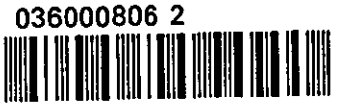


WATER SORPTION AND SHELF-LIFE OF DRY-CURED COD MUSCLE IN HUMID ENVIRONMENTS

by

WILLIAM FREDERIC ANTHONY HORNER

A MASTER'S THESIS

submitted in partial fulfilment of the requirements for the award of Master of Philosophy of the Loughborough University of Technology.

DEDICATION

To my wife, Valerie, and children, Rebecca, Duncan and Abigail, for their love, patience and encouragement in spite of my very gradual progress through this period of study.

ACKNOWLEDGEMENTS

I would like to thank Dr George Hall for his invaluable guidance and supervision of this project.

Particular thanks are also due to Dr Steven Hanson, Dr Peter Quantick and Dr Michael Dillon for their constructive suggestions.

I am also grateful to the staff of the Instituto Tecnologico del Mar, Boca del Rio, Veracruz, Mexico for providing the ignition spark for this project and the Humberside Polytechnic technician, library and secretarial staff for their help in experimental, literature search and thesis presentation. In particular thanks are due to Debbie Taylor of Humberside Polytechnic in Grimsby and Debbi Leake of the Humberside International Fisheries Institute, Hull University for their kind assistance in putting this thesis together.

I am particularly indebted to my fellow lecturers at Grimsby's School of Food Studies for taking on the burden of my teaching load which allowed me to complete this study, and to the staff of the Natural Resources Institute, Chatham for supplying photographs and other relevant material.

ABSTRACT

Dry-cured fish includes products primarily preserved through having a reduced water activity, due to drying or salt addition or both, and occasionally secondarily preserved by smoking or addition of preservative chemicals. The popularity of such products is world-wide, although many of the most significant markets occur in the humid tropics. Storage of dry-cured fish in humid environments considerably reduces the shelf-life of the product otherwise expected in temperate environments and leads to large-scale loss of product or product value.

The investigation was carried out to determine the effect of different processing methods on storage life of cod muscle in humid conditions.

The sorption of water in cod muscle subjected to different curing processes and rehydrated under constant and cycling humidity and temperature storage conditions was examined. Relationships between water content and water activity at different distances from the surface during desorption and resorption were investigated in an attempt to elucidate optimal processing parameters for maximal storage life in humid tropical conditions.

In drying, water movement parallel was faster than that perpendicular to the direction of the myofibrils within the mytomes. This directional difference in drying rate, however, disappeared as drying proceeded, and the convergence of the directional drying rates increased with the severity of the drying conditions.

The extent to which various edible coatings applied to the surface of the product after drying acted as barriers retarding the uptake of water in humid storage was evaluated.

Of the barrier materials applied to the product post-process, the carbohydrate-based materials actually appeared to increase the hygroscopicity of the cured fish in a humid environment. The lipid barriers, however, were effective. Vegetable oil dipping reduced the rate of water uptake to less than 40% of the control rate. This could be significant in extending the mould-free shelf-life in the normal daily cycle of atmospheric conditions in a tropical environment.

CONTENTS

I INTRODUCTION

I (i) Cured fish.

Dried and salted fish are nutritionally important and popular constituents of human diets in many parts of the world.

Marine fish bones found in excavations of known, European, prehistoric dwellings, situated many days foot-journey from the nearest coast, indicate the antiquity of the practice of fish curing around the Mediterranean. Iberian colonization and emigration spread the taste and demand for *bacalao*, salt-dried cod, to the West Indies, America, Africa and the East Indies, although the dry-curing of locally-caught fish species was undoubtedably practised by many of the aboriginal inhabitants of the lands which came under Spanish and Portuguese influence.

Hernan Cortes, the Spanish conquistador of Mexico, probably followed a route, from Veracruz to Mexico City, already well-trodden for centuries by Totonac dried fish traders bringing their goods to their Aztec overlords in Tenochtitlan (Mexico City).

Bacalao and its local immitations are still very popular in Mediterranean countries, the Caribbean and Latin America where it is a traditional constituent of the main meal on Christian festival days.

Stockfish, which is (normally) cod dried naturally, without salting, in the cold, dry winds of Norway and Labrador, is popular in Nigeria.

In fact, many of the regions, where dried and salt-dried fish products are most-consumed, experience humid, tropical climates for much of their year. Rapid uptake of atmospheric moisture results in a foreshortening of the saleable life of the product, as predicted in the paper by Doe et al. (1982) based on the relationship between water activity (a_W) of the product and its mould-free shelf-life.

The impetus for this work was the observation that much of the expensive, imported *bacalao*, for sale in Mexican supermarkets, exhibited signs of spoilage. Contrary to the suggestion of Poulter et al. (1982) that surface *dun* mould (as shown in Figure 1) would probably be the first sign of spoilage, *pink* patches, probably due to the proliferation of red, halophilic bacteria colonies, seemed to be the commonest initial indication of spoilage. When allowed to grow and intensify, these pink patches became the source for putrid smells, reinforcing the suspicion that this was, indeed,

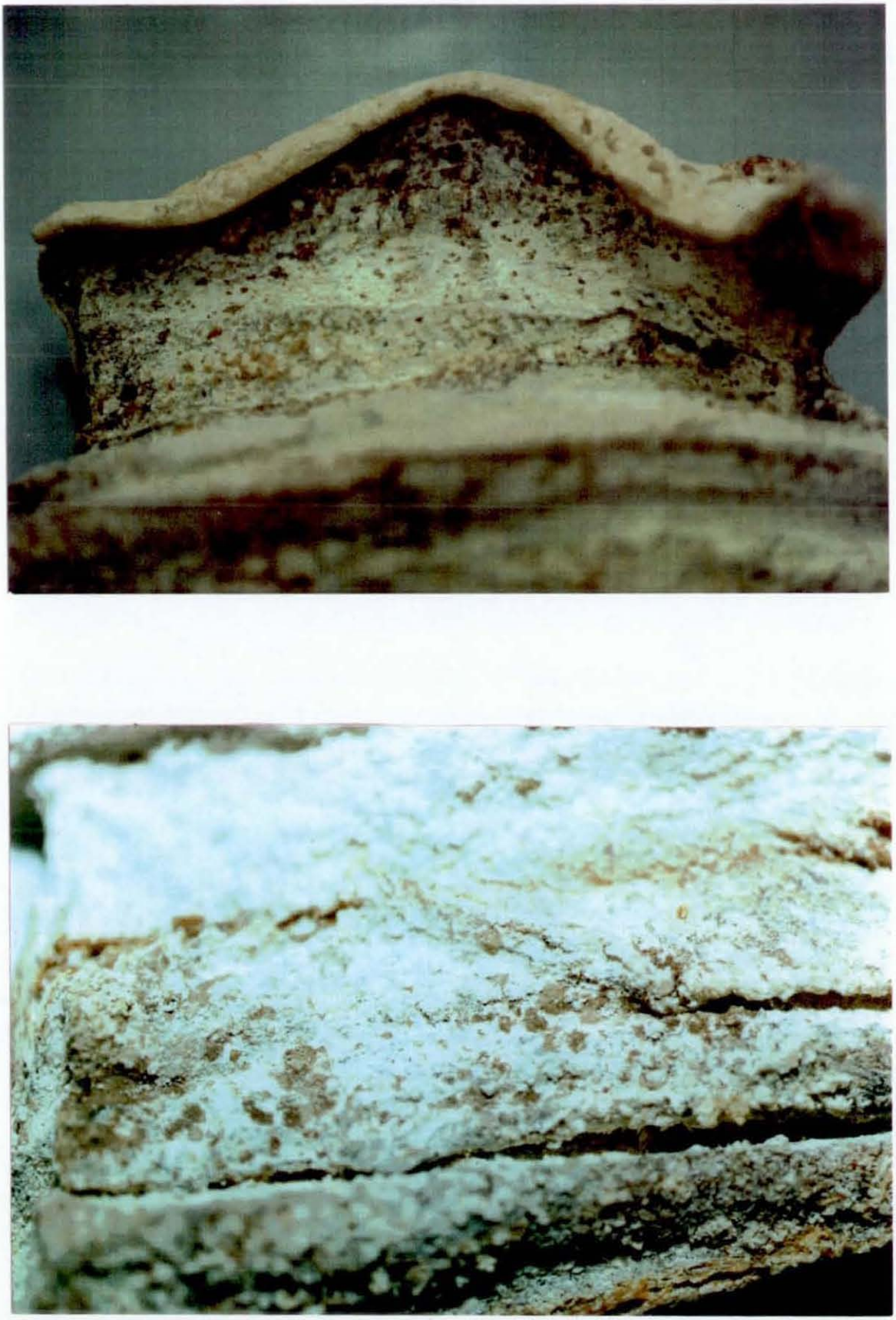


FIGURE 1: Dun mould (**Wallemia sebi**) on dry-salted shark
(photographs courtesy of NRI, Chatham)

(a)

(b)

(c)

(d)

FIGURE 2: Kench-cured salt-cod

("bacalao") (a) sample taken from wet-stack

(b) sample taken from "pining"

(c) sample taken after kiln drying

(d) sample taken from pining showing "pink" (Halinobacterium salinari) infestation

regeneration of the red obligate halophiles, whose growth had been witnessed by the author to be a common occurence in the *wet-stack* stage of the *kench-curing* of cod when ambient temperatures are relatively high, in the summer months of producer-countries. Figure 2 is a photograph of sides of cod (*bacalao*) after wet-stack salt-curing; after wet-stack and 3 months' maturing (*pining*); after wet-stack,pining and kiln-drying; and of a wet-stack sample infected by "pink" before treatment with sodium metabisulphite solution to render it saleable.

I (ii) Spoilage of cured fish in humid climates.

Seiler (1959) expressed surprise at the lack of published data on the effect of a_w and storage temperature on the time taken for mould growth to become visible on foodstuffs. Since this is likely to be the basis for rejection on quality grounds for most solid and semi-solid, intermediate-moisture foods, manufacturers would recognize this as the most obvious criterion in assessing the shelf-life of such foods. An expression was derived to estimate the mould-free shelf-life of cake in days at 27°C:

log(mould-free shelf-life) = 6.42 - (0.0647ERH%) where ERH% is the percentage equilibrium relative humidity.

For flesh foods, however, Macara (1943) had revealed that the mould-free shelf-life of dried meat, which was approximately 30 days at 27'C and 75%ERH, was reduced to only 8 days when the ERH was increased to 90%. Similarly, Shewan (1953) observing the storage of dried cod at 20°C, found that a mould-free shelf-life of approx. 450 days at 65%ERH was reduced to approx. 50 days when the ERH was increased to 75%. It was noted that reducing storage temperature increases mould-free shelf-life, and that the presence of *Wallemia sebi* as the infecting agent, rather than *Penicillium spp.*, decreases it.

With respect to the author's observation that, in Mexican supermarkets, it seemed to be bacterial, rather than mould, spoilage which heralded the end of the shelf-life of salt-dried fish, the findings of Hicks et al. (1955) has relevance. These suggest that the concentration of solutes at the surface during the drying process leads to a lower a_w value here compared to that at the centre of the muscle. Although this depression of surface a_w is partially compensated for by the diffusion of solutes of low molecular weight away from the surface and diffusion of water from lower levels to the surface, large gradients in a_w through the surface layers persist on storage. Nevertheless, it was observed that chilled meats, subjected to severe surface dessication, were usually spoiled by moulds. With salt-dried fish undergoing resorption in a humid environment, therefore, the movement of low molecular weight solutes would be towards the resorbing surface; and the resultant increase in sub-surface a_w could lead to a resurgence in the growth of the red halophiles, which frequently heavily infest the salt used in curing processes, before the appearance of surface mould colonies.

Poulter et al. (1982) showed that an increase in fish water content from 20 to 30% (dry weight basis) increases a_w from 0.69 to 0.83 in unsalted, dried fish, and from

0.69 to 0.73 in salt-dried fish containing 10% salt (d.w.b.). When the water content was further increased from 30 to 40% (d.w.b.), the a_w of the 10% salt-dried fish increased from 0.73 to 0.81; but in 20% salt-dried fish, the a_w only increased from 0.69 to 0.72 for a 30 to 40% increase in water content. The authors also commented that *Halobacterium spp.* begin to grow again as a_w rises above 0.75. Lupin (1978) commented that good hygiene throughout production was the best method of preventing such spoilage, but importing countries had no means of ensuring this. Water, it was stated, was the best disinfectant for the removal of red halophiles should growth occur in production. Its use, however, would require lengthy re-salting and re-drying operations.

Snow et al. (1944) reported (Table 1.) that the main factor controlling mould growth on dry animal feeds was the % relative humidity of the atmosphere in which they were stored:

Table 1. Days before first appearance of mould mycelia on six selected feeding-stuffs when stored at different humidities:

2-3	3-5	5-6	7-14	19-43	32-52	82-227	118-238	314-961
100	90	85	80	77	75	72	71	67%RH

Thus, the authors predicted safe levels for storage humidities required in-store periods over which the feedstuffs would remain mould-free. They also noted that different components of a feedstuff took up water at different rates in a given, constant relative humidity, and that fats and non-hygroscopic ash constituents exert a depressing effect on water-uptake rate.

I (iii) Pathogenesis in spoiling cured fish.

The occurence of spoilage, due to inadequate reduction of water content during production, or to subsequent resorption of water by the product stored in humid conditions, has been considered, thus far, in terms of visual quality likely to be perceived by the consumer. The appearance of *pink* spoilage or surface mould colonies have been regarded as preventable or eliminable nuisances. That such occurences could also herald the possibility of health-endangering changes simultaneously affecting the product, has been revealed in the work reported by several authors.

The presence of colonies of *Aspergillus flavus*, a known producer of carcinogenic aflatoxins, was suspected, having witnessed the nature and environment of spoilage, on many samples of salted, dried fish for sale in S.E. Asian markets by Hanson and McGuire (1982). Fourteen strains of this organism were isolated by Prasad et al. (1987) from 40 samples of cured fish collected from an Indian fish market. Three of these strains were shown to possess aflatoxin-producing capability.

A black fungus, *Exophiala werneckii*, which is the causative agent of the superficial human mycosis, *tinea nigra*, was recovered, as the sole colonizer of spoiling, salt-dried Amazonian fish (*Osteoglossum bicirrhosum*) by Mok et al. (1981). This occured on samples which had been immersed in saturated brine (prepared from previously sterilized salt) for 10 minutes and samples immersed in 20-30% brine for 5 days before shade drying for 30 days.

Measures and Gould (1973) reviewing the work of other authors, concluded that, in general, the minimum water activity which would support growth of microorganisms was lower in environments where glucose or glycerol was the main solute than in those where it was salt. A notable exception to this observation was *Staphylococcus aureus*, which will grow down to a_w 0.83 in salt, but only down to a_w 0.89 in glycerol environments. There seems also to be a pH effect. Riemann et al. (1972) reported growth of this organism in upto 10% sodium chloride solution at pH 4.5, and upto 16% w/v concentration at pH 6.0. For cured fish then, this could be a significant hazard, since Pawsey and Davies (1976) reported the presence of coagulase-positive types in 99.5% of examined samples of fish gutted and frozen at sea.

I (iv) Minimalization of losses due to microbial spoilage of cured fish.

Whittle (1985) quoted that the losses occuring in cured fish during drying and storage in 1983 were estimated to be 2.7M tonnes or 25% of total production.

The key to the extension of the shelf-life of cured fish in humid environments is the prevention of resorption of water from the air which surrounds it. Packaging such products in sealable films has not been found satisfactory because: (i) the rigid, sharp edges of the product tends to perforate the film in transit, and (ii) changes in temperature during storage and distribution lead to condensation on the inner surface of the film which stimulates the growth of moulds on the products' surface.

II THE ROLE OF REDUCED WATER ACTIVITY IN FISH PRESERVATION.

In this section, it is intended to examine the concept of water activity, its relationship with water content and, by exploring its validity as a preservation index in the processing and storage of cured fish, assess its usefulness in the control of quality and safety of such products.

II (i) Defining a_w and its relationship with food spoilage.

According to Gilbert (1986) the term "activity" was first used by G.N. Lewis in 1907 in accounting for the difference between the thermodynamic free energy of a component in a system and that of the same compound isolated from the system.

The difference in free energy is related to a function he termed "fugacity"__ a measure of the excess, rather than total, free energy available for the work of either the system or the component within the system. Reid (1973) defined fugacity as "a measure of the escaping tendency....having the form of a vapour pressure which has been corrected for non-ideality of the vapour." Thus, "activity" implies the "fugacity ratio" i.e. f/f_0, which is defined as:

$$\frac{\text{the escaping tendency of a component from a system}}{\text{the escaping tendency of pure component at the same temp.}}$$

Provided that the non-ideality of the component is not too large, the fugacity can be substituted by measured vapour pressure. Fortunately, this is true for aqueous systems since, at normal temperatures, water vapour approximates to an ideal gas. Hence an aqueous system's fugacity(f) can be approximated by its vapour pressure(p) and, applying Raoult's Law

$$p_A = p_A^0 . x_A$$

where p_A is the vapour pressure exerted by component A in the system;

p_A^0 is the vapour pressure exerted by pure component A; and

x_A is the mole fraction of component A in the system.

More than forty years after Lewis' introduction of the term "activity", Mossel and Westerdijk (1949) used the term "water activity" in the context of food spoilage factors of importance; and Mossel and Ingram (1955) published (as shown in Table 2) the approximate water activity values below which, they believed, growth of different microorganism groups would not occur:

Table 2.

ORGANISM	a_w
Bacteria	0.90
Yeasts	0.88
Moulds	0.80
Halophilic Bacteria	0.75
Xerophilic Bacteria	0.65
Osmophilic Yeasts	0.61

The usefulness of a_w as a preservation index indicator was pointed out by Scott (1957). "The fundamental importance of a_w applies to foods as well as to aqueous solutions studied by physical chemists." Scott substituted the use of a_w in preference to relative vapour pressure (RVP) as used by Walter (1924), since the former is a fundamental property of aqueous solutions, whereas the latter more strictly applies to a substance's surroundings rather than to the substance itself.

$$a_w = p/p_0$$

Refering back to Raoult's Law, a_w has taken the place of the term, x_A, so water activity is the "effective concentration of water" in a substance.

II (ii) The use of a_w as a preservation index.

All substrates tested by Scott were revealed to support growth of *Staphylococcus aureus* down to a_w (in the remarkably narrow range) 0.88 to 0.86; yet water contents in these same substrates varied between 16 and 375% (dry weight basis). This lower limit for growth was not appreciably affected by the nature of the solutes used, provided that they were electrolytes. The substitution of non-electrolytes for electrolytes as solutes, however, did significantly affect the lower growth limit.

When the substrate is a non-hygroscopic material, the lower growth limit may be even further removed from that expected in a simple electrolyte solution. Block (1953) showed the a_w lower limit for yeast growth on glass wool was 0.75. The same yeast, nevertheless, would grow in electrolyte solution down to a_w 0.62.

Returning to Scott's classic chapter on water activity (1957) and the assertion of its usefulness in food preservation, it was stated that microorganisms compete with solute molecules for the water they require for growth over the entire range of a_w for which they are viable. It was acknowledged, however, that factors other than a_w are frequently important in determining whether microorganisms survive and grow. For example, some chemical inhibitors of such growth have little or no effect under optimum a_w conditions and yet an increasingly significant effect as a_w is reduced.

Christian (1962) wondered whether microbial growth and inhibition was most significantly controlled by a_w, water content or the presence of specific solutes. Exploring this question further, Duckworth (1972) and Duckworth and Kelly (1973) decided that, in water/solute/polymer systems, the minimum a_w at which solvent action (and, by implication, Scott's "water competition" between microorganisms and solutes) becomes apparent depends upon the solute not the polymer; on the other hand, the amount of water UNAVAILABLE as solvent in such a system DOES depend upon the nature of the polymers present.

Experiments had indicated, concluded Bone (1973), that there was a large degree of interaction between food structure and the solution phase; a technological breakthrough was needed to exploit this interaction in the control of a_w in foodstuffs.

Mossell (1975) questioned the choice of the term "water activity" in the context of prevention of microbial spoilage. A deterioration reaction, it was said, was always a multi-factor dependent one, in a food system, and a_w was only one of those factors. Only when a_w was the rate-limiting factor, for example: when water content was so

low that the mobility of reactants had been reduced several orders of magnitude, did a_w have a direct influence on spoilage rate.

Reid (1976) cast further doubt on practicability of using a_w. It was reiterated that the ratio p/p_0 denoted relative EQUILIBRIUM vapour pressure, not RVP, in that it applied only to systems at equilibrium where the fugacity was equal in all phases. Apart from the time needed to attain true equilibrium conditions in a system so complex as a foodstuff (which could be days or, even, weeks) many foodstuffs were inherantly unstable systems, eg. multi-phase mixtures, like dough, or supersaturated solutions, like boiled sweets, so their could be no equilibrium. It might, however, be argued that errors, due to the strictly inaccurate assumption of equilibrium attainment, are generally trivial. Gal (1972) had shown that there was only 0.2% difference between a_w defined on fugacities and RVP.

Errors in the measurement of the availability of water for life and, therefore, spoilage which, as Loncin et al. (1968) surmised, had become to be expressed in terms of a_w were much more significantly due to the shear complexity of most foods.

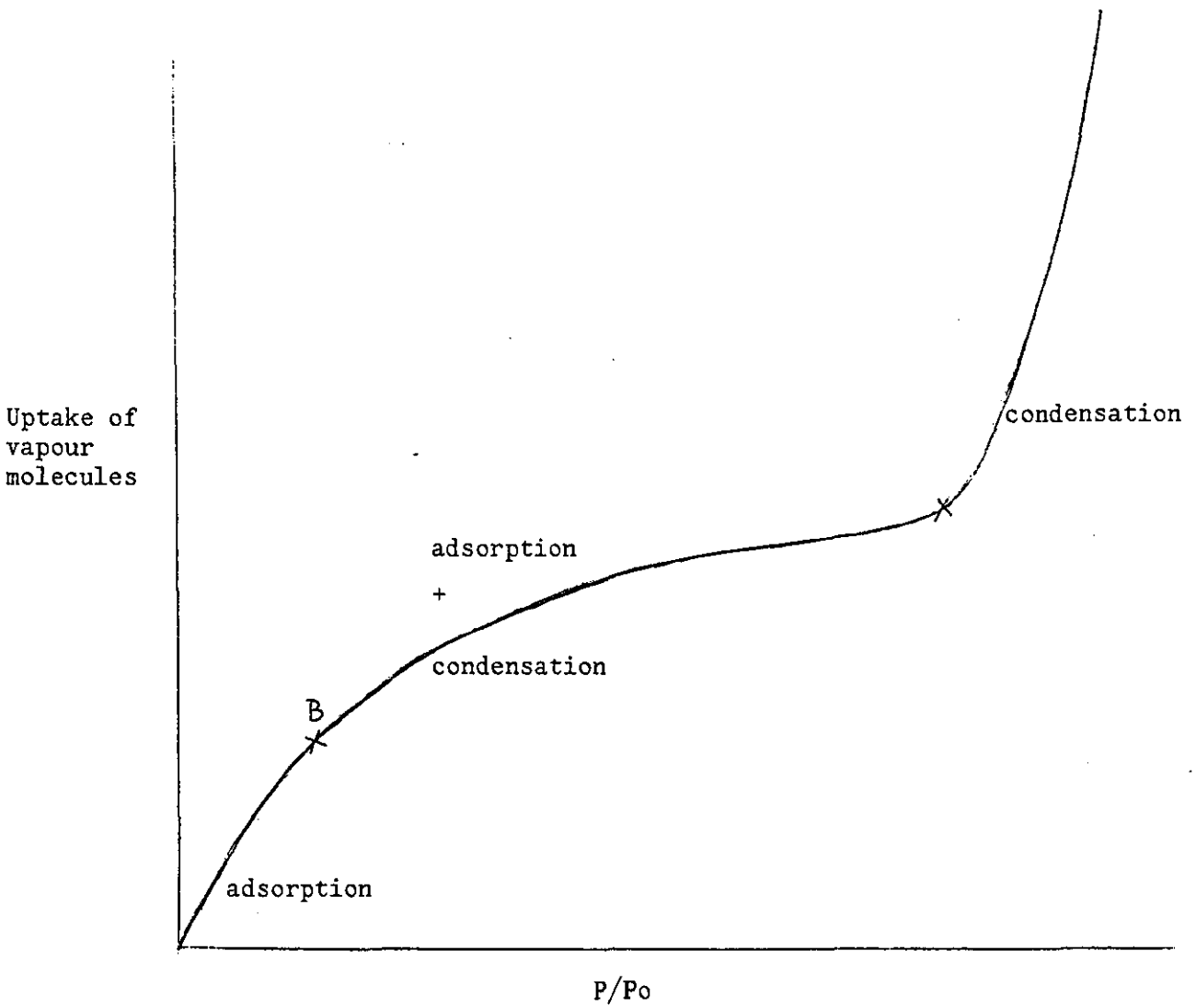
Van den Berg and Bruin (1981) described fish as having a fibrous component, constituting its solid phase, holding a continuous liquid phase together with a continuous or dispersed lipid phase; this system is further complicated by the dissolution and delayed crystalization of other components during sorption. Not surprisingly, hysteresis is exhibited between desorption and adsorption isotherms for the same material which suggested to the authors that no true thermodynamic equilibrium exists. In this situation, it was concluded that what, in effect, was being measured was an empirical pseudo-a_w.

Commenting upon the same paradox, Suggett (1976) had said that the occurrence of a hysteresis loop in the $\%H_2O/a_w$ relationship (obtained through a dehydration-rehydration experiment) immediately indicates that, in at least one arm of the cycle, the system cannot be in equilibrium as the observation of two alternative water activities at a single water content is thermodynamically untenable. There must, therefore, exist, he postulated, certain non-equilibrium ways of binding water; and the reason this water remained effectively inaccessible to microorganisms must be due to kinetic rather than thermodynamic factors.

Despite such criticism of the use of a_w in a food preservation context, Gilbert (1986) reported that it was still regarded as an intrinsic parameter of a food system; whilst ERH was only indirectly related to the food's preservation state, being a property of

the gas phase in contact with the food system. Since a_w was measured as the fugacity in the vapour phase, it could be queried whether such a distinction is, itself, valid in terms of water reactivity within the solid matrix of the food system. Additionally, in a foodstuff which could be represented as a dilute solution, the structural or entropy difference were rated negligible and randomness high enough to obviate the distinction between a_w and ERH as measures of differential free energy.

FIGURE 3: B.E.T. Sorption Isotherm showing the zones in the typical sigmoid curve



II (iii) The relationship between water content and water activity: sorption isotherms.

Brunauer, Emmett and Teller (1938) examined the adsorption of gases onto solid surfaces. Their plots of gas uptake by the solid against the "relative pressure" (p/p_0) of the gas (where p_0 is the saturation pressure) revealed typically sigmoid forms (Figure 3). The first portion of the sigmoid curve (to point B in the figure) was said to represent the filling up of a monomolecular layer of gas adsorbed to the solid's surface. The values for gas uptake by the solid at point B were found not to vary by more than 10% for the range of twelve different gases under standard temperature conditions. The second portion of the graph (after point B) could be fitted to a straight line equation, a modification of the Langmuir Adsorption equation, on the assumption that the forces responsible for monolayer condensation attract subsequent molecular layers to the surface in regular fashion. Between p/p_0 values 0.35 to 0.5, however, the isotherms were found to deviate from linearity in the direction of there being too little adsorption at a given p/p_0 value.

Brunauer, Emmett and Teller's work and their interpretation of it (refered to as B.E.T. theory) has been used to explain the similarly sigmoid shapes obtained when water uptake is plotted against water activity to reveal the sorption isotherms for foodstuffs.

Many researchers have used such isotherms as the basis for empirical equations and/or tables for the computing of water activity and thence to shelf-life given the more widely attainable water and salt contents of the food. Nevertheless, it must be said that the applicability of these predictive devices is very narrowly restricted. The following quotation was taken from van den Berg and Bruin (1981): "In the field of food engineering, it is hardly necessary to stress the importance of a relatively simple equation describing the moisture sorption isotherm of a food reliably with a limited number of parameters.....this equation does not exist at present and is not expected to be found in the near future."

Various workers (Macara (1943) Shewan (1953) and Christian (1962) for example) have, nevertheless, noted the usefulness of a known sorption isotherm, for a given food product, in deciding its storage and packaging requirements. Loncin (1961) also advertised their importance in the prediction of the drying rates of foodstuffs which would be essential in designing and operating drying equipment and processes.

Gane (1950) plotted isotherms for various vegetable materials held at temperatures between 0° and 37°C. He found the variation between isotherms plotted for different

temperatures to be small compared to that between those for different materials at the same temperature. Most of his isotherms did not, however, extend beyond a_w 0.8 because microbiological spoilage intervened before equilibrium could be attained.

This limitation did not persist long after this date and Scott (1953) published isotherms for meat, milk and a soup mix where the a_w range extended from 0.8 to 0.97. The difficulty of accurately measuring a_w at values close to 1.00 was, nevertheless, acknowledged.

Loncin et al. (1968) observed that although vapour pressure varies considerably with temperature, the ratio, a_w, is much less temperature dependent. They emphasised, however, the need to measure water content by a direct method, such as Karl Fischer, rather than by drying methods when looking at the very low water contents (0 to 0.3kg water per kg of dry matter).

Point B in Figure 3 corresponds to the B.E.T. monolayer value at which, theoretically, all solid surfaces are covered in a layer of adsorbed gas (or water, in the case of dried food) one molecule thick. Pauling (1945) put forward the view that water adsorption on proteins would occur predominantly as one water molecule per polar side-chain upto the B.E.T. monolayer value. Shaw (1944) and McLaren and Rowen (1951) suggested that adsorption mainly occured on the polar groups present in the molecular surface of polymers. This view was challenged by Eley and Leslie (1962) whose work suggested that the peptide bond of protein chain was also a significant sorption site. However, to account for the quantity of water adsorbed at the B.E.T. monolayer value, it was calculated that (for haemoglobin) 73% of the polar side-chains and the peptide bonds would have to be at the molecular surface.

The notion that there is a point on the sorption isotherm which corresponds to exact completion of a monomolecular film over the surface of the food's structural molecules had also been questioned by Salwin (1959). Since clusters of water molecules around reactive adsorption sites, rather than a continuous, single-layer film,seemed the more feasible on available evidence, a "statistical" monolayer value

for moisture content was proposed. It could be calculated by modifying the B.E.T. equation:

$$\frac{R}{M(100-R)} = I + SR$$

where R is % relative humidity
M is moisture content in g/100g dry solids
I is the y-axis intercept of the straight-line plot of R/M(100-R) against R
and S is the slope of the same plot.

Values of R and M for two points on the isotherm between R=5 and R=55 were be used to obtain simultaneous equations, the solution of which provided values for I and S. These were then used to calculate the statistical monomolecular layer moisture content, M^m, from the equation:

$$M^m = \frac{I}{I + 100S}$$

Water molecules adsorbed to these reactive sites, were said to protect them against the rapid oxidation they would, otherwise, undergo on exposure to atmospheric oxygen. Klotz and Heiney (1957) had already pointed out that attachment of oxygen to what would, otherwise, be a water binding site on the protein caused an interruption in the aqueous covering sheath which could disturb the hydration structure of neighbouring sites. Therefore, Salwin stressed the importance of not drying beyond the M^m value to avoid oxidative deterioration.

Additionally, the bond energy of the adsorbed water would inhibit interactions between polar groups on adjacent protein molecules which would have the effect of preserving the product's rehydratability and minimising its toughness when reconstituted.

To support these contentions, the author cited the example of dried shrimp. Was it possible that the frequently encountered poor quality of this item could have been due

to overdrying? The U.S.D.A. recommended specification for maximum water content of the product for long-term storage was 2.5% which compared with a theoretical M^m value of 5.56%, and an analysed monolayer value of 3.09%. Salwin's experiments had revealed considerable textural deterioration when shrimp was dried much beyond its monolayer level.

Similarly, Martinez and Labuza (1968) recommended that 32% R.H. allowed freeze-dried salmon the greatest storage stability. This corresponds, on the sorption isotherm, to a water content greater than the M^m value, but below this a_w,adsorption of oxygen and rancidification was much more rapid.

Duckworth and Smith (1962) showed there was no movement of solute at water contents below the M^m value. Tracer studies suggested, however, movement of water into and along cell walls in plant tissue dried to water contents slightly above the M^m value. Such movement implied that physical, chemical and biological changes might also be initiated and, thereby, impair long-term storage.

The mobility of small molecules in many food systems becomes apparent, reported Karel (1973) at water contents higher than the M^m value which correlates with the total number of polar groups able to bind water.

Beyond the monolayer value (point B in Figure 3) there is a section of the isotherm where a_w changes very rapidly for very small change in water content. This includes what Scott (1957) termed the preservation significant zone: a_w 0.7-0.8.

Christian (1962) commented that the zone between the ends of the flattest part of the typical sorption isotherm (Figure 3) where a large change in a_w results from a small change in water content, had been described as "bound" water. This could not possibly hold, he argued, at higher a_w's where the large amount of sorbed water would be beyond the holding capacity of the weak sorption forces.

The concept that water might exist in different states, one being held more tenaciously than the other, has been popular for many years. Tagaki (1973) found three Differential Thermal Analysis peaks associated with the water component of kamaboko (a type of cooked fish jelly popular in Japan). The third of these, that at 150°C, he concluded, corresponded to water which was firmly bound to the protein, whilst the other two, at 100°C and 60°C, represented the "free" water in the system.

Kuprianoff (1958) published the following definitions of "bound" water which reflect varying aspects of the properties of water:

"....water that is retained after a prescribed drying process...."

"....water that fills the first adsorbed monolayer (B.E.T. theory)...."

"....water that remains unfrozen, even if the food is submitted to very low temperatures e.g. -70° to -80°C...."

"....water that is not available for the solvation of solutes...."

The first and third definitions assume that there exists, in food, water which is so firmly attracted to other constituents that it cannot be removed under the normal conditions of the drying process or crystallise on cooling. The problem with the former is that there would be an infinite number of "bound water contents" as there are drying process conditions; and with the latter, according to Hardman (1985), is that during cooling, some of the water forms a glass, rather than crystals, so that thermodynamic equilibrium cannot be established on a normal experimental time-scale. Hence the validity of both these definitions is questionable.

The second and fourth of these definitions should coincide with M^m, the equivalent monolayer value. Salwin (1959) gave an average value for the latter as 4.5g/100g of dry solids for proteins. This was well below what was subsequently quoted, by Duckworth and Smith (1962) to be the range for "bound" water content, i.e. 16-50g water/100g dry solids, and below the maximum hydration water content (where all the binding forces due to hydrophilic sites on macromolecules are satisfied) given as 20g/100g of dry solids for proteins by Hamm (1962). Most flesh foods preserved solely by drying, however, were said to be dried to a water content lower than the range given for "bound" water and Duckworth and Smith thought it unlikely that the water remaining in the dried product would all have been bound to macromolecules. Since enzyme-controlled reactions persisted, even at these low water contents, suggested that films of "free" water still persisted in capillary spaces.

Karel (1973) stated that a_w varies according to capillary radius, as shown in Table 3. Table 3. Assuming a contact angle of 0°:

for capillary radius (cm)	a_w
1	0.9999999
10^{-2}	0.999895
10^{-4}	0.99895
10^{-6}	0.90
10^{-7}	0.20

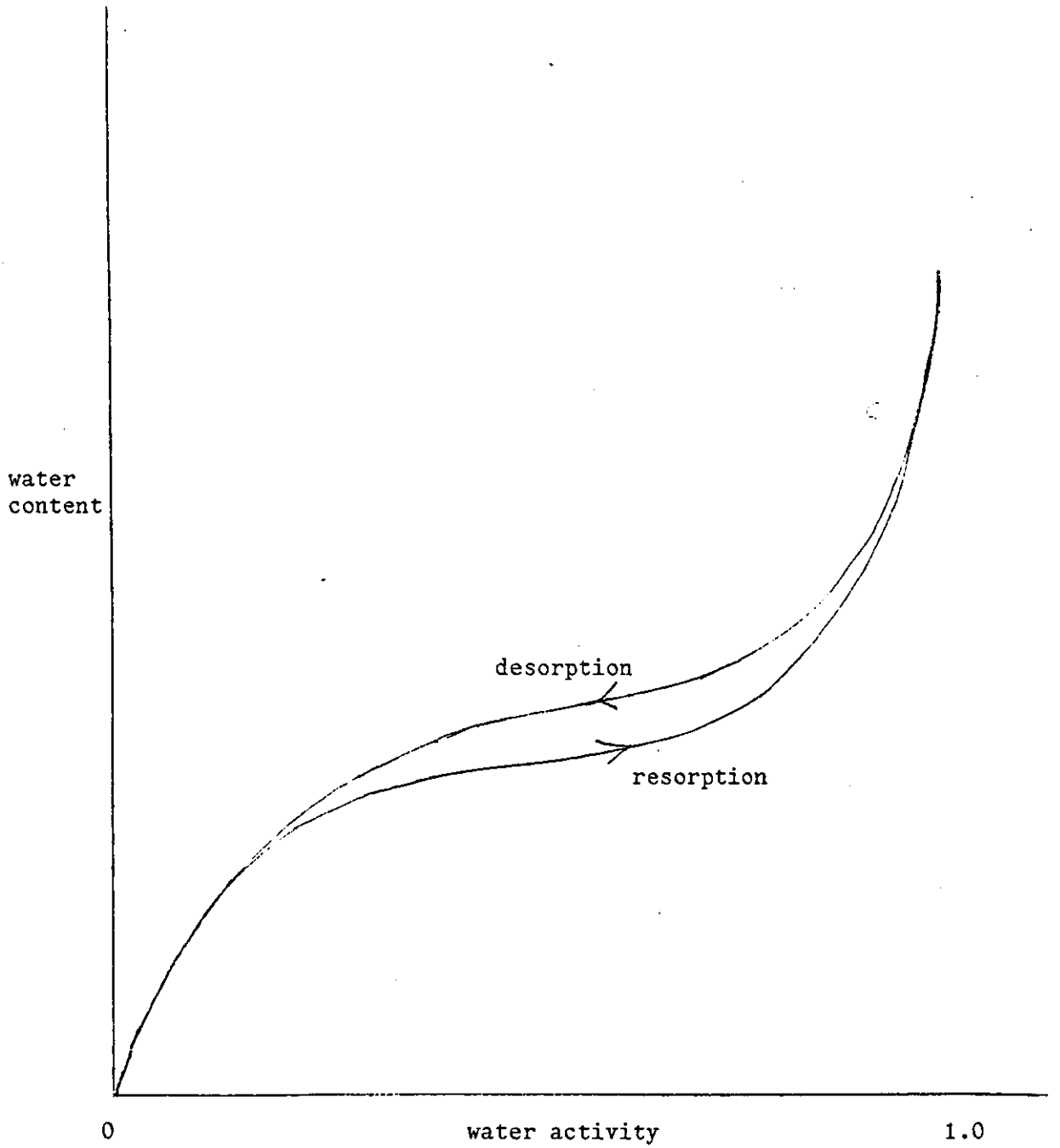
It would be expected, therefore, that a definite capillary effect on a_w would occur in food substances with $a_w > 0.90$ as capillaries with a radius around 10^{-6}cm are probably common in food structures. Films of free water in food capillary spaces existing at a_w levels approaching coincidence with M^m seem less likely, though not impossible.

The concept of "free" water appears to be more credibly definable than "bound" water. Hamm (1962) described it as that removable water which freezes at the same temperature, exerts the same vapour pressure and has the same dissolving power as normal, pure water. It is the sorption of this free water, also involved in solution and capillarity, which is described by the third section of the isotherm; where large differences in water content accompany relatively small changes in a_w. Much of any drying operation therefore involves the removal of the overwhelming majority of the water naturally associated with the fresh food material. Only during the initial stages of drying, however, does this water move freely, as from the surface of a body of pure water. Labuza (1977) reviewed the many methods which had been used to measure the degree of binding of water in foods. These included measuring:

(i) a_w or relative vapour pressure(RVP);

(ii) the degree of vibrational and rotational freedom of a water molecule in a food system by use of NMR (Shanbhag et al.,1970);

FIGURE 4: Typical Food Sorption Isotherm showing Hysteresis Behaviour



(iii) the ease of removal of water from foods by calorimetric methods, like differential spin calorimetry (DSC) and differential thermal analysis (Duckworth,1971);

(iv) the freedom of a water molecule to vibrate within a system at a specific frequency using a dielectric method (Roebuck et al.,1972);

(v) the water holding capacity (whc) and binding of water in meat systems by determining the effect of pressure on squeezing water out of food (Hamm,1963);

(vi) the whc using centrifugation;

(vii) the amount of syneresis under standard conditions;

(viii) freezing point depression and the amount of water unfrozen at the normal freezing point for the foodstuff (Moy et al.,1971);

(ix) the amount of water which can be sucked out of a foodstuff by some dry, inert material, such as filter paper (Lewicki et al.,1978).

Using such empirical methods, it would be possible to obtain "bound" water contents varying from that which coincides with the equivalent monolayer value (M^m) to others which coincide with a_w values very close to 1.00. The latter would be the case using, for example, methods (v) to (ix) for the determination of "bound" water in a gel. In such a system, despite the huge relative distances between macromolecules, and consequent free space for the movement of water molecules, the water is held either by some unusual, long-range forces or by capillary suction in the pores formed between the macromolecules. Methods (i) to (iv), on the other hand, would produce much lower values for "bound" water because by far the most of the water in gels has been shown to have the same thermodynamic properties as pure, bulk water.

The remaining significant feature of the typical sorption isotherm for a foodstuff (Figure 4) is its hysteresis loop, the existence of which casts doubt on the use of the term a_w in a "food" context, as discussed in section II(v). Nevertheless, it appears that a larger amount of water is held, at certain given a_w values, by desorbing food than by resorbing food. In contrast, Labuza et al. (1972) concluded that microorganisms grow more rapidly at given water activities reached by desorption than by adsorption. The work of Plitman et al.(1973) and Sinskey (1976) supported

this in showing that *Staphylococcus aureus* grew in a food system with a_w=0.88 reached by desorption, but failed to grow in the same food system where this a_w had been achieved by adsorption; but, at equal moisture contents, whether achieved by desorption or adsorption, no difference in growth characteristics was discernible.

Food, unlike wet sand, glass fibre and other model systems, does not readsorb water in precisely the reverse manner to its desorption. This hysteresis, Kapsalis (1981) explained, is nature's built-in protective mechanism against climatic extremities, like drought or frost, causing life-threatening water loss. To the lay-observer, it may be obvious that rehydrated food seldom resembles its fresh counterpart. The type of changes encountered upon desorption and adsorption, however, depend on:

(i) whether the sorbent is, initially, amorphous or crystalline;
(ii) whether physico-chemical transitions are taking place during adsorption;
(iii) the speed of desorption; and
(iv) the extent of desorption before resorption.

It has already been mentioned that when food materials are dehydrated below the equivalent monolayer level, the exposed polar groups quickly react with atmospheric oxygen or with one another, which affects their capacity to resorb water subsequently. Adding this chemical complexity to the physical complexity of the different phases present in most food systems, as noted by van den Berg and Bruin (1981),might imply that hysteresis in the sorption isotherms of most foodstuffs was not only to be expected, but also that the hysteresis loop be expected to be wide. Seehof et al. (1953) suggested that the width of this hysteresis loop might be correlated with the number of $-NH_2$, -SH, and -S-S- groups in proteins. These were regarded as the strongest water binders. Kapsalis (1981) observed that, in high-protein foods, hysteresis extended from the capillary condensation region of the sorption isotherm, around a_w 0.85 to zero a_w. Later work by Tanaka (1985) has shown, however, that the cross-linking between the polar groups, exposed during drying operations, for example, has only a very small effect on the water binding capacity of proteins.

Labuza (1977) picked out the capillary structure of food and the supersaturation of carbohydrates, which had changed from crystalline to amorphous state during desorption, as important factors in the extent of the hysteresis phenomenon. Similarly, it has been suggested by Eichner (1986) that a higher water vapour partial pressure is required to fill food capillaries (during adsorption) than to empty them (during desorption). This so-called "ink bottle neck" theory, first expounded by

Kraemer (1931), and the existence of water soluble food components, which can switch between crystalline and amorphous states, were put forward as major causes of hysteresis.

At low temperatures (i.e. temperate ambient and below) and over relatively short periods of time, hysteresis has been said, by Kapsalis (1981), to be reproducible and persistent over many adsorption/desorption scans. At higher temperatures and over longer periods of time (as in the prolonged storage of dried foods) this did not appear to be true.

Cutting et al. (1956) for example, had shown that the temperature used for drying cooked fish samples did affect the shape of the isotherms obtained. The magnitude of this effect, however, may be insignificant in practice. Curran and Poulter's (1982) results showed that neither fish species nor salt concentration nor variation in drying temperature, within the limits of what was commercially normal, had any significant effect on the sorption characteristics of cured, tropical fish. Nguyen and Haard (1987) however, found the salt content range in commercial samples of heavily salted cod to be so wide (37-49% of total solids) that no correlation between a_w and water content could be established.

Nonetheless, the changes in the hysteresis loop with time and temperature has been put forward as a basis for a quantitative index of dried food quality. The implications of this suggestion on objectives of this research programme will be considered further under section III(iv).

II (iv) Sorption isotherm modelling for water activity and shelf-life prediction.

The classic kinetic model for the adsorption of gases onto plane surfaces was expressed in Langmuir's (1918) equation:

$$V = V_m \frac{(cp)}{(1+bp)}$$

where V is the volume of water vapour adsorbed isothermally by the surface at vapour pressure p; V_m is the volume adsorbed when internal surfaces are totally covered with a monolayer of molecules; and c is a constant dependant on the temperature and type of surface.

It seemed to Brunauer et al. (1938) that the same forces producing condensation would, also, be chiefly responsible for the binding energy of multimolecular adsorption.

Accordingly they extended Langmuir's treatment to cover multimolecular adsorption, thus:

$$V = \frac{V_m cp}{(p_0-p)\{1+(c-1)(p/p_0)\}}$$

where p_0 is the saturation pressure of the gas. The constant, c, was found to have a value approximately equal to $e^{E_1-E_L/RT}$, where E_1 is the heat of adsorption of the first monomolecular layer and E_L is the heat of liquefaction.

This gives an S-shaped isotherm, being concave to the pressure axis when p/p_0 is low and convex to the pressure axis when p/p_0 is high, which is typical of the shape of the water vapour sorption isotherms for most proteinaceous, fibrous foods. It does not, however, account for the hysteresis between adsorption and desorption arms of such isotherms obtained empirically.

Hailwood and Horrobin (1946) stated that the typical shape of the experimentally obtained isotherms had previously been explained by postulating two kinds of combined water,attached respectively to the polar side chains and to the polar groups in the polypeptide chains, a well as that which condensed ultimately in the pores of the fibres as liquid water. Counter to this, they chose a model which assumed adsorbed water to exist either in simple solution or combined to form a monohydrate with a definite unit of the fibre molecule.

$$\frac{Mr}{1800} = \frac{ah}{1-ah} + \frac{abh}{1-abh}$$

gives the relationship between uptake (r = total grams of water absorbed per 100g of dry polymer) and relative humidity, h. M is the "molecular weight" of the operative polymer unit, and a and b are constants.

The equation was found to fit experimental sorption data well for nylon, human hair, wool, silk and cotton. . . . Their main criticism of their derived equation, however, was its failure to provide for the hysteresis which was such a noticeable feature of the experimental adsorption-desorption isotherms for the fibrous polymers, except nylon.

Smith (1947) said that the water sorption isotherm could be described by the equation

$$m = A + B.\ln(1-a_w)$$

where m is the water content and A and B are intercept and gradient constants from the plot of m against $\ln(1-a_w)$.

For a casein/water system, Chirife et al. (1985) evaluated B and A at -0.08801 and -0.28657 respectively.

A sorption equation to provide an expression for the condensation of water in multilayers, at a relatively large distance from the surface of the sorbing polymer species, was developed by Halsey (1948):

$$p/p_0 = \exp(-a/RT\theta^r)$$

where $\theta = X/X_m$, the ratio between equilibrium coverage (X) and monomolecular coverage (X_m) in grammes of water adsorbed per gramme of solid, and a and r are constants.

Iglesias et al. (1975) used Halsey's equation for the prediction of water adsorption isotherms for foodstuffs. They put the expression into the form

$$\ln.\ln(p_0/p) = -r\ln\theta + \ln a'$$

to produce a straight line from which values of a' (where a' = a/RT) and r could be obtained. The predicted isotherms, thus produced, matched those obtained through experiment in a wide variety of foods.

A further extension of the B.E.T. model, which derives from the predictive equations of Guggenheim, Anderson and DeBoer, and, therefore, referred to as the "G.A.B." model by Van Den Berg and Bruin (1981) takes account of the modified properties of the fluid which is sorbing (water, in this case) in the multilayer zone of the isotherm.

$$\frac{M}{M_m} = \frac{Cka_w}{(1 - ka_w)(1 - ka_w + Cka_w)}$$

where M/M_m is the ratio of the actual water content to the B.E.T. monolayer water content on a dry basis;

C is the Guggenheim constant = $c'\exp(H_q - H_m)/RT$ with H_q and H_m being the total heat of sorption of the water molecules condensing in multilayers and monolayer on the primary adsorption sites respectively;

and k is a factor correcting properties of the multilayer molecules with respect to the bulk liquid such that $k = k'\exp(H_1 - H_q)/RT$ with H_1 being the heat of condensation of pure water vapour.

Bizot (1983) found this model suitable for the prediction of a_w in the range 0.1 to 0.94, for a wide variety of foods, when only a few (i.e. 4 or 5) points had been found experimentally on the a_w against water content isotherm.

In spite of almost 100 years of research relating the water content and E.R.H. of foods, Van den Berg and Bruin (1981) observe that food products are still dried to a water content empirically found to allow safe storage with minimum quality deterioration. The latter workers have compiled a list of 75 isotherm equations which could be used for mathematical sorption modelling but warn that "we can never prove the validity of a sorption model just by its ability to fit the observed isotherm." For their work on moist mixtures, they selected the equation proposed by Ross (1975) to give a rapid estimate of a_w and, hence, the possible stability of intermediate moisture foods.

The latter proposes that "a_w is simply the product of a_w values for simple solutions of each solute measured at the same concentration as in the complex solution."

$$a_w = (a_w^0)_1.(a_w^0)_2.(a_w^0)_3.....$$

Ross suggested that the errors incurred in calculating a_w using this equation, rather than obtaining a value experimentally, would be less than 1% in the usual concentration ranges of food products.

According to Ferro-Fontan et al. (1980) it is desirable and sufficient to predict a_w within about 0.01 a_w for i.m. foods, as is claimed posible using the Ross equation, and this can be done with pure salt solutions. It is, however, only possible to get within 0.02 a_w when predicting for salt fish in this way.

Lupin et al. (1981) used other research workers' results on salted cod, anchovy and mullet roe to obtain a straight-line relationship between a_w and molality of salt in fish:

$$(a_w)_{sf} = a_w/^0 - bm = 1.002 - 0.042m$$

where $(a_w)_{sf}$ is the water activity of the salted fish product, $a_w/^0$, the water activity at zero salt molality, b, the slope of the line and m, the molality of the salt in the fish.

Temperature, over the range likely to be met in commercial fish salting, had no significant effect on this relationship, and it seemed evident that the water activity of salted fish was the water activity of the salt solution which would be formed by the contained salt in the contained water. The approximate linearity over certain portions of the sorption isotherms for foods allows for some degree of extrapolation in estimating a_w when salt and water content are known. It can be based on the established thermodynamic relation for binary solutions

$$a_w = \exp(- w\, m_1\, m\, v)$$

where w is the osmotic coefficient, m_1, the number of kg per mole of water (i.e. 0.018) m, the molality of the solute and v, the number of moles of all species which give 1 mole of solute in solution (v = 2 for NaCl).

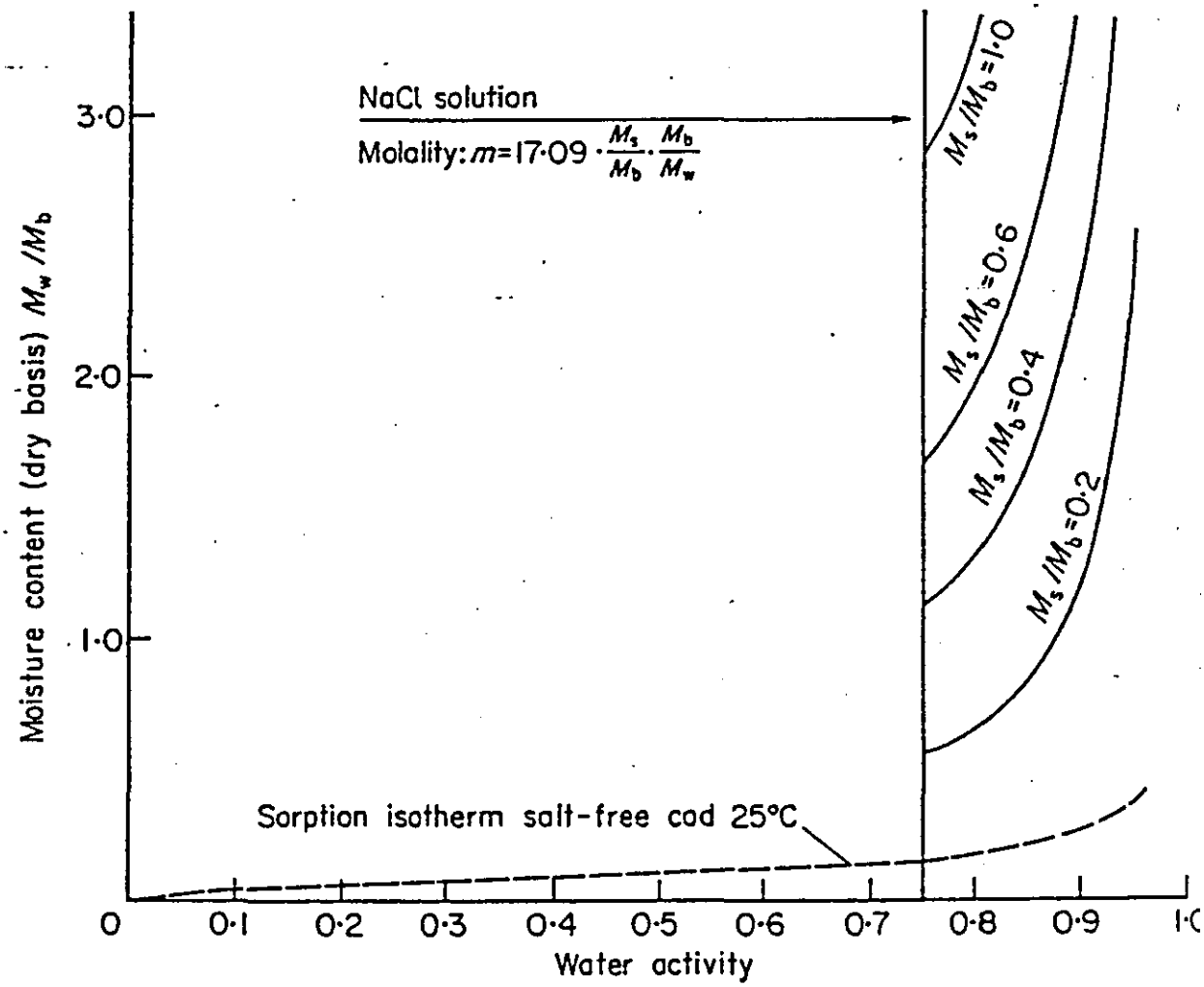
Mean values for the osmotic coefficient for pure salt solution and for salted fish were said to be so close (1.111 +/- 0.028 and 1.167 +/- 0.056 respectively) that they could be taken as equivalent when taking experimental errors into account.

The application of the Ross Equation in predicting the water activity of i.m. foods containing a non-solute solid was found by Chirife et al. (1985) to show differences (experimentally determined - calculated) of between -5.1% and -19.6%. This was thought to be due to the fraction of water (5 to 6% on a dry basis) which was strongly bound to the non-solute solid, in this case, casein. For this reason, the authors added a correction to the Ross prediction, allowing for 5.5g water per 100g of dry casein not being available to dissolve the solute. This gave an average +3.6% error (exp. - calc.) compared to the average -11.5% error obtained using the uncorrected Ross prediction.

Lilley (1987) suggested an extension to the Ross Equation which was thermodynamically valid when attempting to predict the water activity of a three component mixture:

$$a_{w1.2.3} = (a_{w1.2})(a_{w1.3})(a_{w2.3})(a_{w1} \cdot a_{w2} \cdot a_{w3})$$

FIGURE 5: Sorption isotherms for sodium chloride solutions of different salt to dry solids (Ms/Mb) ratios compared with the sorption isotherm for salt free cod



(after Doe et. al., 1982)

Table 4. Water activities from water and salt contents

Salt content (M_s/M_b)

Water content (M_w/M_b)	0	.02	.04	.06	.08	.10	.12	.14	.16	.18	.20	.22	.24	.26	.28	.30	.35	.40	.45	.50	.55	.60	.65	.70	.75	.80	.85	.90	.95	1.0
.02	.04	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03
.04	.09	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07
.06	.17	.13	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12	.12
.08	.26	.21	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19
.10	.47	.41	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35	.35
.12	.59	.53	.46	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44	.44
.14	.69	.63	.56	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51	.51
.16	.75	.69	.63	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56	.56
.18	.80	.74	.68	.61	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60
.20	.83	.78	.73	.67	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62	.62
.22	.86	.82	.77	.71	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65	.65
.24	.89	.85	.80	.75	.69	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67	.67
.26	.91	.87	.82	.78	.72	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68	.68
.28	.93	.89	.85	.80	.75	.70	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69
.32	.95	.92	.88	.84	.80	.75	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71
.36	.96	.93	.90	.87	.83	.79	.74	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72	.72
.40	.97	.95	.92	.89	.85	.82	.78	.74	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73
.44	.98	.96	.93	.90	.87	.84	.81	.77	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74
.48	.99	.96	.94	.91	.89	.86	.83	.80	.76	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74
.52	.99	.97	.95	.93	.90	.87	.85	.82	.79	.75	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74	.74
.56	1.0	.98	.96	.93	.91	.89	.86	.84	.81	.78	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.60	1.0	.98	.96	.94	.92	.90	.87	.85	.82	.80	.77	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.64	1.0	.98	.96	.94	.93	.90	.88	.86	.84	.81	.79	.76	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.68	1.0	.98	.97	.95	.93	.91	.89	.87	.85	.83	.80	.78	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.72	1.0	.98	.97	.95	.93	.92	.90	.88	.86	.84	.82	.78	.77	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.76	1.0	.99	.97	.96	.94	.92	.90	.89	.87	.85	.83	.81	.79	.76	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.80	1.0	.99	.97	.96	.94	.93	.91	.89	.88	.86	.84	.82	.80	.78	.76	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.84	1.0	.99	.97	.96	.95	.93	.92	.90	.88	.87	.85	.83	.81	.79	.77	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.88	1.0	.99	.98	.96	.95	.93	.92	.90	.88	.87	.86	.84	.82	.80	.79	.76	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.92	1.0	.99	.98	.96	.95	.94	.92	.91	.89	.88	.86	.85	.83	.81	.80	.78	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
.96	1.0	.99	.98	.97	.95	.94	.93	.91	.90	.88	.87	.85	.84	.82	.81	.79	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
1.0	1.0	.99	.98	.97	.96	.94	.93	.92	.90	.89	.88	.86	.85	.83	.82	.80	.76	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
1.5	1.0	.99	.98	.98	.97	.96	.95	.95	.94	.93	.92	.91	.90	.89	.89	.88	.85	.83	.80	.77	.75	.75	.75	.75	.75	.75	.75	.75	.75	.75
2.0	1.0	1.0	.99	.98	.98	.97	.97	.96	.96	.95	.94	.94	.93	.92	.92	.91	.89	.88	.86	.84	.82	.80	.78	.76	.75	.75	.75	.75	.75	.75
2.5	1.0	1.0	.99	.99	.98	.98	.97	.97	.96	.96	.96	.95	.95	.94	.94	.93	.92	.90	.89	.88	.87	.85	.83	.82	.80	.78	.77	.75	.75	.75
3.0	1.0	1.0	.99	.99	.99	.98	.98	.97	.97	.97	.96	.96	.96	.95	.95	.94	.93	.92	.91	.90	.89	.88	.86	.85	.84	.83	.81	.80	.79	.77
3.5	1.0	1.0	.99	.99	.99	.98	.98	.98	.98	.97	.97	.96	.96	.96	.96	.95	.94	.93	.92	.92	.91	.90	.89	.88	.87	.86	.84	.83	.82	.81
4.0	1.0	1.0	1.0	.99	.99	.99	.98	.98	.98	.98	.97	.97	.97	.96	.96	.96	.95	.94	.93	.93	.92	.91	.90	.89	.88	.88	.87	.86	.85	.84

(after Doe et. al., 1983)

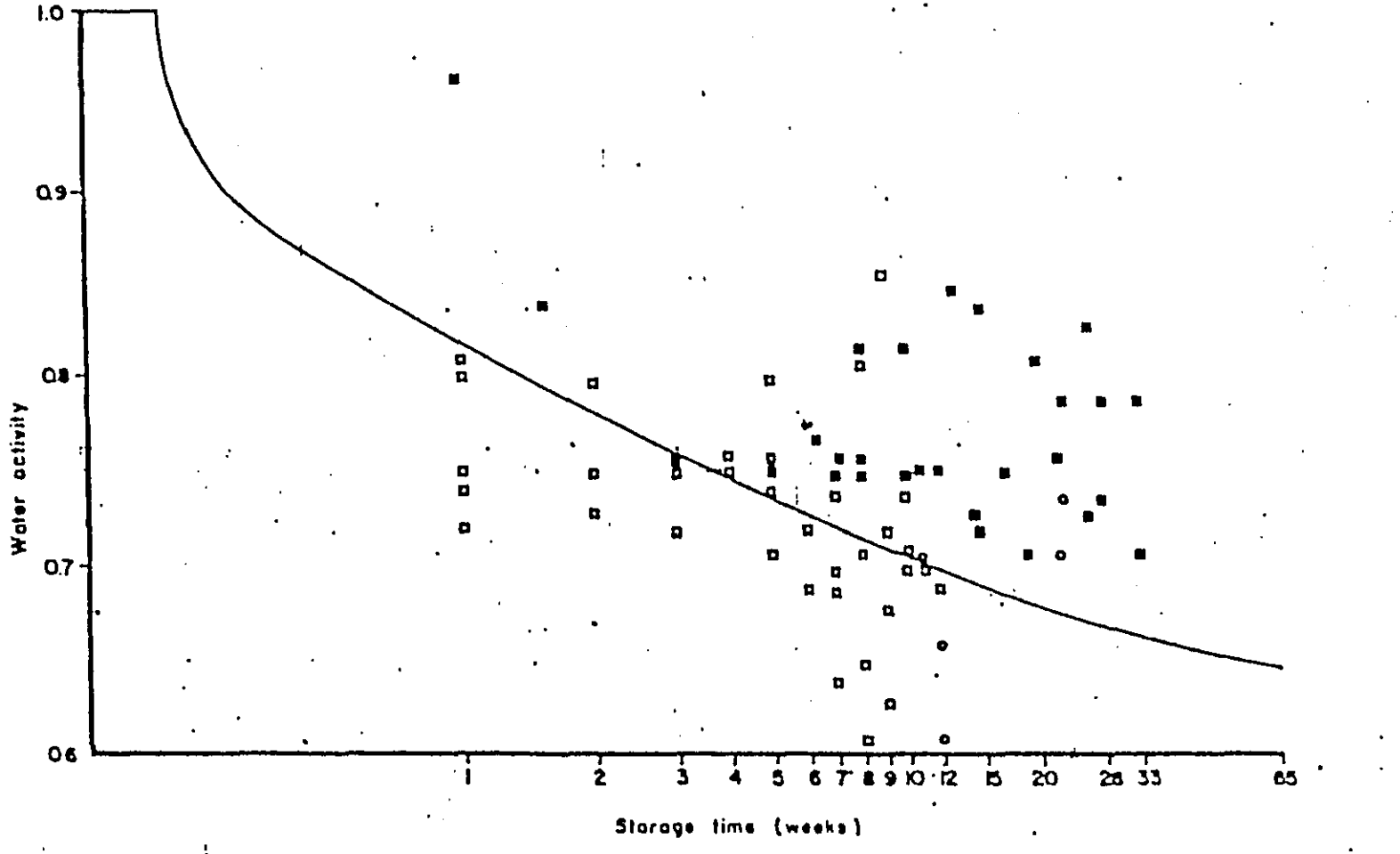


Fig 6 Growth curve for appearance of visible colonies of dun mould, W. sebi, with water activities of stored fish. Results of storage trials: □ no mould or beetles; ■ mould; ○ beetles; ● mould and beetles

(after Doe et. al., 1983)

This gave better agreement with observed results than the unmodified form of the Ross Equation

$$a_{w1.2.3} = (a_{w1} \cdot a_{w2} \cdot a_{w3})$$

Taking the components of dried, salted fish to be water, salt, fat and fish muscle (protein) and assuming that fat took no part in water activity calculations, due to its hydrophobicity, Doe et al. (1983) suggested that the a_w of dried, salted fish could be predicted using the Ross Equation written thus:

$$a_w = a_{w0} \cdot a_{wn}$$

where a_{w0} is the water activity of the fish muscle (i.e. the salt-free, fat-free solid fraction, and a_{wn} is the water activity of the sodium chloride solution present in the muscle. Values for a_{w0} were found by applying the latter equation to the sorption isotherm for unsalted cod from Doe et al. (1982) given in Figure 5.

The latter researchers used the equation to produce a table (Table 4) which predicted the a_w of dried, salted fish given its salt content, M_s, water content, M_w, and salt-free, fat-free dry solid content, M_b, and related it to storage life through empirical observations (Shewan-1953) on the time taken for dun mould (*Wallemia sebi*) colonies to become visible at different a_ws (Figure 6).

II (v) Validity of a_w as a predictor of product performance.

In the preceding section, it has been seen how, through approximations and simplifications, the water activity of substances as complex as foods can be estimated and used to predict shelf-life. The accuracy of a_w estimations is, according to Lilley (1985) however, affected by solute size and hydration, increases in which would reduce a_w below that expected; and by solute- solute association, like the protein-protein and protein-salt associations, so extensive and variable in curing and cured fish, which would increase a_w beyond that expected from the Ross Equation.

Water activity has been used increasingly as a concept in food preservation since the classic review of Scott (1957). The parameter measured and recorded as "water activity", however, is, in fact, the relative vapour pressure (RVP). This was described by Van Den Berg (1985) as an empirical pseudo-a_w, reflected in the system's relative humidity, which exists rather than a thermodynamic a_w. The latter is not possible because foods are NOT usually simple mixtures but complex systems exhibiting often non-equilibrium behaviour exemplified by the hysteresis of their sorption isotherms.

The magnitude of the hysteresis loop, and, therefore, according to Hardman (1985), the potential errors in using a_w as a foodstuff performance predictor, is influenced by the food's chemical and physical structure, its moisture level, temperature and pretreatment, and the method of measurement. Proteins, however are said to exhibit less hysteresis than carbohydrates.

Misinterpretations of food stability as a function of water activity (strictly, RVP) has led, according to Van Den Berg (1985), to calamities in the preservation of i.m. foods recently. Safe conclusions with respect to standards for practical processing and storage for a specific product should be given only after careful consideration of the water relations and shelf-life tests on that product. As was noted by Johnston (1985) the spectacular lack of predictive utility (regarding quality, stability and viability) of RVP, in the absence of a detailed description of the behaviour of i.m. systems, is demonstrated by the hysteresis between the desorption and resorption isotherms.

III THE EFFECT OF CURING PROCESS ON EATING QUALITY.

The curing of fish involves one or more of the following preservation techniques:

(i) the abstraction of water into unsaturated air at normal or reduced pressure;

(ii) the infusion of solute(s) through immersion in solution or direct contact with the solid (usually common salt);

(iii) the infusion of the products of pyrolysis and partial oxidation of wood through contact with smoke or liquid smoke extracts.

It has been seen, in previous sections, that the success of such attempts at preservation (in terms of expected shelf-life of the product) depends on the extent to which its water activity is reduced and kept low. In this section it is intended that the hypothesis:

"the sorption characteristics and the eventual eating quality of fish preserved by dry curing are affected by both the extent and conditions of dehydration"

-be examined.

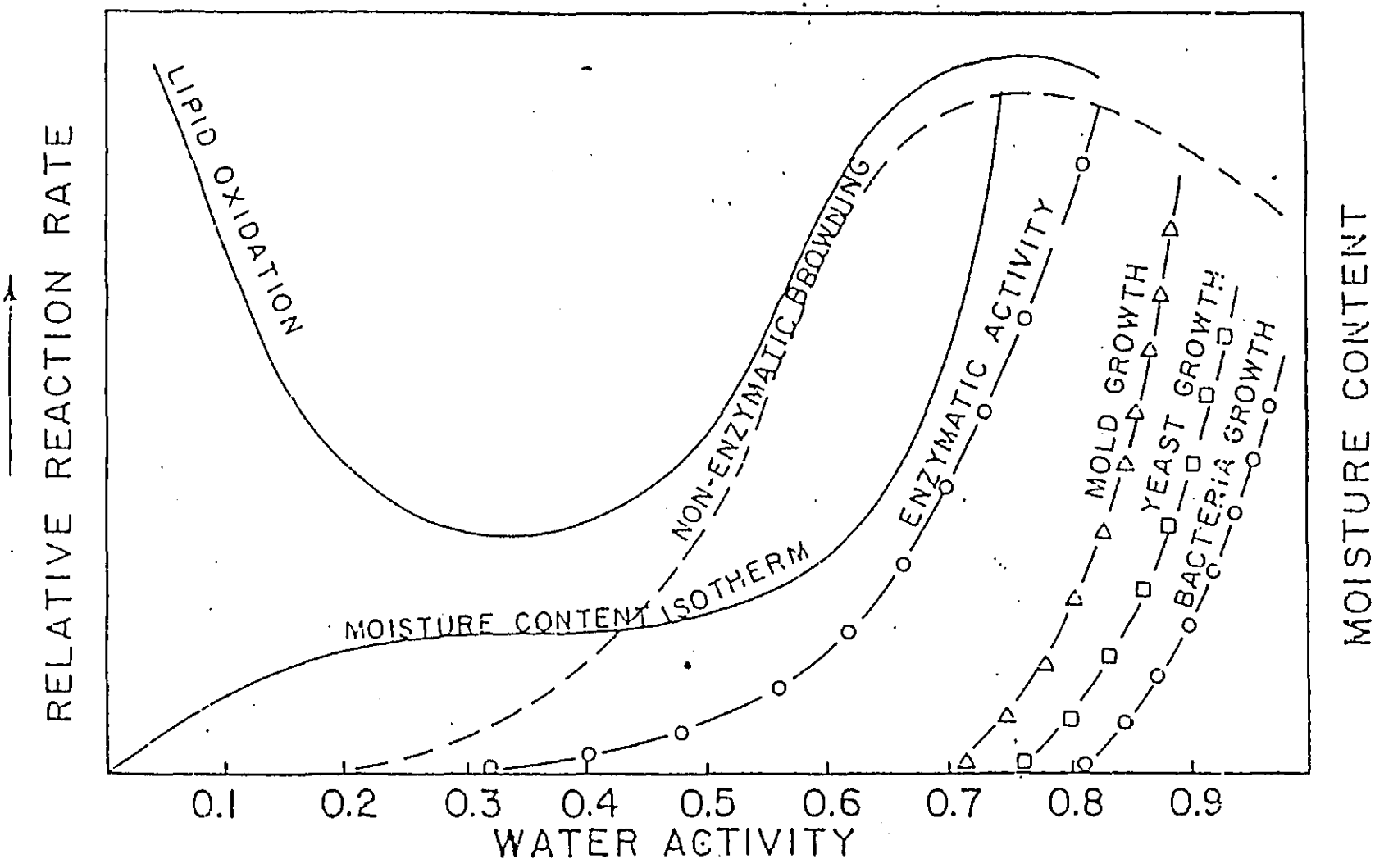
III (i) Drying rates and sorption isotherms.

The rate at which food dries is proportional to the difference between the vapour pressures exerted by the food surface and the air in which it is in contact. Loncin (1961) concluded, therefore, that the curves which related a_w to water content would be important in the prediction of drying rates. The water content which would have to be taken into account, however, was that at the surface. This would, unfortunately, be vastly different from that below the surface throughout all but the very earliest part of the drying process.

Labuza (1972) stated that high speed drying was necessary for the minimization of nutrient and quality loss in liquids, like milk. If this were to be the case, also, with solids, like fish, temperature increase might be the most obvious means of accelerating drying rate and, thereby, maximizing quality retention.

On the other hand, an increase in drying temperature might, itself, affect the manner and extent of water resorption by the dried product, which would also influence its perceived quality. Iglesias and Chirife (1976) showed that drying temperature affected the sorption capacity of dried beef. Conversely, the sorption characteristics, as affected by salt content, for example, influence the drying rate. Berhimpon et al. (1990) found the higher the salt content of fish after brining, the slower was the rate of water loss during, and the higher was the final water content after, drying.

FIGURE 7



STABILITY OF FOODS AS A FUNCTION OF WATER ACTIVITY

(after Labuza, Tannenbaum and Karel, 1970)

III (ii) Storage and sorption isotherms.

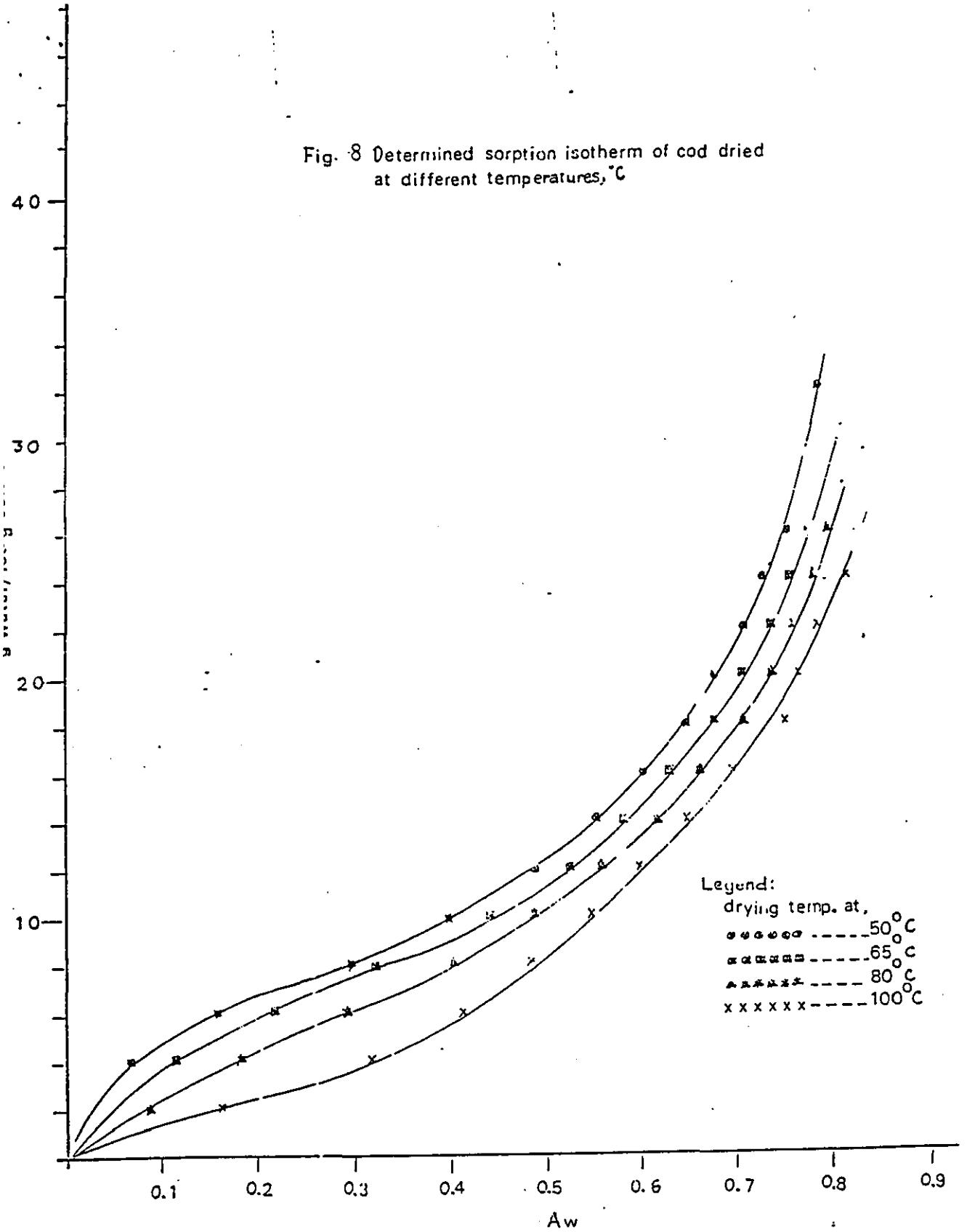
Once produced, dried food is considered, for marketing purposes, to be shelf-stable over the range of temperatures likely to be encountered in storage, distribution and retailing. Cole (1962) and Obanu et al. (1975), however, have shown that storage temperature also affects the sorption properties of dried meat. For example, a 12% loss in recoverable sarcoplasmic proteins, with corresponding reduction in rehydrateability, occured in freeze-dried beef stored at 38°C over a 2-month period.

Similarly, Maruf et al. (1990) found that the temperature at which dried-salted mackerel was stored had a very significant effect on net protein utilization (NPU) and biological value (BV) of the product when fed to rats. After 20 weeks storage at 20°C, NPU was 53.7% and BV 56.8% compared to 75.6% and 77.4% respectively, after the same storage period at 0°C. The authors suggested that one of the more likely explanations for this dramatic decrease in NPU and BV was the formation of complexes which lower the biological availability of some of the essential amino-acids. Some of the products of lipid oxidation, which is much more significant at higher temperatures, might be involved in the acceleration of such complexing reactions.

Duckworth and Smith (1962), stated that the movement of solutes through semi-dry food influences quality changes in the material during storage. Tracer studies had shown that the movement of solutes during air drying is towards the centre of the drying piece, as a concentration gradient is set up due to the rapid water removal from the surface layers. As the sodium and chloride ions compete more effectively than protein for the decreasing amount of available water, the protein, as Hamm (1960) said, increasingly precipitates out. Thus, Rolfe (1976) observed that foods rich in native proteins, like raw meat and fish, were most susceptible to textural changes as abstraction of water proceeded to reach the low levels associated with preservation purely by dehydration.

In contrast to this observation, Labuza (1977) reported that the higher the a_w of intermediate moisture, textured protein foods, during storage, the harder and tougher became the product after reconstitution. This was said to be due to the increased rates of lipid oxidation and Maillard browning reactions at the higher a_w end of the sorption isotherm (see Figure 7), the products of which effected the cross-linking and, thereby, the increasing insolubilisation of the protein.

Fig. 8 Determined sorption isotherm of cod dried at different temperatures, °C



(after Jones and Peralta, 1980)

III (iii) Drying temperature and sorption isotherms.

Sorption isotherms were reconstructed by Jones and Peralta (1980) from empirical observations during the resorption of cod muscle dried at different temperatures, individual results having been adjusted using the Clausius-Clapeyron relationship. These showed (Figure 8) that the sorption isotherm was displaced progressively with rising temperature of drying so that the same water content material had progressively higher a_w the higher the temperature at which it was dried.

In the limited range of temperatures likely to occur in natural, commercial drying operations in the tropics, this would be insignificant, according to Curran and Poulter (1983). Using mechanical driers, however, the range of temperatures which could be selected for a process could have a noticeable effect on the sorption characteristics of the products. The work of Berhimpon et al., on the salting and drying of yellowtail (*Trachurus mccullochi*) has shown that treatment with lower brine concentrations followed by drying at relatively low (35°C) temperatures and high (50%) relative humidity gave better quality products (as judged by sensory panel) than treatment with stronger brines or more severe drying conditions.

III (iv) Sorption hysteresis as a quality index.

Wolf et al. (1972) investigated the usefulness of sorption hysteresis as an index of the quality of dried foods. It was found that the width of the hysteresis loop increased with storage time. For beef and haddock, there was almost 3% difference in water content, for a given a_w value, between freshly dried and stored samples. Foods which exhibited such a large increase in hysteresis upon storage, showed a drastic decrease in their capacity to adsorb water, and furthermore, upon sensory examination, showed a decrease in the quality attributes of colour, taste and rehydrateability. This latter could be due to the cross-linking reactions which, according to Obanu et al.(1975), occur between protein molecules as storage, particularly at tropical temperatures, proceeds.

It had already been suggested by Labuza (1970) protein-protein aggregation in dried foods would lead to poor solubility and toughening engendering a gradual loss of quality through the storage period. Such change might be the result of the products of oxidising lipids interacting with proteins to produce protein radicals. These could further react to form scission products of sufficiently low molecular weight to become mobile in the limited free water present in dried foods. Particularly, if dried food water contents vary, as a result of humidity changes in the store, this increased protein reactivity and mobility would increase the tendency for them to aggregate. This would have the effect of widening the hysteresis loop.

Water contents between 30 and 80% (dry basis) were found by Chou and Labuza (1973) to coincide with the maximum rate of oxidation and consequent protein aggregation. Such water contents are encountered most frequently in i.m. products, but might also occur in dry-cured fish taking up water vapour from a humid storage environment. The width or area of the hysteresis loop might, therefore, be used to indicate that a dry product with apparently satisfactory water content at the time of testing, had, in fact, at some stage during its storage, been subjected to unsuitable conditions.

Hysteresis loop size may not, however, be indicative only of less-than-ideal drying and storage conditions. Williams (1976) work showed that the quality status of fish at the commencement of drying could affect the sorption characteristics of the product and how these might change during storage. High levels of nucleic acids, as might be encountered in spoiling fish, signified high levels of ribose which readily reacted with proteins in the Maillard browning reaction. In turn, this promoted the cross-linking of proteins causing the product to be tougher and less digestible. The

maximum rate of Maillard browning was reported by Labuza (1970) to occur between a_w 0.6 and 0.9. Such a_w levels would coincide with the later stages of drying and with the storage of the dried food.

These factors responsible for hysteresis loop widening, such as the levels of reducing sugars and lipids in the flesh, are subject also to variations which are associated with physiology, season and the method by which the fish is captured. Thus, although it might be a basis for a quality index, the extent to which dried fish might exhibit hysteresis would not be indicative of the causative agents of quality deterioration.

IV THE STORAGE AND SPOILAGE OF DRY-CURED FISH

From the author's own observations, refered to in the introduction, dry-cured fish rapidly takes up water, when exposed to a humid environment, becoming susceptible to microbiological spoilage long before the expected expiry of the shelf-life normally projected for such products.

IV (i) Packaging, Distribution and Storage.

Throughout the world, the production of dry-cured fish continues to be an overwhelmingly low-technology operation, employing little or no equipment and, most frequently, utilizing the no-cost energy of sun and wind. Packaging, if used, has tended to fulfil only containment and unitizing functions compatible with mere conveyance of product between producer and wholesaler. The retailer has been content to purchase, display and sell such products without any form of package. Indeed, large split fish, which have been salted and dried, are traditionally displayed unpackaged so that the retail customer can select his "cut" from the "side". In Spain and Portugal, cuts of *bacalao* retail at different prices just like cuts of beef (Giles-1990).

Wooden crates, hessian sacks and, more recently, fluted cardboard boxes have been used for the bulk containment of dry-cured fish. Where it has been intended to export the product from temperate producer to tropical wholesaler, bituminized paper liners have been used retard uptake of atmospheric moisture.

Flexible films, although possessing greater moisture barrier properties, have not been significantly exploited since they are easily punctured by the hard, sharp protruberances of the product and prevent the escape of water from the product. Such water condenses on the inner surface of the film, if the ambient temperature falls, and mould-growth quickly ensues.

More recently, Antony et al. (1988) tested the suitability of four different packaging systems for storage and transportation of dried, salted fish. It was found that high density polyethylene (HDPE) woven sacks laminated with a 100 guage low density polyethylene (LDPE) inside performed most satisfactorily over a 7-month period of trial. The contents, dried, salted shark, showed insignificant water uptake over the storage period, whereas the control, packed in palmyrah mat and jute sack, rapidly took up water in the wet season and spoiled within two months. Drop and roll testing caused puncturing of the inner lining, permiting subsequent water uptake by the contents, in other package systems tested, whilst the HDPE/LDPE sacks remained intact. The latter were, therefore, recommended in spite of their 20% higher cost, as a measure to improve the quality of dried, salted fish exported from India.

Kench-cured (i.e. dry-salted) white fish, having been taken out of wet stack (Figure 9) after two weeks' contact with dry salt, can be stored for a year or more at 5°C. In fact, the processes of water and salt equilibration and flavour maturation

FIGURE 9: Kench curing of cod (wet stack)

FIGURE 10: Kench curing of cod (pining)

FIGURE 11: (a) Arranging 'pined' cod in the drying kiln

(b) kiln dried salt-cod

FIGURE 12: The production of pindang

during such storage is traditionally held to be beneficial to the eventual quality of the fully-dried product. This "pining" process (Figure 10), so-called after the Scandinavian pine sheds used to store the semi-cured fish, involves prolonged storage even though the water content and a_w remain considerably above the minima at which the growth of xerotolerant moulds might be expected. Maintenance of a low temperature throughout, however, ensures the non-occurence of such spoilage.

In the U.K., the vast majority of this "pined" fish is tunnel-dried (Figure 11) to reduce its a_w to a level sufficiently low to prevent spoilage during distribution to tropical markets. A small, but increasing proportion nonetheless, is being portioned and plastic film-packaged without drying for chill distribution and display on the home market.

The salt-boiled fish or *pindang* of S.E. Asia is edible without the desalination required before consuming kench-cured fish but its shelf-life (Figure 12) is only a few days. Ibrahim (1986) however reported that packing the product in a carbon dioxide enriched atmosphere could incease its shelf-life by at least 50% over that of controls packed in a normal atmosphere.

IV (ii) Agents and symptoms of spoilage.

Before an estimation of shelf-life can be made, it is necessary to know the likely manner of deterioration under given conditions and how these relate to human sensory perceptions of "quality".

The generalized food sorption isotherm with superimposed relationship between reaction rates and a_w (Figure 7) produced by Labuza (1970) gives an idea of the likely predominant spoilage agency at different a_w levels. In the range of water activities at which dry-cured fish are packaged for export (i.e. <0.7) chemical changes are the major causes of quality loss. In the case of dry-cured fish being stored in the tropics, however, fragmentation and attack by beetles and mites would be regarded as much more significant causes.

Above a_w 0.7, attack by blow-fly larvae is seen as a major cause of loss in quality and yield in the tropics (Esser 1988) but spoilage by microorganisms arguably predominates in shelf-life limitation as it might be judged by the consumer.

According to Giles (1990) the predominant type of spoilage occuring during the process of kench curing is due to the proliferation of "pink" halophilic bacteria present in virtually all curing salt supplies. Generally, such spoilage becomes noticeable in the wet-stack stage of salting during the warm summer months. (Figure 2).

The variety of microbial spoilage most frequently associated with spoilage of the finished dry-salted products, as they take up atmospheric moisture in humid tropical conditions, is fungal. The appearance of visible colonies of the dun mould, *Wallemia sebi*, (Figure 1)has been used by Doe et al. (1982) as the criterion of storage life determination. This approach has been reviewed in section II(iv) where the relevant table (Table 4) and graph (Figure 6) are reproduced.

IV (iii) Shelf-life studies

Kalaimani et al.(1988) who used the method of Doe et al. (1982b)to determine a_w of samples of commercially-prepared dry-salted fish (i.e. by using Table 4 to find a_w from analysed water, salt and dry, fat-free, salt-free solids contents) found extensive mould growth after 3 weeks' storage of samples ranging in a_w between 0.74 and 0.95. The use of mould visibility as the "limit of shelf-life" criterion seemed justified since sensory inspection revealed that wherever mould growth on a sample was significant, it was overwhelmingly rejected. In comparison, there was a certain degree of tolerance for samples which bore evidence of insect attack.

Unlike the Kalaimani et al. samples, which were sealed in polythene bags and, therefore, probably remained at their initial a_w during storage, the dry-salted fish control samples of Antony et al.(1988) picked up moisture through their packaging during storage. Their water content increased from 29.6% to 43.0% in 60 days. Using the table and graph from the report by Doe et al.(1982) this represents an increase in a_w from 0.74 to 0.77; that is the difference between 7 weeks and 16 days (visibly) mould-free shelf-life.

These samples, however, had been surface-treated with the antimycotic, calcium propionate (0.1%) and spoilage was characterised by "discolouration, intense ammoniacal smell and appearance of insects." The former two suggest advanced "pink" spoilage.

This tallies with the author's observations noted in section I(i) and the findings of George Joseph et al.(1988) who, on examining 358 samples of sun-dried/dry-salted fish, noted evidence of pink ("red") spoilage in 62% of them. It was not stated whether antimycotic agents had been used in their preservation.

The studies of Young et al.(1973) on tunnel-dried abalone revealed a mould-free shelf-life of several months in temperate climatic conditions, even though samples had been dried only to 40% water content (wet basis) so that their a_w, at the start of storage, was considerably higher than 0.7, the minimum a_w for mould growth. During storage (9 months at temperate ambient) however, it was found that the water content had decreased to 8 - 13% and a_w to 0.3.

The reverse seems to be true when dry-cured fish is stored in tropical ambients: even when the a_w is reduced in processing to levels considerably below 0.7, uptake of water from the humid surroundings is such that spoilage very quickly ensues. Hence,

as Troller and Christian (1978) observed, the susceptibility of tropics-produced, dried, salted fish to spoilage by mould, particularly the dun species, *Wallemia sebi*, is high.

V AIMS OF PRESENT WORK

Means are available for extending the storage life of cured fish under humid environmental conditions. The options are:

(i) the prevention of water uptake from the humid ambient, and

(ii) the inhibition or destruction of the agents of spoilage in the product.

These are not mutually exclusive, indeed (i) might be the means of accomplishing (ii), and (ii) might be employed whether or not the measures taken to achieve (i) are successful.

V (i) Barriers to water uptake

Packaging has been considered in section IV(i) but its major drawbacks are that:

(a) its cost would very significantly increase the unit cost of the product and put it beyond the means of much of its present market;

(b) the market is unfamiliar with the product in packaged form and might be suspicious of the motives behind packaging, for example, whether it was to conceal inferior quality;

(c) the product being often hard, rigid and angular, the package might puncture and tear, thus quickly becoming useless, during transit handling.

A more practical solution to the rapid uptake of water in humid climates might be to surface coat the finished product by dipping or spraying with some edible substance which would subsequently retard the ingress of water.

Previous work has shown the effectiveness of this proposition. Motlagh (1982) showed that the dipping of lime fruits in an emulsion of polysaccharide and sucrose esters of fatty acids retarded moisture LOSS compared to that from control samples. Similarly, Fargher (1987) showed the varying barrier-to-water-vapour properties of some edible fats in different layer thicknesses.

Fish has been described as a fibrous gel (Hamm 1962) and since these fibres are organised in bundles in the muscle, it seems likely that there would be some directional differences in the rate of water movement. The rate of water uptake, or removal, might then depend upon whether it was moving across or along muscle fibres. Hence, the manner in which the fish was cut and prepared for drying would affect the rate at which it dried and the rate at which it resorbed moisture from a humid atmosphere. Trying to explain the very slow rate of solutes diffusion in food (one-fifth of the rate in water) Guerts et al. (1974) suggested that obstructions due to the tortuosity of pores could account for the retardation. If there is a difference between "across" and "along" fibre drying rates, this "pore tortuosity" might be responsible.

V (ii) Spoilage inhibition

Small uptake of water by dried fish leads to a disproportionately large increase in water activity and, therefore, its propensity to spoil unless other preservative measures are, or have previously been, taken.

Refrigeration, natural or mechanical, is used to preserve salted, partially-dried fish during the "pining" stage of the traditional "kench" cure process for *bacalao*. The product a_w at this stage is too high to delay the growth of xero-tolerant moulds or halophilic bacteria for more than a few days at temperate ambient, but below 5'C, the product remains unspoiled for upto a year (Giles-1990).

Modified Atmosphere Packaging (MAP) of salt-cured fish has also been demonstrated to extend its shelf-life by Ibrahim (1986).

These techniques of supplementary preservation will not be considered in this study, however, because they are not frequently available options in most of the tropical markets where the end-product is sold.

Destruction of the agents of spoilage by heat treatment, as a supplement to preservation by drying, has the drawbacks of being lastingly effective only when recontamination can be prevented and being additionally deleterious on textural quality.

The preservation of fish by salt-boiling is, nevertheless, widely practised in South-East Asia. *Pindang* is produced in Indonesia by covering and sealing fish (which are placed on a bamboo-slat trivet to prevent them from adhering to the base and scorching) with salt in earthenware pots which are heated over naked flames until all the contents are thoroughly cooked. The amount of salt used is upto 25% of the fish weight and a small amount of water (upto 12% of fish weight) enables more efficient heat distribution initially. The pots and their contents (Figure 12), after decanting surplus brine and cooling, can be stored, for upto two weeks under tropical ambient conditions, 26-29'C and 70-90% RH, provided the sealing layer of salt remains undisturbed.

Ibrahim (1986) found that *pindang,* produced in this manner changed in composition during storage thus:

Table 5.

	water content (% wet basis)	salt content	a_w
after 1 day	58.5	3.0	0.97
after 14 days	44.5-48.0	8.5-9.5	0.82-0.87

The expiry of shelf-life, which in tropical markets was heralded by the occurrence of ammoniacal and putrid odours and slimy appearance, coincided, in Ibrahim's experiments, with the sensory detection of rancidity, over-saltedness and dryness. This reflected the compositional difference between the laboratory-prepared samples and those purchased on tropical markets which had water and salt contents of 60 to 67% and 0.7 to 3.9% respectively. Differences in preparational hygiene standards and individual tolerance to rancid flavours would also have affected the results such that the laboratory-prepared samples remained free from the normal signs of microbial spoilage, even after 42 days in tropical storage conditions, although they had been pronounced sensorially unacceptable after 14 days.

Rolfe (1976) suggested the addition of humectants which would permit relatively high water levels in cured products so that all bound water would be retained and structural change affecting texture would be reduced or eliminated. Sodium lactate, for example, was said by Loncin (1975) to depress a_w more than predicted by Raoult's Law for a given concentration and, for preservation purposes, was even more synergistically effective in combination with sodium chloride.

Considerable stability could also be achieved by the removal of lesser amounts of water from food supplemented by suitable adjustments in pH and/or addition of antimycotic agents.

Karel (1976) classified i.m. food production techniques as "moist infusion", "dry infusion" and "blending". In this work it was intended to examine dry infusion, whereby already-dried fish would be infused by dipping it into a brine solution followed by redrying, as a method of extending shelf-life in tropical storage.

Inhibition of spoilage may, also, be effected by the destruction of the microorganisms still present in the product or contaminating it after the curing process. Dipping the product in a weak solution of sodium metabisulphite before the final drying stage has the effect of killing most of the microbes present and preventing the growth of post-process contaminants, particularly the causative agents of "pink" spoilage (Giles-1990). Attitudes and legislation on the use of this chemical, however, precluded its investigation as an option in this work in order that possible, "non-additive" solutions to the problem could be tested.

For similar reasons, the option to retard proliferation of spoilage flora during humid storage by irradiation treatment of the cured product has, likewise, been ignored. As a future investigation, however, the use of additional preservative measures for cured fish destined to be stored in a humid environment might be considered.

V (iii) Experimental Objectives

1. To determine the sorption isotherms for cod dried in air under different conditions and compare observed sorption behaviour with that predicted from mathematical models.

2. To examine the effect of drying conditions and extent on the sensory quality of dried cod.

3. To discover whether there is a difference in drying rate due to whether water moves predominantly ACROSS or ALONG muscle fibres.

4. To discover whether the method of curing (drying, salting and/or smoking) affects the rate of water uptake in humid storage.

5. To evaluate the efficacy of different barrier substances, applied to cured fish samples prior to storage, in reducing their rate of water uptake in humid, tropical conditions.

VI MATERIALS AND METHODS

VI (i) Sorption isotherms: water activity and water content

Fresh cod fillets, purchased from a Grimsby Fish Dock wholesaler, were dried in a current of warm (40°C dry bulb) air, skin side down on plastic-covered metal racks with the air impinging on the fillet surface in a perpendicular direction from above.

The fillets were weighed and samples incised with a scalpel from the flesh surface (yellow, opaque surface - YOS) and from deep within (translucent centre - TC) the fillet, close to the under-surface of the skin at intervals during the drying period. Using plastic gloves to avoid transferring moisture from the hands to the samples, the latter, having been chopped in a thoroughly dried liquidiser, were placed and sealed in three "Novasina" water activity sample boxes. The % Relative Vapour Pressure (R.V.P.) for each of the triplicate samples was recorded after equilibration of the headspace atmosphere in the opened box at 25°C under the "Novasina" sensor for one hour. (Water activity was taken as R.V.P./100.)

After recording a_w, the triplicate samples were used for water content tests. Each was dried , in a 105°C moisture oven, over the period found necessary to achieve constant weight (18+/-6h), and moisture content estimated by difference.

The mean of the triplicate values for the above was used for plotting the sorption isotherm and for checking the predictive usefulness of the sorption models of Halsey (1948) and Lupin et al. (1981) and the table of Doe et al. (1982) discussed in section II(iv).

When the fish samples had dried to almost equilibrium with the drying air, that is, to a level where weight change with drying time was very small, they were placed in a 25°C (dry bulb) 80% RH controlled ambient Fison's BR 185 climatic cabinet. Weight gain in each individual fillet were recorded, as had been the weight losses during drying, at intervals. Samples were similarly incised from the fillets and treated for water activity and water content determinations as before.

This experiment was repeated many times for fish samples which had been dehydrated under different conditions:

30°C dry bulb = cool (C)
60°C dry bulb = hot (H)
0.45 m/s air velocity = slow (S)
1.3 m/s air velocity = fast (F)

- which, after drying, had been dipped in an intended surface barrier substance before storage, and

- which had been salted before or after drying.

VI (ii) Ash and salt contents

The samples treated with salt or brine were analysed for ash and salt contents after a_w and water content measurements had been made on them.

The weighed dried samples were gently charred over a bunsen burner and then placed in a muffle furnace at 550°C to incinerate until the sample was completely free from carbon particles. The dish was then placed in a dessicator to cool for 30 minutes before re-weighing to find the ash content.

The ash from each sample was dissolved in 100 cm^3 distilled water in a graduated flask. 10 cm^3 of the solution was pipetted into a small conical flask and titrated against 0.05M silver nitrate using potassium chromate as indicator.

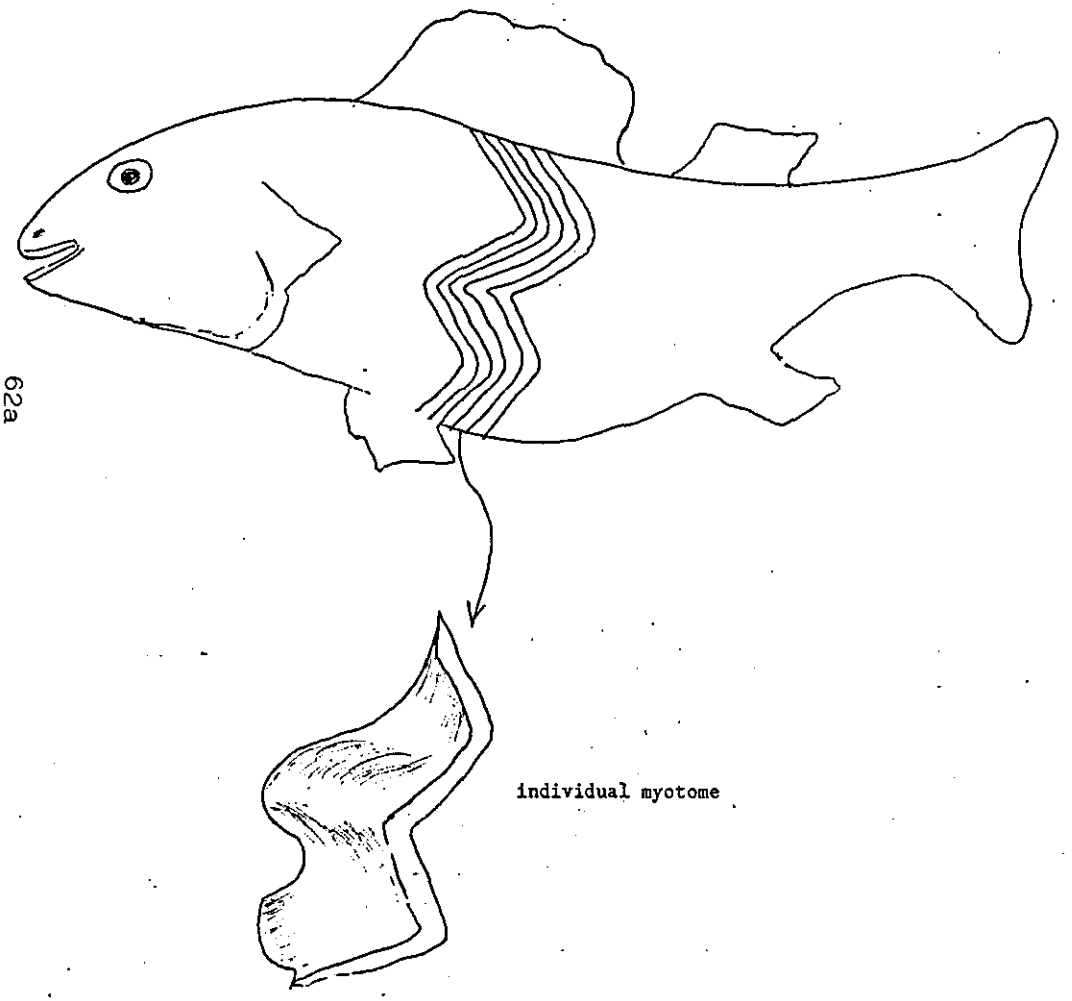
Both ash and sodium chloride contents were expressed as % of the dry solids content of the samples.

VI (iii) Sensory assessment

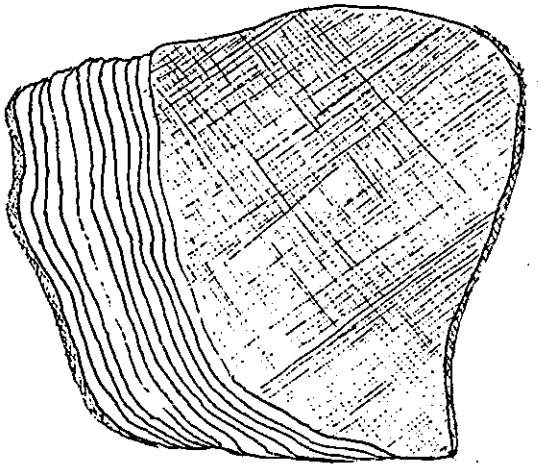
After drying to different water contents, some of the dried fish samples were immediately rehydrated by immersing them overnight in a refrigerator and cooked by simmering them in 90°C water for 30 minutes.

A panel of three tasted, discussed and agreed upon the characteristics of the samples presented before they were recorded.

FIGURE 13: Diagram of arrangement of W-shaped myotomes of fish muscle



(a)



vacuum grease

(b)

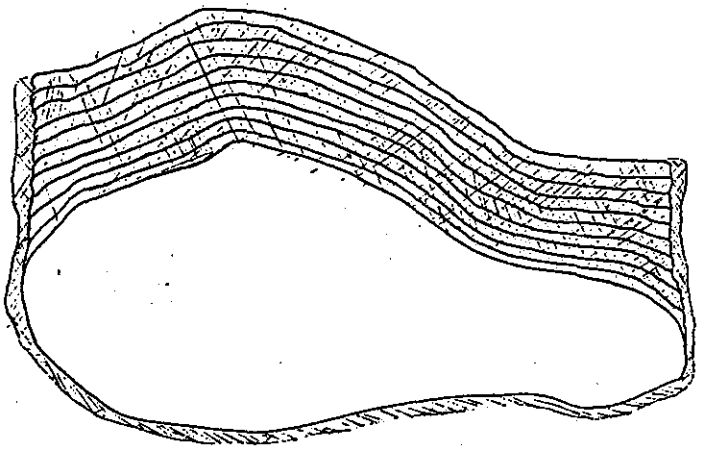


FIGURE 14: Diagram to show application of vacuum grease to fish myotome block to water flow (a) parallel and (b) perpendicular to the myofibrils

VI (iv) Direction of water movement during sorption processes

Fish muscle is organised into W-shaped (Figure 13) myotomes, hence its flakiness when cooked. Columns of such flakes were incised from the raw fillet by carefully sectioning along the connective tissue septa separating muscle columns. And by sectioning between individual myotomes, blocks with approximately equal height and diameter dimensions were obtained.

Prior to drying under the temperature and humidity conditions of VI(i), a thick layer of vacuum grease was applied, as shown in Figure 14:

- either to the upper and lower faces of the myotome block (thus attempting to arrest the movement of water parallel to the axes of the myofibrils within the myotomes)

- or to the circumference of the cylindrical block (thus attempting to arrest the movement of water perpendicular to the myofibrillar axes).

The samples were arranged in the basket of the experimental tunnel drier with their exposed faces (i.e. those not coated with vacuum grease) parallel to the air flow.

Observations of drying rate with time of drying were recorded on quadruplicate samples for two drying runs under the following conditions:-

1. Air velocity 1.3m/s; Air temperature 39°C dry bulb; 22°C wet bulb.

2. Air velocity 0.75m/s; Air temperature 52°C dry bulb; 25°C wet bulb.

VI (v) Drying surface area

The surface area of fish muscle blocks and fillets before drying were measured by contacting each surface with greaseproof paper, drawing around the wetted "imprint", tracing onto squared graph paper and counting squares. Using these, drying rates were computed and quoted per square metre of surface exposed, ignoring the shrinkage which occured during the drying operation because the increased irregularity and rigidity of the surface made such measurement too difficult.

VI (vi) Water uptake in humid storage as affected by curing method

Batches (P,Q,R and S) of skinned cod fillets were dried at air velocity 1.3m/s, dry bulb temperature 40°C and RH 20% over an eleven hour period, recording the weight of each fillet on eight occasions over this time.

Batch P, the control batch was dried only for a further 5h 20 min under the same conditions as before.

Batch Q was buried in dry salt overnight (16 hours) after which the salt was brushed from the surfaces and the batch was dried for a further 5h 20 min under the same conditions as before.

Batch R was immersed in saturated sodium chloride brine for 15 seconds,dried back to its pre-brining weight and then dried for a further 5h 20min under the same conditions as before.

Batch S was immersed in saturated sodium chloride brine for 15 seconds, hot smoked at 80°C for one hour and dried for a further 5h 20min under the same conditions as before.

Half of the samples P,Q,R and S were placed in a climatic cabinet (Fison's BR 185) at 29°C dry bulb temperature, 80% RH for 72.3 hours after which the conditions were altered to 24°C dry bulb, 90% RH and storage contiued for another 120.7 hours. Batch weights were recorded periodically over the storage trial until indications of incipient spoilage (i.e mould growth, surface slime formation, "pink" spoilage, putrefaction) became obvious. Water content, having been determined for the fresh fillets prior to the first drying stage,was expressed for each batch on the basis of 1 square metre of surface initially exposed.

The other half of samples P,Q,R and S were subjected to a storage environment of alternate periods of 8 hours at 29°C dry bulb, 70% RH and 16 hours at 25°C dry bulb, 90% RH in an attempt to model the daily cycling nature of temperature and humidity in the tropics. Batch weights were recorded at each climatic changeover until signs of spoilage became obvious as before. Water contents for each batch, on the basis of one square metre of surface initially exposed, were computed.

At each weighing a sample fillet was taken for a_w, water, ash and salt content analyses.

Repeat batches of fillets which had been dried (only) under different conditions, namely:

fast/cool = 1.3 m/s; 30'C dry bulb; 30% RH

slow/cool = 0.45 m/s; 30'C dry bulb; 30% RH

fast/hot = 1.3 m/s; 60'C dry bulb; 10% RH

slow/hot = 0.45 m/s; 60'C dry bulb; 10% RH

were subjected to cycling humid storage as before and the same analyses performed to discover whether drying conditions showed any effect on product sorption characteristics.

VI (vii) The efficacy of barrier substances applied to cured fish surfaces in retarding water uptake

After dried fillets had been prepared in the manner of batch P in the preceeding section, batches were coated in different barrier substances:

5% "Prolong": (a suspension of carboxymethyl cellulose combined with sucrose esters of monoglycerides) : one dip followed by drying to the original dry weight.

2% "Prolong": 3 dips interspersed by drying to the original dry weight.

2% "Capsul": (a solution of a waxy maize dextrin modified by treatment with octenyl succinic acid and dry roasting): 3 dips interspersed by drying to the original dry weight.

HPKO (Hydrogenated Palm Kernal Oil): one dip in cool but liquid HPKO which was allowed to solidify on the surfaces before further treatment.

Vegetable Oil (Craigmillar "Saladin"): one dip with agitation to avoid air bubbles which might have given rise to bare patches on the fillet surfaces.

Barrier coated batches of fillets were stored in the climatic cabinet set at 25°C dry bulb and 80% RH. Weight changes (on a 1 square metre original surface area basis) were recorded throughout the storage period and compared with the changes in control samples without barrier coatings.

VII RESULTS AND DISCUSSION

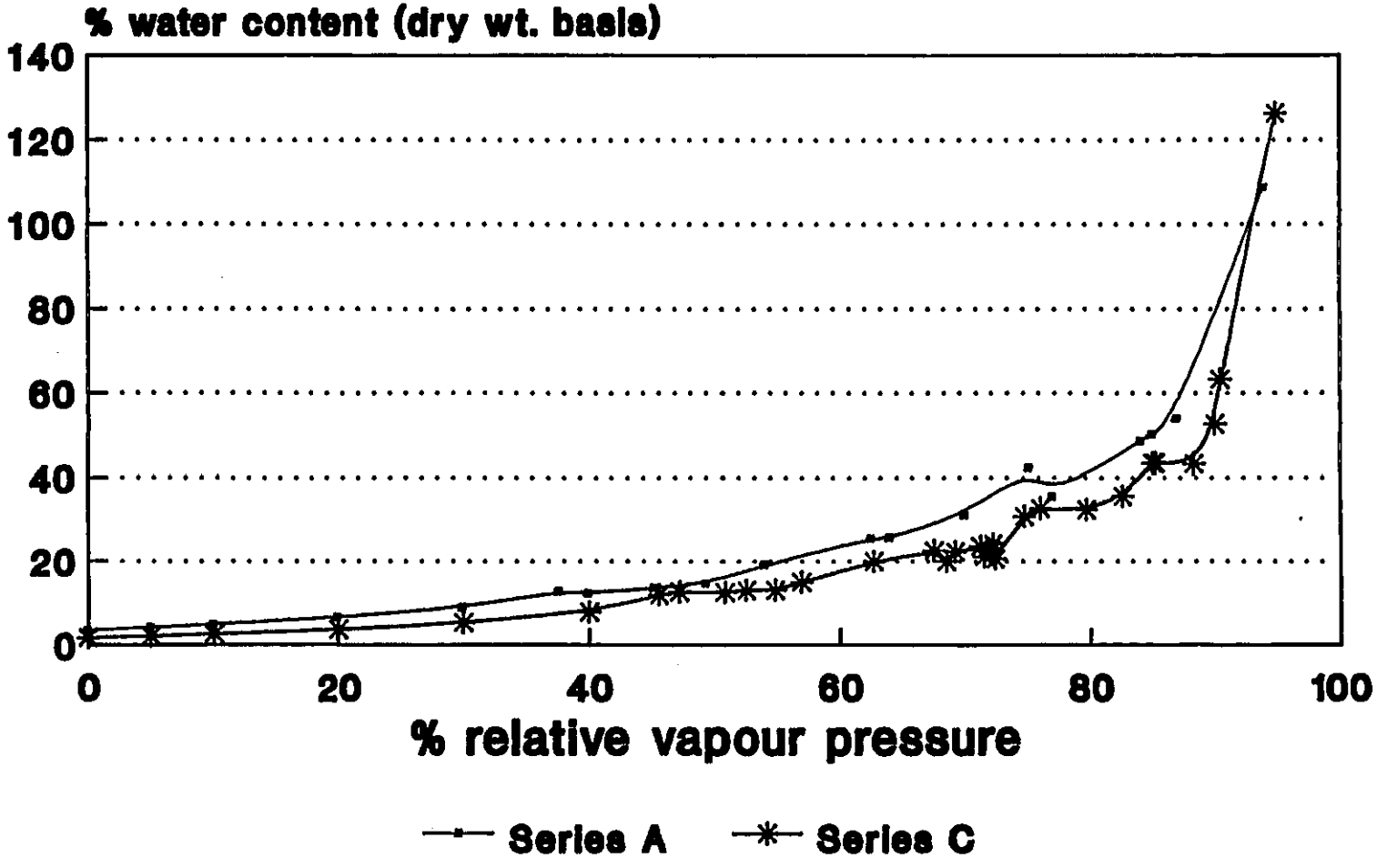
To what extent fish during sorption acts as a gel or a bundle of fibres (to use Hamm's 1962 protein organization extremes) affects the rate of water movement and the sensory characteristics of the product upon rehydration and consumption. The sorption isotherms presented (Figures. 15 to 20) suggest that sorption characteristics depend upon:

(a) whether initial desorption or subsequent resorptions and desorptions are taking place (Figure 15);

(b) the set of parameters (e.g. air temperature, air velocity and salt content as covered by Figures 19 and 20) used in the curing operation; and

(c) the extent to which desorption has taken place (illustrated by the differences between the isotherms YOS and TC in Figures 16, 17 and 18).

FIGURE 15: WATER SORPTION ISOTHERM
% water (d.w.b.) v. %RVP (*Gadus morrhua*)



A=desorption/C=resorption

Exponential Regression
A: r=0.99 C: r=0.93

FIGURE 16: WATER SORPTION ISOTHERM
COD (yellow, opaque surface)

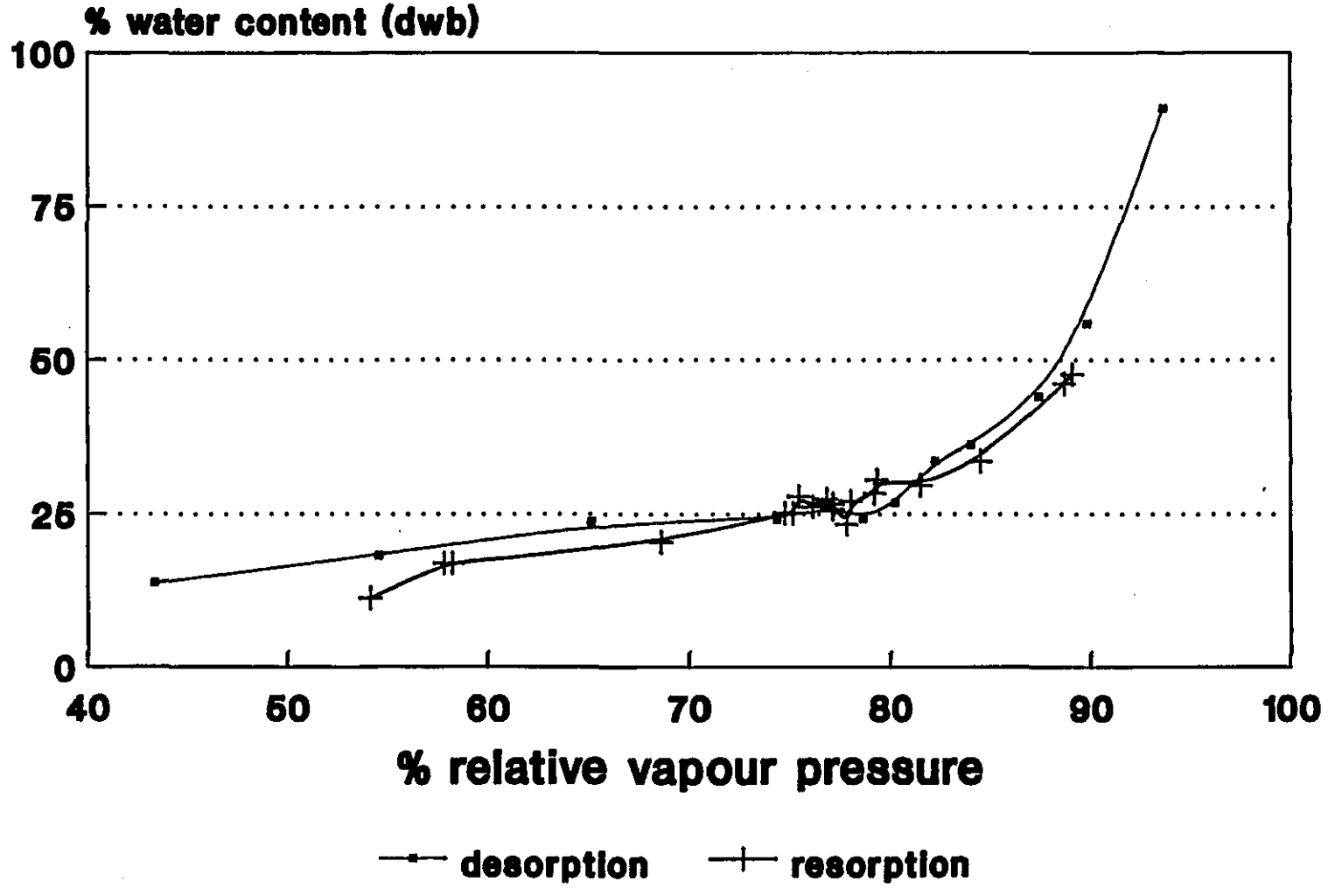


FIGURE 17: WATER SORPTION ISOTHERM
COD (translucent, centre)

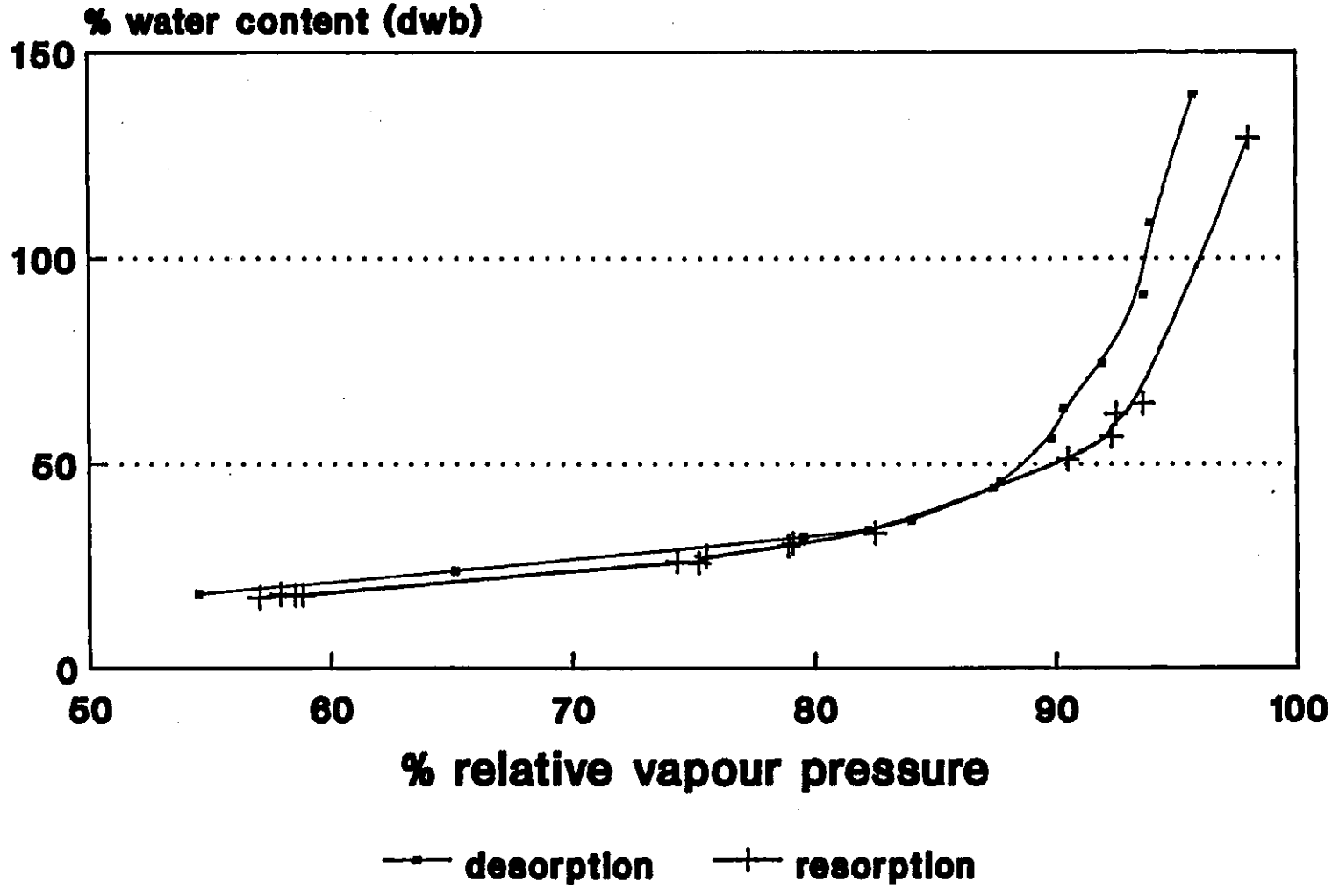


FIGURE 18: RESORPTION YOS v. TC
data from Figures 16 & 17.

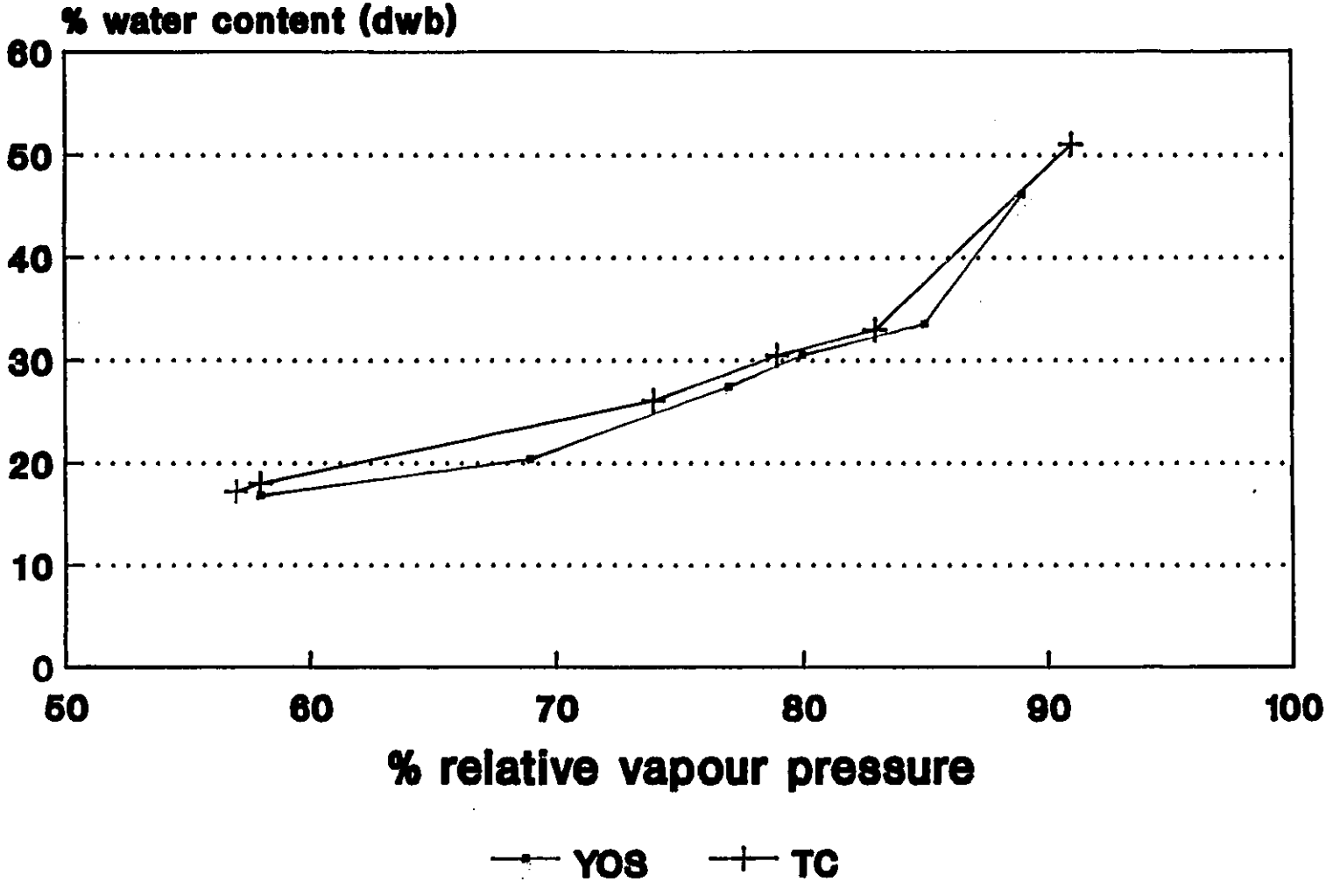


FIGURE 19: WATER SORPTION ISOTHERM
COD (dried then salted) RESORPTION
% WATER - % SATD. BRINE v. % RVP

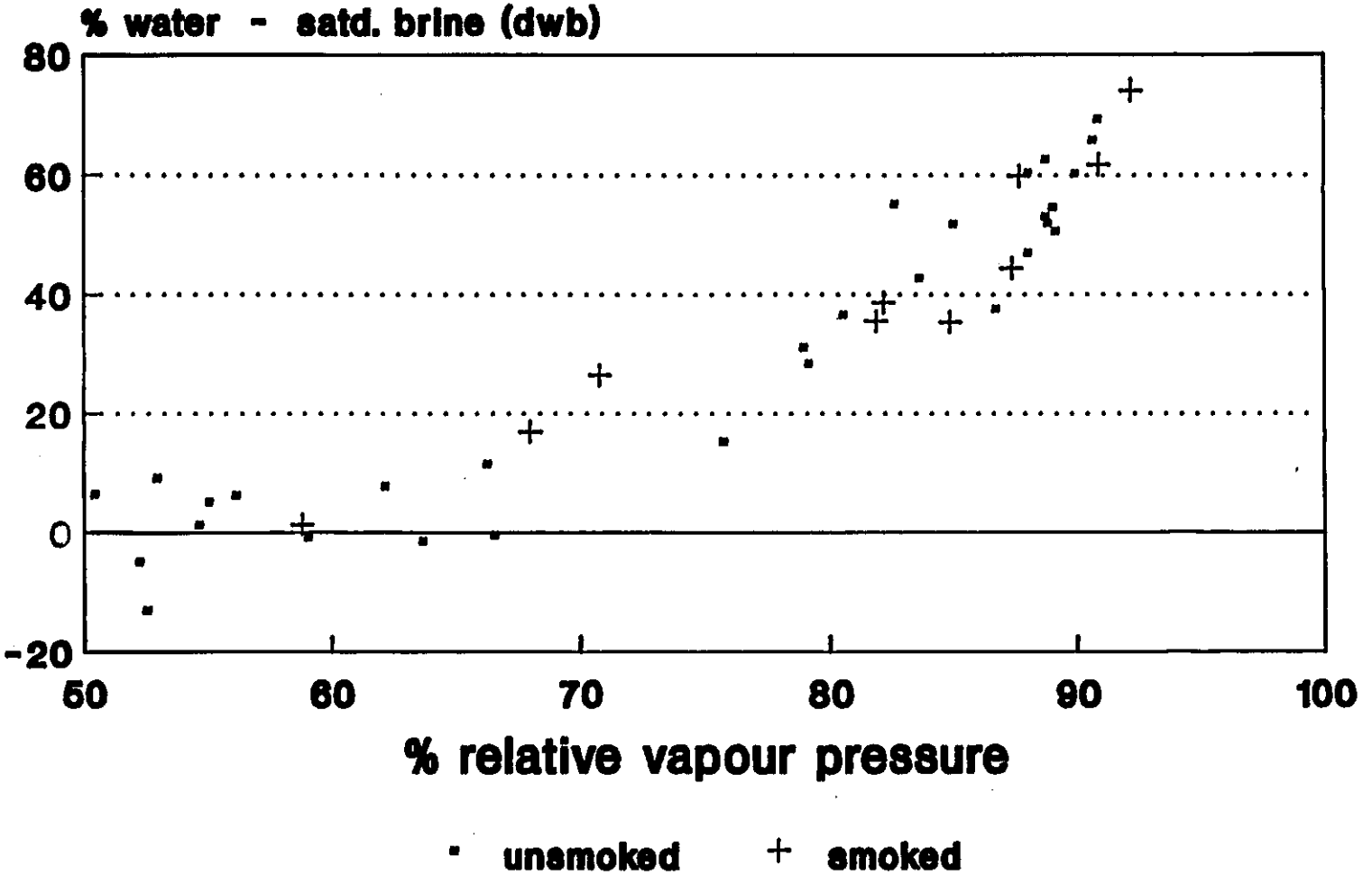
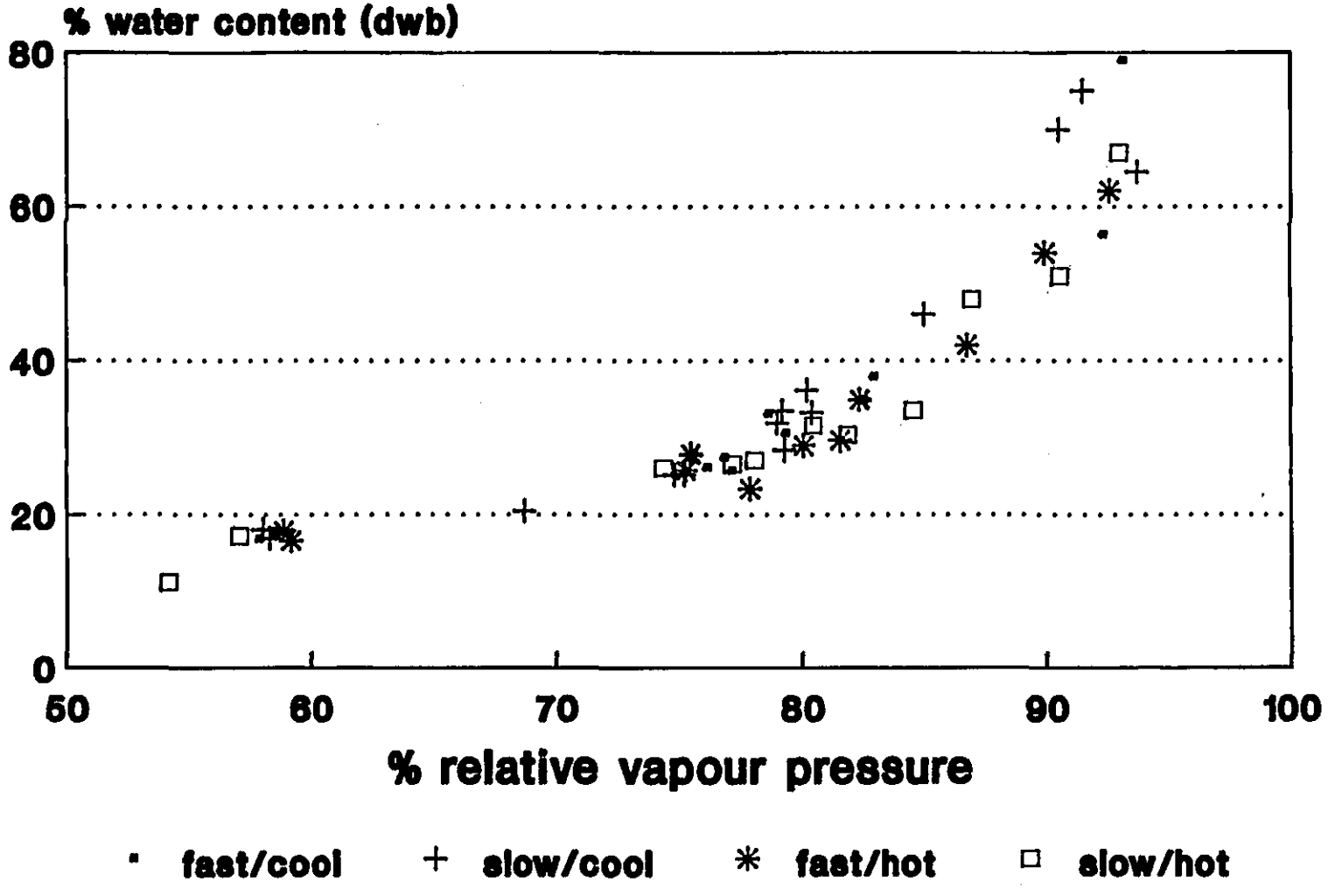


FIGURE 20: WATER SORPTION ISOTHERMS
COD(dried fast/slow/hot/cool)RESORPTION



VII (i) Sorption isotherms for cod: the effect of the extent of curing on hysteresis and quality.

Micro-organisms have been found to grow more rapidly at given water activities reached by desorption than by adsorption (Lubuza et al. 1972) and it has therefore been suggested (Kapsalis, 1981) that this phenomenon might be exploited in the preparation of intermediate moisture foods. This does not mean that the water added to a dehydrated material may excede that present in the same material as it is dehydrating for the same anti-microbial effect. The hysteresis of the water content against relative vapour pressure sorption isotherm indicates that the same relative vapour pressure may be reached during resorption at a considerably lower water content than when it was reached during initial desorption (Labuza, 1977).

That there is a degree of hysteresis between the sorption isotherms for drying fresh fish and rehydrating dried fish was evident in this work (Figures 15, 16 and 17).

[Exponential regressions ($y = Ae^{Bx}$) on the desorption and resorption curves, in Figure 15, reveal their differences, thus:

	A	B	Correlation Coeff$^{t.}$
Desorption	3.43	0.032	0.99
Resorption	1.69	0.039	0.93

The extent to which the "sorption hysteresis" effect counterbalances the "microbial spoilage hysteresis" effect may depend upon the degree to which the fish is dehydrated before resorption takes place. For example, the "yellow, opaque surface" patches, which seemed to occur in places where relative vapour pressure (RVP) had been reduced below 50%, exhibited higher relative vapour pressures for the same % water (dry weight basis) readsorbed than the "translucent centre" (Figure 18). It would be expected, from the foregoing argument, that the YOS samples would begin to spoil at lower water content than TC samples, but further work is needed to verify and quantify this.

Hardman (1985) notes that a different value of RVP (water activity) is obtained for the same moisture content depending on whether the system is sorbing or desorbing water. This hysteresis effect must be due to decreased ability of the water binding sites on fish component molecules to bind water after dehydration. A combination of the two reasons discussed in the following two paragraphs may be responsible.

Working on water sorption by textile fibres, Hailwood and Horrobin (1946) assumed that water, associated with a fibrous material, existed either in solution or combined to form a hydrate with a definite unit of the fibre molecule. Noting that their derived sorption model equation (given in Section II(iv)) failed to account for hysteresis, they explained the phenomenon in terms of the changing accessibility of polar groups throughout the absorption process. Thus, as adsorption and swelling of the polymer fibres proceeds, some portions of the crystallite regions open, exposing more polar sites. Since these newly exposed sites also become hydrated, the adsorbed water exceeds that calculated on the assumption that M, the molecular weight of a polymer molecule capable of combininig with one molecule of water, is constant. Hysteresis, then, is due to the fact that when the saturated fibre is desorbed, there is a lag in the reformation of crystallites.

The crosslinking of protein molecules noted by Obanu et al. (1975) in stored intermediate moisture beef would, if the same occurs in dehydrating and dehydrated white fish, block some water binding sites. Salwin (1959) noted that once a food material has lost the amount of water required to sheath reactive sites on its molecules the latter become accessible to oxygen attack. The attachment of an oxygen molecule to a binding site of a protein produces, according to Klotz and Heiney (1957), an "incongruity in the aqueous covering sheath" which could affect the hydration characteristics of this and neighbouring binding sites.

Although no attempt was made to determine whether a statistically valid relationship existed between sensory appreciation of textural quality and the increased loss of water binding capacity apparent when fish is dehydrated below RVP 50%, the loss in succulence of the yellow, opaque surfaces compared with the translucent centre portion was noted in sensory testing. This coincides with the observation by Young et al. (1973) that, for dried abalone, translucence is associated with a quality product.

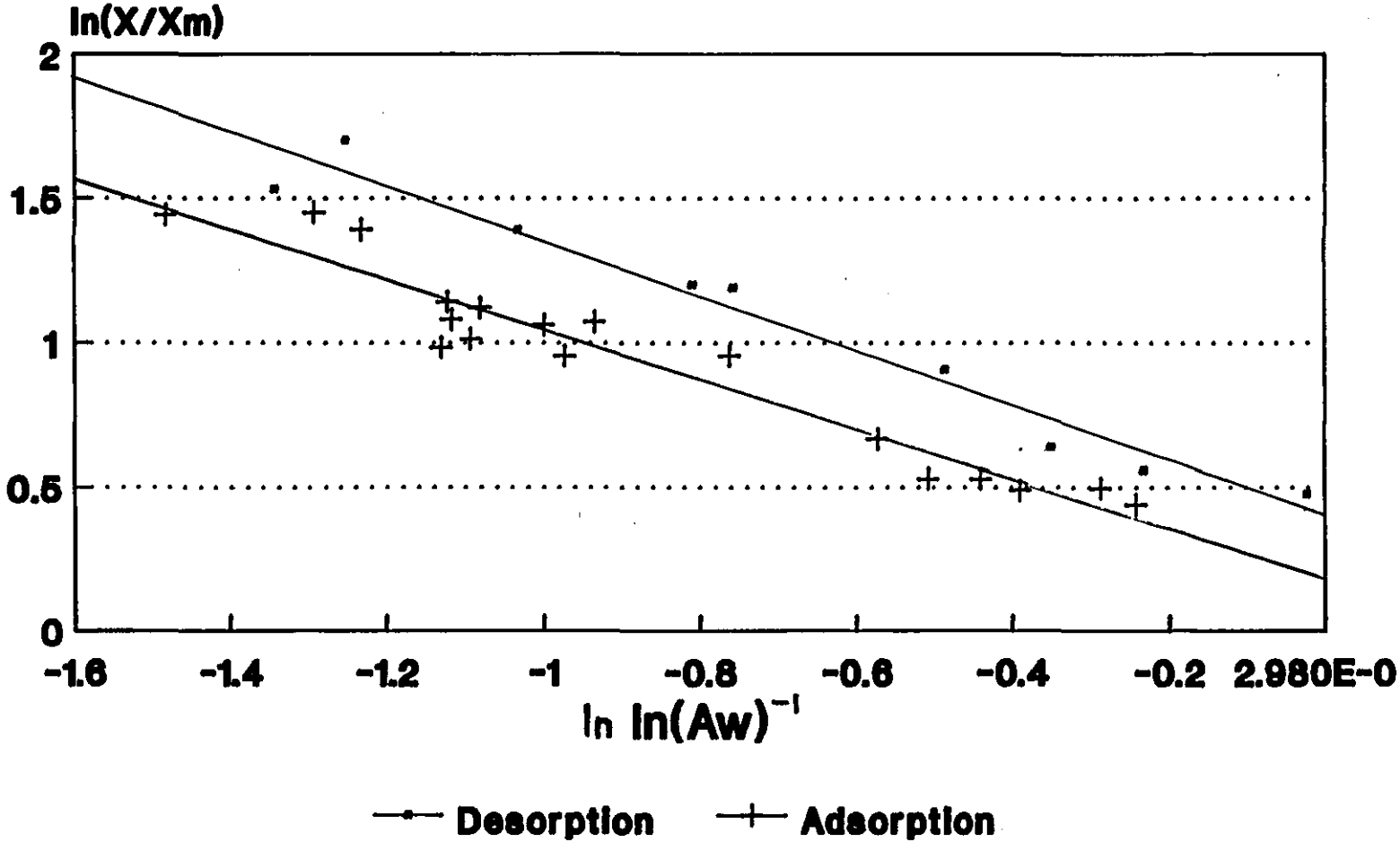
Wolf et al. (1972) found that dried haddock showed a large decrease in its capacity to sorb water with storage time which corresponded to decreases in colour, taste and rehydrateability quality attributes. The hysteresis in such sorption isotherms as that shown in Figure 15, as quantified by measuring the area enclosed by the loop or its width at certain RVP values, for example, might, therefore, provide an "index of quality" for dehydrated fish.

It seems that the textural eating quality of rehydrated white fish can be adversely affected by air drying to unnecessarily low RVP levels, and this, to some extent, can be perceived visually. Combining salt addition with the dehydration process allows

preservation to remain effective at higher water contents. Salting prior to drying, although it retards the rate of water removal, yields a product with a much higher equilibrium water content (Berhimpon et al.- 1990).

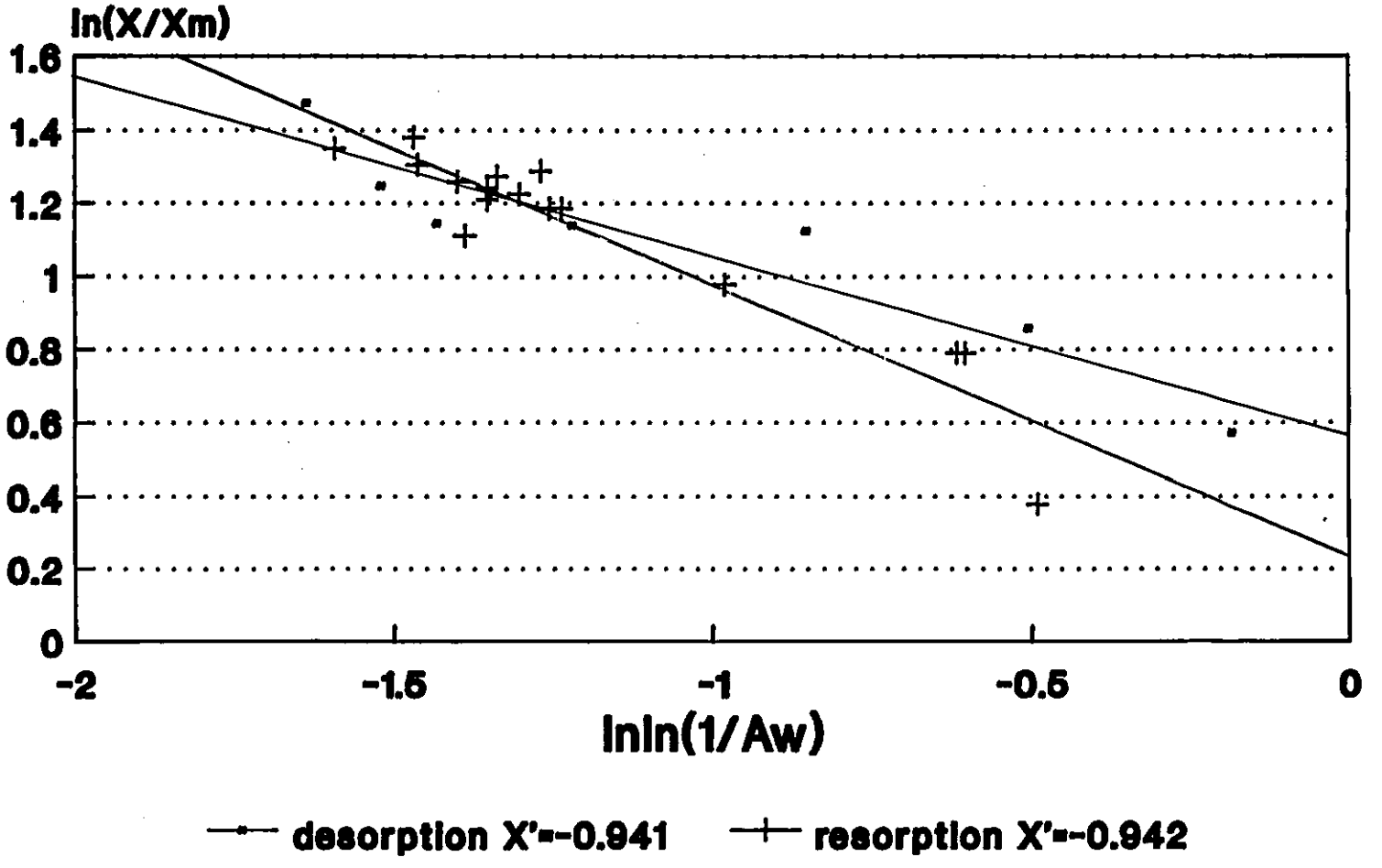
The drying process, therefore, need not be so extensive as to cause over-drying. The deleterious effect on texture associated with over-drying may, therefore, be largely avoided by limiting drying times and/or by combining the processes of drying and salting.

FIGURE 15a: HALSEY SORPTION MODEL
on data from Figure 15



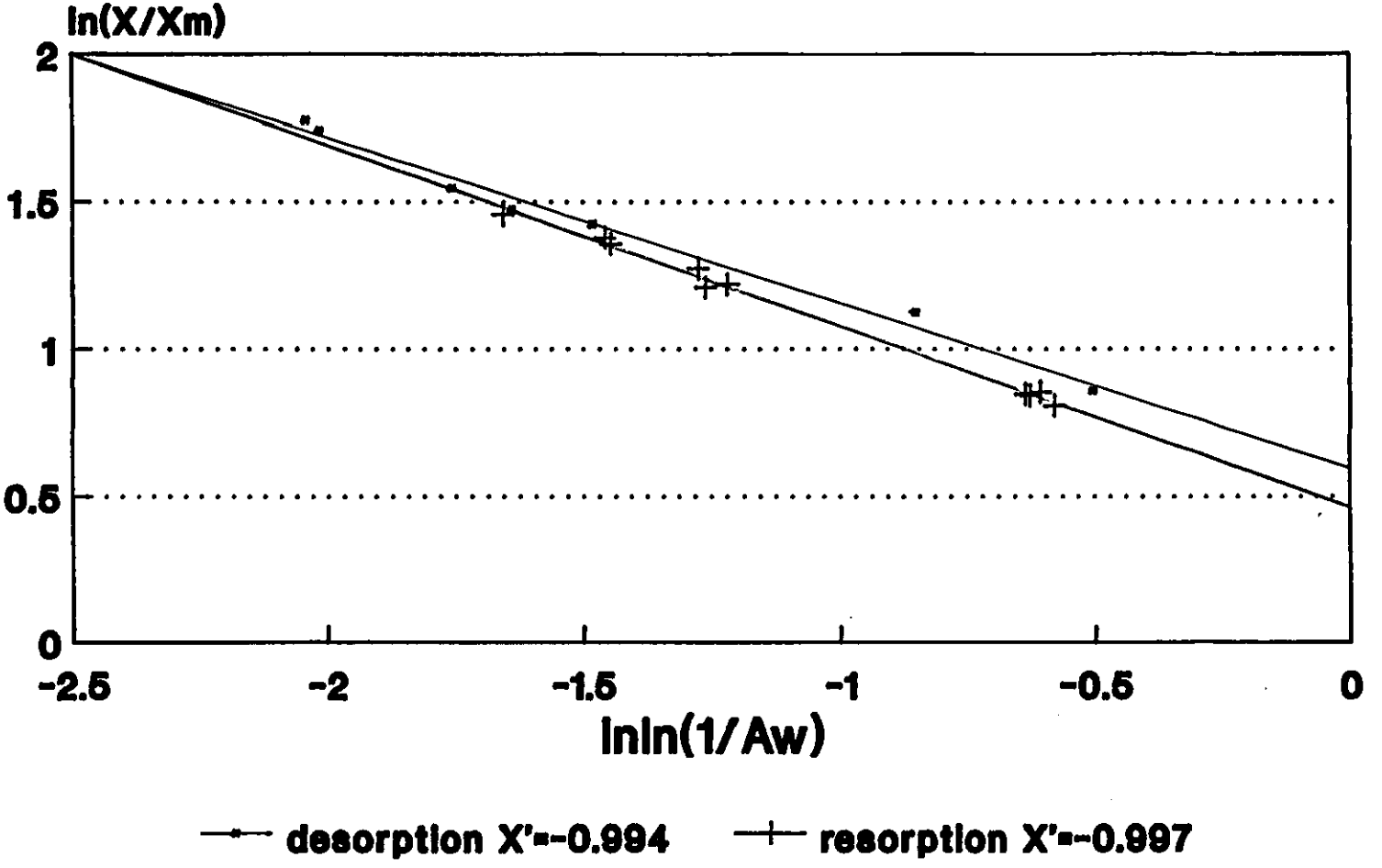
lnln(1/Aw)= -rln(0)+ ln(a')
Desorption: a'=1.510, r =0.9395,X'=-0.98
Adsorption: a'=1.197, r=0.8646,X'=-0.964

FIGURE 16a HALSEY SORPTION MODEL
on data from Figure 16



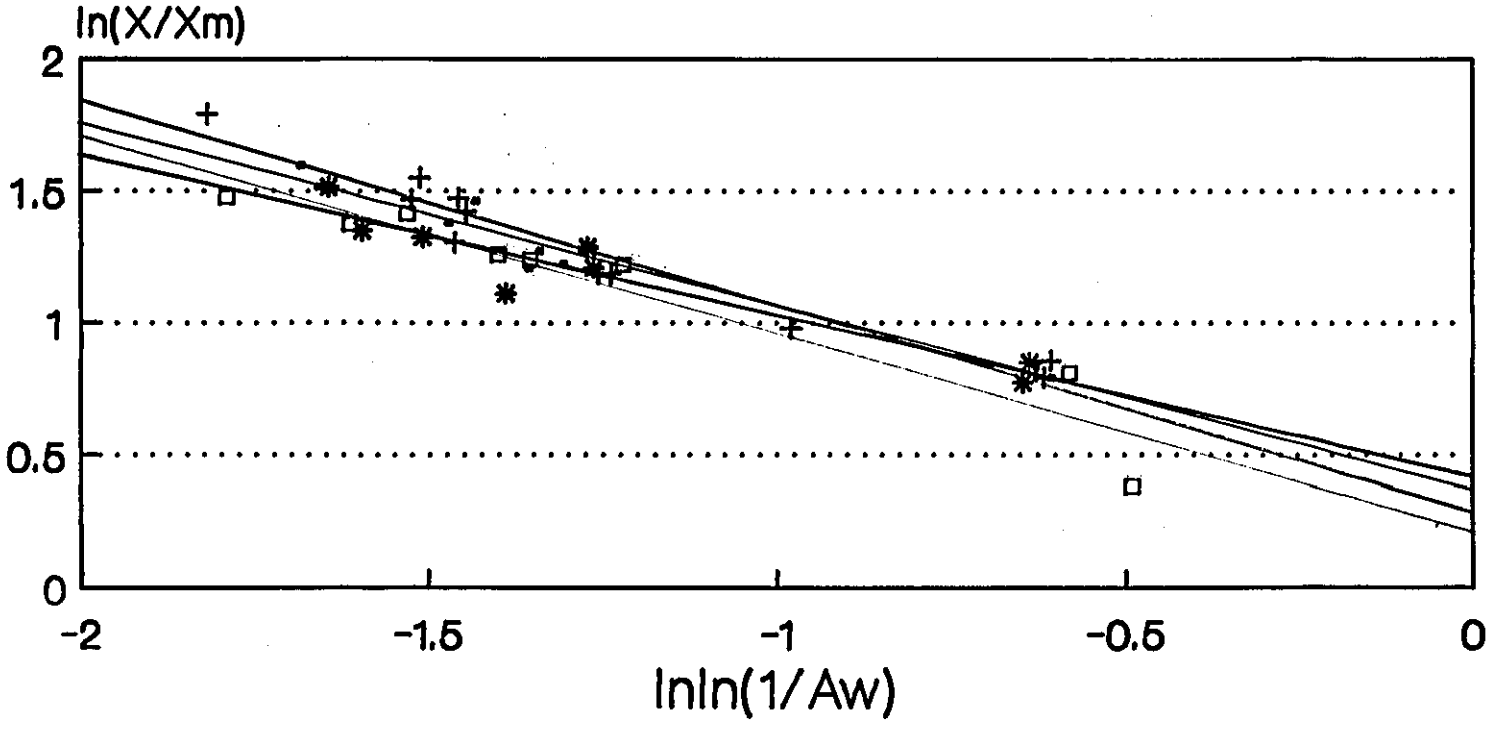
lnln(1/Aw) = -rln(0)+ln(a') and 0=X/Xm
where Xm=7.68g/100g (dry basis)
A)a'=1.81;r=-0.56 B)a'=1.59;r=-0.62

FIGURE 17a: HALSEY SORPTION MODEL
on data from Figure 17



lnln(1/Aw) = -rln(0) + ln(a') and 0=X/Xm
where Xm = 7.68g/100g (dry basis)
A)a'=1.81;r=-0.56 B)a'=1.59;r=-0.62

FIGURE 20a: HALSEY SORPTION MODEL
on data from Figure 20
RESORPTIONS



lnln(1/Aw) = -rln(0) + ln(a') and 0=X/Xm **where Xm=7.68g/100g (dry basis)**
A)a'=1.48;r=-0.70 B)a'=1.39;r=-0.73
C)a'=1.52;r=-0.61 D)a'=1.24;r=-0.75

VII (ii) Comparison of observed sorption behaviour with that predicted from mathematical models.

The gradient and intercept differences on the Halsey sorption models (obtained from the data used to produce the isotherms in Figures 15, 16, 17, and 20) for desorption and resorption (Figures 15a, 16a, 17a and 20a) could be used in the control of fish drying processes and, hence, product quality. This would be an alternative, requiring much fewer data, to the laborious experimental procedure of ascertaining the sorption isotherm and gauging its extent of hysteresis against specified standards. The introduction of salt into the curing process, however, might complicate this approach.

In Figure 19, the relationship between the percentage (d.w.b.) water excess to the requirements of that needed to constitute a saturated brine in the fish tissues, and percentage relative vapour pressure is shown. The curve appears very similar to that of the resorption isotherm for dried white fish (Figure 15). Exponential regression (where $y = Ae^{Bx}$) gives values of A to be 0.72 and 1.69 respectively for salted and unsalted with values of B, 0.075 and 0.038 respectively, and coefficients of correlation 0.92 and 0.93 respectively. Both resorption isotherms pass through the preservation significant zone (Scott, 1957) RVP 70-80% as the amount of water (above that required to make a saturated brine of the contained salt) increases from approximately 20 to 35% on a dry weight basis. Halsey sorption models using data thus modified could still, therefore, be used.

Table 6. Comparison of Measured and Predicted a_w.

Mw/Mb	Ms/Mb	aw(Doe)	aw(Lupin)	aw(measured)
0.193	0.046	0.69	0.83	0.51
0.170	0.078	0.58	0.67	0.52
0.158	0.103	0.56	0.53	0.53
0.223	0.047	0.76	0.85	0.53
0.217	0.073	0.67	0.76	0.55
0.254	0.072	0.74	0.80	0.55
0.213	0.054	0.73	0.82	0.56
0.233	0.086	0.67	0.74	0.59
0.171	0.056	0.60	0.77	0.59
0.261	0.066	0.77	0.82	0.62
0.300	0.112	0.72	0.73	0.64
0.325	0.075	0.82	0.84	0.66
0.390	0.141	0.74	0.74	0.67
0.272	0.037	0.85	0.90	0.68
0.420	0.056	0.90	0.91	0.71
0.277	0.045	0.83	0.89	0.76
0.352	0.025	0.92	0.95	0.79
0.403	0.033	0.93	0.94	0.79
0.467	0.036	0.94	0.95	0.81
0.490	0.037	0.95	0.95	0.82
0.433	0.028	0.95	0.96	0.82
0.701	0.053	0.96	0.95	0.83
0.544	0.042	0.95	0.95	0.84
0.652	0.048	0.95	0.95	0.85
0.434	0.029	0.94	0.95	0.85
0.471	0.034	0.95	0.95	0.87
0.538	0.034	0.96	0.96	0.87
0.714	0.040	0.97	0.96	0.88
0.717	0.043	0.96	0.96	0.88
0.688	0.056	0.96	0.94	0.89
0.617	0.039	0.96	0.96	0.89
0.805	0.064	0.96	0.95	0.89
0.632	0.040	0.96	0.96	0.89
0.651	0.041	0.96	0.96	0.89
0.728	0.045	0.96	0.96	0.90
0.896	0.072	0.96	0.94	0.91
0.819	0.057	0.96	0.95	0.91
0.706	0.032	0.97	0.97	0.91
0.655	0.031	0.97	0.97	0.92
0.851	0.039	0.98	0.97	0.92

Concerning the prediction of a_w from a knowledge of cured fish salt and water content, it is evident, from Table 6, that the measured %RVP values for dried and lightly salted fish were consistently lower (by amounts ranging from 3 to 23%) than values predicted purely from salt and water content in Table 4 taken from the paper by Doe et al. (1983).

The latters' results revealed much closer agreement between calculated and actual water activity. These results, however, were for heavily salted and dried fish where

the salt content ranged between 45 and 150% of the dry matter content, whereas the results in this study were for dried fish which were only lightly salted with salt 2.8% - 14% of the dry matter content. Perhaps not surprisingly, some of the largest discrepancies between "calculated" and "measured" in both sets of results occur in the middle, "plateau" zone of the sorption isotherm where very small changes in water content provoke very large changes in product RVP or water activity. Additionally, considering the hysteresis in the isotherm and its sensitivity to drying conditions and extent, prediction of a_w from salt and water content in this region is unlikely to coincide with measurement.

A least squares linear regression fit of the "calculated" against "measured" data for lightly salted dried cod gave the relationship:

$$RVP(calc) = 0.849\ RVP(meas) + 22$$

with a correlation coefficient, r = 0.94 which compares

with the Doe et al. (1983) relationship:

$$a_w(calc) = 0.977\ a_w(meas) + 0.021$$

with a correlation coefficient, r = 0.99.

A comparison of observed (using the "Novasina" a_w meter) against expected (using the Doe et al. table) results for more heavily salted cod samples, revealed the following:

WATER (on % wet basis)	SALT	EXPECTED a_w	OBSERVED a_w
3.2	45.8	0.12	0.23
44.5	38.0	0.75	0.75
57.4	9.2	0.89	0.91

-suggesting better agreement than obtained with the lightly salted and dried samples, at least over the a_w range of most commercial samples bought on tropical markets (Hanson and McGuire, 1982).

Prediction of a_w using the equation obtained by Lupin et al. (1981) viz.:

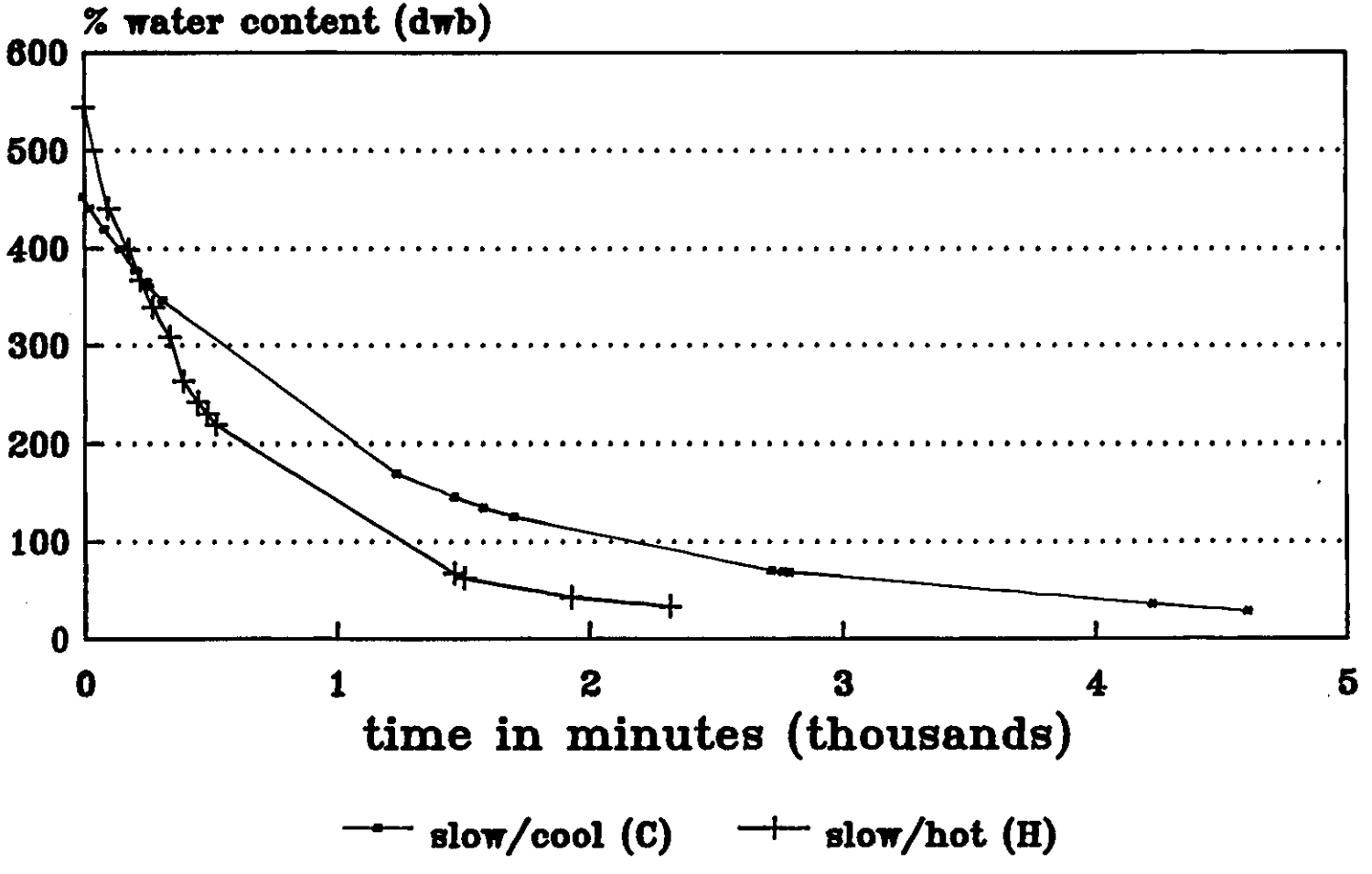
$$(a_w)_{sf} = 1.002 - 0.042m$$

(where $(a_w)_{sf}$ is the water activity of salted fish and m is the molality of the solute, i.e. of the salt, in the water of the fish)

-revealed (Table 6) an even greater deviation upwards of the measured values. This equation was given, however, for moist salted fish products like anchovy; and it was remarked that any drying which accompanied the brine-salting process resulted in measured values of a_w being less than those predicted using the equation. Even the small amount of drying effected by pressing resulted in such deviation between observed and expected.

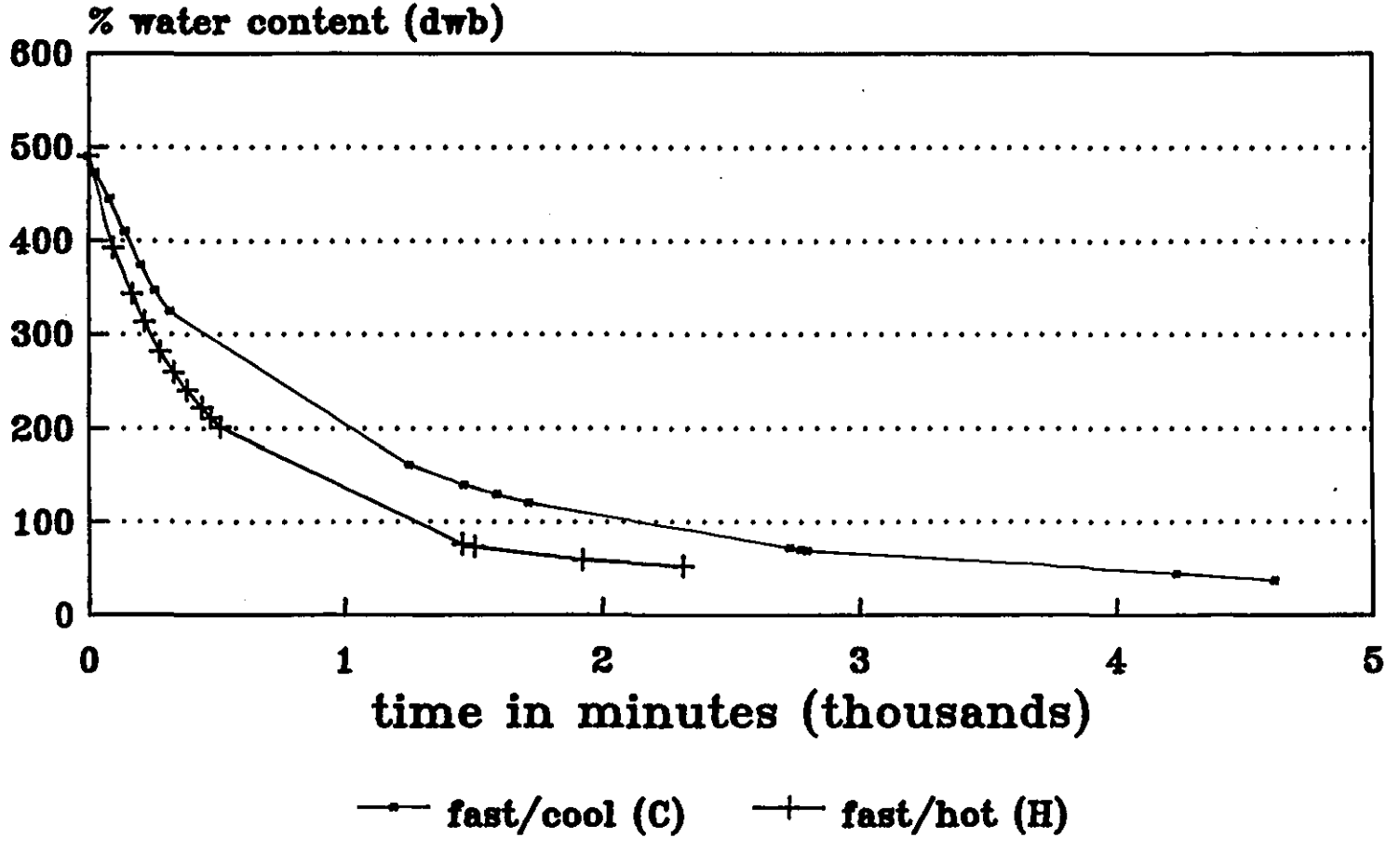
It appears, then, that the medium in which the salt/water system exists has an increasingly significant effect on a_w as its proportion increases beyond that of salt/water as drying proceeds.

FIGURE 21: COD(slow, cool/hot)DESORPTION
% water (dwb) v. time (min)
air vel.0.45m/s; d.b.30C(cool) 60C(hot)



Initial Mass (having a surface area
= 1sq.m) C = 5.582kg H = 5.927

FIGURE 22: COD(fast, cool/hot)DESORPTION
% water (dwb) v. time (min)
air vel.1.3m/s; d.b.30C(cool) 60C(hot)



Initial Mass (having a surface
area = 1sq.m)C=5.495kg H=5.395kg

VII (iii) The effect of drying conditions on the sorption isotherm and cured fish quality.

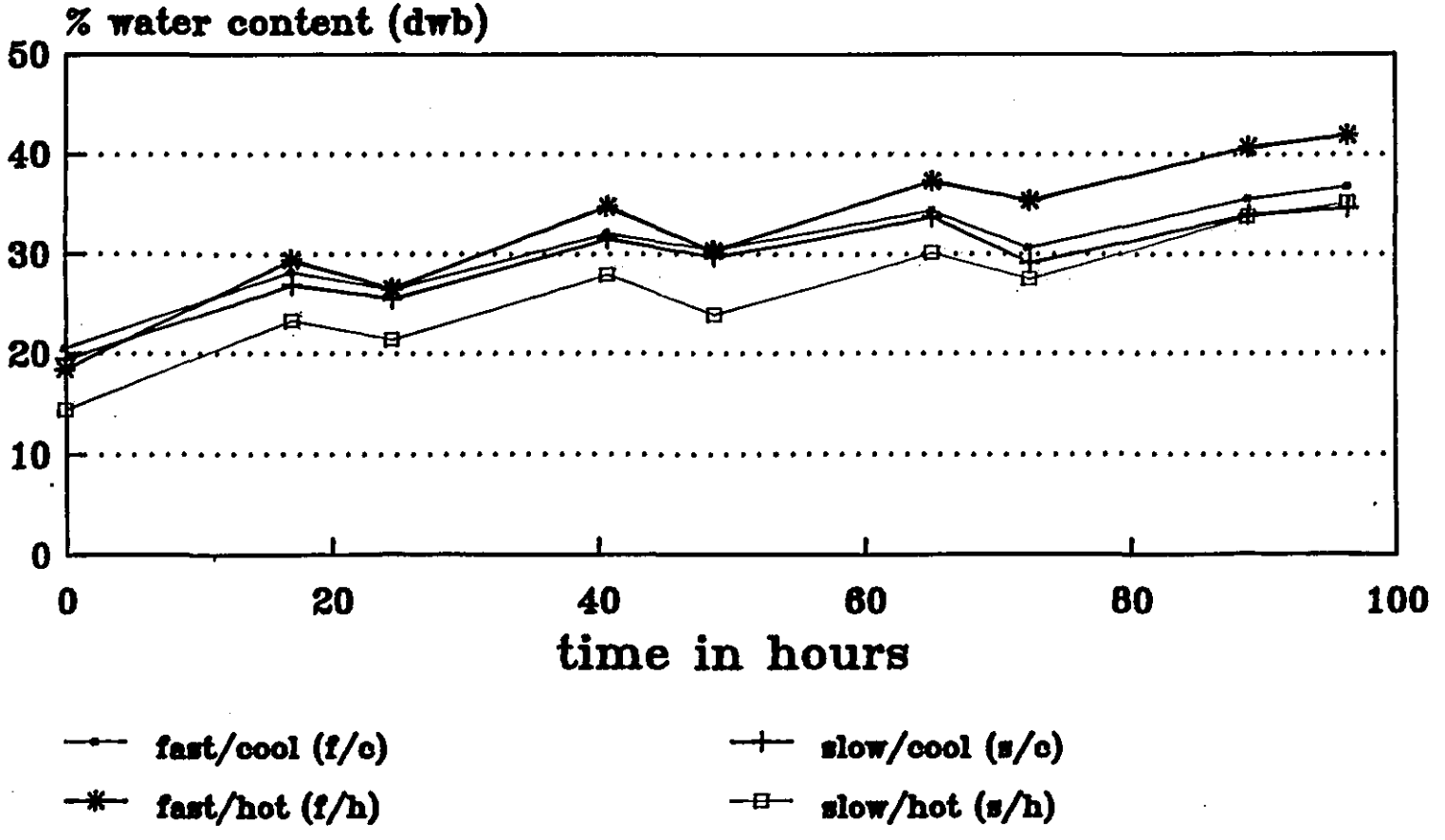
The effects of the drying air temperature and velocity on the rate of water sorption by the product were investigated and the results were summarised in Figures 20 to 23.

Iglesias and Chirife (1976) found that the temperature of drying affected the sorption capacity of dried beef. The higher the drying temperature the lower the sorption capacity appeared. That is to say, the higher had been the drying temperature, the less water needed to be re-adsorbed to achieve a given water activity. They pointed out, however, that this observed lowering of the isotherms with drying temperature did not necessarily indicate a reduction in the product's rehydration capacity, as most water, taken up on rehydration, became loosely bound water, associated in a three-dimensional bonded network, rather than water which was tightly bound to specific sorption sites.

It appears reasonable to suppose that the rate of rehydration, where water taken up is to become "loosely-bound", would be faster where the structure is open and fibrous, lending itself more readily to capillary condensation. Certainly, in this work, fish samples dried at higher temperatures exhibited a greater proportion of yellow, opaque, fibrous area to translucent gel-like area and sorbed water faster than low temperature-dried samples. Additionally disadvantageous, the cross-linking of proteins, which occurs in flesh products to a greater extent with increasing temperatures, leads to a lower water holding capacity (Obanu et al., 1975) so that larger water activity variations result from smaller water content changes.

The effect of air velocities and temperatures, used during desorption, on the sorption isotherm were also investigated (Figure 20). Although Doe et al. (1982) conclude that the sorption isotherms are probably influenced by temperature of drying, more results would be needed than available from this work to make a definitive statement upon the effect of varying these parameters on the sorption isotherm. However, Jones' and Peralta's work (1980) where temperatures, only, were varied between 50 and 100°C, was similarly inconclusive. Only the sorption isotherm for 50°C dried cod (Section III(iii) - Figure 8) showed greater water adsorption capacity for given water activities consistently over the whole range compared to those for cod dried at higher temperatures (65, 80 and 100°C). No mention is made, however, of what effects these higher drying temperatures would have on the sensory properties of the reconstituted product.

FIGURE 23: COD SORPTION CYCLES
after drying under different conditions
% water (dwb) v. time (min)



IM(kg/m):1.124; 1.210; 1.085; 1.053
res.● 92%RH;25C(db) des.● 70%RH;29C(db)

Curran and Poulter (1983) decided that the sorption characteristics of fish muscle were unlikely to be affected by the limited range of temperatures extant in commercial dehydration processing in the tropics.

Only two dry bulb temperatures 30°C (cool) and 60°C (hot) in combination with air velocities 0.45 (slow) and 1.3 ms^{-1} (fast) were used in this work.

For a given a_w value, a higher water content suggests that less damage has been sustained by the structural components of the food material during the drying operation.

Interestingly the slow drying process produced dried cod seemingly capable of adsorbing greater quantities (Figure 20) of water to achieve a given RVP than dried cod samples produced by the other 3 temperature/air velocity variations. This coincides with Doe et al. (1982) view that fish dried at temperatures above 31.5°C show a higher water activity for a given moisture content than would apply for fish dried below 31.5°C.

Figure 23 shows the water sorption behaviour of the differently dried samples subjected to alternate exposure to high and moderate humidity ambients (modelling the daily cycling of climatic conditions in the humid tropics). In general, through the storage period of alternate high (92% RH) and moderate (70% RH) humidites, the samples dried at the higher temperature (60°C) showed steeper gains and losses of water with time than the samples dried at the lower temperature (30°C).

Drying air velocity seemed to have a lesser effect on subsequent sorption rates in samples subjected to cycling humidity storage than drying air temperature. Although slightly faster sorption rates for the fast/hot dried samples compared to the slow/hot dried samples were discernible.

From this, in terms of water content changes, it appears that the slow/cool dried (s/c) sample was least affected, and the fast/hot dried (f/h) sample most affected by the cycling climatic conditions.

In the opinion of the author, this "slow, cool" sample was the only one of the four with satisfactory sensory attributes.

Observations during these experiments, suggest, that for best sensory quality and longest shelf-life in a typical, humid tropical environment, air drying temperatures and velocities be as low as is compatible with achieving a preservation adequate a_w in the centre of the flesh before undesirable spoilage or toxification occurs. This concurs with the findings of Berhimpon et al. (1990) that the lower the temperature at which fish is dried, the higher the taste panel scores obtained on the reconstituted product.

VII (iv) Parallel and perpendicular to myofibril water movement during sorption processes.

The cod used in these investigations contained between 450 and 550 g water per 100 g of dry solids of which most is protein. But protein cannot bind more than 20 g water per 100 g dry matter as water of hydration (Hamm, 1963). Haurowitz (1950) suggests that the rest of the water which is held in fresh flesh is "free" water immobilised by a flexible network of filaments and membranes.

In fish muscle blocks, these filaments and membranes are not directionally random but ordered linearly as myofibrils and their associated membranes.

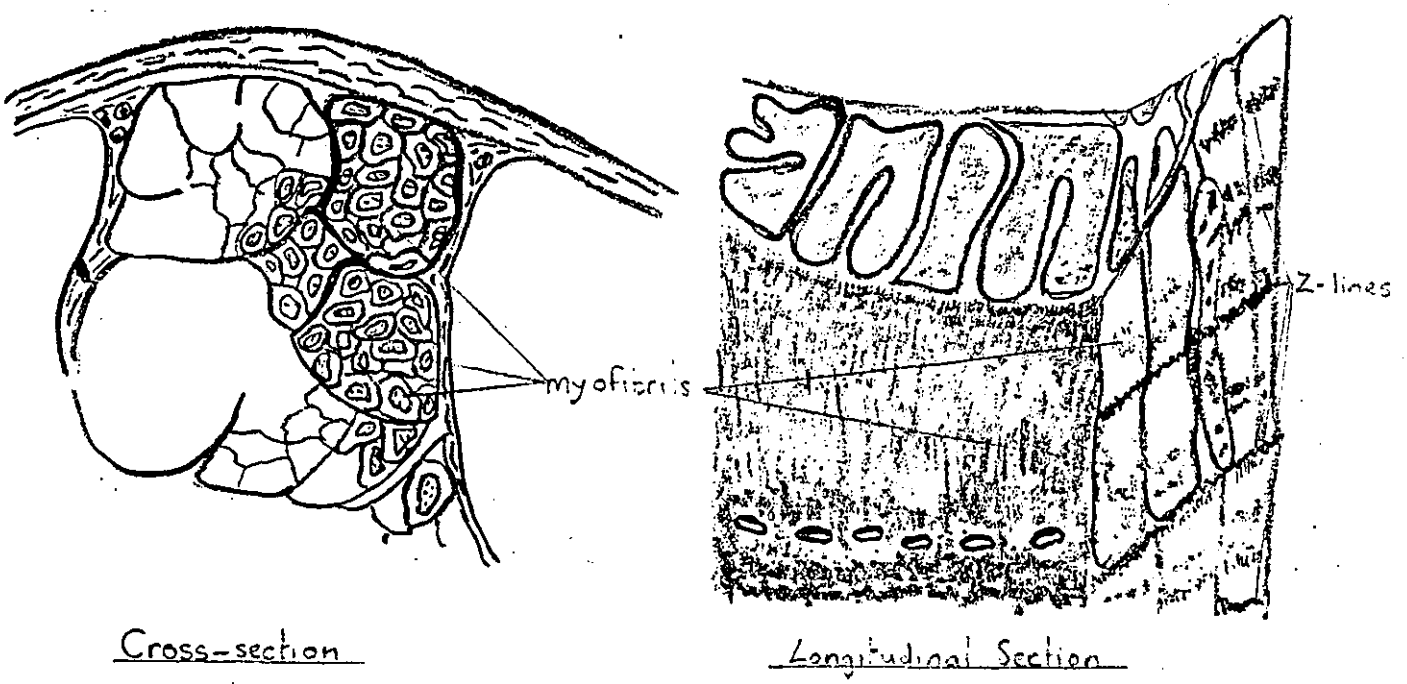
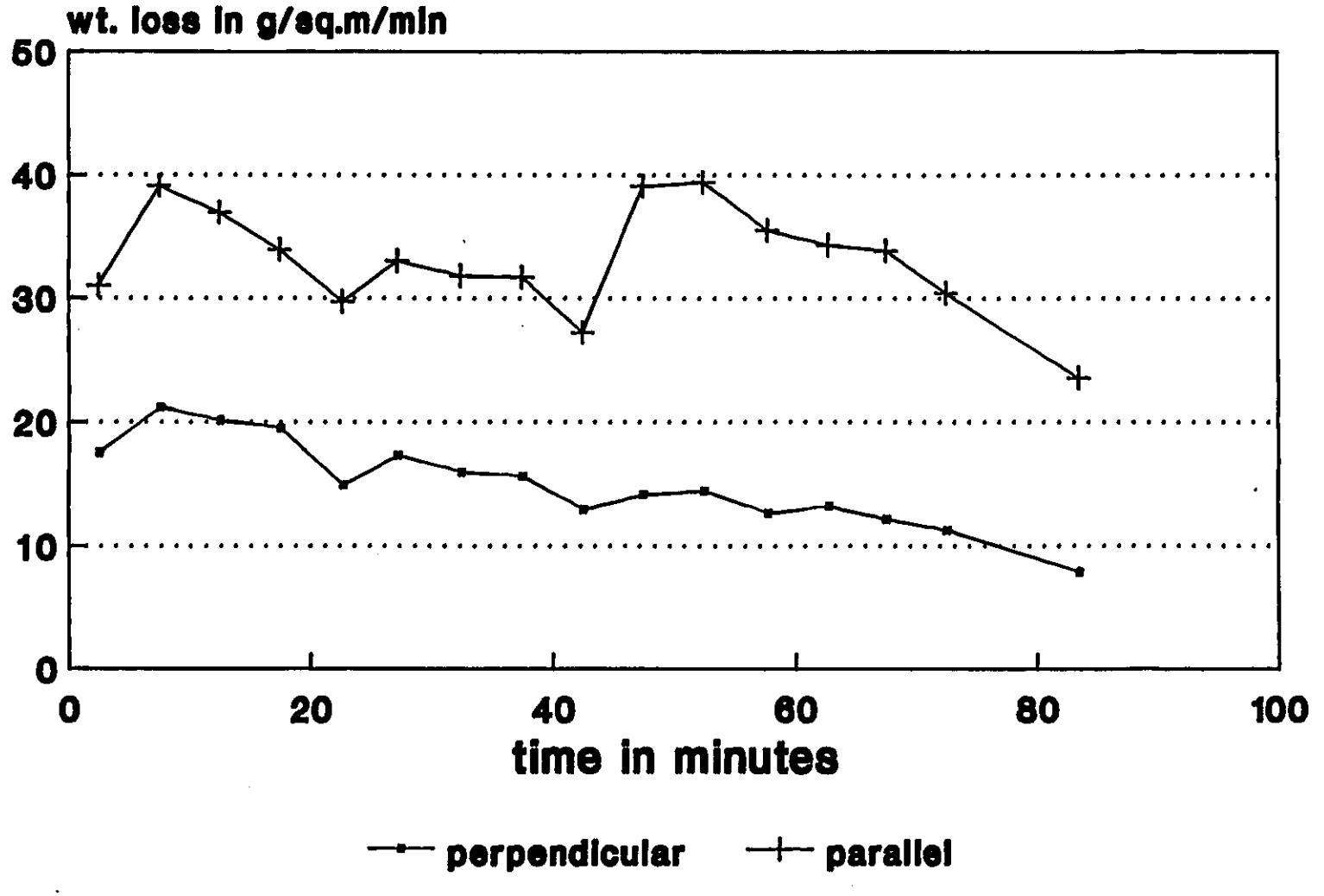


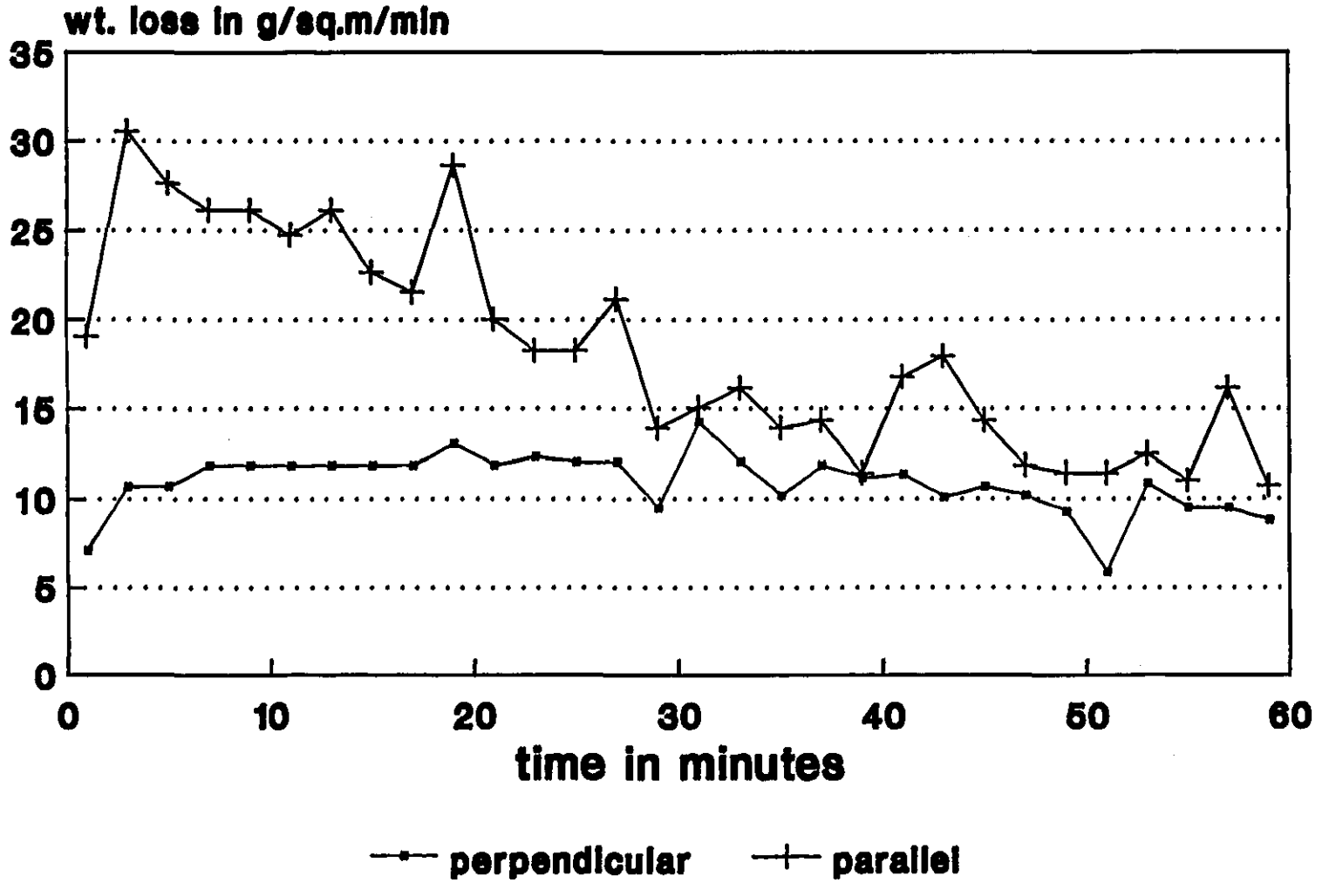
Figure 24. Diagram of a section of fish muscle to show the myofibrillar arrangement.

FIGURE 25: COD DESORPTION
A = perpendicular; B = parallel



air vel. 1.3m/s; temp. 39C(d.b.); RH 20%

FIGURE 26: COD DESORPTION
A = perpendicular; B = parallel



air vel. 0.75m/s; temp. 52C(d.b.);RH 10%

Water desorbed from the muscle blocks during drying will move in all directions towards the surface, in pathways ranging from parallel to perpendicular to the direction of the myofibrils. But, when cut, little of the water leaks out from the cut surface because it is held by weak, long-range forces or by capillary suction in the pores formed between the macromolecules (Labuza, 1977). The suction pressure, due to interaction of pore surfaces with water, can draw water columns to significant heights. Taking 10-100μm as being the range of pore sizes typical in most foods, Lewicki et al. (1978) suggested that to remove this "free" water, it would take an external force equivalent to a head of water ranging from 150 to 1500 cm (i.e. the height to which 100μm and 10μm pore diameters respectively could draw water).

Figures 25 & 26 show a significant difference in the rate of desorption from fish muscle in directions parallel and perpendicular to the arrangement of myofibrils within the fish muscle block. This difference only persists through the initial stages of drying. After 1 to 2 hours, depending upon drying conditions, the difference disappears. This suggests that initially the mean diameter of pores running parallel is greater than that of those running perpendicular to the myofibrils.

As drying proceeds this directional difference in desorption rate quickly disappears. Possibly this coincides with the flesh surface becoming dry and desorption rate coming to depend upon the rate at which water vapour diffuses across the ever-widening dry zone rather capillary suction created by evaporation of water from the surface.

Alternatively, what is referred to by Guerts et al. (1974) as "pore tortuosity" increases as drying proceeds and shrinkage occurs such that the difference between "across" and "along" water movement rate decreases.

FIGURE 27: COD (P,Q,R,S) DESORPTION
Air vel. 1.3m/s Dry bulb 40 C R.H. 20%
Mass 1sq.m surface=3.602kg (0.712kg(ds))

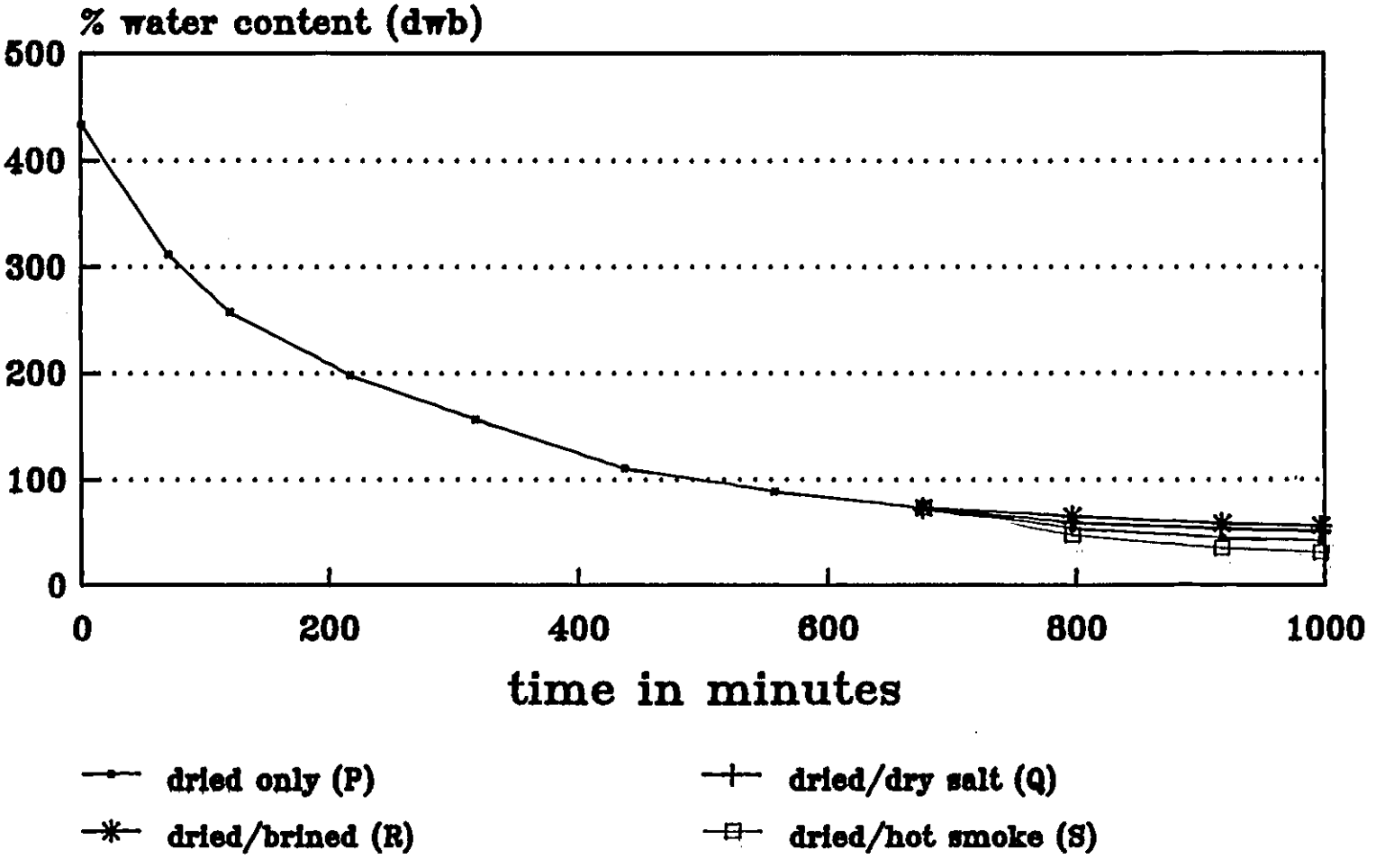


FIGURE 26: COD (P,Q,R,S) RESORPTION 1
in humid storage at 80%RH 29 C(db) 72.3h
then at 90%RH 24 C(db) 72.3h to 193h.

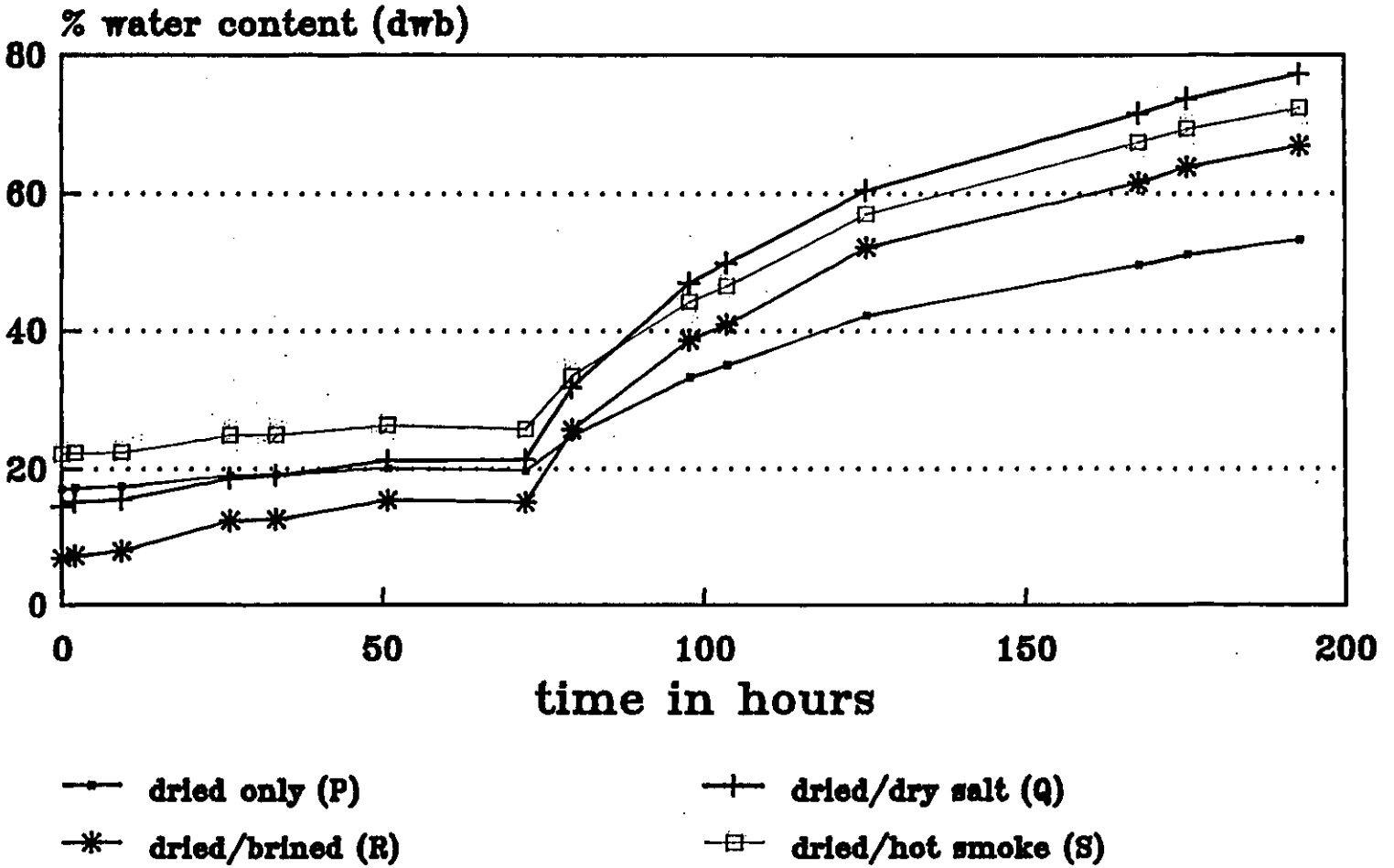
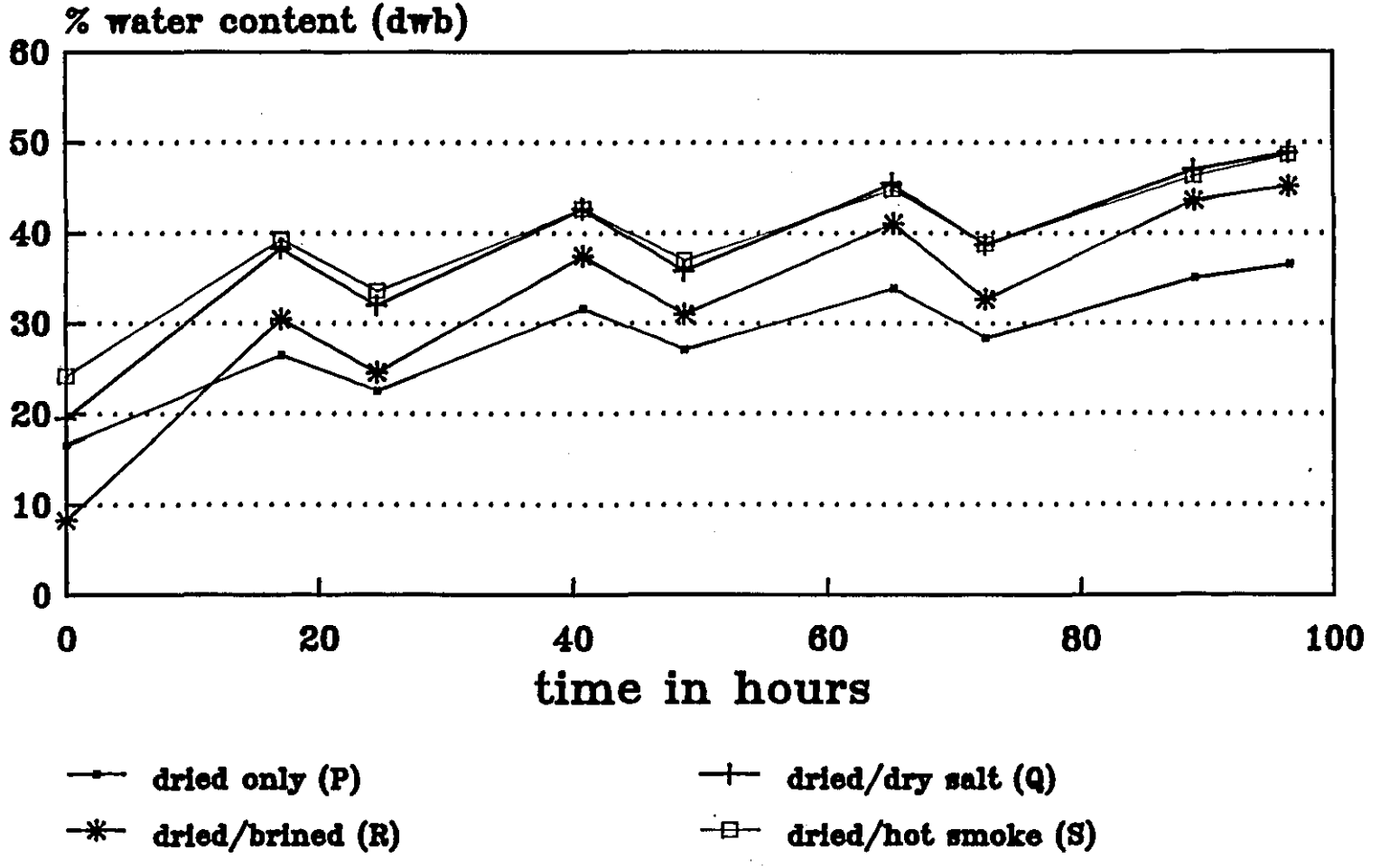


FIGURE 29:COD (P,Q,R,S) CYCLING SORPTION
res. 92%RH 25C(db); des. 70%RH 29C(db)
Itl.Mass(kg/sq.m)P0.83;Q0.85;R0.77;S0.89



VII (v) Effect of curing method on rate of water uptake and humid storage life.

The desorption history of cod fillet samples which had been dried for 11 hours and then subjected to further drying only (P) dry-salting & drying (Q) brining & drying (R) and brining, hot-smoking & drying (S) as described in section VI(vi) is shown in Figure 27.

Figures 28 and 29 show that salt addition increases the rate of water uptake by dried fish in humid storage. Although the relative vapour pressure of salted, dried fish was considerably lower than that for fish which was dried only at a given water content, the increased rate of water uptake occasioned by the salted or brined samples rendered them more rapidly susceptible to microbial spoilage. This took the form of the appearance of mould colonies on the surface of mildly salt cured samples and the appearance of pink patches (due to growth of halophilic bacteria) in heavily cured samples.

Hamm (1963) noted that addition of sodium chloride strongly increased water holding capacity and swelling capacity of meat at its normal pH value. This chloride ion is largely responsible for the increased water imbibing power as they effectively screen the positively charged sites on the protein molecule, weakening the salt cross-linkages between polypeptide chains, and, thereby, loosening the protein structure to facilitate water uptake. This could explain the Berhimpon et al. (1990) observation that the more heavily the fish has been salted, the slower the drying rate and the higher the final water content. It could also explain the increasing rate of water uptake with increasing salt content in the product noted in this work.

Given the difference in initial water contents of samples Q and R (Figures 28 and 29) there seemed to be no apparent difference in their water uptake rates. The hot-smoked samples S, however, appeared to take up water more slowly than either of the latter two in the more humid storage conditions. The heat of the smoking process changing the surface structure and/or the deposition of hydrophobic substances from the smoke in the surface layer may account for such a difference.

[Although smoking after drying is an unconventional order of process, it is used commercially in Malawi and other parts of East Africa (Mkandawire, 1985)].

Table 7. Movement of water and salt in samples Q and R under humid then dry storage conditions.

Stage 1 - after drying and dry-salting (Q)/brining (R) treatments and 2 hours of storage at 25°C(db), 80%RH. [samples s taken from the surface of the cured fillets, samples m, from mid-way between surface and centre and samples c from the centre].

	Q			R		
	s	m	c	s	m	c
% water (dwb)	13.7	17.8	20.3	17.0	19.3	22.3
% salt (dwb)	10.3	7.3	7.2	7.6	4.6	4.7
% RVP	5355	55	52	51	53	

Stage 2 -after a further 7 days' storage at 25°C(db), 80%RH.

% water (dwb)	59.9	68.8	80.5	47.1	61.7	71.4
% salt (dwb)	7.1	8.1	8.9	5.4	5.9	6.0
% RVP	8889	89	86	89	90	

Stage 3 -after a further 2 days' storage at 15°C(db), 40%RH.

% water (dwb)	23.3	30.0	39.0	21.3	26.1	32.5
% salt (dwb)	6.69.2	12.1	5.4	6.6	7.5	
% RVP	5964	67	56	62	66	

Movement of salt during water sorption by fish often appeared (Table 7) to be in the opposite direction to the movement of water. Although after stage 1, the greatest proportion of the salt remained at the surface, a considerable quantity had diffused towards the centre counter to the movement of water. After humid storage, stage 2, it appeared that salt had diffused towards the centre, where the water content had remained highest, bringing about osmotic equilibration and the direction of this movement had remained the same, again counter to the movement of water, during a further cycle of desorption.

Throughout, the solute moved from zones where it was more concentrated to those where it was less concentrated.

That there seemed to be considerable diffusion of solute, even at relatively low moisture levels, was expected from the work of Duckworth and Smith (1963), where significant movement of solute was detected even when food had been dried almost to a level equivalent to the theoretical B.E.T. monolayer water content calculated by Salwin (1959).

Young et al. (1973) noted the tendency of salt to diffuse towards the surface during rehydration at high humidities counteracting the tendency for RVP to rise rapidly due to water adsorption from the humid environment. Such movement of salt from centre to surface would allow RVP to rise at the centre perhaps faster than at the surface.

This coincides with observations in this work where centre RVP's were higher than surface RVP's during rehydration in humid storage and with frequent occurences of halophilic bacterial spoilage at the centre of salted dried fish which otherwise showed no signs of mould spoilage at the surface.

Lupin (1978) suggested that red obligate halophiles, which will grow at 75% RVP, can best be prevented from proliferating by good hygiene during the production of dried salted fish. However these organisms, which lie dormant in the product whilst the RVP remains well below 75% or the store temperature below 10°C, rapidly proliferate once again when humid storage conditions have caused an uptake of water, accelerated by the increasing surface hygroscopicity owing to outward salt migration, sufficient to raise the sub-surface RVP above 75%.

Salted, dried fish are painstakingly de-salted before presentation in edible form in numerous traditional dishes based upon this ancient commodity. In many countries, particularly in Africa, salting is not a popular means of preservation due to the

locally-high price of salt and palates unaccustomed to saltiness. So why bother to produce heavily salted fish products?

Microbial growth has been demonstrated to occur over the RVP range 62 to 99.9% (Scott, 1957). Reduction of RVP in fish to below this range involves the removal of approximately 95% of the water originally present in the fresh fish, if drying is to be the sole method of preservation. This operation is lengthy and, in regions where sun drying needs to be supported or even replaced by mechanical drying, very costly. It is also an operation which is difficult to control.

Frequently over-drying leads to the formation of opaque, yellow patches in the fish which rehydrate poorly, yielding tough, fibrous parts with a dry mouthfeel. This could be due to the removal of "firmly bound" water referred to in sections II(iii) and III(ii) leading to irreversible textural changes. Furthermore, dried-only fish need only absorb 10% water (dry weight basis) for its RVP to change from 40 to 80% (Figure 16). In comparison, lightly-salted fish required to absorb 30% water (dry weight basis) (Figure 19) whereas heavily salted fish required to absorb over 300% (dwb) water to occasion similar rises in RVP (Doe et al., 1982).

Thus, it can be seen that in cool, wet climates common in N. Europe, heavy salting (or 'hard curing') became popular because:

(i) salt was cheap and energy costs to achieve preservation by drying only were too high;
(ii) by using salt there was less chance of over-drying and consequent texture deterioration as the removal of such a large proportion of the water became unnecessary;
(iii) the product remained well-preserved even when it absorbed water when stored through periods of high humidity.

Such heavily-salted products became popular in Europe and their popularity was extended to all parts of the globe colonised by Europeans. Heavy salting, however, may not be the most suitable technology as a lowered water activity preservation method for transferal to all countries.

It was hoped that a light salting process after most of the drying had taken place would yield a salty-surfaced, dried fish which would resist attack by surface moulds. Table 7 shows that the salt did not remain at high concentration at the surface but diffused rapidly to the centre as the dehydration process was completed. Within a

week of storing the lightly-salted, dried fish had equilibrated throughout with respect to salt content.

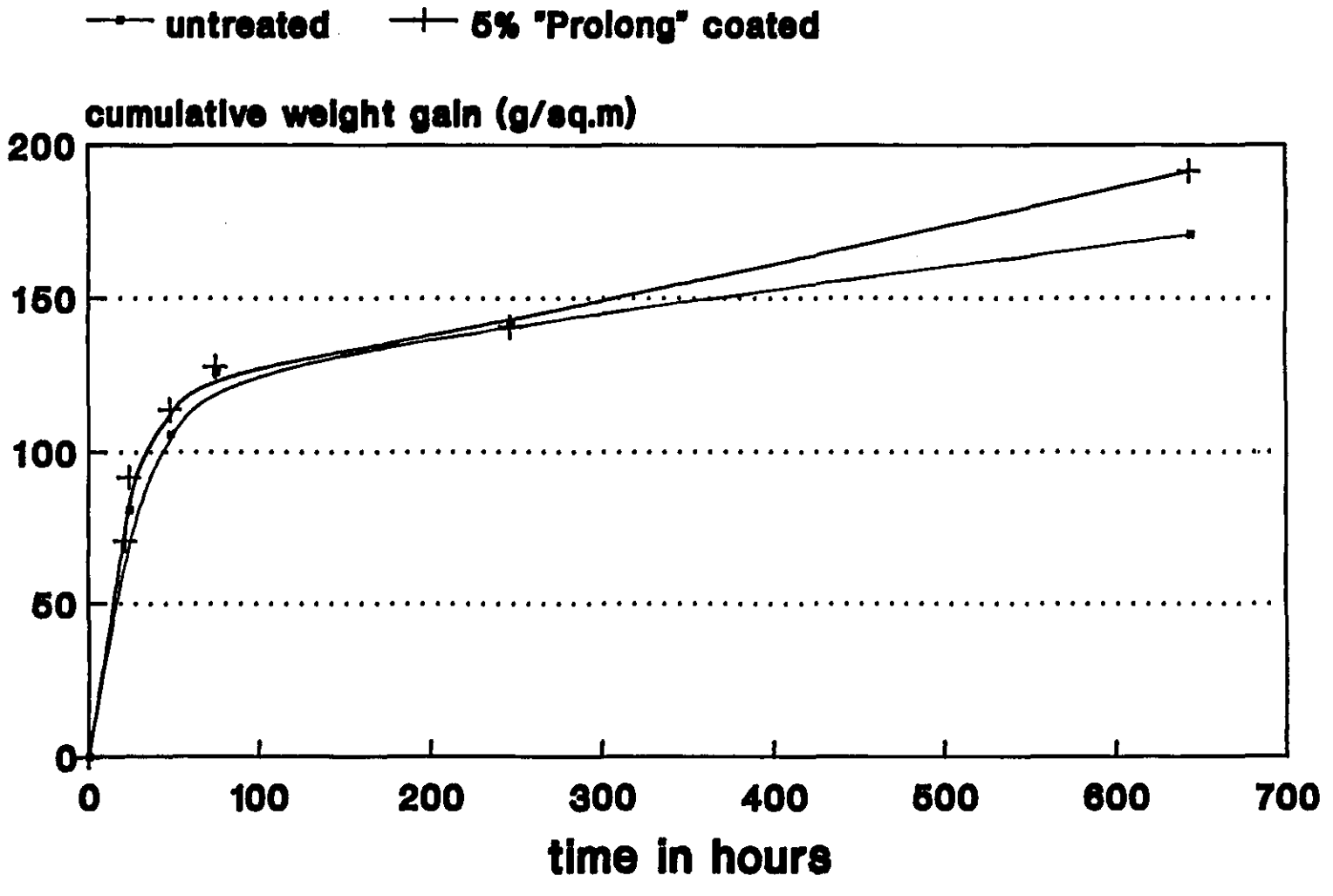
Salted samples Q,R,S (Figures 28 and 29) adsorbed atmospheric moisture at a greater rate than unsalted sample P.

Figure 29 shows the salted samples' desorption rate during the low humidity storage periods was also faster than that of the unsalted samples. It was, nevertheless, noted that mould colonies first appeared on the salted samples Q and R.

In the tropics the daily cycling of relative humidity would lead to similar product weight fluctuations which might show a steady upward or downward trend depending upon the proportion of the day that the relative humidity is higher than product RVP.

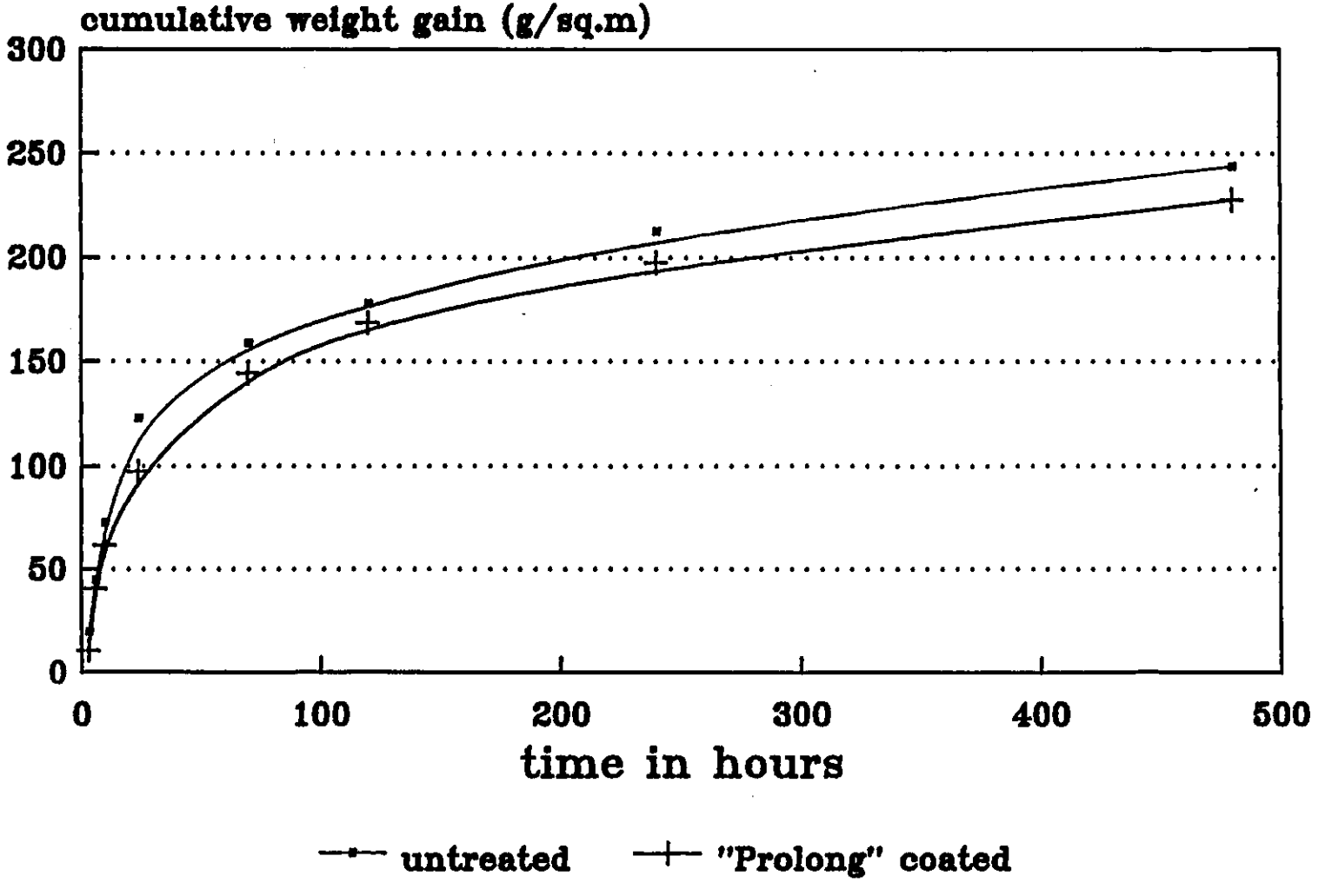
It seems, therefore, that a light salting of the product late in the process will not be effective in extending the shelf-life of dried fish in high humidity storage, as the extra lowering of RVP is more than off-set by the product's increased hydroscopicity. However, this type of process might be recommended on the grounds that less water removal is required to achieve preservation, so there is less likelihood of over-drying producing a poor-textured product. The products are also more convenient to use in not requiring long leaching processes to remove salt, like heavily salted products, before incorporation into meals.

FIGURE 30: COD RESORPTION
5% PROLONG COATED v. UNTREATED



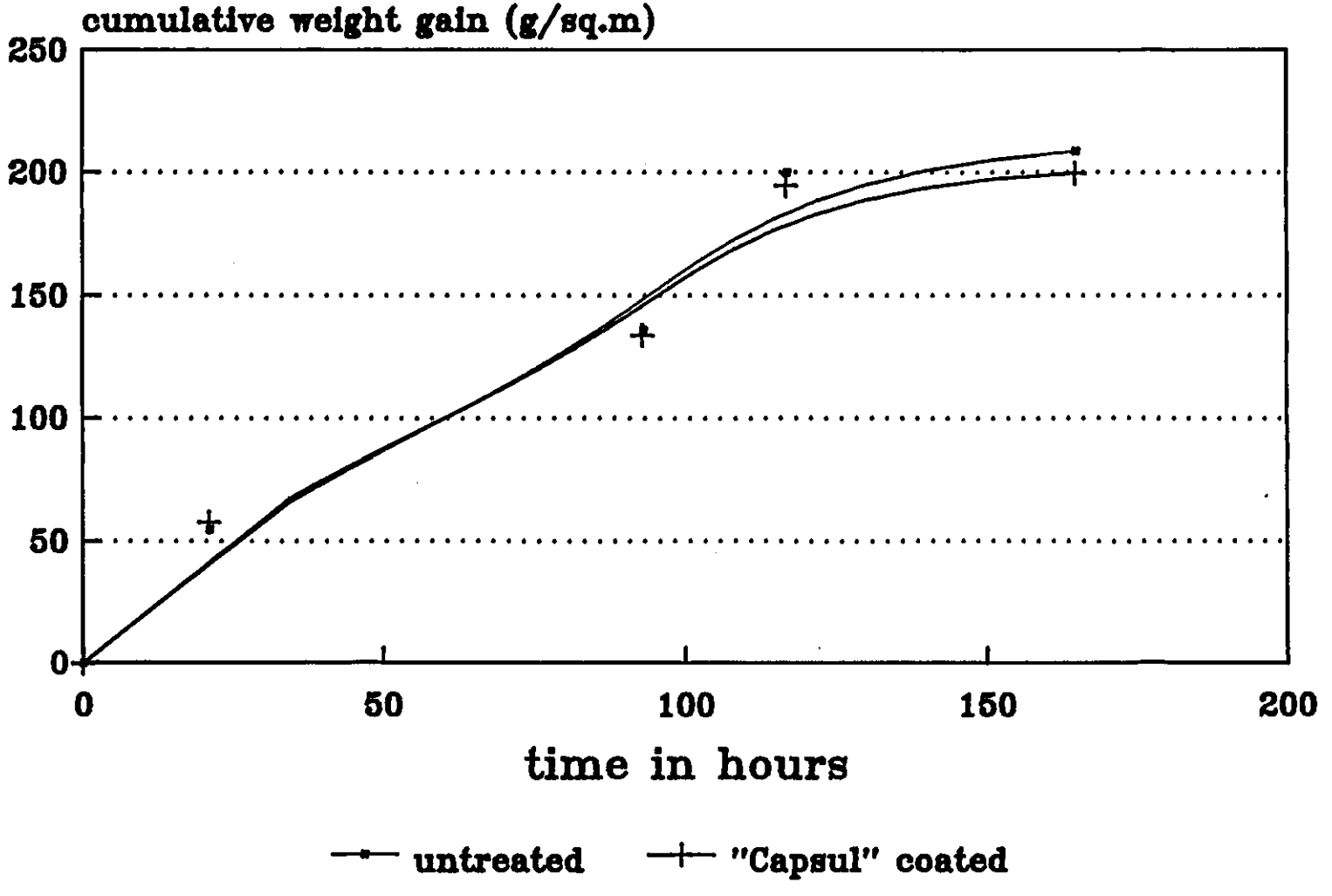
25 C (db) 80% RH

FIGURE 31: COD RESORPTION
2% PROLONG COATED (3 dips) v. UNTREATED



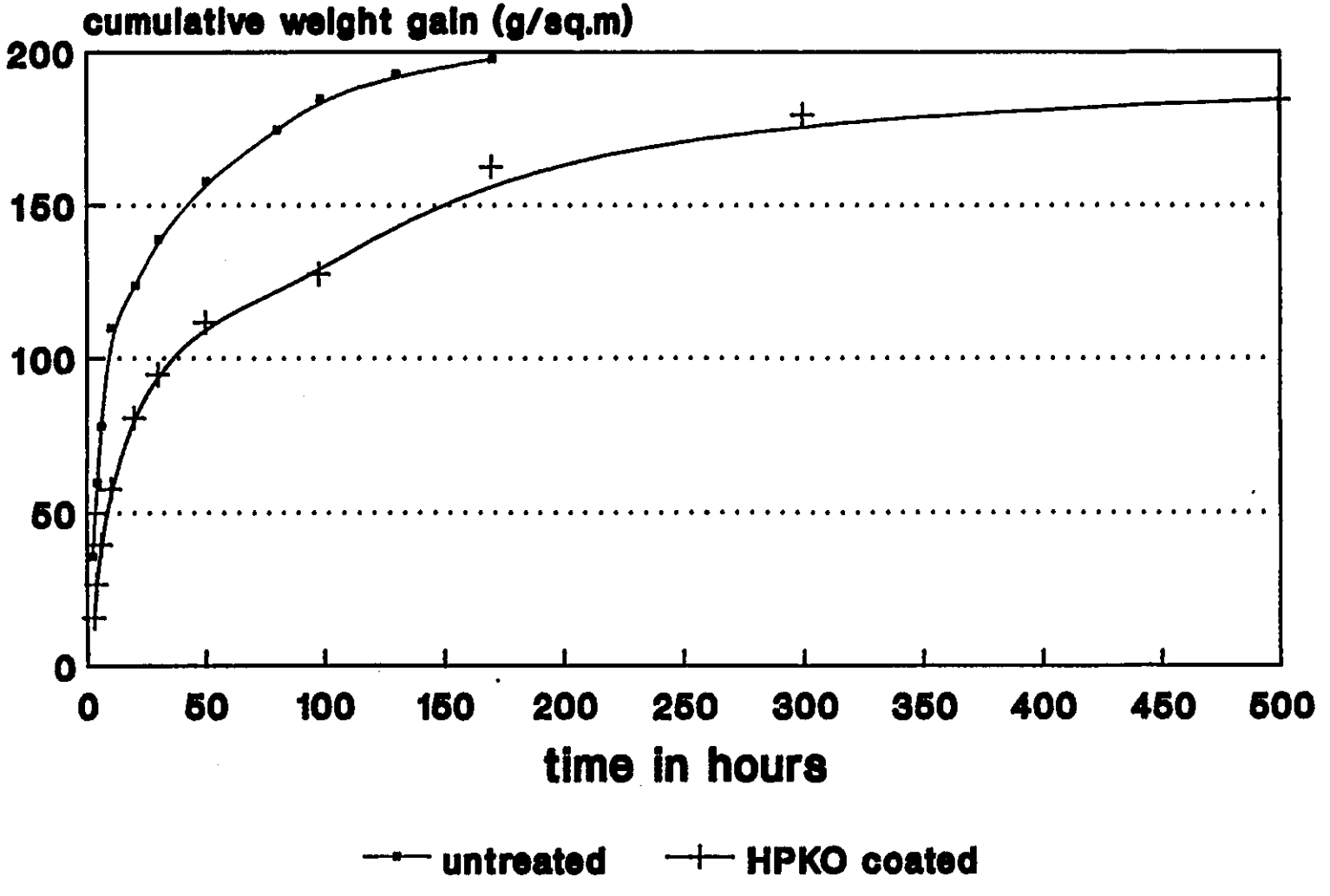
27 C (db) 80% RH

FIGURE 32: COD RESORPTION
2% CAPSUL COATED (3 dips) v. UNTREATED



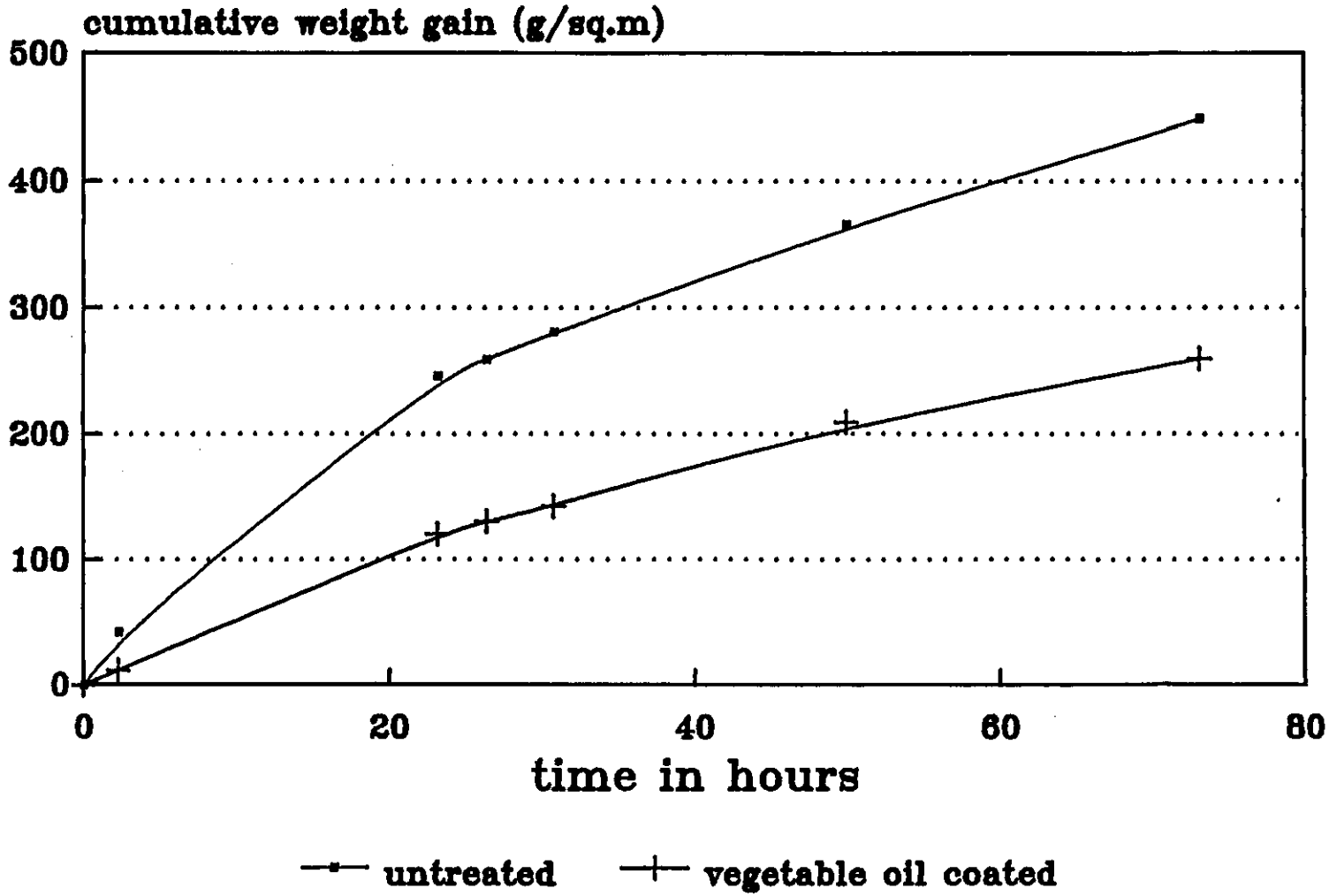
27C (db) 80% RH

FIGURE 33: COD RESORPTION
HPKO COATED v. UNTREATED



25C (db) 80% RH

FIGURE 34: COD RESORPTION
VEGETABLE OIL COATED v. UNTREATED



25C (db) 92% RH

VI (vi) Surface barriers

Dry-cured fish has been traditionally packaged for containment only in hessian bags, wooden and cardboard crates and boxes. Some attempts have been made to limit moisture uptake by the product, which is hydroscopic in humid environments, through lining boxes and crates with bituminised paper. Local dry salted fish processors had found plastics unsuitable because they are easily punctured by the hard, sharp corners of the product and retain moisture evaporating from it when exported from temperate to tropical regions. Condensation thus formed on the inside of the pack encouraged mould growth.

Figures 30,31,32,33 and 34 illustrate the effectiveness of the surface coatings investigated as barriers against the uptake of moisture by dry-cured fish in humid storage. The two lipid barriers proved the most effective. During the first four hours of humid storage, HPKO and vegetable oil coatings respectively halved and quartered the rate of water uptake by the dried fish. The HPKO coating was probably less effective than the vegetable oil coating because it had cracked extensively on setting providing numerous channels for water ingress.

The rate of water uptake by the fish depends upon the humectancy of the product and the vapour pressure gradient between product surface and store atmosphere. In experiments summarised by Figures 30, 31, 32, and 33, the initial surface vapour pressure was approximately 50% compared with the 80% RH of the surrounding atmosphere. For the oil-coated fish experiment represented by Figure 34, this initial surface vapour pressure was 40% compared with the 92% RH of the surroundings.

Greater vapour pressure gradients and greater humectancy of the product (for example, when it is salted as well as dried) may give rise to much greater rates of water uptake in spite of the lipid barrier. Fargher (1987) reported a water vapour transfer rate of slightly more than 1 g per minute per m across greaseproof paper coated with a 0.003 mm layer of lard between a 92% RH atmosphere and anhydrous silica gel. This compares with the 0.3 g per minute per m maximum rate across the oil coated dried fish.

The two carbohydrate-based coating materials, "Prolong" and "Capsul" proved ineffective barriers to the passage of water vapour (Figures 30, 31 and 32). There appeared to be an improvement to the barrier properties of "Prolong" when a triple coating of 2% solution was substituted for a single coating of 5% solution (Figures 30 and 31). However the barrier properties of the latter (Figure 30) were actually

negative. The coating merely increased the hydroscopicity of the product and thereby initially increased the rate of water uptake.

The improvement due to triple rather than single coating was not so significant as to recommend it as a worthwhile exercise. The barrier provided was only slightly better than having no barrier at all. This lack of barrier effectiveness was also shown with the "Capsul" triple-coated samples (Figure 32) where the rate of water uptake was almost undistinguishable from that in the untreated samples.

Of the barriers tested, the vegetable oil seems the most likely to produce a significant shelf-life extension. The sorption isotherms presented earlier in this section discussing results obtained in this work (Figures 15 and 16) show that unsalted, dried fish needs only to take up 10% water on a dry weight basis for the water activity to increase from 0.4 to 0.8. The dry mass of fish presenting 1 m of surface in these investigations was found to be approximately 1 kg (Figure 23) so a water uptake of approximately 100 g in Figure 34 would cause the product water activity to increase to a level which would support surface mould proliferation. In this respect, the surface oil barrier increases the period over which 100 g of water is taken up from approximately 8 to approximately 20 hours in a 92% RH environment. Obviously in a typical, tropical situation with the humidity cycling daily between maximum and minimum values, this improvement could represent a considerable extension of shelf-life.

VIII CONCLUSIONS

This project was instigated by the observation that a sizeable portion of a valuable commodity, dried-salted white fish, was deteriorating to rejection level during production and storage in tropical environments.

The sorption isotherms, elucidated in this and previous work, show that very small amounts of water uptake by cured fish in humid storage can render them susceptible to microbial spoilage within days not weeks. This susceptibility can be aggravated or reduced by the selection of drying conditions or the use of salt in addition to drying.

During production in high humidity, however, the selection of "slow/cool" conditions or the use of salt may mean that the drying of fish takes place so slowly that microbial proliferation at the centre of the flesh would render it putrid before the water activity had fallen to an inhibitory level.

Raising drying air temperature, and, to a lesser extent, air speed increases drying rate, but at the expense of product textural quality and raised susceptibility to resorb water in high humidity storage.

Scoring the flesh to expose greater surface areas and reduce the distance which water must travel to escape the surface is a common practice. Results presented in this work suggest that scoring across, rather than along, muscles would accelerate initial drying rates as water movement parallel in general direction appears faster than water movement perpendicular to the myofibrils.

Considering the protection of the product against water uptake in humid storage environments, high salt contents have long been known to be effective in allowing for considerable water uptake without the danger of reinitiating microbial growth and spoilage. This is because, in sorption isotherms for salted fish, the water activity reaches 0.75 and stays there (Doe et al. 1982) until the water contained becomes less than saturated by the salt contained. However, this depends upon the purity of the salt; impure salts will exhibit this plateau at higher water activities which may exceed the minimum growth levels for certain spoilage organisms. Unfortunately, the addition of salt also increases the hygroscopicity of the product. It was observed in this work that lightly to moderately salted and dried products began to spoil sooner than unsalted controls in humid storage.

Smoked products however were observed to resorb water from humid atmospheres at a slower rate than products dried in identical fashion but without the smoke.

Of the barrier materials applied to the product post-process, the carbohydrate based materials ("Prolong" and "Capsul") were ineffective if not negative in effect. Initial rate of water resorption was faster in some of the "Prolong" treated samples than in their corresponding untreated controls. The lipid barriers, however, were effective. Vegetable oil dipping reduced the rate of water uptake to less than 40% of the control rate.

IX REFERENCES

ANTONY K.P., RAO C.V.N. & GOVINDAN T.K. (1988) "Bulk packages for storage and transportation of salted and dried fish." Fishery Technology (India) 25, pp.112-116.

BERHIMPON S., SOUNESS R.A., BUCKLE K.A. & EDWARDS R.A. (1990) "Salting and drying of yellowtail (*Trachurus mccullochi*) Int. J. Food Sci. Technol. 25, pp.409-419.

BIZOT H. (1983) "Using the G.A.B. model to construct sorption isotherms." in "Physical Properties of Foods." Eds. Jowitt R., Escher F., Hallstom B.,Meffert H.F.T., Spiess W.E.L. & Voss G. pp.43-54. Applied Science, London.

BLOCK S.S. (1953) "Media influence onmould and yeast growth at low water activities." Appl. Microbiol. 1, p.287.

BONE D. (1969) "Water Activity: Its Chemistry and Applications." Food Product Development. August.p81.

BONE D. (1973) "Water Activity in Intermediate Moisture Foods." Food technol. 27, pp71-76.

BRUNAUER S., EMMETT P.H. & TELLER E. (1938) "Adsorption of gases in multimolecular layers." J. Am. Chem. Soc.60 p.309.

CARDEW M.H. & ELEY D.D. (1958) "Fundamental aspects of the dehydration of foodstuffs." Soc. chem. Ind. , pp24- .

CHIRIFE J., RESNIK S.L. & FERRO-FONTAN C. (1985) "Application of Ross' equation for prediction of water activity in intermediate moisture food systems containing a non-solute solid." J. Food Technol. 20, pp.773-779.

CHRISTIAN J.H.B. (1963) "Water activity and the growth of microorganisms." Rec. Adv. Food Sci.3, pp.248-255. Eds. Hawthorne & Leitch. Butterworths.

CHU F-F. (1984) "The relationship of water content and composition of food to mold spore germination." M.Sc. Thesis, Rutgers Univ., New Brunswick, N.J., U.S.A.

COLE L.J.N. (1962) "The effect of storage at elevated temperatures on some proteins of freeze-dried beef." J. Food Sci. 27, pp.139-143.

CURRAN C.A. & POULTER R.G. (1983) "Isohalic sorption isotherms 3: Application to a dried, salted tropical fish (Xenomugil thoburni)." J. Food Technol. 18, pp.739-746.

CUTTING C.L., REAY G.A. & SHEWAN J.M. (1956) G.B. Dep. Sci. Ind. Res., Food Invest.Board Spec. Rep. N^{o}·62 H.M.S.O. London.

DE LEIRIS J.P. (1986) "Water Activity and Permeability." in "Food Packaging and Preservation: Theory and Practice." pp.213-233. Ed. Mathlouthi M. Elsevier Applied Science.

DEL VALLE F.R. & NICKERSON J.T.R. (1967) "Studies on salting and drying fish. 1.Equilibrium considerations in salting." J. Food Sci. 32, pp.173-179.

DEL VALLE F.R. (1965) "The diffusion of sodium chloride in fish muscle." Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, Mass. U.S.A.

DOE P.E., CURRAN C.A. & POULTER R.G. (1983) "Determination of the water activity and shelf-life of dried fish products." In "Production and Storage of Dried Fish." Ed. D. Jaines, FAO Fish Report 279, pp.202-221.

DOE P.E., HASHMI R., POULTER R.G. & OLLEY J. (1982) "Isohalic sorption isotherms. I. Determination for dried, salted cod." J. Food Technol. 17, pp.125-134.

DUCKWORTH R.B. & KELLY C.E. (1973) "Studies of solution processes inhydrated starch and agar at low moisture levels using wide-line nuclear magnetic resonance." J. Food Technol.8, pp.105-113.

DUCKWORTH R.B. (1963) "Diffusion of solutes at low moisture levels." Rec. Adv. Food Sci.3, pp.230-238. Eds. Hawthorne & Leitch. Butterworths.

DUCKWORTH R.B. (1971) "Differential thermal analysis of frozen food systems. 1.The determination of unfreezable water." J. Food Technol. 6, pp.317-327.

DUCKWORTH R.B. (1972) "The properties of water around the surfaces of food colloids." Proc. Inst. Food Sci. Technol.5, pp.60-67.

EICHNER K. (1986) "The Influence of Water Content and Water Activity on Chemical Changes in Foods of Low Moisture Content." in "Food Packaging and Preservation: Theory and Practice." Ed. Mathlouthi M. Elsevier Applied Science. pp.67-92.

ELEY D.D. & LESLIE R.B. (1963) "Hydration of solid proteins with special reference to haemoglobin." Rec. Adv. Food Sci.3.pp.215-217 Eds. Hawthorne & Leitch. Butterworths.

ESSER J.R. (1988) "Assessment of reduction of insect infestation of cured fish in South East Asia, with laboratory studies on *Chrysomya megacephala* (Fab.), a principle causative agent. Ph.D. Thesis, University of Durham.

FARGHER R. (1987) "Investigation into the factors affecting the transport of water through spreads." B.Sc.(Hons) Thesis, Humberside College of Higher Education.

FERRO-FONTAN C., BENMERGUI E.A. & CHIRIFE J. (1980) "The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. III. a_w prediction in multi-component strong electrolyte solutions." J. Food Technol. 15, pp.47-58.

GAL S. (1972) "Die praktiishe Bedeutung von Wasserdampf-Sörptions -Messungen in der Lebensmittelindustrie." Alimenta 6, p213.

GEORGE JOSEPH K., MURALEEDHARAN V., UNNIKRISHNAN NAIR T.S. & KALAIMANI N. (1988) "Quality of cured fish from the Maharashtra Coast." Fishery Technology (India) 25, pp.120-123.

GILBERT S.G. (1986) "New concepts on water activity and storage stability." in "The Shelf Life of Foods and Beverages." pp.303-334 Ed. G. Charalambous. Elsevier Science Publishers B.V. Amsterdam.

GILES N. (1990) Personal communication.

GUERTS J.J., WALSTRA P. & MULDER H. (1974) "Solutes diffusion in cheese." Neth. Milk Dairy J. 28, p.102.

HAILWOOD A.J. & HORROBIN S. (1946) "Absorption of water by polymers: Analysis in terms of a simple model." Trans. Faraday Soc. 42B, pp.84-92.

HALSEY G. (1948) "Physical adsorption on non-uniform surfaces."J. chem. Phys. 16, pp.931-937.

HAMM R. (1960) "The biochemistry of meat hydration." Adv. Food Res. 10, p.356. Eds. Mrak E.M. & Stewart G.F. Academic Press.

HAMM R. (1963) "The water imbibing power of foods." Rec. Adv. Food Sci.3, pp.218-229. Eds. Hawthorne & Leitch. Butterworths.

HANSON S.W. & McGUIRE K.J. (1982) "Investigation into post-harvest losses of cured fish in S.E. Asia." Report R3693. Overseas Development Administration.

HARDMAN T. (1985) "Interaction of water with food components." A paper given at the International Symposium "Interactions of Food Components" University of Reading, 3 April 1985.

HICKS E.W., SCOTT W.J. & VICKERY J.R. (1955) "The influence of storage behaviour on the water content of the superficial tissues of meat." Bull. Intern. Inst. Refrig. Annexe 1, pp51-56.

IBRAHIM R. (1986) "Production and storage of salted-boiled fish (*pindang*) using atlantic mackerel (Scomber scombrus)." M. Phil. Thesis, University of Technology, Loughborough.

IGLESIAS H.A. & CHIRIFE J. (1976) "Equilibrium moisture contents of air-dried beef. Dependence on drying temperature." J. Food Technol. 11, pp.565-573.

IGLESIAS H.A., CHIRIFE J. & LOMBARDI J.L. (1975) "An equation for correlating equilibrium moisture content in foods." J. Food Technol. 10, pp.289-297.

JOHNSTON K. (1985) "Water-sorbent-solute relationships in dry food model systems." Paper given at a Royal Society of Chemistry Discussion Conference. "Water Activity: A Credible Measure of Technological Performance and Physiological Viability." Girton College, Cambridge.

JONES N. & PERALTA J. (1980) "Preliminary report on the effect of drying temperature on the sorption isotherm of cod muscle." Fisheries Section N.R.I., Chatham, Kent ME4 4TB.

KALAIMANI N., GOPAKUMAR K. & UNNIKRISHNAN NAIR T.S. (1988) "Quality characteristics of cured fish of commerce." Fishery Technology (India) 25, pp.54-57.

KAPSALIS J.G. (1981) "Moisture Sorption Hysteresis." in "Water Activity: Influences on Food Quality." Eds. Rockland L.B. & Stewart G.F. Academic Press. pp.143-177.

KAREL M. (1973) "Recent research and development inthe field of low and intermediate moisture foods." Critical Reviews in Food Technology 3, pp.329-358.

KAREL M. (1976) "Technology and application of new intermediate moisture foods.". in "Intermediate Moisture Foods." pp.4-31. Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

KLOTZ I.M. & HEINEY R.E. (1957) "Changes in protein topography upon oxygenation." Proc. Nat. Acad. Sci. U.S.A. 43,717.

KUPRIANOFF J. (1958) Conference on "Fundamental aspects of the dehydration of foodstuffs." Soc.Chem.Ind. London p22.

LABUZA T.P. (1970) "Properties of water as related to the keeping quality of foods." Proc. 3rd Int. Congress Food Science & Technol. Inst. Food Technol. pp.618-635.

LABUZA T.P. (1972) "Processing and storage effects on nutrients in dehydrated foods." Critical Reviews in Food Technology 3, pp.217-240.

LABUZA T.P. (1977) "Properties of water in relationship to water binding in foods: a review." J. Food Processing and Preservation 1(2) pp.167-190.

LABUZA T.P., CASSIL S. & SINSKEY A.J. (1972) "Stability of intermediate moisture foods: 2)Microbiology." J. Food Sci. 37, pp.160-162.

LABUZA T.P., TANNENBAUM S.R. & KAREL M. (1970) "Water content and stability of low moisture and intermediate moisture foods." Food Technol. 24, pp.543-550.

LANGMUIR I. (1918) "The adsorption of gases on plane surfaces of glass, mica and platinum." J. Am. Chem. Soc. 40, pp.1361-1365.

LEWICKI P.P., BUSK G.C., PETERSON P.L. & LABUZA T.P. (1978) "Determination of factors controlling accurate measurement of Aw by the vapour pressure manometric technique." J. Food Sci. 43, pp.244-246.

LEWIS G.N. (1907) Proc. Am. Acad. Sci.43,p.259.

LILLEY T.H. (1985) "Water activity as a thermodynamic device; definitions and assumptions." Paper given at a Royal Society of Chemistry Discussion Conference. "Water Activity: A Credible Measure of Technological Performance and Physiological Viability." Girton College, Cambridge.

LILLEY T.H. (1987) "Technical Note: Criticism of a proposed corrected form of the Ross Equation and a suggested extension of the equation." Int. J. Food Sci. Technol. 22, pp.563-567.

LONCIN M. (1961)" Les Operations Unitaires du Genie Chimique." Ed. Dunod Paris, Desoer Liege.

LONCIN M. (1975) "Basic principles of moisture equilibria." in "Freeze Drying and Advanced Food Technology."pp.599- . Eds. Goldblith S.A., Rey L. & Rothmayr W.W. Academic Press.

LONCIN M., BIMBENET J.J. & LENGES J. (1968) "Influence of the activity of water on the spoilage of foodstuffs." J. Food Technol. 3, pp.131-142.

LUPIN H.M. (1978) "Technical consultation on the Latin American Hake Industry." FAO Fisheries Report No.203,suppl.1 p.161.

LUPIN H.M., BOERI R.L. & MOSCHIAR S.M. (1981) "Water activity and salt content relationship in moist, salted fish products." J. Food Technol. 16, pp.31-38.

MACARA T.J.R. (1943) "Dried meat II. The growth of moulds on dried meat." J. Soc. Chem. Ind. 62, pp.104-106.

MARUF F.W., LEDWARD D.A., NEALE R.J. & POULTER R.G. (1990) "Chemical and nutritional quality of Indonesian dried-salted mackerel (*Rastrelliger kanagurta*)." Int. J. Food Sci. Technol. 25, pp.66-77.

McLAREN A.D. & ROWEN J.W. (1951) "Sorption of water vapour by proteins and polymers: A review." J. Polymer Sci. 7, pp.289-324.

MEASURES J.C. & GOULD G.W. (1973) "Interactions of Microorganisms with the Environment of Intermediate Moisture Foods." in "Intermediate Moisture Foods." pp.281-297. Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

MOK W.Y., CASTELO F.P. & BARRETO DA SILVA M.S. (1981) "Occurence of *Exophiala werneckii* on salted freshwater fish *Osteoglossum bicirrhosum*." J. Food Technol. 16, pp.505-512.

MOSSEL D.A.A. & INGRAM M. (1955) "The physiology of microbial spoilage of foods." J. Appl. Bact. 18:232.

MOSSEL D.A.A. & WESTERDIJK J. (1949) "The physiology of microbial spoilage of foods." Antonie van Leeuwenhoek 15, pp.190-202.

MOTLAGH F.H. (1982) "Storage of Brazilian limes using "Pro-long"." M.Phil. Thesis, Univ. Technol. Loughborough.

MOY J.H., CHAN K-C. & DOLLAR A. (1971) "Bound water in fruit products by the freezing method." J.Food Sci. 36, pp498-499.

NGUYEN C.D. & HAARD N.F. (1987) "Evaluation of methods for determination of moisture content of heavy salted cod, *Gadus morhua.*" Can. Inst. Food Sci. Technol. J. 20, 2, pp.65-69.

OBANU Z.A., LEDWARD D.A. & LAWRIE R.A. (1975) "The protein of intermediate moisture meat stored at a tropical tempeature." J. Food Technol. 10, pp.657-666 & 667-674.

PAULING L. (1945) "The adsorption of water by proteins." J. Amer. chem. soc. 67, pp.555-557.

PAWSEY R. & DAVIES R. (1976) "The safety of IMF with respect to *Staphylococcus aureus.*" in "Intermediate Moisture Foods" ed. Davies R.; Birch G.G.; Parker K.J. Applied Science Publishers. pp182-202.

PLITMAN M., PARK Y., GOMEZ R. & SINSKEY A.J. (1973) "Viability of *Staphylococcus aureus* in intermediate moisture meats." J. Food Sci. 38, pp.1004-1008.

POULTER R.G., DOE P.E. & OLLEY J. (1982) "Isohalic sorption isotherms. II Use in the prediction of storage life of dried, salted fish." J. Food Technol. 17, pp.201-210.

PRASAD B.N., SINHA B.K. & SINHA A.K. (1987) "Aflatoxigenic fungi isolated from fish and its public health importance." Indian J. Comp. Microbiol. Immunol. & Infect. Diseases. 8, pp135-136.

REID D.S. (1973) "Water Activity Concepts in Intermediate Moisture Foods." in "Intermediate Moisture Foods." pp.54-65 Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

RIEMANN H., LEE W.H. & GENIGEORGIS C. (1972) "Control of *Clostridium botulinum* and *Staphylococcus aureus* in semi-preserved meat products." J. Milk Fd.Technol. 35 pp.514-523.

ROEBUCK B.D., GOLDBLITH S.A. & WESTPHAL W.B. (1972) "Dielectric properties of carbohydrate-water mixtures at microwave frequencies." J. Food Sci. 37, pp.199-204.

ROLFE E.J. (1976) "A place for intermediate moisture foods." in "Intermediate Moisture Foods." pp.1-3. Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

ROSS K.D. (1975) "Estimation of water activity in intermediate moisture foods." Food Technol. 29, pp.26-30.

SALWIN H. (1959) "Defining minimum % water content for dehydrated foods." Food Technol.13, pp594-597.

SALWIN H. (1959) "Defining minimum % water for dehydrated foods." Food Technol. 13, pp.594-595.

SCOTT W.J. (1957) "Water relations of food spoilage microorganisms." Adv. Food Res.7, pp.84-127. Eds. Mrak E.M. & Stewart G.F. Academic Press.

SEEHOF J.M., KEILIN B. & BENSON S.W. (1953) "The surface area of proteins. V. The mechanism of water sorption." J. Amer. chem. soc. 75, pp.2427-2430.

SEILER D.A.L. (1973) "Stability of Intermediate Moisture Foods with Respect to Mould Growth." in "Intermediate Moisture Foods." pp.166-181. Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

SHANBHAG S., STEINBERG M.P. & NELSON A.I. (1970) "Bound water defined and determined at constant temperature by wideline N.M.R." J. Food Sci. 35, pp.612-615.

SHAW T.M. (1944) "The surface area of crystalline egg albumen." J. chem. Phys. 12, pp.391-392.

SHEWAN J.M. (1953) "The bacteriology of dehydrated fish. 2. The effect of storage conditions on the bacterial flora." J. Hyg. 51, pp.347-358.

SINSKEY A.J. (1973) "New Developments in Intermediate Moisture Foods: Humectants." in "Intermediate Moisture Foods." pp.260-280. Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

SMITH S.E. (1947) "The sorption of water vapour by high polymers." J. Amer. Chem. Soc. 69, pp.646-651.

SNOW D. (1949) "The germination of mould spores at controlled humidities." Appl. Biol. 38, pp.1-13.

SNOW D., CRICHTON M.H.G. & WRIGHT N.C. (1944) "Mould deterioration of feeding-stuffs in relation to humidity and storage." Ann. appl. Biol. 31, pp.102-110 & 111-116.

SUGGETT A. (1973) "The Significance of Water, Hydration and Aqueous Systems in Intermediate Moisture Foods." in "Intermediate Moisture Foods." pp.66-74. Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

TAGAKI I. (1973) In "Water in Foods", Japan. Soc. Sci. Fish (Ed.), Kosei-sha Kosci-Kaku Inc., Tokyo.

VAN DEN BERG I.C. & BRUIN S. (1981) "Water activity and its estimation in food systems: Theoretical aspects." in "Water Activity: Influences on Food Quality." Eds. Rockland L.B. & Stewart G.F. pp.1-62. Academic Press.

VAN DEN BERG I.C. (1985) "On the significance of water activity in low moisture systems; water vapour sorption equilibrium and hysteresis; the starch/water system as a model." Paper given at a Royal Society of Chemistry Discussion Conference. "Water Activity: A Credible Measure of Technological Performance and Physiological Viability." Girton College, Cambridge.

WALDERDORF M.G. (1924) "Ueber Kultur von Pollenschlauchen und Pilzmycelien auf festem substrat bei verschiedener Luftfeuchtigkeit". Bot Arch 6 pp 84-111.

WALTER H. (1924) Z. Bot. 16,353.

WHITTLE K.J. (1985) "A vital role for aquatic resources in feeding a hungry world." Proc. Nutr. Soc. 44, pp.19-29.

WILLIAMS J.C. (1973) "Chemical and Non-Enzymic Changes in Intermediate Moisture Foods." in "Intermediate Moisture Foods." pp.100-119. Eds. Davies R., Birch G.G. & Parker K.J. Applied Science.

WOLF M.,WALKER J.E. & KAPSALIS J.G. (1972) "Water vapour sorption hysteresis in dehydrated food." J. Agr. Food Chem. 20, pp.1073-1077.

YOUNG F., JAMES D.G., OLLEY J. & DOE P.E. (1973) "Studies on the processing of abalone 4: Dried abalone: products, quality and marketing." Food Technol. Aust. 25, pp.142-149.

ZUGARRAMURDI A. & LUPIN H.M. (1980) "A model to explain observed behaviour on fish salting." J. Food Sci. 45, pp.1305-1317.