities above about 0.5.
- D: Addition of sodium chloride at 10 ppm had no effect on lipid oxidation at any of the water activities studied. In contrast the addition of 10 ppm copper chloride had a marked pro-oxidant effect at all water activities. Therefore, chemically, on a weight to weight basis, sodium chloride is far less active as a catalyst of lipid oxidation than copper chloride. It would, therefore, appear that sodium chloride is not directly involved in the chemistry of rancidity but influences the reaction by its effect on the physical nature of the reaction conditions.
- E: Copper (in the absence of sodium chloride) in amounts at or above 1.5 ppm readily catalyses lipid oxidation at $a_w = 0.53$ and, presumably, based on conclusion D, at all water activities. This effect is greatly reduced below the 0.5 ppm level and may only become apparent in long term storage. Hence salt with low copper content should be used in salted-dried fish production.

- F: Copper in amounts above 0.5 ppm actively catalyses the production of colour in 8 days and is most apparent at water activities above 0.7.
- G: The water activity of the salted-dried fish is paramount in the development of rancidity. Lipid oxidation is greatly enhanced at the low (i.e. $a_w = 0.14$) and high (i.e. $a_w = 0.84$) water activity ranges and is slowest in the range of $a_w = 0.32$ and 0.55 . This finding applies even in the presence of the additives studied. The additives (copper chloride and sodium chloride) had more apparent effect on lipid oxidation at the higher water activity range. Hence in salted-dried fish production the water activity should be reduced as quickly as possible to 0.7 or less. Water activities as low as 0.5 are normally not practical because of the effect on texture.
- H: In the presence of high levels of salt there is more colour production at the higher levels of water activity. In contrast, in the absence of salt more colour is produced at the lower water activity levels. Thus at the lower water activities a high concentration of salt can actually limit the production of colour both as visually observed and as extracted.
- I: Freeze-dried mackerel mince gives rise to much higher TBA values than have previously been reported for foodstuffs (see Section 4.7).

6.

SUGGESTIONS FOR FURTHER WORK

- A: The changes in fatty acid structures during oxidation should be studied by capillary gas chromatography and related to the PV and TBA changes, colour production and loss in nutritive value of salted-dried fish.
- B: The influence of malonaldehyde and other products of lipid oxidation on browning should be studied, initially in system at a wide range of water activities. The possibility of using colour production as an index of oxidative rancidity in salted-dried fish should be examined and assessed using taste panels.
- C: The extent of rancidity development of salted dried fish in relation to the toxicity of the products, e.g. malonaldehyde, should be assessed.
- D: The relationship between malonaldehyde production and the method of fish mince drying (e.g. freeze drying or thermal drying) should be studied.
- E: The relevance of the thiobarbituric acid value (TBA) for assessing the extent of rancidity in systems undergoing extensive rancidity should be re-examined.

7.

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APPENDIX

Colour Plates 1, 2 and 3 - Simulation of S.E. Asian fish
salting and drying process.

Colour Plate 4 - Storage at constant a_w in presence of
high levels of sodium chloride.

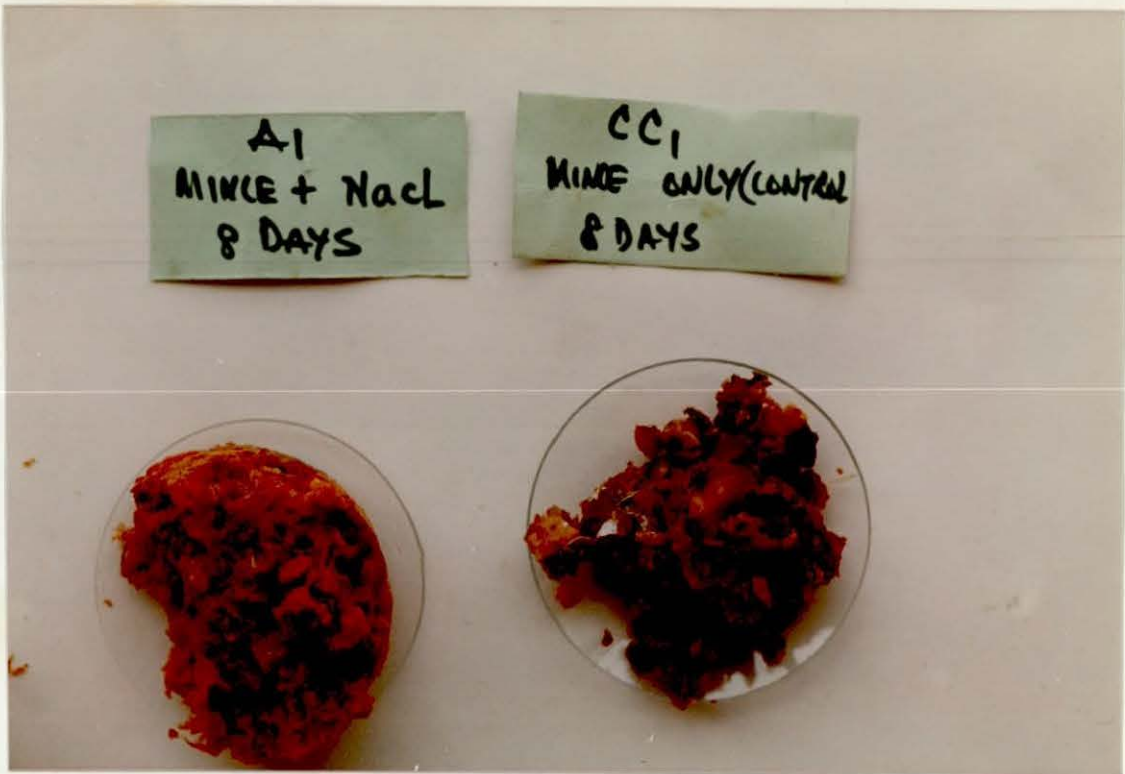
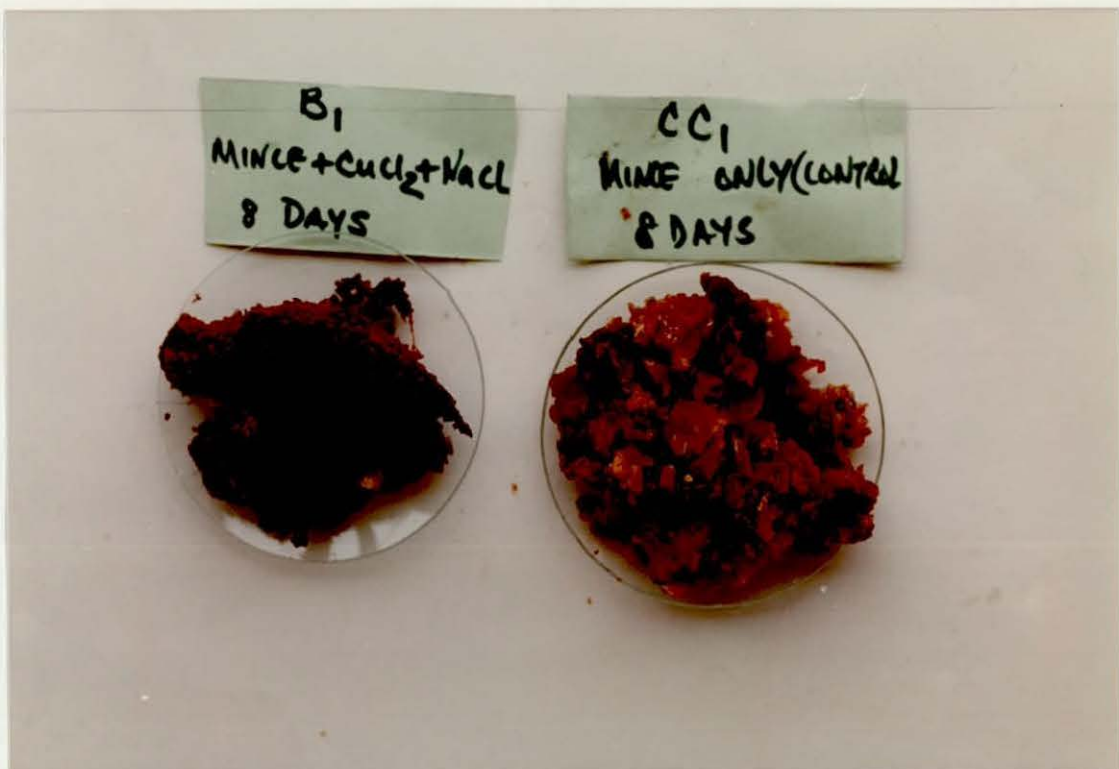
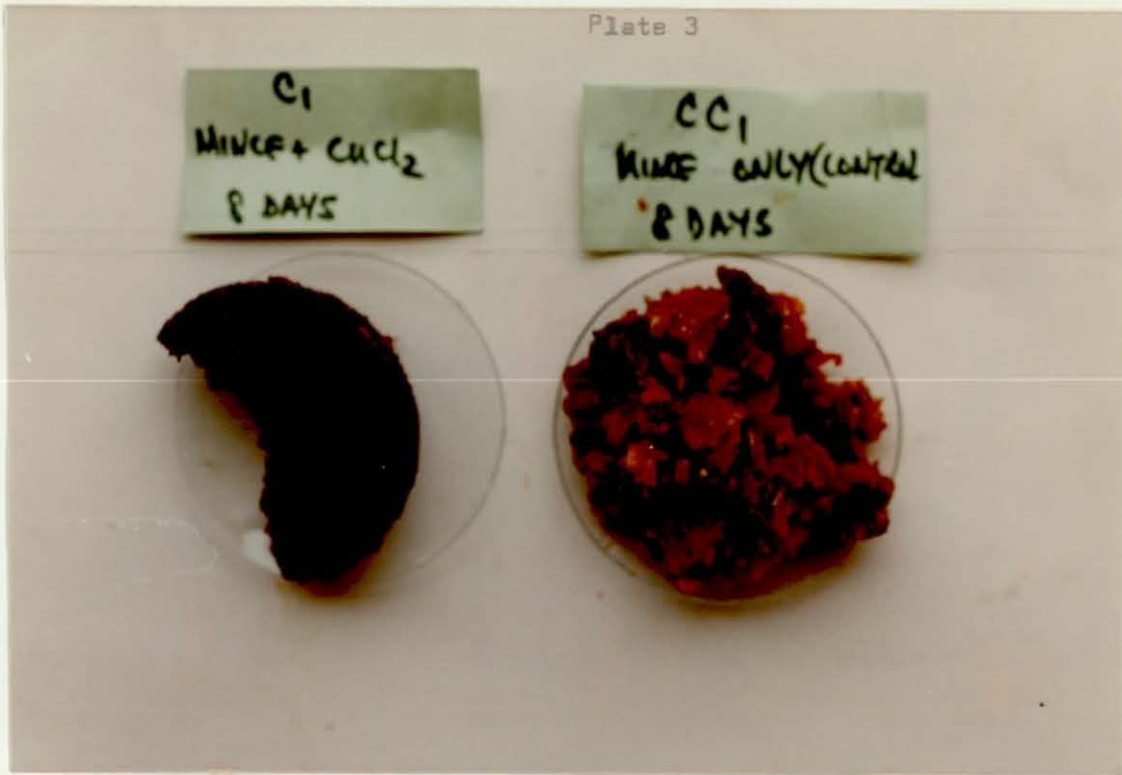


Plate 2

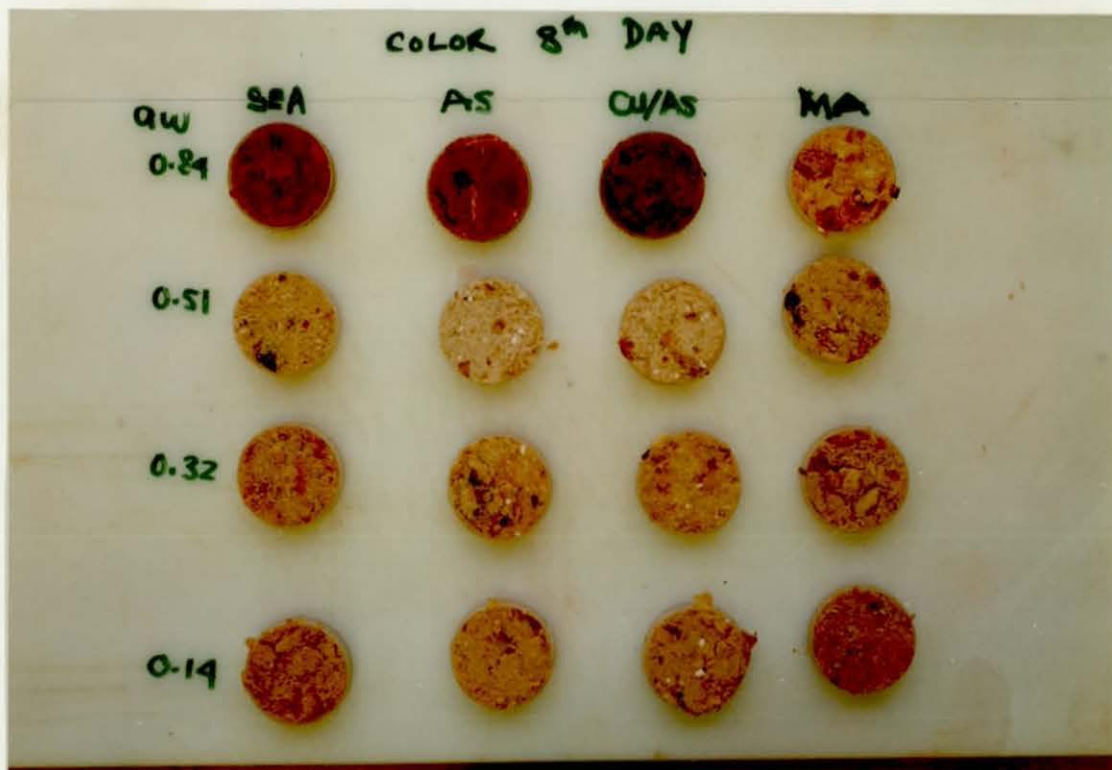


Simulation of S.E. Asian fish salting and drying process



Storage at constant a_w in presence of high levels of salt

Plate 4



SEA - South East Asian NaCl

AS - Aristar NaCl

Cu/AS - Aristar NaCl + CuCl_2

Ma - Mince alone

