Physical Changes During the Heating of Herring Muscle

by

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Dedicated to Tatiana and Vera Silvia

MEMORANDUM

This thesis is submitted to the Loughborough University of Technology in support of my application for admission to the degree of Doctor of Philosophy. It contains an account of my own original work performed at Torry Research Station in Aberdeen in the period September 1976 to December 1979 under the supervision of Professor J Mann and Dr A Aitken. The work is the result of my own independent research except as specified in acknowledgements or in the text. Neither the thesis nor the original work contained therein has been submitted to this or any other university for a higher degree.

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ABSTRACT

This work embodied three approaches to a study of the effect of heating on herring muscle at two fat levels. The first approach involved the determination of water and fat release during cooking and pressing of the muscle as a function of temperature and holding times, for the temperature range 35-113°C. Temperature had a significant effect on fat and water release, but holding times of up to 1 hour had no significant effect. Losses of water and fat were not proportional to temperature. Outstanding findings were that water losses during cooking at 45°C were as high as those at 85°C, and minimum water release occurred around 60°C. Concerning fat losses during cooking and pressing, temperatures as low as 55-60°C were as efficient as temperatures around 100°C in easing the liberation of fat. Losses of water during pressing were generally inversely related to those during cooking.

The second approach involved the design, construction and operation of an isoperibol calorimetric system to determine the influence of composition and temperature on the apparent specific heat of the muscle. The apparent specific heat of the low fat content (LFC) muscle was significantly higher than that of the high fat content (HFC) one. The influence of temperature on the specific heat was small in the HFC samples and absent in the LFC samples.

The third approach encompassed subjective observations on changes in translucency, texture and palatability, and optical and electron microscopic examinations of the heated muscle. Major changes in texture and translucency occurred below 60°C. At 50°C the muscle was completely palatable. Optical microscopy revealed progressive degradation of the connective tissue with temperature up to 60°C, above 45°C there was increased deposition of granulated material on the connective tissue and on the fibre surfaces. Electron microscopy showed that even at 85°C the sarcomere structural organisation was still visible although the Z-line had disappeared and the H-zone had been disrupted.

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CHAPTER 1

INTRODUCTION

Except in a few regions of the world where it is eaten raw, all fish receives some form of heat treatment before its consumption. This heat treatment may be the culinary preparation of the fish in the home or part of processes like drying, canning, smoking and fish meal manufacture for animal feed.

An initial review of the literature on the effects of heat on muscle revealed that while there is considerable information on the changes in meat muscle caused by the action of heat, particularly with reference to water holding capacity (Hamm, 1966, 1977), very little scientific effort has been devoted to studying the effects of heat on fish muscle. A substantial part of the work available, mainly by Japanese workers, has been devoted to the effects of heat on proteins in situ or in suspensions. Most of these studies were concerned with the manufacture of kamaboko (fish sausage), and are not very relevant to other industrial processes.

According to Aitken and Campbell (1969a) a phenomenological description of cooking of fish would include the following changes: a) loss of water (and fat) holding capacity, b) increased muscle firmness, c) increased ease of separation of fibres, d) separation of flakes, e) development of opacity, f) development of flavour, g) development of colour, h) preservation by destruction of microorganisms and inactivation of enzymes. The most obvious macroscopic effect of heat on fish muscle is the first of these phenomena, the release of water and fat, if present in significant amount. This effect is of major importance in the technological processes mentioned above, while changes in water and fat have an important bearing on consumer attributes of the cooked fish,

such as succulence, juiciness, tenderness, flavour and colour. As Karmas and Turk (1976) pointed out, one reason why the left over parts of fish carcass, after the fillets have been removed, are underutilised as a source of fish flesh,is the poor water binding quality during cooking of such flesh when recovered.

The lack of systematic work on the effects of heating on water and fat release from fish muscle leads to the conclusion that established industrial processes of cooking fish have been developed empirically. It seemed worthwhile, therefore, to carry out some systematic work which could help to clarify the factors and mechanisms affecting the release of water and fat from muscle, and might benefit heat processing of fish. As it is generally accepted that water retention in muscle is directly related to the properties of the muscle proteins (Hamm and Deatherage, 1960; Simidu and Takeda, 1951) it was initially planned to do fundamental work by studying the effects of factors such as pH, salts, denaturing and stabilising agents on fat and water release during heating of herring muscle. Herring, a fatty fish, was selected for study since most species that are canned or smoked are fatty, while the bulk of the world fish meal production is prepared from fatty fish. However, a preliminary experiment gave an indication that the weight loss of herring muscle during cooking was not a simple function of temperature. Consequently, it was thought justifiable to centre investigations on a careful examination of the effects of temperature on the release of water and fat, examining also the role of holding time.

Some previous investigators used methods which did not prevent evaporation losses or which did not clearly distinguish release of water and fat by heating from release by mechanical stress applied during heating. Since the aim of the work was to measure primarily the effects of heat on water and fat release, it was necessary to devise a method of

cooking to avoid these problems. The effect of pressing the cooked muscle, which is relevant to fish meal manufacture, was also studied because it could serve as a criterion for estimating the degree of binding of the water and fat retained in the muscle after cooking. Temperatures studied in previous work did not exceed 100°C; as the present work was intended to be relevant to fish canning, higher temperatures have been examined.

During cooking fish muscle is transformed from a soft, translucent gel-like structure to a firm, opaque friable one. As the muscle structure plays an obvious role inretaining water, observations on changes in these sensory properties may help to elucidate changes in water retention.

Our understanding of the heat processing of fish could benefit from basic information on its thermal properties, namely the thermal conductivity, specific heat and thermal diffusivity. Although there is a shortage of data on these properties for foods in general, values for fish muscle for temperatures above ambient are particularly fragmentary. Specific heat is an important thermal property to be measured since, in addition to being useful for equipment design and process evaluation, it can serve to detect energy changes occurring during heating of muscle. These energy changes, which almost invariably accompany physical or chemical changes, may be evidenced by peaks or troughs in the specific heat curve as a function of temperature.

Direct measurement of specific heat requires the use of calorimeters. The most common method used for measurement of specific heat of foods is the method of mixtures. In this method the material, of known mass and temperature, whose specific heat is to be determined, is dropped into a calorimetric system containing water at a known temperature, or, conversely, water at a higher temperature is added to the material. The unknown specific heat is then calculated from a heat balance equation between the heat gained or lost by the water and calorimeter and that lost or

gained by the sample. The heat capacity of the system is usually obtained from calibration runs. Mohsenin (1975) gives a detailed description of such a system and of the calculations involved. Since the method allows direct contact between food materials and the heat exchange medium the food may be rendered useless for further study. For this reason, the method of mixtures was not used in the present work, although there are ways of overcoming the difficulty. The sample could be encapsulated in a metallic container (Kulacki and Kennedy, 1978) but, for accurate work, a water tight container would be required and special devices to heat and drop the sample to ensure that there is no appreciable drop in temperature before it reaches the fluid inside the calorimeter.

The calorimetric system adopted was similar to the types used for measuring heat capacity of pure substances. Such calorimeters are usually designed for a specific purpose and it is not easy to find one in the literature exactly suited to a particular set of conditions such as nature of sample and temperature range of interest. Aitken and Campbell (1969a) had made use of an isoperibol system to determine the specific heat of cod muscle. In an effort to improve their system and to increase the possibility of detecting any major thermal transition during the heating of herring muscle, a modified system was designed and built and used to determine the apparent specific heat of herring muscle. This work is described in this thesis as is the study of the influence of temperature and holding time on the release of water and fat during the cooking of herring muscle discussed earlier. Observations on sensory and histological changes, of a preliminary nature, are also presented.

CHAPTER 2

LITERATURE REVIEW

A. Water and Fat in Fish Muscle

1. The role of water and fat in foods

Water and fat are ubiquitous macrocomponents of flesh foods so that the consequences of their release or retention during food processing are not always immediately perceived. Therefore it is useful to start by considering briefly and broadly the roles of water and fat in foods.

a. Water

Water, the universal solvent and transport medium, is such a common and abundant constituent of foods that its effect on their properties is not often realised. The amount of water present in a food influences its sensory quality, its behaviour during processing and storage, and its keeping quality, all of which have some part to play in its final nutritional value.

The interactions between water and other food constituents of biological systems have been the subject of a recent review (Duckworth, 1975) and, based on this review, Duckworth (1976) has presented a detailed description of the factors influenced by water in foods of various levels of moisture content; most of the remainder of this section is based on his observations on high moisture content foods such as fish muscle.

The principal chemical roles of water are as a solvent, accounted for in part by its high dielectric constant, and as a reaction medium; as a solvent it dissolves salts, pigments, odoriferous and sapid compounds, and macromolecules such as polysaccharides and proteins; as a reaction medium it permits the development of flavourous compounds both desirable and undesirable, and hydration of gelatin, starch or gluten.

Table 2.1 taken from Duckworth (1976), summarises the chemical roles of water in high moisture (above 0.9 water activity) foods and the mechanisms by which quality attributes are affected.

Table 2.1 Chemical roles of water in foods

Role	Mechanism of effect	Quality attributes affected
1. solvent	solution	all
2. reaction medium	facilitation of chemical change	all, especially keeping quality
3. reactant	hydrolysis of lipids, proteins polysaccharides	flavour, texture, keeping quality
4. pro-oxidant	main source of free radicals in irradiated foods	flavour and colour after irradiation
5. structural (intramolecular)	maintenance of integrity of protein molecules	texture and other attributes influenced by enzymes
6. structural (intermolecular)	influence on the conformation and interaction of gel-forming polysaccharides and proteins and influence on the structure of emulsions through interactions with surface-active lipids	textural properties of gels

From Table 2.1 above it may be noticed that most of the chemical mechanisms influenced by water affect some physical property of the food.

Major influences of the physical properties of water on food are related to textural quality, by maintaining turgor in muscle tissue, and performance of a material during a processing operation: the volume increase as water solidifies is one source of damage to foods during freezing. At high moisture levels, water dominates the physical behaviour of a food material, and physical parameters such as specific gravity, specific heat and dielectric constant are largely determined by their values for pure water (Duckworth, 1976).

Water has also an important role to play in the biological stability of foods by influencing the growth of microorganisms (Mossel, 1975), and the possibility of enzyme activity (Multon and Guilbot, 1975); apart from thermal processes, most techniques to preserve food are based on the removal of water.

b. Fat

Lipids occur in all parts of plant and animal tissues but are present in large quantities in specific fat tissues, in seeds and in some fruits;

in fruits	:	in olives, in avocado, in coconut
in seeds and nuts	:	cotton seed, sunflower seed, soy-bean, palm nut, peanut etc.
in animals	:	body fats, milk, fish livers (Braverman, 1963).

In the form of triglycerides, phospholipids, cholesterol, and cholesterol esters, lipids are important for the structure, composition and permeability of membranes and cell walls in living tissues. They are major components of adipose tissue, which serves as an energy store, as thermal insulation for the body, as protection against shock to internal organs, and as contributor to body shape (Dugan Leroy, 1976).

Lipids in foods are a source of energy, serve as a vehicle for the fat soluble vitamins, influence flavour and taste, modify texture through interaction with water and protein, and contribute to the sensations of mouth feel and satiety (Morton, 1977; Bennion, 1972). Although essential for these desirable nutritional and sensory properties of meat, poultry and fish, animal fats and other lipids may create problems by virtue of their propensity for undesirable odour and flavour changes.

Lipids play a very important role in the physical, chemical and biological properties of foods during manufacturing or storage. They influence mechanical properties (fatty fish is much more liable to damage during handling than lean), act as a barrier to moisture diffusion during drying of fatty fish (Jason, 1965), may decrease the heat conductivity of thermally processed fish (Fujita and Kishimoto, 1956) and act as an insulator during freezing. Of the chemical reactions leading to flavour deterioration in frozen fish, the most important is often the development of oxidative rancidity in the lipid constituents (Banks, 1952). There is some evidence, though not conclusive, that free fatty acids produced by lipolysis, can cause textural deterioration during frozen storage of fish. Refrigerated cooked meats may present the so-called "warmed-over flavour" which describes the rapid development of oxidised, rancid or stale flavours on reheating (Pearson et al., 1977). Lipid oxidation limits also the shelf life of dried products. The oxidation of lipids may not only decrease the biological value of fish meals but even cause combustion (Karrick, 1976).

2. Hydration water of proteins

To understand the interaction of water with protein foods and changes in such, it is necessary to consider the ways in which water

is held by proteins. The number of techniques utilised to study hydration of proteins and polypeptides, which were reviewed by Kuntz and Kauzmann (1974) and are summarised in Table 2.2 demonstrates the difficulty researchers have faced in trying to establish a definite picture of the various states of water that are believed to exist in proteinaceous systems.

Table 2.2 Methods utilised to study hydration of proteins and polypeptides

A. Thermodynamic Methods	
1. Absorption isotherms	
2. Calorimetry	
B. Kinetic Methods	
1. Hydrodynamic Measurements	viscosity, diffusion, sedimentation
2. Rotational Diffusion Constants	NMR dispersion, fluorescent depolarisation, dielectric relaxation
3. High Frequency Dispersion Techniques	rotary diffusion of water - dielectric dispersion, magnetic resonance of water mobility.
4. Self-Diffusion of water molecules	
C. Spectroscopic Techniques	infrared and raman spectroscopy, magnetic resonance spectroscopy
D. Diffraction Techniques	low angle X-ray scattering, high resolution X-ray studies

From: Kuntz and Kauzmann, 1974

This difficulty is easily appreciated when it is considered that each technique measures different properties of the system. However, Fennema (1977) found a consensus in the most reliable results and suggested

the following classification.

Constitutional water is that located within the protein molecule, or bonded to specific active sites, or simply located in interstitial regions (Kuntz, 1975). Protein-water and water-water binding energies are much greater than those existing in normal water. This water amounts to about 0.003g H_2O/g of protein, which means that in a 20% protein solution about 0.1% of the total water is constitutional water.

Interfacial water is the water situated at or very near the protein water interface and may be subdivided into two categories, vicinal and multilayer water. The first one or two layers of water adjacent to the protein molecule would constitute the vicinal water whose properties are strongly influenced by the nature of the protein surface (Kuntz et al., 1969). Water confined in crevices or pores of molecular dimensions, e.g. in membranes or between protein filaments, will exhibit reduced mobility and altered structure as compared to normal water (Clifford, 1975). This water is tightly held in place, largely by physical forces, and amounts to some 0.3-0.5g H_2O/g protein (Kuntz and Kauzmann, 1974), i.e. 5-10% of the total water content of a 20% protein solution.

With properties between those of vicinal water and bulk water some investigators believe there is a multilayer water which however, cannot be sharply differentiated from vicinal water through the use of the techniques enumerated in Table 2.2.

When compared to normal water, interfacial water shows reduced vapour pressure, reduced mobility and a large proportion of it is non-freezable (Finch et al., 1971; Hazlewood et al., 1969; Kuntz and Kauzmann, 1974). Interfacial water, with respect to structure, is different from and probably more structured than normal water (Fennema, 1976).

Bulk phase water represents at least 90% of the water contained in dilute protein suspensions and in cellular systems. In cellular systems this water is physically entrapped whereas in a dilute solution it can be referred to as "free" when the system behaves like a sol, or regarded as entrapped if it becomes physically restrained when the system changes to a gel structure.

In muscle it is the bulk phase water that principally affects the properties of muscle in relation to processes such as mincing, drying, salting and heating and influences the quality attributes such as juiciness, flavour, tenderness and colour of the cooked muscle.

3. The binding of water to muscle

A brief description of the basic muscle structure may help to understand how water is held by it.

Vertebrate fish muscle is composed of striated muscle fibres. Within the fibre and running the length of it are fibrils surrounded by sarcoplasm which is a solution of proteins and other substances. A muscle fibre is a single cell and the cell wall is referred to as the sarcolemma. The fibrils consists of interdigitating thin and thick filaments of highly ordered molecular chains of the proteins actin, tropomyosin and troponin, constituting the thin filaments, and myosin, constituting the thick filaments (Howgate, 1979). The filaments are associated with water and dissolved substances probably in the form of a gel or gel solution (Blanshard, 1975; Hamm, 1975) and the water is obviously immobilised in some way for it does not flow freely from the cell even when the cellular system has been severely disrupted (Hamm, 1963; Ling and Walton, 1976). For meat, and the position should not be much different from fish muscle, Ranken (1976) has given the amount of water associated with the main components of muscle (Table 2.3).

Table 2.3 Percentage of water associated with the muscle components

Composition of muscle		% of muscle	% water
Fibres	Fibrils	60	45
	Sarcoplasm	25	19
Extra-cellular space including connective tissues		15	11
	Total	100	75

Thus, as in other gels, water is held in the muscle tissue by forces such as hydrogen bonds between water and appropriate groups in the molecules, and by capillary attraction in the interstices of the gel structure. Many of the types of molecules found in muscle have a considerable degree of attraction for water: sarcoplasmic, myofibrillar and connective tissue proteins, phosphates such as ATP, glycogen and the non-protein nitrogenous compounds (Paul, 1972). As described above, the muscle is a complex of membranes, fibres, fibrils and filaments, cross bonded in a variety of ways providing spaces where water is immobilised mechanically, probably even by cross linkages and electrostatic forces between the molecular chains (Hamm, 1960). According to Hamm (1963), again based on work on meat, the amount of free water immobilised within the tissue is strongly influenced by the spatial molecular structure of the muscle tissue. Factors that cause tightening of the network of proteins decrease the amount of immobilised water and those causing loosening of the protein structure have the opposite effect.

To complement these broad concepts of water retention by muscle it is necessary to mention that there are two opposing views on the properties of this physically entrapped water concerning the mechanism controlling the holding of water in the cell. In one view some investi-

gators (Chang et al., 1972; Ling and Walton, 1976; Hazlewood et al., 1969) postulate that cell water is ordered sufficiently to make it behave differently from a dilute salt solution. Bulk phase water would exist in the cell in the form of oriented multilayers due to (1) favourable positive-negative charge distribution on fibrous proteins, and (2) the small intervening space between protein molecules.

According to an alternative hypothesis (Cooke and Kuntz, 1974; Drost-Hansen, 1971; Finch et al., 1971) the properties of entrapped bulk water in cells would be similar to those of a dilute salt solution, and with the exception of a small percentage in the form of water of hydration, the previously-referred-to constitutional and interfacial water, on proteins and other macromolecules, cell water is considered to be in the free state. In this view, the integrity of the cell membrane is essential for the retention of cell water.

4. The physical state of lipids in fish muscle

In fish meal processes, as will be pointed out more extensively later, one of the reasons for a cooking stage is the need to cause fat to separate from the muscle structure. For this purpose some knowledge of where fat is located and how it is retained in the muscle tissues is fundamental in devising more efficient means to promote the separation of fat from muscle. Furthermore since proteins and fat are brought together during heat processing due to disruption of muscle tissues and fat cells, it is of interest to have some idea of what are the consequences that can develop when fat comes close to proteins in the presence of water.

The amount of lipids present in fish tissues varies enormously in different parts of the body. Yamada (1964), who carried out extensive histological examinations of the anatomical distribution of lipids in

the tissues of many species of fish, found:-

1. The fat contents of fish muscle differ remarkably in different species, and the great part of the fat contained in fish muscle consists of fat cells. The fat of fish exists mainly in the subcutaneous adipose tissue, in the connective tissue, and distributed around the muscle fibres;
2. within the same species large fish have larger fat cells and muscle fibres than small fish;
3. the fat layer is thicker in oceanic migratory fish, e.g. skipjack, bluefin tuna, jack mackerel, common mackerel, yellowfin tuna, than in fish living on the bottom, or in littoral or fresh waters. The former group have a large amount of fat scattered among the muscle fibres which is particularly abundant in the ventral flesh. These findings are in accord with Marshall (1965) who reported that fat tissues in the surface layers of tunnies and marlins, fast swimming fish, are sandwiched between layers of connective tissue. Karrick and Thurston (1964) also found that the belly wall of fatty species like salmon often shows the highest concentration of lipid. In the latter group studied by Yamada (1964) comprising sea bass, flat fish, carp, the fat is very scarce among the muscle fibres, being located in the subcutaneous adipose tissue with a small portion present in the connective tissue;
4. bigeye tuna, eel and conger eel were found to have a fat distribution similar to that of meat.

More recent work has been carried out by Mohr (1979) who examined the distribution of fat in the body of fatty species like capelin, mackerel and herring, the study being more detailed for capelin. He found that in capelin the major lipid depots are located in two distinct tissues,

namely under the skin and in the connective tissue lining the body cavity. These two depots account for 55.7% of the total lipid content of the fish. A particularly interesting phenomenon that can be observed from his results is that, although the actual fat content of the fish varies considerably with season, the relative distribution of the lipid in the tissues remains constant.

His work showed also that in mackerel and herring, skin and light muscle constitute major fat depots, accounting for approximately 70% of the lipid of mackerel and 54% of the lipid of herring. In the latter a substantial amount of fat, 27% of the total lipid content may also be deposited around the intestines. For herring Mohr (1979) gives the following percentage distribution of fat:-

Skin	Light Muscle	Dark Muscle	Lining of Body Cavity	Intestines	Other parts of the body
30	20	4-5	6-7	29	7-9

The lipid present in large proportion in the muscle tissues of herring and mackerel, is found in special fat cells which are dispersed between the white muscle cells (Mohr et al., 1976). This finding is in line with an analysis for herring by Ross (1977) which indicated that white muscle lipid is almost totally extra-cellularly stored along the connective tissue, but that lipids of dark muscle are both intra- and extra-cellular. However, George (1962) states that many pelagic fish which must swim continuously in order toreceive enough oxygen (e.g., herring and mackerel) store lipid in the flesh both intra- and extra-cellularly.

Still according to Mohr et al. (1976), capelin and herring fat cells are of a round to oval shape, containing one or more droplets of fat which take up a considerable part of the volume of the cell. His obser-

vations indicate that the cell capsule, in addition to cell membranes, has collagen fibres which contribute to binding the cells in the tissues.

As seen above, the striking characteristic of fatty fish species is the storage of most of its fat in the muscle. This fat is accumulated during the feeding season, to be used as a source of energy when food is not abundant (Iles and Wood, 1965). In consequence, great changes in the concentration of lipids occur in muscle during a single year. Obviously these changes in the fat content of the muscle will be reflected in its mechanical properties which are important to its technological utilisation. Fish of different fat content need different degrees of heat treatment during canning or fish meal production, for instance. For herring, whose total fat content covers a range of 1-30% according to season, there is a rapid rise in its fat content during May and June corresponding to a period of intensive feeding. Maximum fat content is attained by July with a gradual decrease until reaching minimum values by April. The speed of the drop in fat content depends on the spawning season, a relatively high fat content being maintained until spawning takes place (Lovern and Wood, 1937). In herring muscle there is another striking relationship between the percentages of fat and water; as one rises the other falls, at all levels of fat accumulation, so that their sum is approximately constant (Lovern and Wood, 1937; Brandes and Dietrich, 1953; Iles and Wood, 1965). The proportions of lipid and water are similarly related in the flesh of other fatty fish species as found by Hardy and Keay (1972) for mackerel and as reported by Sen and Revankar (1972) for sardines.

It is interesting to point out that in relation to the seasonal variation in fat content of herring it was found that the total collagen content of the fish varies inversely with the fat (Hughes, 1963). It is well known, especially to fish canners, that high fat content summer

herring are more liable to physical breakdown. Hughes (1963) suggested that the phenomenon could be associated with the need to maintain the fish shape contour after fat depletion. As it seems that to take up more fat the fish increases the size of its fat cells (suggested by the fact that the triglyceride content increases but the phospholipid remains constant), the reduction in collagen content could be attributed to the need to increase the elasticity of the cell wall to accomodate more fat.

5. The binding of lipids by proteins

Lipids and proteins may interact forming stable complexes of lipid and proteins which behave as discrete units and resist separation into lipid and protein by physical methods.

Chapman (1969) summarised the various consequences that can develop if lipids and proteins are brought together in water. These are:

a) no interaction and no complex formation, i.e., both lipid and protein retain their original configurations;

b) interaction, with the lipid losing all, or part, of its configuration, but with the protein retaining its configuration; charged groups on the protein may be neutralised by a few molecules with the protein largely retaining its original configuration;

c) interaction, with the protein losing all, or part, of its configuration, but lipid retaining its configuration; it is conceivable that a protein chain may unfold at the lipid surface, with the lipid configuration remaining largely unchanged;

d) interaction, with both lipid and protein losing all, or part, of their original configurations: a gross re-arrangement of the long chain moieties of the lipid is conceivable so that they are within the hydrophobic central region of the protein, i.e., associated with the non-polar amino acids;

e) interaction, with both lipid and protein largely retaining their original configuration; a small interaction might occur involving a sheet of lipid and protein in which both largely retain their original configuration.

Lipid protein complexes may be described as (Burley, 1971):

Lipoproteins - complexes soluble in aqueous media and consisting of neutral lipids, phospholipids and proteins.

Phospholipoproteins - complexes of protein and phospholipids that are soluble in aqueous media.

Proteolipids - complexes that are soluble in certain organic solvents but are not soluble in aqueous solutions.

The same author reviewed the types of bond usually considered to contribute to forming and stabilising lipid-protein complexes.

a) Covalent bonds. Covalent bonds between lipids and proteins in biological systems are uncommon (in lipoproteins most of the lipids are readily removed by organic solvents at low temperature).

b) Non-covalent: ionic. Ionic binding is theoretically possible between lipids with an electric charge and appropriately charged groups of protein. The stability of ionic binding depends on the dielectric constant of the medium and for aqueous media on the ionic strength and pH as well.

c) Non-colavent bonds: weak secondary forces and hydrogen bonds. Although van der Waals forces are individually weak, collectively they confer great strength provided that close contact is possible. Hydrogen bonding is reported to be of less importance for binding lipids and proteins because most lipids have a low proportion of labile hydrogen atoms. However, their presence may help explain the binding of non-polar lipids to proteins.

d) Non covalent bonds: hydrophobic interaction. The basis of the hydrophobic interaction concept is the fact that some hydrocarbons do not dissolve in water in spite of an expected enthalpy change that is prevented by a very favourable entropy which could arise only from a less random arrangement (ordering) of water molecules; consequently, in aqueous media molecules of these hydrocarbons are assumed to minimise contact with water by congregating at interfaces (Shenouda, 1974; Franks, 1975).

It would seem that hydrophobic interactions are highly significant in stabilising complexes formed between lipid and protein in aqueous media (Cornwell and Harrocks, 1964).

B. Effects of Heating on Muscle

Heating is the most common treatment of foodstuffs and it brings about a series of chemical and physical changes which, depending upon the aim of the process, can be considered desirable or not. Chemical, physicochemical and biological changes in foods caused by the action of heat have been the subject of two recent books (Priestley, 1979; Høyem and Kvale, 1977). In general terms a list of effects brought about by heat, depending on moisture content and the history of the process, would include (Thijssen, 1975):

a) material losses in the form of volatile substances,

b) physical changes in structure which affect density and colour like denaturation of proteins,

c) chemical reactions such as,

- enzymatic reactions
- oxidation of lipids, amino acids and development of flavour components
- development of flavours, both adverse or beneficial, by reaction products
- cross linking reactions of proteins which affect the water holding of the product.

Since much more work has been carried out on the heating of meat than on heating of fish and since the two materials have much in common, the literature on heating of meat will be briefly discussed first.

1. Changes in meat muscle

Heating of muscle has been extensively studied for meat. It is well known that heat produces marked changes in meat muscle, due to denaturation and coagulation of proteins, melting of fat, alterations in pH and in water holding capacity, and chemical changes in heat labile components (Paul, 1972). The most drastic changes in meat during thermal treatment are those that involve muscle proteins. Shrinkage of tissue and the release of juice are caused by changes mainly in the fibrillar proteins whereas discolouration of muscle and the loss of many muscle enzymes are the result of denaturation mainly of the sarcoplasmic proteins (Hamm, 1966). According to Lawrie (1968) who reviewed the chemical changes in meat due to processing, the most obvious change when meat is heated is loss of water holding capacity, due to denaturation of both the sarcoplasmic and myofibrillar proteins.

Hamm (1960, 1966, 1970, 1977) has given extensive reviews of the effects of heat on muscle proteins. In the following discussion the main topics of his reviews will be pointed out, emphasis being given to those influencing water holding capacity that may be relevant to fish muscle.

a. Changes in myofibrillar and sarcoplasmic proteins

Most of the myofibrillar and sarcoplasmic proteins are coagulated by the time the temperature reaches 62°C, the degree of coagulation depending on pH and ionic strength (Bendall, 1964). Hamm (1966) reported that at 65°C most of the myofibrillar proteins are coagulated; sarcoplasmic proteins coagulate at temperatures between 40-60°C, and all become

insoluble at 80°C. These statements agree with results obtained by Roberts and Lawrie (1974) but Davey and Gilbert (1974) found that heat denaturation of the sarcoplasmic proteins extended from 40 to 90°C. Penfield and Meyer (1975) found that heating meat at 45-60°C for 10 hours causes some solubilisation of the myofibrillar proteins which was attributed to the effect of proteolytic enzymes present in the muscle tissue.

The denaturation of proteins during heating of muscle tissue up to 70°C is connected with a release of protein-bound alkaline-earth ions (El-Badawi et al., 1964) attributed to a reduced sequestering power of the protein upon unfolding.

Heating muscle causes a shift in pH. If the pH of the tissue, previous to heating, is smaller than 4.5 or higher than 7.0, cooking will cause a decrease in the tissue pH. In the range of pH 4.5-7.0, heating will cause an increase in pH (Hamm, 1960).

Hamm and Hoffman (1965) concluded that the process of heat coagulation of beef muscle taking place between 40-60°C is not accompanied by the formation of new disulphide bonds between protein molecules. But at higher temperatures, 110-112°C, there is a considerable decrease in the number of sulfhydryl groups, attributed to an oxidation process caused by such high temperatures. Of all reactions which may be responsible for the destruction of sulfhydryl groups, the formation of H_2S, which begins at 80°C, is the most prominent. It is interesting to point out that the heating of meat with higher fat content produced significantly more H_2S than the heating of lean meat (Kunsman and Riley, 1975).

Based on these findings and others not reported here, Hamm (1977) concluded that the decrease in solubility of myofibrillar proteins between 30 and 60°C is characterised by an unfolding of the protein chain; the association of the unfolded peptide chains causes protein coagulation.

b. Changes in connective tissue

When a collagen fibre is heated it shrinks. The temperature of shrinkage depends on pH. At lower pH values, shrinkage occurs at lower temperatures. Collagen is considered to be denatured at this shrinkage temperature Ts, but higher temperatures are required to break the molecules apart into soluble gelatin. The thermally contracted fibres show optical changes, becoming transparent or "glassy". Ts of collagen in meat is about 60°C, whereas for extracted collagen it was found to be 65°C, (Lawrie, 1968).

Connective tissues located in different parts of the muscle structure behave differently in relation to heating. Shrinkage and partial solubilisation of endomysial connective tissue is initiated at 50°C and completed at approximately 70°C. Perimysial connective tissue required internal temperatures of 70°C or higher before any significant changes in the fibres were observed by microscopy (Schmidt and Parrish, 1971). Solubilisation of collagen below 60°C during prolonged heating might be in part due to proteolytic activity by collagenases (Penfield and Meyer, 1975).

The general concept that heat alterations in the connective tissue have the main role in softening or tenderising meat does not always apply since Paul (1973) found evidence that the increasing coagulation of the contractile proteins is more important than the breakdown of the collagenous tissue in controlling tenderness changes between 58 and 82°C.

c. Changes in water holding capacity

Water holding capacity can be simply defined as the ability of the food, when subjected to physical or chemical treatments, to retain its natural water content or to retain added water; research on water holding capacity of muscle has explored these two approaches to the definition.

Two terms that are always quoted in connection with water holding capacity of foods are "free" and "bound" water. The commonest definition of bound water is water that is so tightly held on the protein molecules, that it no longer has the characteristic freezing point, vapour pressure or solvent ability of normal water; this is the water described as constitutional and interfacial in previous sections. "Free" water is that present in the food, which keeps the characteristics of pure water, the physico-chemically defined bulk phase water. In research in food, authors have established their own concepts of "free" and "bound" water in relation to the particular processes applied to the food. For example, water lost on cooking may be described as free, whereas water released by pressing or centrifugation may be described as "bound"; in terms of the definitions discussed earlier in Section A.2, both forms of water are in fact physico-chemically "free" water. The terms "free" and "bound" water used in this way are correlated with changes in the food being studied as a result of different treatments. It is thus not possible to relate the amounts of "free" and "bound" water measured by one author with those measured by another.

The understanding of the ways in which heat denaturation of myofibrillar and sarcoplasmic protein and heat changes of connective tissue proteins, discussed previously, are related to tenderness and water holding capacity is important for the optimisation of muscle cooking processes.

The results reported by Hamm (1966), Bouton and Harris (1972) and Roberts and Lawrie (1974) support the view that, concomitant with myofibrillar protein coagulation, the decrease of water holding capacity begins at about 35°C and occurs primarily between 40 and 50°C. Laakkonen et al. (1970) however, disagreed with these results since they found that, using meat slices and slow heating, 43% of the total weight loss occurred at temperatures

between 60 and 80°C. The difference between them may lie in the fact that the former authors measured in their experiments the ability of disintegrated meat muscle to hold added water whereas the latter measured the ability of intact muscle to retain its own water. At 50 to 55°C, Hamm (1970) reports a delay in the decrease of water holding capacity with temperature which he attributed to a re-arrangement of the myofibrillar proteins, but this statement is not substantiated by other authors.

Sherman (1961) tried to explain the mechanism of fluid release with temperature by suggesting that an increase of water retention at 50°C was due to soluble nitrogen compounds coagulating within the meat and inter-linking the protein molecules, thus forming a barrier to further fluid loss. At higher temperatures this coagulated structure weakens, possibly because of the breakdown of the molecules to units of lower molecular weight, and the entrapped fluid is released.

Toughening, shrinkage and weight loss are closely associated phenomena; Davey and Gilbert (1974) found two separate phases of toughening with increasing cooking temperature. In the first cooking phase between 40 and 50°C, associated with loss of myosin solubility indicating denaturation in the contractile system, up to a four-fold toughening occurs. Between 65 and 75°C further toughening occurs which they found to be associated with collagen shrinkage. All this causes shortening of the meat fibre, forcing out meat juice.

Hamm (1977) concluded that the change in water holding capacity during heating of meat takes place in two stages:

a) in the first stage, between 30 and 50°C, the reduction in water holding capacity would be governed by the heat coagulation of the actomysin system,

b) in the second one, between 60 and 90°C, reduction in water holding

capacity would be controlled by the denaturation of the collagenous system and/or by the formation of new stable cross linkages within the coagulated actomysin system.

2. Changes in fish muscle

The findings outlined in the above study of heating of meat muscle are certainly relevant to research in heating fish muscle. Many of the works quoted refer to the heat treatment of meat or poultry muscle as "heating of muscle" on the assumption that all skeletal muscle has basically the same structure and composition and that, therefore, results found for one type of muscle can be generalised. The behaviour of meat and fish muscles when exposed to certain physical and chemical treatments must be fundamentally the same; however, there are strong indications that results obtained with meat cannot be directly transposed to fish muscle.

According to Connell (1964) three of the reasons why fish muscle proteins should receive separate attention from meat, in relation to technological utilisation, are:

1. Processing of fish presents certain difficulties that stem from the particularly high instability of its proteins, that is, a propensity to undergo denaturation, coagulation and degradation which result in large changes in overall physical properties (Connell, 1970).
2. With present day fishing methods pre-treatment of the live fish is impossible and intrinsic factors largely control the quality of the landed fish (Burgess and Shewan, 1970).
3. Post rigor biochemical events in fish muscle are significantly different from those occurring in warm blooded animals.

Like meat proteins, the technological importance of fish muscle proteins resides in the fact that the myofibrillar proteins determine to a large

extent the physical properties and textural eating quality, the sarcoplasmic proteins function as enzymes influencing flavour, odour and colour, and those proteins with haem prosthetic groups affect colour and may also act as pro-oxidants.

The large amounts of material having a low sedimentation coefficient would be an indication that the average molecular weight of the fish sarcoplasmic proteins is lower than in mammalian muscle (Connell, 1958).

In fish muscle, the ratio of the myofibrillar proteins, constituting 65% of the total muscle protein, to the sarcoplasmic proteins, contributing 26-30%, is characteristically different from mammalian muscle which contains approximately 40% and 35-40% of myofibrillar and sarcoplasmic proteins respectively. The proteins of the connective tissue range from 3% for teleosts to 10% for elasmobranchs against an average of 17% in mammals (Jebsen, 1962).

There are several reasons justifying increased effort in studying changes brought about by heat in fish; fatty fish, in particular, deserves special attention.

a. Technological significance

According to FAO (1976) the catch of clupeoids, herring-like fish, sardines and anchovetas, amounted to 15 million metric tons in 1976. This figure represents 20% of the total world catch of aquatic animals and 36% of the total world catch of marine fishes. Like other fatty fish species, most of the clupeoids are destined to processing involving heat treatment e.g., canning, hot smoking and fish meal manufacture. This last process still uses the largest proportion of the available fatty fish despite widespread suggestions by the scientific community for greater use of fatty fish for direct human consumption.

In the fish canning process the main purpose of the pre-cooking operation is the release of enough water, in order that the water liberated during the later sterilising stage does not cause strong convection currents, breaking the fish into pieces, nor give to the packing oil a watery appearance. On the other hand overcooking in this stage may result in excessive exudation of water, fat and soluble proteins with the consequence that, if the fish is lean, the sterilisation treatment may toughen the fish muscle which will then have a chewy and dry texture.

In fish meal manufacture optimum breakage of cell walls and protein denaturation of the fish tissues are required during the cooking procedure, for maximum release of water and oil in the following pressing stage. If the material is undercooked too much oil will be retained in the press cake causing the dried and powdered end product to have an undesirably high oil content. However, if the fish is overcooked its texture becomes too soft and mushy to permit ready straining of the expressed liquid through the fibres of the tissues during pressing. The proportion of the dissolved and finely suspended solids in the press liquor is increased, hindering separation of the oil from it (Burgess et al., 1965).

As in the above mentioned processes, in hot smoking the moisture content of the fish is a very important variable influencing the quality of the final product. Pietrzik (1977) states that the critical temperature of collagen heat changes is in the range 60 to 70°C. The increase of collagen strength at high temperature is dependent on decrease of water content and to prevent the smoked fish becoming too soft and friable, even falling from the racks, it is necessary to remove water at lower temperatures (40-50°C) followed by denaturation of the muscle proteins. Although these are the correct principles for hot smoking fish the temperatures given by Pietrzik may be too high. Changes in fish muscle collagen occur

at 37-45°C (Gustavson, 1955) and ideally the initial step of removing water should be carried out at about 30°C.

These examples give some indication of the technological significance and possible applications in industrial processes of a better understanding of the mechanisms of fat and water holding by fatty fish muscle if it brings about the possibility of choosing optimum thermal regimes for heat processing of fish.

In general, there has been little work on studying the effects of heating on the release of water and fat from fish. Baker (1943) reviewed the work up to 1943 and a more recent review, by Wagenknecht and Tulsner (1974), relied on work on meat when dealing with the effect of heat on water binding. In their review, Aitken and Connell (1979) pointed out that, in addition to the fact that little fundamental work has been done on the mechanisms of the release of liquor from cooked fish, studies on heat treatments seldom include systematic examination under completely well-defined conditions which would include sample composition. They state further that the practice of not describing the cooking procedures in detail is almost universal.

One feature of the work carried out to examine how heat affects fish muscle is that most of it has been directed to immediate and restricted technological application, in the sense that temperatures and times studied, even cooking procedures, were those commonly used in commercial processes. Thus one of the earliest works on cooking of fish, that by McCance and Shipp (1933), determined the percentage loss of weight when catfish, herring, plaice and whiting were steamed for periods of time varying from 7 to 45 minutes. Average weight losses for fatty and lean species were about 20% although there were great variations between species.

In their comprehensive report they also gave the detailed composition of fish analysed in the cooked state. Tarr (1941) found that canned halibut, processed at 120°C lost 3-4% more weight than when processed at 100°C. Dollar et al. (1974), studied changes in temperatures, loss of weight through evaporation and as drip of tuna during precooking under commercial conditions. Their results suggested that drip loss accounted for about half of the average 27% weight loss during the operation. Meesemacker and Sohier (1959) found that precooking by various methods of sardines for canning caused a weight loss in the range 20-30% but fat losses were very small. Ward et al. (1977) reported that weight losses on cooking sprats, cod frames and sand eels at 60, 80 and 100°C increased with temperature, but losses due to pressing the cooked fish were practically independent of the cooking temperature.

Other work may be said to have been catering oriented since it was focused on studying parameters such as drip loss, flavour, texture and "doneness" in relation to the palatability of the cooked product. So, Charley (1952) baked one inch salmon steaks to internal temperatures of 70, 75, 80 and 85°C in an oven at 205°C. In a parallel experiment, using oven temperatures of 177, 204, 232 and 260°C, she baked steaks to an internal temperature of 75°C. For the first experiment she found that cooking losses increased with increasing internal temperatures and that steaks cooked at 80 or 85°C generally received higher palatability scores than did those cooked to the two lower temperatures. She also noticed that to increase the temperature from 70 to 75°C and from 75 to 80°C took 3 minutes whereas an increase from 80 to 85°C took 6 minutes. In the second experiment she found that differences in total cooking losses associated with oven temperature were not significant nor

were the effects on palatability. As in the previous experiment she observed apparently anomalous behaviour in the rate of temperature increase since an increase in oven temperature from 177 to 204°C reduced the cooking time by 9 minutes while an increase in oven temperature from 232 to 260°C reduced the cooking time by only 2 minutes. No attempt was made to interpret the supposedly anomalous behaviour of rise of temperature of the steaks in both experiments.

In contrast to the above results, Charley and Goertz (1958) reported that, using oven temperatures of 177, 204, 232 and 260°C for baking two-pound salmon steaks, the average cooking losses due to evaporation increased significantly with increasing oven temperature.

Armstrong et al. (1960) working on cooking of frozen Atlantic cod fish fillets stated that fillets cooked uncovered had the higher moisture content (attributed to the action of the layer of dried-out flesh covering the exposed surfaces) and were rated more attractive and more palatable than those cooked in a covered vessel.

Few systematic attempts, like those of Kushtalov and Saduakasov (1971) and Tulsner (1976), have been made to evaluate how the release of water is influenced by time and temperature of thermal treatment. In these more basic works the temperatures studied were up to 90°C which is understandable since most of the changes in the fish muscle proteins are expected to occur below 85°C. Another feature in these works is the use of minced fish, as in the classic experiments of Hamm and Deatherage (1960) and Wierbicki et al. (1957) on meat, in spite of the fact that the mincing operation in itself alters drastically the water holding characteristics of the muscle. None of these authors has reported on the release of fat during heating although fat is generally acknowledged to act as a protective barrier against loss of water by the muscle.

On the other hand, much more fundamental work has been conducted to investigate the influence of heating on the chemical and physical properties of fish muscle proteins. This work like that on meat muscle proteins has been developed in two different ways. In one of them, changes in the proteins were measured after the fish had been heated and in the other approach muscle suspensions or extracted proteins were heated and the changes observed directly.

b. Changes in isolated muscle proteins

Suyama (1951), based on a curve of relative viscosity versus temperature for salt extracts of myofibrillar proteins, found indications that carp fish myosin flocculates around 40°C and is coagulated at 45°C. Ueda et al. (1963) reported that isolated fish actomyosins when heated to different temperatures showed an initial fall in viscosity at about 30°C, which was attributed to the start of denaturation of the proteins. A much greater fall in viscosity occurred at 40 to 45°C, probably associated with the coagulation of the proteins. They extended this work (Ueda et al., 1964) to study the phenomenon for several fish species and concluded that the decrease in intrinsic viscosity caused by the heat denaturation differs considerably from species to species. From their results it was possible to infer that the denaturation temperature of actomyosin is related to the normal environmental temperature of the living fish. The denaturation of actomyosin ATPase from carp muscle, incubated at 35°C, proceeded at a uniform rate as protein concentration increased above 5mg/ml, while it accelerated as the protein concentration was reduced below 4mg/ml, suggesting that the protein is more susceptible to heat in the presence of higher amounts of water (Arai and Fukuda, 1973). Simidu and Takuma (1951) placed into salt solutions minces of mackerel, horse mackerel and tuna and heated these solutions to different temperatures. The temperature at

which insolubility, as measured by the amount of soluble nitrogen remaining, started to develop was about 35°C; half the protein became insoluble at 40-45°C and at 65°C all protein had been denatured. Warrier et al. (1973) however, using protein extracts of Bombay Duck found that heat treatment at temperatures of 60, 80 and 100°C for 10 minutes caused no precipitation in the sarcoplasmic proteins.

c. Changes in the proteins of the heated muscle

Burdina and Mel'Nikova (1974) measured the amount of extractable proteins of fish and cuttlefish heated at several temperature. They found that the muscle protein coagulation started at 35-40°C and was complete between 60 to 70°C. The amount of extractable myofibrillar proteins decreased markedly during heating at 30°C for 40 hours for cod and hilsa (Howgate and Ahmed, 1972), but only 25% of the sarcoplasmic fraction became inextractable. The falls in extractability were generally higher for cod than for hilsa. Half the myofibrillar fraction of carp and pike became inextractable upon boiling in water for 20 minutes. This heat treatment has only a slight effect on the extractability of the sarcoplasmic fraction (Aman and Smirnova, 1972).

A consensus of the works quoted leads to the suggestion that most of the myofibrillar fraction of fish muscle proteins starts to denature at about 30°C and coagulates around 40 to 45°C. The sarcoplasmic proteins seem to be more stable to heat and the greatest change in their solubility occurs at 60 to 65°C. Another conclusion to be drawn is that there are significant differences in the stability of proteins of different fish species, and this may explain differences in behaviour when the muscle is heat processed.

d. Microscopic changes

Howgate (1979) reviewed the existing work on fish microscopy in general. Little information, however, is available on the histological changes in heated fish muscle.

Charley and Goertz (1958) studied the effect of baking salmon steaks to an internal temperature of 70°C, using different oven temperatures, on the microscopic structure of the muscle. Effects of heat were disintegration of the muscle fibres, increase in the width of fibres, precipitation of granular material on the fibres, granulation of the connective tissue which became gel-like and increase in the dispersion of fat globules within the flesh. In another study Schaller and Powrie (1972) examined by scanning electron microscopy the appearance of beef, chicken and rainbow trout muscles heated to 60 and 97°C. The normal appearance of the myofibrils, apart from disintegration at the level of the I-zone, was retained even for the 97°C treatment. A gap in the myofibril at the level of the H-zone was only noted for trout, in addition to transverse fractures at the Z-discs which were found for all muscles studied. At 60°C trout muscle was different from the other muscles in showing extensive damage to its structure whereas changes in beef and chicken muscles were small. Aitken and Campbell (1969b) noticed in the microscope that when thin slices of fish (cod) were heated from one edge, successive translucent and opaque bands were observed. They suggested that protein reactions could be underlying these changes in opacity.

C. Thermal Properties of Fish Muscle

The calculation of heat transfer in foods requires knowledge of (Dickerson and Read, 1968):

1. Thermal properties
2. Dimensional characteristics of the food
3. Thermal processing conditions:
   a. temperature of heat source
   b. initial temperature of food
   c. temperature difference between heat source and food surface.

Consequently, figures for the thermal properties, specific heat, thermal conductivity and thermal diffusivity are of primary importance in establishing heat processing specifications for a partciular food. Values of thermal properties are essential in order to make analytical studies of heating processes in which food substances are involved. The analytical studies are useful for attaining the optimum design of equipment used for canning, drying and other processes involving heat transfer. Hill et al. (1967) and Jason and Jowitt (1969) pointed out that the lack of fundamental information on the thermal properties of food has to be overcome in order that food processing equipment may be designed to bring about thermal changes in a desired manner. The problem has persisted, since more recently Rha (1975) can be quoted: "temperatures of food products during heating and cooling are not generally, in practice, predicted or calculated for the process. The main reason for this is because of diversity of the conditions in which the thermal data is obtained and unavailability due to ineffective coordination and tabulation of the existing data, as well as lack of data".

1. Thermal diffusivity

In food processing the temperature of the system most commonly changes with time, that is the heat transfer conditions involved are unsteady state. In this case, it is of interest to know what temperatures changes are brought about and how fast the system transfers heat when submitted to a temperature

gradient. The thermal property concerned is the thermal diffusivity which may have its value defined in terms of other physical properties of the system: thermal diffusivity Y is the ratio of thermal conductivity, K, to the product of heat capacity, C, and density, ρ, that is:-

$$Y = K/C\rho \qquad (2.1)$$

Units of thermal diffusivity are cm^2/s. Values of thermal diffusivity of fish are scarce. Annama and Rao (1974) measured thermal diffusivity of mackerel and sardine minces at different moisture levels and found that for fresh mackerel and sardine values were 1.332×10^{-3} and 1.136×10^{-3} respectively. Cooper (1937) reported the value of 1.6×10^{-3} for cod, mackerel and tuna and Aitken and Campbell (1969a), from their direct measurements of specific heat and thermal conductivity, found the value of 1.47×10^{-3} for cod.

Descriptions of apparatus to measure thermal diffusivity have been given by Annama and Rao (1974) and Dickerson (1965).

2. Thermal conductivity

Thermal conductivity, K, is defined as the quantity of heat transferred in the steady state across unit area of the sample of unit thickness for a temperature difference across the sample of one degree. (In steady state condition, the temperature distribution across the sample does not vary with time).

In mathematical form, the thermal conductivity, K, is the proportionality factor in Fourier's heat conduction equation,

$$K = Q\,A\,dT/dx \qquad (2.2)$$

where Q is the quantity of heat, A is surface area and dT/dx is the temperature gradient normal to the surface. (The units of thermal conductivity are J/cm $s^{\circ}C$).

Thermal conductivity of solid materials varies with the material, temperature and moisture content. Since biological materials vary in cellular structure and composition, their thermal conductivity is expected to exhibit greater variation than that of the non-biological materials (Mohsenin, 1975). The dependence of thermal conductivity of beef on moisture content, temperature and direction of heat flow has been reported by several authors, (Hill et al., 1967; Lentz, 1961; Miller and Sunderland, 1963). For fish the little data available for ambient temperatures is represented by the work of Lentz (1961), Jason and Long (1955), Kawakami (1935), and Tatsumo and Nito (1936). In spite of the importance of composition on thermal conductivity only the two latter works give the composition of the fish used in their experiments.

Dealing with the influence of temperatures above ambient there is only the work of Aitken and Campbell (1969a) for cod and Fujita and Kishimoto (1956) for mackerel. The former found that thermal conductivity was not influenced by the length of previous ice storage of the fish used in their measurements, but reported a temperature effect, thermal conductivity values being 5.448×10^{-3}, 5.580×10^{-3} and 5.763×10^{-3} for 30, 50 and 70°C respectively. The latter derived a mathematical method for determining the temperature dependence of the thermal conductivity from temperature data obtained with the sample packed in a flat can. From a graph, their thermal conductivity figures for 30, 50 and 70°C would be 4.43×10^{-3}; 5.23×10^{-3} and 6.02×10^{-3} respectively. It must be pointed out, however, that in their calculations they assumed the specific heat of the mackerel flesh to be equal to that of water at 15°C. Neither of the quoted papers gave the raw material composition although Fujita and Kishimoto found that samples to which oil had been added had lower thermal conductivity

values. Annama and Rao (1974) determined the thermal conductivity of mackerel and sardine minces at different moisture levels. For the fish in the fresh state, values of thermal conductivity were found to be 5.8×10^{-3} and 6.0×10^{-3} for mackerel of 78.9% moisture content and sardine of 77.6% moisture respectively, at 45-50°C. They also report that thermal conductivity increased with moisture content.

Methods for determining thermal conductivity of foods were reviewed by Woodams and Nowrey (1968) who gave extensive literature values for a large number of foodstuffs.

3. Specific Heat

Enthalpy can be generally defined as the total heat contained in a body, including sensible and latent heat. Based on this concept the specific heat of a substance at constant pressure is defined as the change of enthalpy with respect to temperature.

In heating processes, specific heat is a very important unit as it determines the amount of heat to be supplied in order to bring the food material to the desired temperature. In addition, knowledge of specific heat allows the calculation of thermal diffusivity when the thermal conductivity and density of the foodstuff are known. In simple mathematical terms, specific heat,

$$C = Q/m\ \Delta T \qquad (2.3)$$

where,

Q, amount of heat input

m, weight of the food material

ΔT, temperature difference caused by the heat input

The international units of specific heat are J/g°C, but cal/g°C is still in widespread use.

Many of the physical and chemical changes in fish muscle caused by heating discussed previously might be associated with energy changes. One of the most important phase changes in heat processing of fish muscle is protein denaturation which influences water retention and other physical and chemical alterations. The heat of denatuation of protein may vary up to several hundred kilocalories per mole, depending on the protein and the conditions (Rha, 1975).

As the protein denaturation related to muscle processing is usually endothermic (Hagerdal and Martens, 1976; Karmas and Di Marco, 1970) heat has to be supplied to the protein to induce the denaturation change and thus the apparent specific heat will increase in the region where the change takes place. In the same way the apparent specific heat as a function of temperature can serve as a useful parameter for the detection of any reactions taking place with uptake or liberation of heat if they occur over a narrow temperature range. Here one ought to mention that Cover (1957) reported that to increase the temperature of oven broiled steaks from 61 to 80°C, although involving only an increase of 19°C, more than doubled the total time of cooking necessary to heat the steaks from 0 to 61°C. Haughey (1968), quoted by Paul (1972), who came across the same phenomenon suggested that this energy is supplied for mechanisms other than increasing temperature, being utilised to release bound or immobilised water but not for water evaporation.

There is very little data on specific heat of fish in general, (Jason and Kent, 1979) and virtually no data on the influence oftemperature above 30°C. The only exception as an inspection of Table 2.4 shows, is the work of Aitken and Campbell (1969a). The specific heat of cod, varying little with temperature, was 3.65 J/°C over the range 30 to 90°C.

The fact that there is a substantial difference between the thermal properties of water and other food constituents has led to many formulae being suggested to estimate the specific heat of the food mainly from its moisture content. These formulae rely on the assumption that specific heat is an additive property. Lamb (1976) has reviewed the several equations put forward to determine specific heat and other thermal properties of foods from their composition.

Table 2.4 Literature values of specific heat of fish above freezing temperatures

Fish	Temperature range (°C)	Percentage composition		Specific heat value (J/g°C)	Source
		moisture	fat		
Cod	2-20	80.3	-	3.68	Riedel (1956)
Haddock	2-20	83.6	-	3.72	Riedel (1956)
Fish (!)	0-100	80	-	3.60	Ordinanz (1946)
Cod	30-90	-	-	3.65	Aitken and Campbell (1969a)
Cod	0-60	-	-	3.72	Langstroth (1931)
Herring	0-60	-	-	3.72	Langstroth (1931)
Mackerel	0-36	-	-	3.26	Langstroth (1931)

In relation to specific heat, probably the first formula to be brought forward was that of Siebel (1892) who suggested that specific heats above freezing point of high moisture foods like eggs, meat, fruits and vegetables could be calculated from the following equation which like all other equations given below, gives specific heat in cal/g°C:

$$C = 0.008\ M + 0.20 \qquad (2.4)$$

where M is the water content of the foodstuff in percent wet basis and 0.20 is a constant assumed to be the contribution of the specific heat of the dry solid.

Lamb (1976) suggested instead, based on data of Earle (1966) and Charm (1971), the following equation for all foods:

$$C = 0.0065\ M + 0.35 \qquad (2.5)$$

and Charm (1971) suggested that a practical equation to calculate specific heat is

$$C = 1.0\ X_w + 0.3\ X_s + 0.5\ X_f \qquad (2.6)$$

where X_w, X_s and X_f are the weight fractions of water, solid and fat respectively of the food.

An alternative equation was given by Riedel (1956) who studied the specific heat of fresh beef, veal, haddock, cod and perch and found that, for water contents higher than 28%, their specific heat could be expressed by:

$$C = 0.006\ M + 0.4 \qquad (2.7)$$

The above equations are useful in estimating specific heat in practical situations when experimental data is not available, but it is obvious that the different equations will give different specific heat values and in addition, with the exception of equation 2.6, they do not make allowance for large amounts of fat in the foodstuffs. Furthermore, although there is enough evidence to indicate that the equations may be of general use, investigations using direct measurement of specific heat for particular foodstuffs have shown substantial discrepancies from the calculated figures. Berlin and Kliman (1974), quoted by Lamb (1976), found an apparent specific heat of the water component of 0.965 above 50% moisture content but as low as 0.121 below 50%. Karmas (1970) reported that the

water present in the food (muscle) had different heat capacities at different levels of moisture content. Mohsenin (1975) stated that experimental values of specific heat of undried material are always larger than those obtained by adding the specific heat values of the dry solid and the water.

These indications of inconsistency of calculated values of specific heat based on equations, justify the direct measurement of specific heat for particular foodstuffs when a precise prediction is required.

Direct measurement of specific heat requires the use of calorimeters. As can be deduced from the hundreds of designs described in the literature, calorimeters are generally specific purpose instruments and, many times, one is faced with the need to build a calorimeter to suit particular experimental conditions like those demanded by the type of sample used and temperature range to be covered. The design, construction and uses of calorimeters is the subject of calorimetry.

D. Calorimetry

Calorimetry is concerned with the experimental measurement of the thermal energy change accompanying a physical or chemical change in a given material. The change can be in phase, temperature, pressure, volume, chemical constitution or any other property of the material which is associated with the change in heat.

For measuring specific heat of foods temperature change is usually the significant variable and therefore any body to which heat can be supplied and whose temperature change can be measured can be considered a calorimeter.

A calorimeter can measure the heat involved either directly by comparison with a known amount of electrical energy or indirectly by comparison with materials with known properties i.e., standard reference substances. All calorimeters, even those designed to be adiabatic, gain or lose some heat to their surroundings; this so-called heat leak, which must be accounted for, is responsible for the characteristic features of most of the calorimetric apparatus.

In most of the calorimetric techniques, the energy Q absorbed or generated in the calorimeter vessel produces a measured temperature change ΔT. If these two quantities Q and ΔT, are precisely measured then, given the mass m of the specimen, its specific heat Cp can be determined:

$$Cp = Q/m\ \Delta T \qquad (2.3)$$

In relation to fish, making use of straightforward calorimetry, the works of Riedel (1956) and Jason and Long (1955) for low temperatures ought to be mentioned. Moline et al. (1961) tried to obtain results of general application by determining the specific heats of fats, gelatin gels and water, again at low temperatures. From these data they proposed to obtain the specific heat of foods based on their composition. Ordinanz (1946) gave a table of the specific heat of foods during cooking, but did not specify how they were obtained, and one suspects they were obtained through Siebel's equation.

Most of the recent work studying enthalpy changes in food materials or proteins from foods makes use of differential calorimeters and, has been used to evaluate heats of transition e.g., collagen ⟶ denatured collagen (Leward et al., 1975), degree of irreversible transitions in a model protein (Hagerdal and Martens, 1976), denaturation thermo-profiles of proteins (Karmas and Di Marco, 1970) and amount of bound water in

foodstuffs (Betchel et al., 1971), rather than to the determination of the specific heat of the foodstuffs.

Although differential calorimeters, particularly differential scanning calorimeters, permit the fast accumulation of data in a short time, they are not free from some relatively serious drawbacks, (Flynn, 1971); apart from their high cost, the use of samples of 14-30 mg could not be representative of, for example, fatty fish which show wide variation in composition. A good review on constructional details of differential microcalorimeters has been given by Reid (1976). This discussion will be centred on macrocalorimeters.

1. Types of calorimeters

Calorimeters may be classified in several ways. One method of classification is according to which physical variables are kept constant and thus calorimeters are classified under two major headings (Spink and Wadso, 1976): adiabatic calorimeters and heat conduction calorimeters.

The ideal adiabatic calorimeter is perfectly insulated, thermally, from its surroundings, so that heat developed inside the calorimeter is totally retained there. With this calorimeter one measures the temperature changes brought about by the process under investigation.

By contrast, the ideal conduction calorimeter is perfectly connected, thermally, with its surrounding heat sink, so that heat developed in the calorimeter is totally transferred from it. With this type of calorimeter one measures the heat flow from the calorimeter to the heat sink. Ideally, the heat sink is of infinite capacity, and there is no final change in temperature of the calorimeter resulting from the process under investigation (Skinner, 1969).

An arrangement lying between these two extremes is the isoperibol, or isothermally jacketed, calorimeter. A simple diagrammatic description of this type of calorimeter, which is basically similar to other types of calorimeter is given in Figure 2.1. The calorimeter vessel (a) is separated from the surrounding thermostatic bath or metal block (b) by an isothermal jacket (c). Between the calorimeter and jacket there is a thermal insulating space (d) filled with air or evacuated. The calorimeter vessel is also equipped with a device for temperature measurement (e). According to the purpose for which the calorimeter is designed, the vessel contains additional components. Commonly, it is provided with an electrical heater that for some applications is only used for its calibration, but that may be also used for heat capacity measurements. When the calorimeter has a heater, constructional details must allow for bringing current and potential leads to the heater. Current leads are those conducting electrical current to the heater and, potential leads are those used to measure the potential drop across the heater during electrical energy input. These potential drop measurements are used to calculate the power introduced into the calorimeter. Additional details in the calorimeter vessel may include a device for starting a reaction (for instance, an ampoule-breaking mechanism), a stirrer and so forth. In this system the calorimeter is imperfectly insulated from the surrounding jacket, which is kept at a constant temperature. Heat developed in the calorimeter brings about a temperature rise of the calorimeter, which is followed, due to the imperfect insulation, by a gradual return to the temperature of the environment. One measures the calorimeter temperature before, during and after the change being studied. From these data the heat transfer between calorimeter and jacket is calculated.

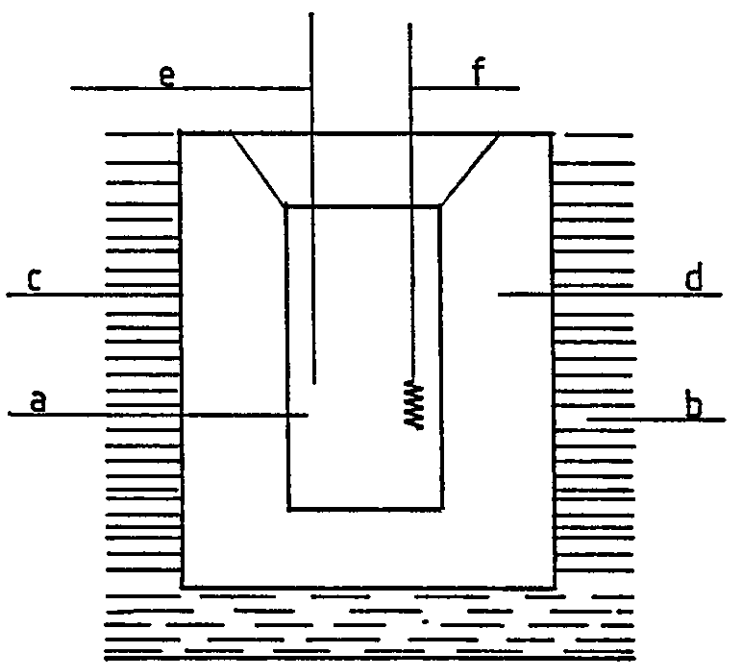


Fig 2.1 Isoperibol calorimeter

a- calorimeter vessel b- thermostat bath or metal block

c- isothermal jacket d- air gap or vacuum

e- thermometer f- heater

The names of several calorimeters describe a characteristic property or function of the calorimeter. A microcalorimeter refers to a calorimeter used to measure small heat effects. A flow calorimeter is one used with flowing samples. An aneroid calorimeter is interpreted as one which depends on solid conduction rather than fluids to distribute heat.

In the field of chemical reactions, such names as combustion bomb, reaction, flame and solution calorimeters are used to describe the various types of reactions studied.

The calorimeter surroundings has a varying terminology and receives names like: shield, jacket, guard, envelope and so forth.

2. Standard reference substances

In order to minimise errors in calorimetry, it may be a great advantage to check the reliability of the calorimetric measurements with a "reference material" whose state is reproducible and known. This requires the availability of results of accurate measurements made on this reference by standard laboratories. When moderate calorimetric accuracy, is adequate, as in this work, it is not difficult to find a reference material whose thermal properties have been measured with higher accuracy.

Three substances have been recommended as "standard" reference material: benzoic acid, n-heptane and aluminium oxide. In the range 0-100°C, water usually will serve as the most useful material, due to its universal availability in a pure state, and because of the numerous measurements on its heat capacity. In the range -25 to 77°C benzoic acid will serve if a solid is needed while n-heptane will serve up to 127°C if a liquid is needed. In the range 27 to 197°C diphenyl ether serves as a liquid with relatively low vapour pressure (Ginnings and Furukawa, 1953).

Although it is true that a comparison method reduces certain errors, the degree of reduction depends upon the experimental conditions. The comparison method is most effective in reducing errors when the physical properties and amount of the material being investigated are as close as possible to those of the standard reference material. Even when the material has identical physical properties, there is always the possibility of variation of the performance of a calorimetric apparatus from day-to-day (Ginnings, 1968).

3. Choice of calorimetric method

Obviously, one of the most important factors to be considered is the accuracy required. If the accuracy required is not as high as the accuracy obtained in published calorimetric measurements on a certain material, then this material can be used advantageously to calibrate a less complicated calorimeter. If possible, this reference material should be chosen to have thermal properties similar to those of the material being investigated. It should be kept in mind that, as pointed out by White (1928), materials may require different times to reach equilibrium in the calorimeter, and difference in time may cause a difference in the evaluation of heat loss during the process; furthermore, different materials may raise the calorimeter temperature by different amounts, with the same effect.

Other general considerations are that the physical properties of the sample have considerable influence on the choice of method and calorimetric design; temperature and pressure range involved in the measurements are important, as well as the amount of sample available.

In measurements for which the time of the experiment is very long, a method having small heat loss should be chosen e.g., adiabatic calorimetry.

According to West and Westrum (1968) two methods are widely used for making heat capacity measurements above room temperature: drop calorimetry and adiabatic calorimetry.

From the large amount of literature on adiabatic calorimetry it seems that this method is the one most used to determine heat capacities. Possibly this is due to the availability of modern electronic instrumentation for temperature control and recording. Karasz (1963) recommended the adiabatic method for experiments in which the heat loss would be too large for the isoperibol one, that is:

1. experiments in which thermal energy in the calorimeter is liberated or absorbed relatively slowly,
2. experiments at high temperatures when radiative heat transfer becomes too large,
3. experiments in which a series of measurements is carried out successively over a wide temperature range.

Additional advantages of the adiabatic method are:

a) errors due to variation in k, the leakage modulus, and to deviation of heat exchange from Newton's Law, are reduced in the adiabatic method, since the multiplier of k, the thermal head, is zero or small;

b) maintenance of small thermal heads reduces convection in the air gap between calorimeter and jacket;

c) troubles from evaporation from an imperfectly sealed calorimeter are less serious (Sturtevant, 1971). Furthermore, using the continuous heating method more data in a given time is collected from an adiabatic calorimeter than from an isoperibol.

On the other hand, perfect conditions are never realised and heat leak corrections are still necessary. The design is more complicated since shield mass, position, means of heating and temperature control are critical (Stansbury and Brooks, 1971). Large thermal lags in parts of the apparatus can make it difficult, if not impossible, to control an adiabatic calorimeter properly (Ginnings, 1968). Ideally the continuous heating method requires instantaneous distribution of heat to the calorimeter and its contents (Westrum et al., 1968). To fulfil this requirement the calorimeter vessel is typically designed for small samples of high thermal conductivity or is provided with means of achieving rapid thermal equilibrium.

The isoperibol calorimeter or isothermally jacketed calorimeter is one of the simplest and most common types of calorimeters. One advantage of this calorimeter is the simplicity of temperature control of the shield by a thermostat. Since the shield is at a constant temperature, another advantage is that it is free from variable thermal contact. Only simple electric equipment is necessary for the measurement of temperature and introduction of energy into the calorimeter.

On the other hand, the success of the isoperibol method requires that the heat losses are kept small permitting the correct evaluation of the leakage modulus; but as pointed out by Ginnings (1968) it is only the uncertainty in the heat leak correction which affects the accuracy of the results, not the magnitude of the correction.

The most commonly used method of heating for adiabatic calorimetry is the intermittent heating method (Staveley et al., 1955), the same as used with the isoperibol calorimeter. In this method of heating the temperature of the calorimeter at steady state is measured before the power input. The calorimeter is then heated at a constant power for a

known time interval. After the heat input the temperature is followed until a steady state is reached again. From these temperature measurements the heat leak is evaluated. So, with the necessity of heat leak corrections, (even with adiabatic calorimetry, for highest accuracy, heat leakage corrections must still be made) and intermittent heating the superiority of the adiabatic method is not so clear-cut, if with the isoperibol method the heat leak is kept small and measured reasonably precisely.

As a practical matter, however, the choice of method is frequently determined by available apparatus and personnel.

Design considerations in building a calorimeter

Anticipating the fact that the calorimeter used to do the work described in this thesis was of the isoperibol type it is appropriate to point out that a detailed description of the design of an isoperibol calorimeter is given by Stout (1968). Additional descriptions are given by Hubbard *et al.* (1954), Skinner (1969), White (1928) and Sunner and Wadso (1959). An apparatus able to work as adiabatic or isoperibol was described by MacLeod (1967b).

As previously discussed the choice of calorimetric method depends upon temperature range, accuracy desired, properties of the sample, amount of heat involved, duration of the experiment, available apparatus and personnel, cost of the apparatus and possibly other factors. But after a method is chosen, mechanical, chemical, electrical and heat flow considerations arise. Most of the general discussion that follows has been based on the work of Ginnings and West (1968).

a. Chemical and Mechanical

Chemical

The problems of whether or not the sample material reacts with the calorimeter wall and how the reflectivity of the outside wall of the

calorimeter will be impaired by the chemical atmosphere existing in a laboratory, can be easily avoided by choice of material.

Mechanical

The mechanical design is necessarily a compromise between mechanical and other considerations, such as thermal and chemical. The calorimeter must be strong enough to withstand any pressure and temperature encountered, not only avoiding rupture but also keeping down any change in dimension due to pressure.

A spherical shape might be ideal mechanically, but a cylindrical section having hemispherical ends is the most frequently chosen shape. Internal vanes can give added strength and also serve to distribute heat to reduce temperature gradients. Special attention must be paid to soldered junctions: thick layers of solder should be avoided. The design of soldered joints is specially important in calorimeters used over large temperature ranges when differential expansion may cause large stresses in the joint.

b. Heat Flow

For calorimetry the essential quantities to be measured are: mass, temperature and energy.

In most experiments the major part of the energy is either derived from or compared with electrical power which, with precautions, can be measured very accurately. However, the energy change also includes heat losses or gains from other sources, such as that resulting from heat leak. It must be re-emphasised that it is the absolute uncertainty in the heat leak that results in error, rather than the magnitude of the heat leak.

The steps to minimise errors due to heat leak are: a) design the calorimeter to have a large thermal resistance to its surrounding shield, b) design the calorimeter and shield to have adequate temperature sensing

devices at suitable locations to measure the effective temperature difference between the calorimeter and its surroundings even with temperature gradients, c) make corrections for any measurable heat leak in an effort to account for it.

One of the most important problems in accurate calorimetry results from temperatures gradients inside the calorimeter. To avoid temperature differences on the surface, the heater should be located centrally; further improvement is obtained through the installation of metal vanes running from the heat source to the surface. Where a single thermocouple is used to measure the calorimeter surface temperature it should be located at a point whose temperature is as near as possible to the average surface temperature.

To minimise these gradients use of high conductivity materials is recommended. Gold, copper and silver have high thermal conductivity and are the most frequently used in the construction of calorimeters. Even when using materials of high thermal diffusivity it is still necessary to position the heater centrally within the calorimeter to minimise temperature gradients (Westrum et al., 1968).

In accounting for the power in the current lead segments, the convention assumes that the heat developed in a current lead segment (that part of the current lead situated between the calorimeter and its jacket) divides equally between calorimeter and shield, so that the potential lead is attached to the centre of the current lead segment. The use of potential lead at mid-points is valid with calorimeter and shield at different temperatures only if a heat leak correction is made for the "apparent heat flow" along the current lead segments assuming their ends are at the temperatures of calorimeter and shield (Ginnings and West, 1964).

Summarising, the general requirements of a calorimeter vessel are that it be strong enough to withstand working pressure, that it not react chemically

with the calorimetric sample, and that it also provide good thermal contact throughout the heater-calorimeter-sample system. In addition, it is desirable that the calorimeter heat capacity be relatively small compared to that of the sample.

c. Heat exchange between calorimeter and environment

Heat exchange between a calorimeter and its surrounding jacket is due to:

a) conduction via solid connections between them e.g., electrical lead wires;

b) convection and conduction by gas molecules occupying the interspace;

c) radiation;

d) evaporation losses from an unsealed calorimeter containing liquid.

The size of the first three is dependent upon the temperature difference between calorimeter and jacket. Evaporation losses are eliminated by using a perfectly sealed calorimeter.

Provided the thermal head, the temperature difference between the calorimeter and its jacket, is no more than a few degrees, the heat exchange by radiation, by gas conduction and conduction by solid, obeys Newton's Law (Skinner, 1969), so that:

$$dT/dt = k\,(T_j - T) \qquad (2.8)$$

where, T, calorimeter temperature at time t,

T_j, jacket temperature,

k, leakage modulus of the system.

A well designed isoperibol calorimeter aims at a constant and small value for the leakage modulus, at a given temperature. To achieve this, in addition to the recommendations already given, the following design features should be met:

1) the outer surface of the calorimeter and the inner surface of the jacket should be highly polished, or plated, to minimise radiative heat transfer between them.

2) the air gap between the calorimeter and its jacket should preferably be evacuated to minimise conductive and convective heat transfer.

If copper is the material of construction of the calorimeter one has to remember that ordinary copper hardens much faster than oxygen free copper. O-ring material should not be likely to suffer permanent alterations which could affect the sealing leaving the calorimeter not vacuum tight.

All parts of the calorimeter vessel must be easily removable for testing and repairing. Ease of operation is an important requirement for successful calorimetry (Cruickshank et al., 1968).

d. Electrical

1. Size of thermocouple wire

The minimum size is that which can handled safely during assembly and disassembly. In deciding how large to make the leads, one must compromise between electrical resistance and thermal conductance. If heat flow is important one uses the smallest thermocouple wire compatible with the requirements of strength for convenient handling.

In the use of thermocouples in accurate calorimetry it is necessary that the thermocouple emf be a true measure of temperature difference. Precautions to achieve this are: a) the thermocouple should be used under conditions which do not invalidate its calibration; b) spurious emf's due, for example, to inhomogeneities in the thermocouple material should be prevented; c) it should be made certain that the junctions are really at the temperatures of the components whose temperature they measure.

Much of the heat flow from the thermojunction is along wires connected to it. To reduce heat flow along these thermocouple wires and any other wire coming into contact with the calorimeter, a technique called "tempering" is used. In this technique, wires are fastened in thermal contact with the calorimeter and/or with the surrounding jacket by using epoxy resins to attach them, thereby providing both thermal contact and electric insulation. In addition to ensuring that the thermocouple emf is a true measure of temperature difference, there is the problem of proper location of the junction to evaluate the effective temperature or that correct account can be taken of both heat leak and temperature change, even if temperature gradients do exist.

2. The calorimeter heater

The calorimeter heater resistance should be large compared to its lead resistance in order to minimise the heat generated in the current leads (Westrum et al., 1968); to minimise problems of temperature distribution in the calorimeter, its heat capacity should be small.

Since the heater has some heat capacity, usually an appreciable time is required for the initial rise of temperature of the heater to take place. In addition to this initial transient the slower change of resistance must be considered; usually this is not bothersome, except in very accurate calorimetry, provided the current and the potential drop across the heating element are used to calculate the heat supplied to the calorimeter. The obvious solution to the problems resulting from change in resistance of the calorimeter heater and its leads is: a) to minimise the thermal transient by keeping the heater in good thermal contact with the calorimeter; b) to construct the heater with a material having a small temperature coefficient of resistance e.g., constantan, manganin, etc.; c) either make the heater leads of low electrical resistance or use a material with small temperature coefficient.

3. Potentiometer and null detector

The potentiometer enables a very precise comparison of an unknown voltage with that of a standard. In commercial forms the instrument consists of a system of resistors and their controls mounted on a panel.

In conjunction with the potentiometer a galvanometer or an electronic amplifier is used as null detector. Galvanometers present two disadvantages: 1) they are susceptible to mechanical vibration; 2) if a relatively large voltage is applied to the galvanometer the moving coil system may be damaged so that a new suspension wire is required. Electronic amplifiers are less sensitive to vibration and less easily damaged by overloading. Zero drift should be very low.

CHAPTER 3

MATERIALS AND METHODS

A. Water and Fat Holding Capacity Studies

1. Raw material

Fresh herring from a single catch, not more than one day in ice and selected to be of the same size to the nearest 0.5cm, were quick frozen in blocks of 15 fish in a blast freezer at -32°C. After freezing, the blocks were glazed with tap water and packed in pairs in polyethylene bags and placed in wooden boxes to be stored at -30°C. Packing procedures were designed to prevent dehydration and retard oxidation.

When required, samples were left overnight at ambient temperature to thaw. The fish to be used in each experiment were hand filleted and skinned, fillets of each individual fish being identified for reasons that will be made clear later. After this filleting operation additional trimming of the fillets was carried out for two reasons.

First, special attention was paid to removing any remains of the belly flap, which contains a very large amount of fat in comparison to the rest of the fillet, and therefore could be a serious cause of inconsistencies in the results. Second, to obtain fillets of similar size, portions of the fillet at head and tail positions were removed in such a way that a typical fillet used in these experiments had the shape of that shown in Fig. 3.1.

2. The cooking operation

a. Temperature measurement

As commercial thermocouple probes were too large or too long and therefore unsuited to the experimental conditions it was necessary to improvise and produce suitable ones in the following fashion. Twin

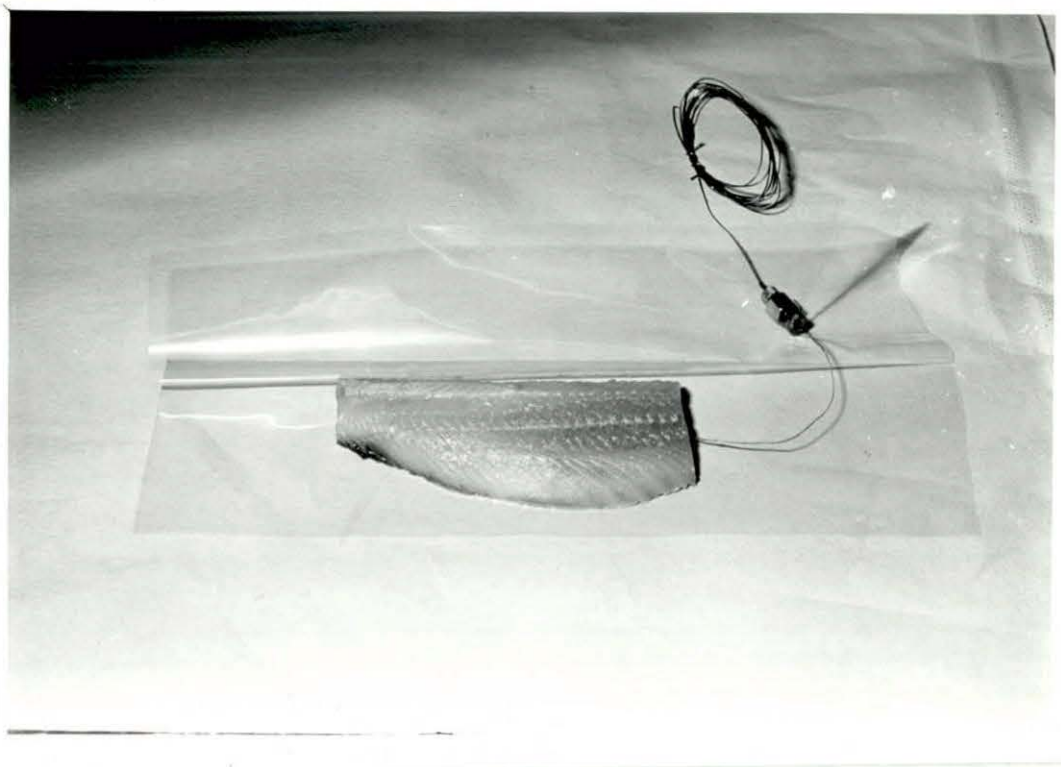


Fig 3.1 A typical herring fillet sample and the scheme used to measure its temperature during cooking

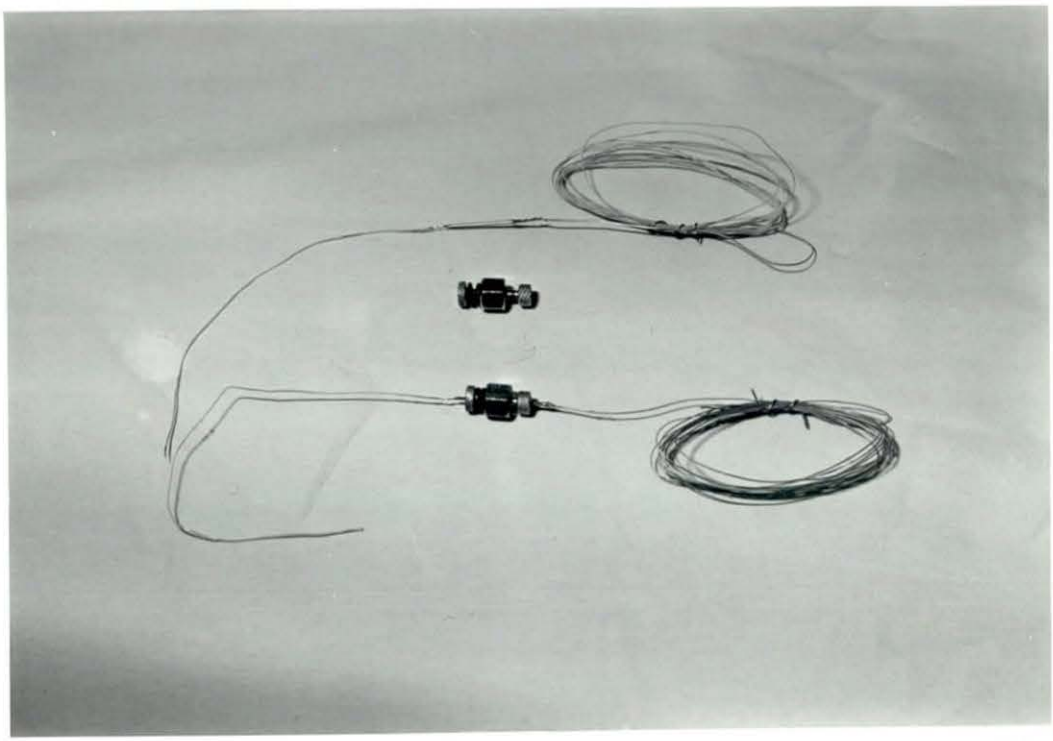


Fig 3.2 The purpose-made thermocouple probes and the sealing glands used with them

thermocouple wire, 32s.w.g. was put through a short stainless steel tube of 0.8mm external diameter, which was then sealed at both ends with Araldite. The stainless tube could then be retained in the thermocouple glands, which in turn were sealed into the nylon bags, allowing the system to be vacuum tight (Fig. 3.2). For temperatures above 85°C a thermocouple was passed through the can by means of the gland and then sealed through the nylon bag with Araldite.

The fish internal temperature was normally measured by three copper-constantan thermocouples as described above, inserted longitudinally into the centre of three fillets, (Fig. 3.1), brought in contact with them through the longest path, to reduce the influence of conductive heat on the thermocouple readings.

Temperature was read at 30 second intervals with an electronic thermometer, Comark type 1624, reading to 0.1°C, in series with a multiple switch selector unit to which the thermocouples were attached (Fig. 3.3). When three consecutive readings in each thermocouple were constant, which normally occurred when the difference between the fish internal temperature and the bath temperature was approximately 0.2°C, the fish was considered to be at the bath temperature.

b. The cooking apparatus

The cooker (Fig. 3.3) consisted of a rectangular bath of aluminium of dimensions 50 x 40 x 30cm^3, fitted with a lid and completely covered by a 5cm thick layer of insulating glass wool. The bath was filled with water for temperatures below 90°C or mineral oil for higher temperatures. Apart from differences in dimensions and geometrical shape, the principles and design followed were the same as for the calorimeter thermostat bath shown in Figure 3.13. Heating of the fluid medium was provided by two 300 watt immersion heaters, current being supplied through two

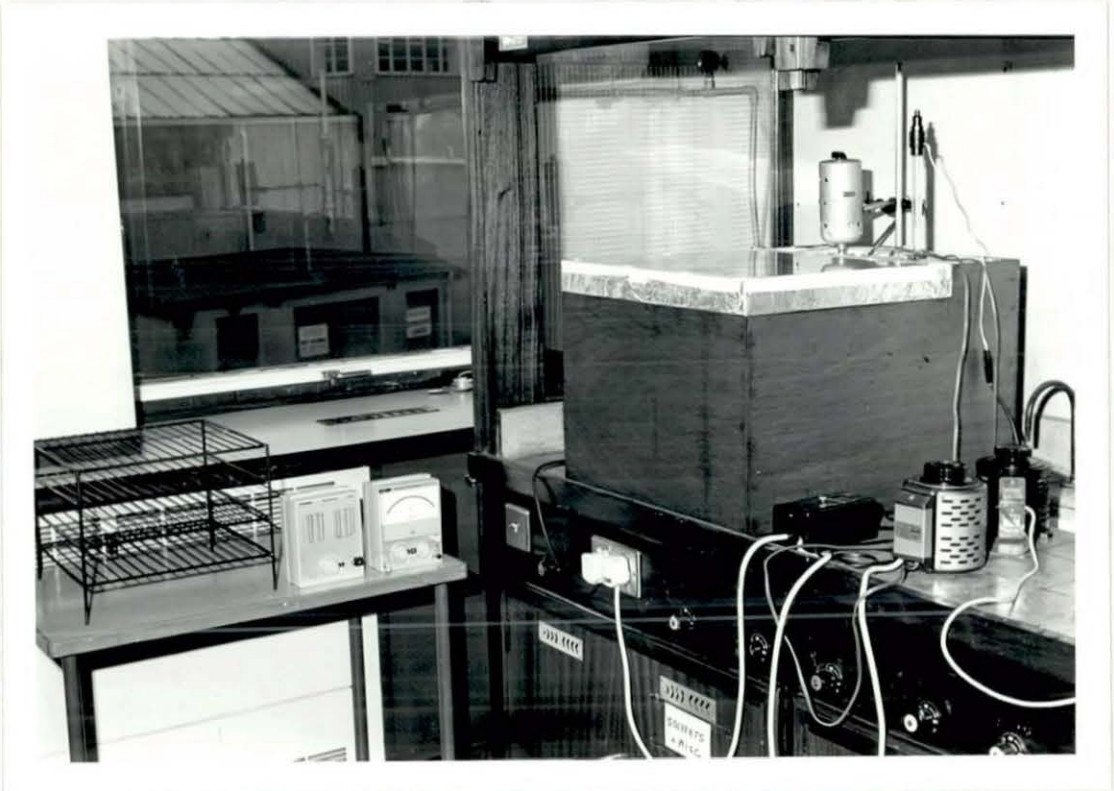


Fig 3.3 Cooking and temperature measurement apparatuses

From left to right: rack, multiple point switch, electronic thermometer, cooker and thermostat control devices

autotransformers with one heater permanently on, and the other being controlled by a mercury contact thermometer in conjunction with an electric relay. Good circulation of the fluid was obtained by a stirrer positioned inside a cylindrical well and driven by a variable speed electric motor. The system, when in equilibrium, allowed a temperature control better than $\pm$ 0.25°C.

c. Cooking procedures

Eleven temperatures were studied in the range 35°-113°C approximately, and for each temperature under study the influence of the duration of heating was verified for treatments lasting 0, 15, 30 and 60 minutes. Time 0 was taken as the time when the fish internal temperature reached the bath temperature.

For a given temperature, six fillets were used to study the influence of each of the different times of heat treatment on weight, water and fat losses. The individual fillets were placed inside tared nylon bags and sealed under vacuum. The bags with the fish, for each time of thermal treatment, were randomly distributed and hung on the wire rack before the latter was immersed in the thermostatted bath. For temperatures above 85°C, the bags were put inside vegetable oil filled cans that were vacuum seamed and placed into the bath at the desired temperature.

The cooked samples were collected when they reached the desired temperature or when they had been in the bath for the necessary time, allowed to cool for 15 minutes held vertically, and then the bags were punctured to allow the cooking liquids to run off. This draining operation lasted 45 minutes after which the samples were weighed. Part of the cooked sample was submitted to a pressing stage, and the change in weight measured.

3. The pressing operation

Pressing was carried out with a hydraulic press (Fig. 3.4), pressure exerted being $3 \times 10^7 N/cm^2$ and residence time 3 minutes. The press chamber (Fig. 3.5) consists of a stainless steel cylinder of internal diameter 2.5cm and of wall thickness 1.1cm. The base is a perforated screen of brass which is removable to facilitate cleaning. The filter element (Fig. 3.5) consisted of a layer of four nylon mesh discs of aperture 20 microns, supplied by Henry Simon Ltd, Stockport, England. The plunger is a stainless steel cylinder, fitted with an O-ring near its front end which prevents leakage through the top of the press.

4. Sampling procedures

Special attention was paid to sampling procedures. As indicated by Brandes and Dietrich (1953), herring from the same catch selected to have the same length, and the same body and gonad weights might present differences in fat content up to 11%. According to the same authors, moisture and fat content vary along the fillet, the highest fat content occurring at the head position and the lowest near the tail.

In spite of the above statements, as no standardisation can worsen the variations, the fish used in these experiments were obtained from the same catch and selected to be approximately of the same size. To minimise the effect of the variations in moisture and fat distribution along the fillets the following methodology was adopted for all experiments.

From the six remaining fillets not used in cooking, for each time of heating, portions of about 15g were taken with a cork borer from head and tail positions, one at each position. These portions obtained from the six fillets were then bulked, passed through a mincer twice and then thoroughly stirred with a glass rod. From this fish two samples weighing approximately 10g were taken and used in the determination of the fat and moisture content of the raw material.

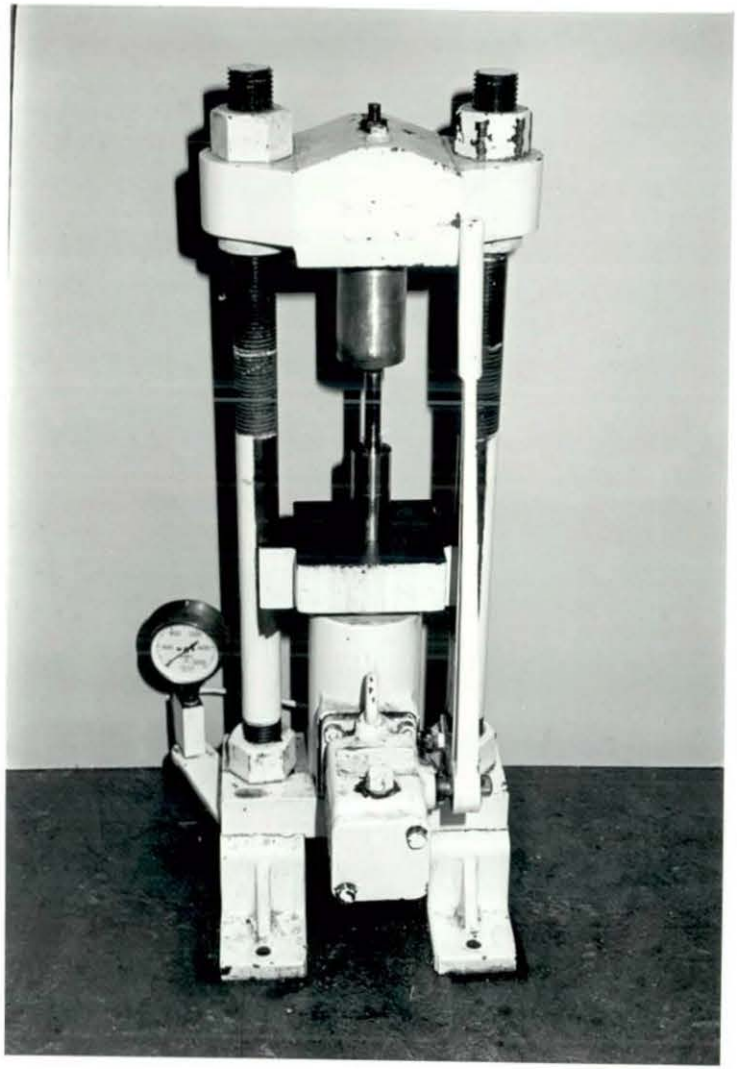


Fig 3.4 The hydraulic press with the small stainless steel press in position

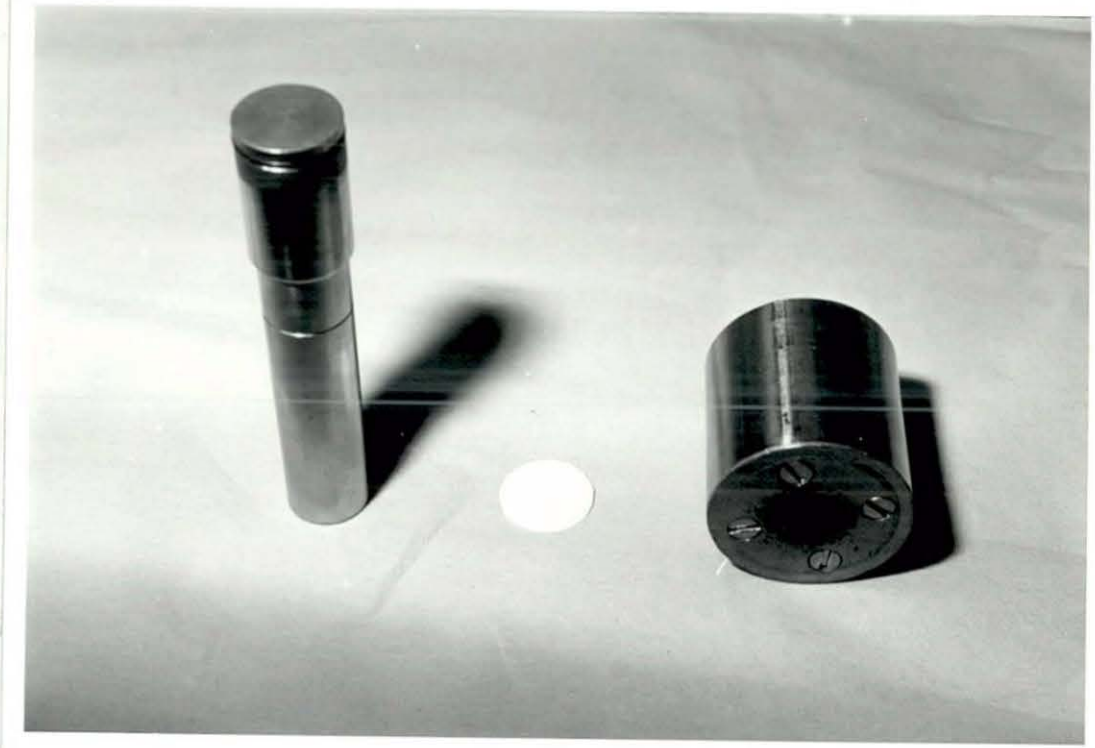


Fig 3.5 The plunger, filter element and press chamber used to study the effect of pressing on the release of liquor from cooked fish muscle

Samples to be used for the moisture and fat analysis of the six cooked fillets were collected as above from head and tail positions. From the 12 pieces thus obtained two samples were used for the analysis concerned with the cooked fish, one sample consisting of 2 pieces from tail positions and one piece from the head position, the other sample being composed of 2 pieces from head positions and one piece from the tail position.

The six remaining pieces were submitted to the pressing stage and combined in the same fashion to give two pressed samples which were used for fat and moisture determinations.

For each treatment, cooking, and cooking plus pressing, the results of the analysis of the two samples were averaged and considered to represent the moisture and fat content of the cooked or of the cooked and the pressed fish.

The percentage weight loss during pressing was used to calculate the losses due to pressing, had the whole lot of cooked fish been pressed. The amounts of liquors released during cooking and during pressing were determined by measuring the difference in weight before and after cooking or pressing respectively, with a balance able to discriminate to 10^{-4} g.

Losses of water in relation to the original water content were obtained through the formula:

$$H_L = \frac{W_1 m_1 - W_2 m_2}{W_f m_f}$$

where,

H_L, % of water loss

W_f, weight of the raw fish

m_f, percentage of moisture of the raw fish

W_1, weight of sample before treatment

m_1, percentage of moisture of sample before treatment

W_2, weight of sample after treatment

m_2, percentage of moisture of sample after treatment

5. Moisture and fat determinations

a. Fat determination

The traditional and standard method of fat determination in fish and other solid foodstuffs is the soxhlet extraction method, which consists of extracting the dried and ground samples with a suitable solvent which normally is diethyl ether. There is a very large number (Smith, 1969) of claimed rapid methods for the estimation of total fat. For fish the Bligh and Dyer (1959) method modified by Hanson and Olley (1966) and the commercial Foss Let (Usher et al., 1973) method which is based on the specific gravity of the extracting solvent, can be included in this category.

The Bligh and Dyer (1959) method is fast in the sense that results can be obtained within a smaller time interval than the soxhlet one, but the fact that it involves solvent volume measurements, homogenisation and centrifugation operations makes it too time consuming.

The Foss Let method has some advantages in relation to the soxhlet one considering that fat determinations can be carried out on the wet material and the operations are rationalised, an average time for one determination being 10 minutes. So a comparison was made between the soxhlet and Foss Let methods for two fat levels, and a t-test statistical analysis on the data presented on Table 3.1 showed that the difference between the means are not significant.

Table 3.1 Comparison between the soxhlet and Foss Let methods of fat determination

Sample	Soxhlet mean*	Soxhlet Standard deviation	Soxhlet No. of samples	Foss Let mean*	Foss Let Standard deviation	Foss Let No. of samples
1	13.79	0.21	6	13.65	0.29	5
2	2.68	0.11	5	2.75	0.20	5

1 Determination in wet samples
2 Determination in dried samples
* Percentage of fat

In spite of the good agreement between the two methods, for this work the chosen method of analysis was by Soxhlet, because with the need to determine moisture the advantage of the Foss Let method of using wet material is lost and, in addition, the requisites of cleaning and temperature stabilising of the apparatus makes it not so flexible for the lone worker as the Soxhlet method is.

As a result the fat content was determined on the dried samples by extraction with diethyl ether for 6 hours.

b. Moisture determination

The need for obtaining the fat content of the same sample used for moisture determination practically conditioned the method used to be that of drying the samples for 24 hours in an oven at 105°C, which is the procedure used at Torry Research Station.

B. Studies of Visual Changes

1. Subjective observations

Gross changes in translucency, texture and palatability of the cooked fillets were noted in relation to the raw fillets and to the different temperatures studied. Changes in translucency were observed

only visually. Changes in texture were evaluated visually and by handling of the fillets. The alterations occurring in the skin of the unskinned control fillets were also noted, especially in relation to the ease of removal of the skin from the flesh as a function of temperature.

2. Microscopic observations

a. Optical microscopy

Histological samples of approximately 1cm in width were taken from the cooked or raw fillets at about 3cm from the head end. These samples were then fixed in neutral formalin for at least 48 hours, but normally for 1 week. The fixed specimens were then dehydrated in dioxan and embedded in paraffin wax. Sections were prepared 7 to 10um thick and stained according to Sweat et al., (1968). Muscle fibres are stained orange and red and connective tissue is coloured blue.

b. Transmission electron microscopy

The tissue, raw or cooked, was cut into pieces approximately $1mm^3$ and fixed for 12 hours at 4^oC in 2.5% glutaraldehyde in cacodylate buffer at pH 7.4. It was then post-fixed in 1% osmium tetroxide in barbitone buffer (pH 7.4) for 4 hours at 4^oC. After fixation the tissue was washed in barbitone buffer and dehydrated through graded ethanol. From 100% ethanol it was transferred to 1.2-epoxypropane for 4 hours, then to 50:50 mixture of 1.2-epoxypropane and Emix resin in small unstoppered wide-mouthed vials. The tissue was left in this mixture overnight, during which time most of the 1.2-epoxypropane had evaporated. The small pieces of tissue were then placed in pure Emix resin for 4 hours and individually embedded in this resin in capsules. The resin was polymerised by heating the capsules to 60^o for 2 hours.

Sections of the resultant blocks were cut on an LKB Ultratome III using a diamond knife. Suitable sections (giving a silver interference colour ≡ approximately 60 to 70nm thick) were harvested on uncoated 200 mesh copper grids and stained with lead oxide (Karnovsky, 1961). They were examined in a Siemens Elmiskop 102, using a 200u condenser aperture, a 50u objective aperture and an accelerating voltage of 60kV. Micrographs were recorded on Kodak Electric Image Film, 4463.

C. Specific Heat Studies

1. Testing of an existing calorimeter

a. The calorimetric system

The calorimetric system was to a large extent an isoperibol one previously used at Torry Research Station (Aitken and Campbell, 1969a) Fig. 3.6, which was based on a design by Sterret et al. (1965).

1. Calorimeter

The calorimeter itself was a copper cylinder 7cm long by 5cm outer diameter, with 0.5mm wall thickness. Three eyelets, through which ran loops of nylon thread to suspend the calorimeter from hooks beneath the shield were soldered to the upper part of the calorimeter. A silicone rubber O-ring top sealed the calorimeter. A coaxial reentrant tube, 0.5cm internal diameter held the heater. Removable circular vanes were tightly fitted to the heater tube to improve heat transfer. A copper-constantan thermocouple firmly screwed to the calorimeter surface at half height, measured the calorimeter temperature.

2. The calorimeter heater

The heater was made of a rod of tufnol with a non-inductive nichrome winding. The surface of the coil was carefully smoothed to constant diameter, insulated with Araldite and again smoothed to fit the reentrant heater tube closely. Its resistance was about 51Ω.

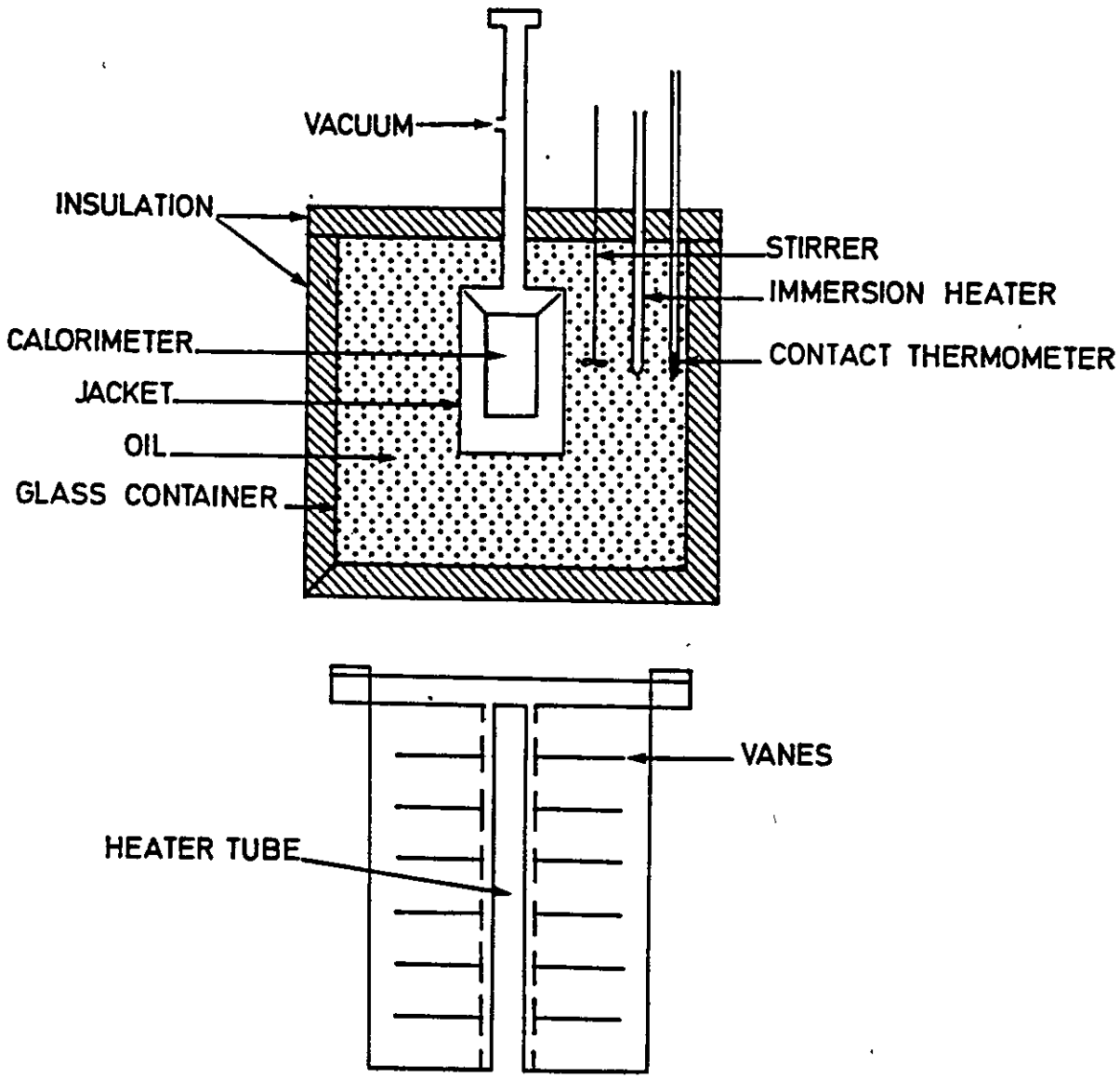


FIG 3.6 SCHEMATIC DRAWING OF THE CALORIMETRIC SYSTEM AND CALORIMETER

3. The calorimeter jacket

The jacket, of heavy brass, designed in a submarine fashion, 12cm long by 8cm internal diameter, surrounded the calorimeter with a gap of 1.5cm from the side walls and gaps of about 3 and 2cm from the top and bottom walls respectively, and was immersed in an oil bath. All leads of the calorimeter circuit were brought into thermal contact with the internal surface of the jacket lid through the use of epoxy resin. A hollow tube brazed to the jacket lid through which ran the electrical and thermocouple leads, allowed the space between jacket and calorimeter to be evacuated.

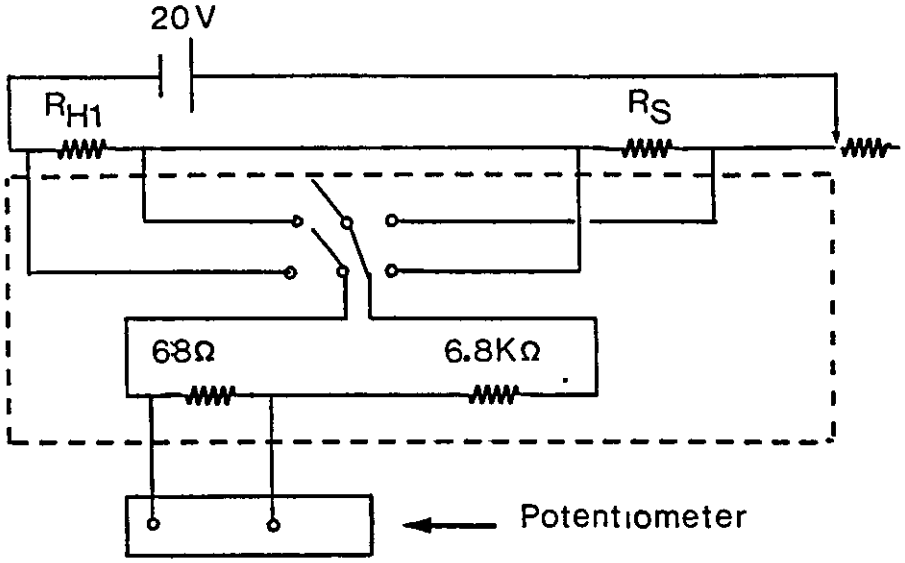
4. The thermostat bath

The thermostat bath consisted of a glass tank filled with mineral oil and completely surrounded by a 5cm layer of expanded polystyrene. This stirred oil bath had its temperature controlled by a mercury contact thermometer in conjunction with a hot wire relay; up to 80°C the temperature control could be as good as $\pm$ 0.01°C.

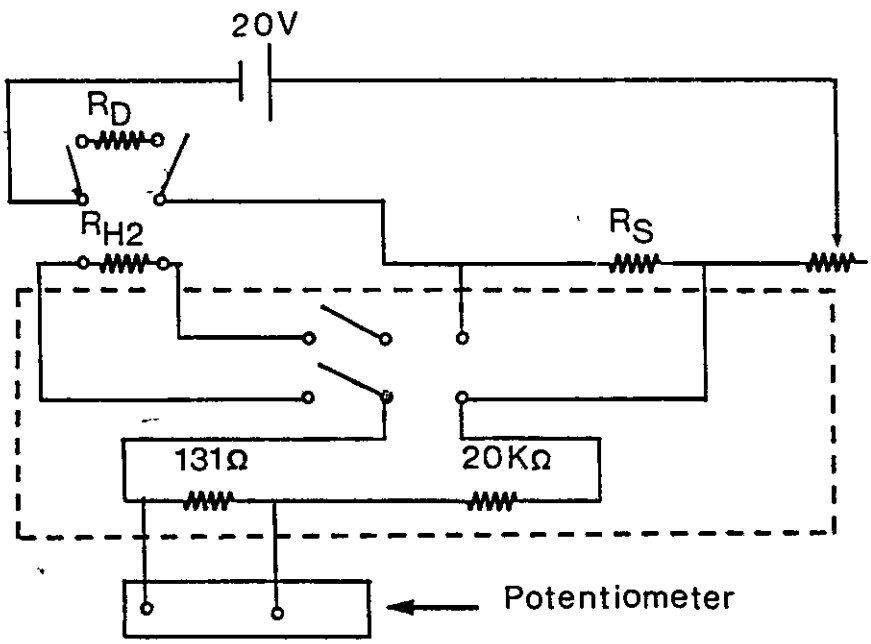
b. Measurement of energy

The calorimeter electric circuit in this first series of experiments is shown in Figure 3.7a. To measure the emf of the calorimeter thermocouple, the heating current and the potential drop across the heater, a Cambridge Vernier potentiometer was utilised. The potentiometer current was held constant by use of a 2V accumulator and standardised against a Weston standard cell maintained in an oil bath. A galvanometer amplifier was used for null detection. The potentiometer could be read in steps of 0.1μV.

The heating power, supplied by a 20V d.c. supply through a rheostat, was evaluated by measuring the potential drops across a standard resistor of 10Ω and the heater in series. As the potentials were outside the range of the potentiometer, they were applied to a calibrated potential divider



a- Existing calorimeter



b- Modified calorimeter

Fig 3.7 Calorimeter energy measurement circuit ; dotted lines enclose the potential divider

R_H = Heater : $R_{H1} = 51\Omega$, $R_{H2} = 40\Omega$

R_S = Standard Resistor

R_D = Dummy Resistor

attached to the potentiometer. The energy E, in joules, liberated inside the calorimeter is given by

$$E = \bar{V}_h \bar{I} t \qquad (3.1)$$

where t = time of heating, in seconds

$\bar{V}_h$ = average potential drop across the heater, in volts

$\bar{I}$ = average current through heater, in amps

$$= \frac{\bar{V}_s}{R_s}$$

$\bar{V}_s$ = average potential drop across standard resistor, in volts

R_s = resistance of standard resistor (= 10Ω)

c. Method of experiment

The heat capacity of the calorimeter and/or sample was determined by supplying it with a known amount of electrical energy. The total time of energy input was measured on a stop watch reading to 1 second; the potential drops V_h and V_s across the heater and the standard resistor respectively, were measured at approximately 2 minute intervals.

In each individual experiment the calorimeter, filled with a known mass of sample, was heated till its temperature was 3-3.5°C below that of the jacket, and then left to reach thermal steady state. When this condition was fulfilled the calorimeter temperature would be 1.5-2.0°C below that of the jacket, and from this point its temperature was followed during 20 minutes, constituting the so-called fore, or initial period. The temperature drift observed in this period is used to calculate the heat loss corrections of the heat capacity measurements. Following the completion of the fore period, electrical energy was supplied to the calorimeter for 15 minutes at a preset rate. During and after this heating, the temperature of the calorimeter was followed as a function of time till it again reached a thermal steady state. This temperature at which thermal steady state was again achieved marked the end of the

main period; at this point the calorimeter temperature was 1.5-2.0°C above the shield temperature. Temperatures were then measured for another 20 minutes, the after rating or final, period, to determine the temperature drift which is also used to calculate the calorimeter heat loss. All the temperature measurements in the three periods were made at 1 minute intervals.

d. Calculation of the heat loss due to heat exchange

The term 'corrected temperature rise' is defined as the temperature change which the calorimeter would have experienced in the absence of heat loss. Regardless of the method used to calculate a corrected temperature rise the validity of this rise as a means of comparing two experiments depends upon certain characteristics of the design of the calorimeter and of the experiment. These include particularly the requirements that temperature gradients within the calorimeter during the initial and final periods be sufficiently similar during the two experiments, and that the thermometric device measures a temperature which gives a valid measure of heat exchange with the environment during the main period, at least on a relative basis for the two experiments (Gunn, 1971).

Several authors, Coops, Jessup and Van Nes (1956), Gunn (1971), King and Grover (1941), MacLeod (1967) and Wadso (1966) have discussed in detail the methods used to calculate the corrected temperature rise during a calorimetric experiment.

The measurements made when using an isoperibol calorimeter are presented graphically as a time-temperature curve, Figure 3.8, which is divided into three periods described above: 1) a fore period during which the temperature change of the calorimeter is entirely due to heat transfer between jacket and calorimeter; 2) a main period in which energy

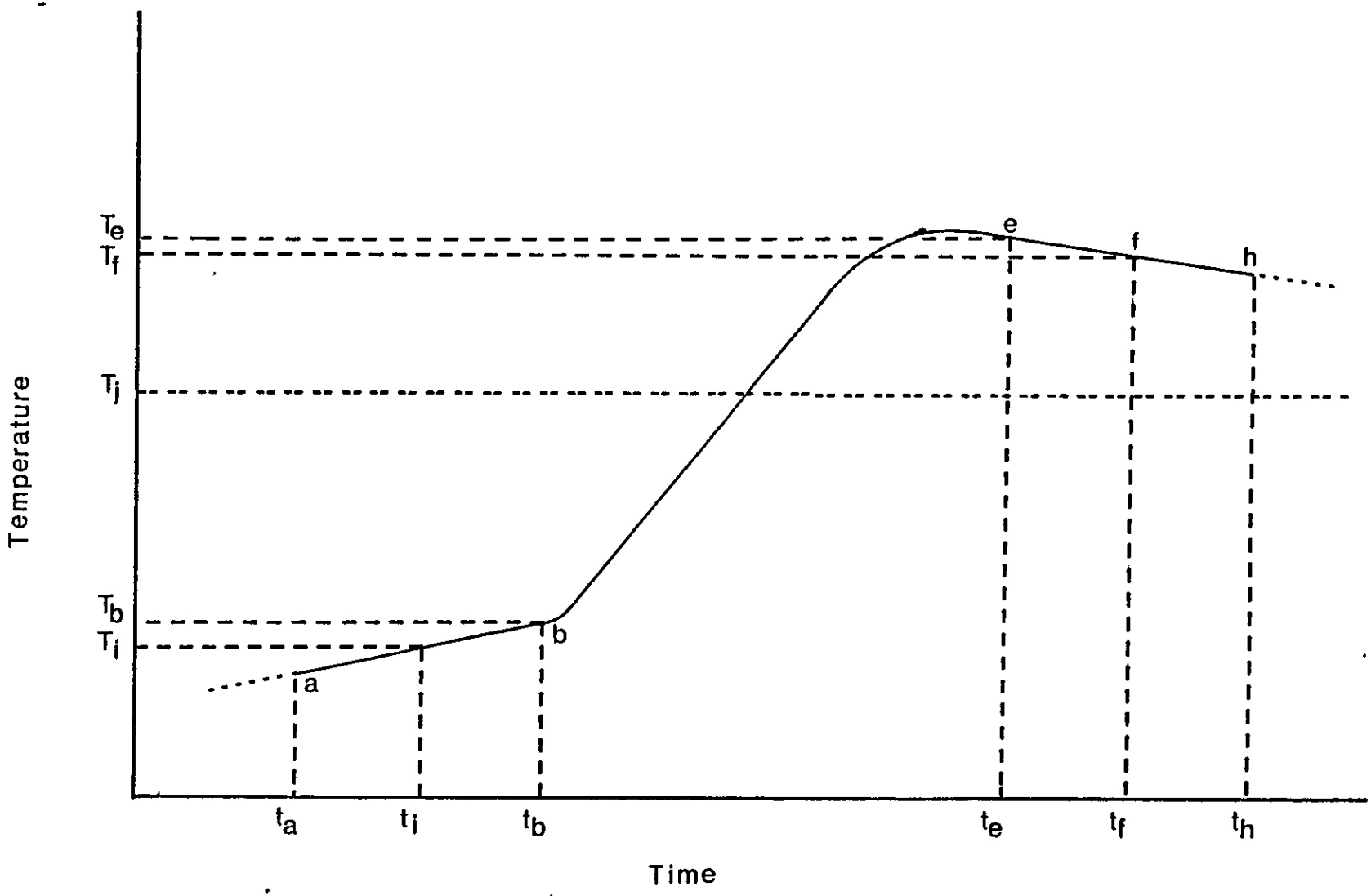


Fig 3.8 Typical time-temperature curve for a calorimetric experiment

is added to the calorimeter (or heating period plus the time necessary for the calorimeter to reach thermal steady state, for an electrically heated calorimeter); 3) an after period in which the calorimeter temperature change is again entirely due to thermal leakage.

Temperature-time readings may start when the calorimetric system has reached a thermal steady state. Starting point, Figure 3.8, is a and, during the fore period to b, a linear behaviour is observed. Input of electrical energy begins at b and has been completed before point e. During the after period, from e to h, the calorimetric curve is linear again.

In the discussion that follows the nomenclature used is that of Coops, Jessup and Van Nes (1956).

T_j, temperature of the surrounding thermostat bath (jacket temperature)

T , temperature of the calorimeter at time t

T_∞, temperature which the calorimeter will approach if the final period is prolonged to infinity

k, is the leakage modulus

μ, is a constant representing heat transfer across any leads extending out to room temperature

g, slope of the initial or final linear periods.

μ, k and T_j are assumed to be constant and although this is never strictly true, the net effects of deviations can be made very small if similar conditions are used for the calibration and the experimental measurements (Gunn, 1971).

Supposing the calorimeter heat exchange follows Newton's Law then the rate of temperature change caused by thermal leakage is proportional to the temperature difference between the calorimeter jacket and the vessel, $T_j - T$. The rates of temperature change during the fore and after periods can thus be written as:-

$$\frac{dT}{dt} = \mu + k\,(T_J - T) \qquad (2.8)$$

when,

$T = T_\infty$, $\frac{dT}{dt} = 0$ therefore,

$$g = \frac{dT}{dt} = k\,(T_\infty - T) \qquad (3.2)$$

for the slopes of the linear parts of the calorimetric curve. Thus for the initial and final periods we have respectively:

$$g_i = \frac{dT}{dt} = k\,(T_\infty - T_i); \quad g_f = \frac{dT}{dt} = k\,(T_\infty - T_f) \qquad (3.3)$$

where,

T_i, mean temperature of the initial period,.

T_f, mean temperature of the final period.

If g_i and g_f represent the values of $\frac{dT}{dt}$ at the mean temperatures T_i and T_f respectively, then:

$$k = (g_i - g_f)/(T_f - T_i) \qquad (3.4)$$

k is a constant, at a given temperature, provided the heat capacity of the system does not change (Wadso, 1966).

The temperature change during the main period of the calorimetric experiment Te-Tb, may be considered to be made up of two terms:

$$Te - Tb = \Delta T_{corr} + \Delta T \,, \qquad (3.5)$$

ΔT_{corr} is the corrected temperature rise and ΔT is the temperature loss due to heat leakage.

ΔT is obtained if equation 3.2 is integrated between the time limits t_b and t_e:

$$\Delta T = \int_{t_b}^{t_e} k\,(T_\infty - T)\,dt \qquad (3.6)$$

this integral equals (Wadso, 1966),

$$\Delta T = k\,(T_\infty - T_m)\,\Delta t, \qquad (3.7)$$

where T_m is the calorimeter wall mean temperature during the main period and Δt is the length of the main period.

Combining equation 3.7 and equation 3.3 we obtain

$$\Delta T = - \left[g_f + k (T_f - T_m) \right] (t_e - t_b) \qquad (3.8)$$

When n temperatures, T_r, are measured (or obtained by interpolation between observed values) at equal time intervals δt, during the main period, the average temperature is given by the Regnault-Pfaundler method:

$$T_m = \left\{ \left[\sum_{r=2}^{n-1} T_r + (T_b + T_e)/2 \right] \delta t/(t_e - t_b) \right\} \qquad (3.9)$$

e. The evaluation of the existing calorimeter

Twenty individual experiments, using temperature increments of approximately 3°C, were carried out to calibrate the calorimeter over the temperature range 30-80°C. Water was used as a reference substance to calibrate the calorimeter and the heat capacity of diphenyl ether was determined to test the calorimeter accuracy. Both substances have their specific heats known with great accuracy (Ginnings and Furukawa, 1953).

2. Design and construction of an improved calorimeter suitable for the determination of the specific heat of intact fish muscle

The construction of a calorimetric apparatus is not merely a question of establishing the most suitable design from mechanical, electrical and heat flow considerations and drawing a list of specifications for the meterial to be utilised. Although the best design can possibly be reached from theoretical considerations, it is often necessary to compromise by adapting it to suit the materials available, and by taking account of cost and ease of manufacture. It was with these three last conditions in mind that the new calorimeter was designed.

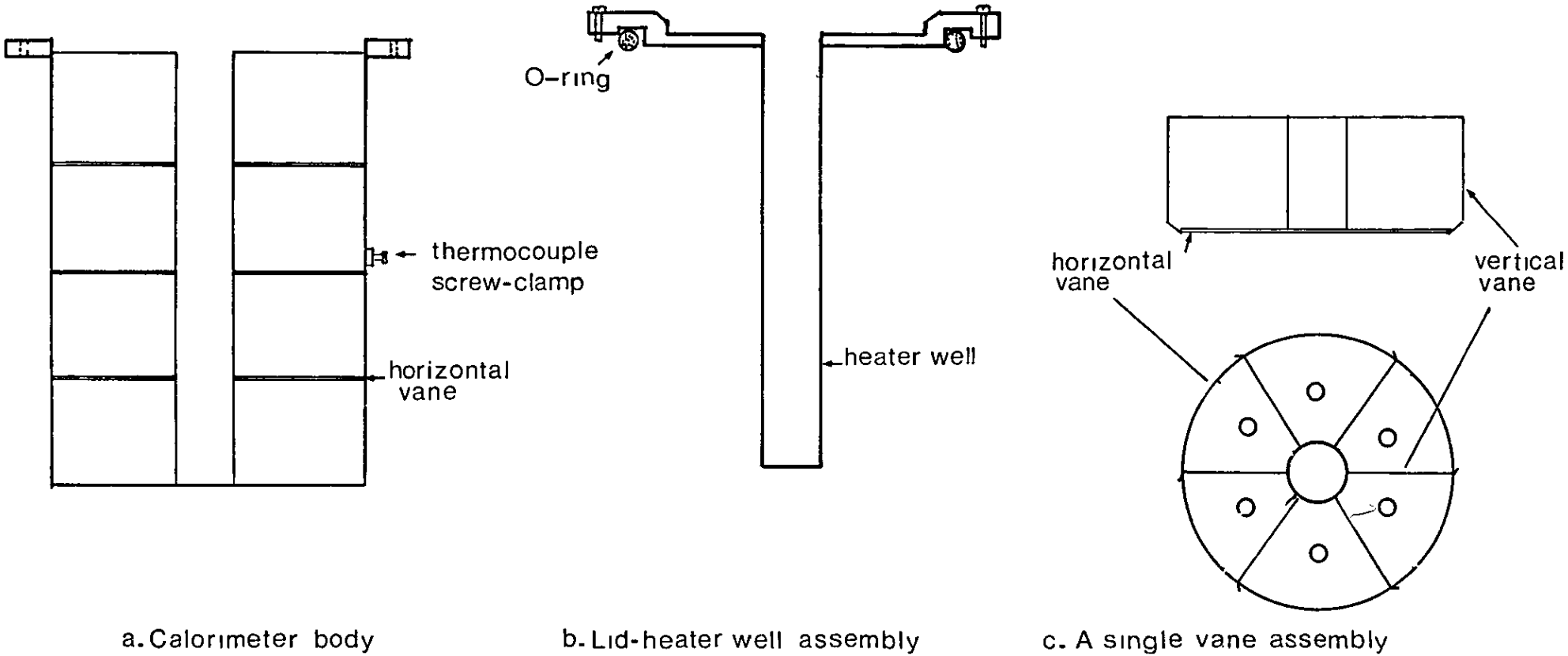
a. The modified calorimetric system

1. The calorimeter body

In order to obtain correct measurements of heat loss and temperature increase during an experiment, it is necessary to minimise temperature gradients inside and over the surface of the calorimeter. Maintaining temperature uniformity over the calorimeter external surface is obviously facilitated by making the vessel from a material having high thermal diffusivity. A metal-like copper, frequently used in the construction of calorimeters, that does have a high thermal diffusivity allows rapid temperature distribution, thus reducing the time required for the calorimeter to reach thermal equilibrium after a heat input. Thus, the calorimeter vessel, Figure 3.9a, was designed to be a copper cylinder approximately 7.5cm long by 5.0cm outer diameter and 0.8mm side wall thickness. The bottom and top walls are 1.2mm thick. A photograph of the calorimeter is shown in Figure 3.10.

Convective heat transfer between calorimeter and jacket which does not obey Newton's Law is eliminated evacuating the space between them. Radiative heat transfer accounts for most of the calorimeter heat leak, and so it is important to make the emittance as low as possible. Polished copper has a higher reflectivity than chromium plated copper, but tarnishes rapidly. As repolishing between experiments is not a convenient solution, apart from altering the calorimeter weight, the internal and external surfaces of the calorimeter vessel and the inside of the jacket are chromium-plated.

The requirement that the calorimeter heat capacity be relatively small compared to that of the sample is not very difficult to fulfil at moderate temperatures; the dimensions of the calorimeter described above are sufficient to meet this requirement while allowing it to withstand moderate internal pressure. The elastic limit of copper



a. Calorimeter body

b. Lid-heater well assembly

c. A single vane assembly

Fig 3.9 The calorimeter body, lid-heater well and vane assembly

Fig 3.10 Calorimeter vessel, vanes, lid and sample punch

is around 15,000lb/in^2 at room temperature. Using a length L of copper in a cylinder of W wall thickness and diameter D, an internal pressure P will cause a force PDL, operating on an area 2WL, so that the stress is PDL/2WL = PD/2W. Supposing from our case, an unlikely pressure of 43.5lb/in^2 and with that D = 1.97in and W = 0.03in the stress is approximately 1428lb/in^2, indicating that we have a safety factor of 10.5 before the stress will cause a permanent set.

2. The lid-heater well assembly

Provision must be made for convenience in filling and emptying the calorimeter of sample and for ensuring a vacuum-tight seal over the temperature range to be employed. Two main considerations arise when designing a calorimeter lid:

1. it must be completely leak-proof when the surrounding space is evacuated,
2. the total heat capacity of the seal assembly must be small in comparison with that of the vessel proper.

This last requirement is connected with the need to ensure temperature uniformity over the whole external surface of the calorimeter vessel.

Normally, as in the previously described calorimeter where the heater was attached to its body, and even if the lid has a small heat capacity, most of the heat required to raise its temperature must come from mechanical contact with the calorimeter body. With this in mind, the seating must be made to give some metal-to-metal contact. To avoid this requirement the new calorimeter lid has the heater well brazed to it; in this manner the need for a metal-to-metal contact between lid and calorimeter body is not critical.

The heater sleeve is a hollow copper tube with wall thickness of about 0.2mm. The lid, which was machined from a copper plate, provides the seat for the silicone-rubber O-ring.

A schematic view of the calorimeter lid-heat well is presented in Figure 3.9b, its photograph is in Figure 3.10.

3. The vanes assemblies

Four removable vane assemblies of thin copper aid in the distribution of heat from the heater tube and in the subsequent re-establishment of a thermal steady state. Three of the four assemblies (Figs. 3.9c and 3.10) have six vertical vanes of 1.8cm height which are soldered on top of a horizontal circular one. The vane assembly that is positioned on the bottom of the calorimeter does not have the horizontal circular vane. These vane assemblies fit the calorimeter body and the heater tube snuggly.

4. The calorimeter jacket

The calorimeter jacket (Fig. 3.11), remained essentially the same as that used in the experiments described in section C.1. To suit the new experimental conditions however, the jacket lid was altered. The length of the tube bringing the current and potential leads out of the calorimetric system was extended. Another 1mm internal diameter copper tube, through which passed the thermocouple attached to the calorimeter vessel, was also brazed to the jacket lid. This arrangement facilitated the replacement of the calorimeter thermocouple without disturbing the assembled current and potential leads. All leads before coming outside the calorimeter system, were brought into close thermal contact with the internal surface of the jacket lid through the use of epoxy resin. This wire covered internal surface was then covered with a thin layer of aluminium foil to reduce radiative heat exchange between jacket and calorimeter. For the same reason the jacket body was chromium plated.

Fig 3.11 Jacket lid with the calorimeter heater ; jacket body

Fig 3.12 Calorimeter in position suspended from the jacket lid

5. The improved bath design

The thermostat bath described previously could not be controlled even to $\pm$ 0.5°C for temperatures above 80°C. As the modifications necessary to improve this thermostat bath could be as laborious as the design of a new one, the decision was made to construct a completely modified thermostat bath.

The following points that must be observed in the design of thermostat baths containing heating elements controlled by thermostats were applied in the construction of the new calorimeter bath.

1. The bath should be compact and of about the same height and diameter. Better circulation with more uniform temperature is obtainable with a round bath than with a rectangular one. The bath should be well lagged through the use of insulation, and precautions taken to diminish thermal leakage to the bath from external sources such as stirrers.
2. Thorough stirring, with rapid circulation of the liquid past the heater and temperature sensing element is the primary requisite for good control. The thermostat must be placed where it can derive full benefit of the circulation. Placing a controlling element midway between a controlled mass and a heater may solve the problem of providing both moderate excursions during control cycles and fast response to thermal disturbances (Kutz, 1968). In addition, it is important that the temperature sensing element or thermoregulator be sensitive and rapid in response.
3. A well diffused primary heater is required to bring the temperature of the bath to a value just below that at which it is to be controlled. The thermostat should control a secondary heater which should be lag free, i.e., heat up and cool down rapidly, as diffused as possible and capable of supplying the additional heat to bring the

bath liquid to the desired temperature. The use of this secondary heater reduces local overheating of the liquid when the heater is on and avoids overshooting of the control system. A general recommendation is that the wattage of the heater should be as low as will maintain the bath at the desired temperature. When using oil as thermostat fluid, control is not usually as good as with the use of water, because of the low heat capacity of oil compared to that of water, so that special attention must be paid to the points mentioned above. Figure 3.13 is self-explanatory in presenting the design of the bath constructed. Figure 3.14 shows thermostat controls.

b. Energy measurement

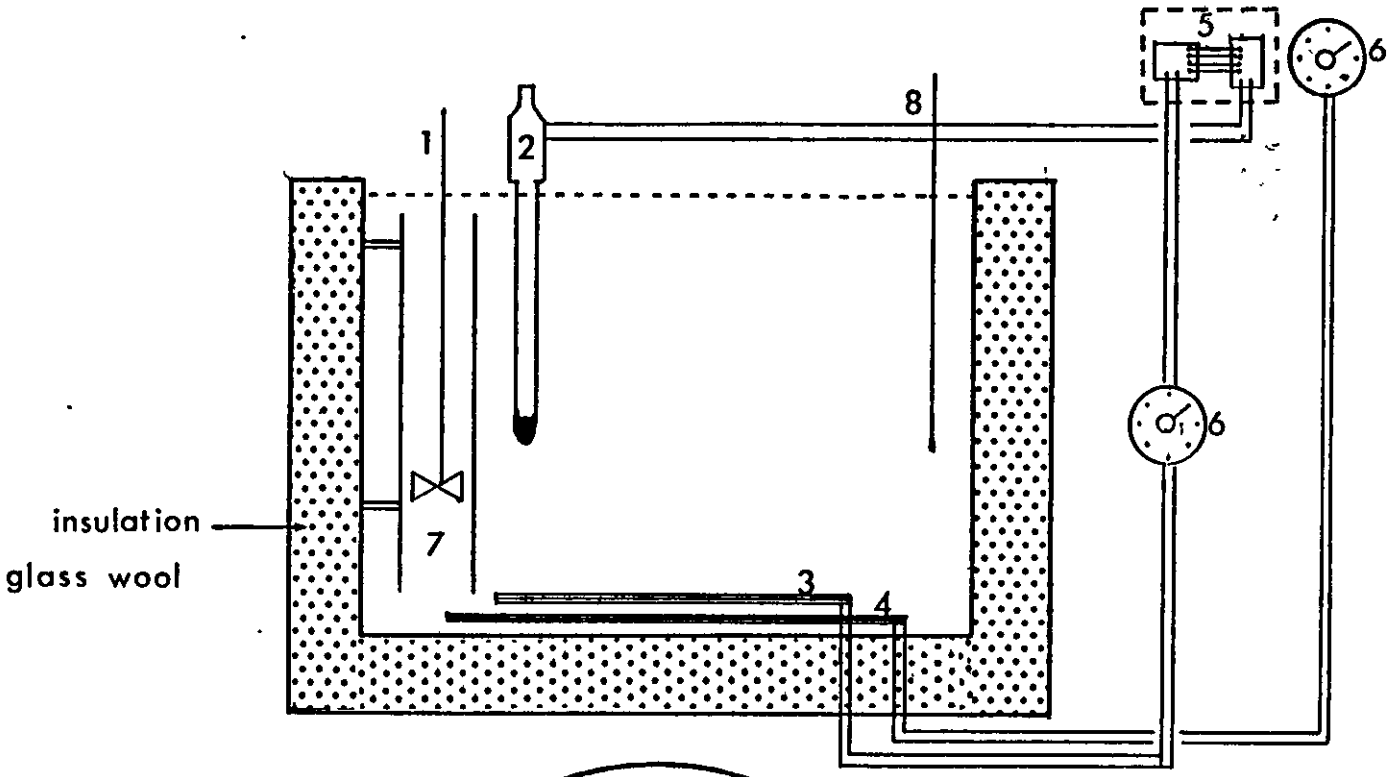
1. The heating element

One basic principle is to use the greatest possible length of wire, to minimise the heat transfer rate per unit length of wire. However, this has to be balanced against the need to keep the heat capacity of the wire and its mandrel small, to reduce thermal lags which influence the thermal equilibration time of the whole calorimeter vessel. The heater resistance wire should preferably have a small temperature coefficient of resistance, and so manganin, constantan, evanohm or other nichrome alloys are suitable. The resistance of the heater should also be large enough to ensure that the power developed in the current leads is only a very small fraction of that developed in the heater resistor.

The above are the basic principles that were followed in the manufacture of the new calorimeter heater. The heater (Fig. 3.11) was made of enamelled nichrome wire wound bifilarly, to prevent reactance effects, in helical grooves on a hollow copper mandrel.

2. Energy measurement ciruitry

The following modifications were introduced in the set-up for energy measurement (Fig. 3.7b):



1. stirrer
2. mercury contact thermometer
3. low wattage heater
4. high wattage heater
5. electric relay
6. variac
7. stirrer tube
8. monitoring thermocouple

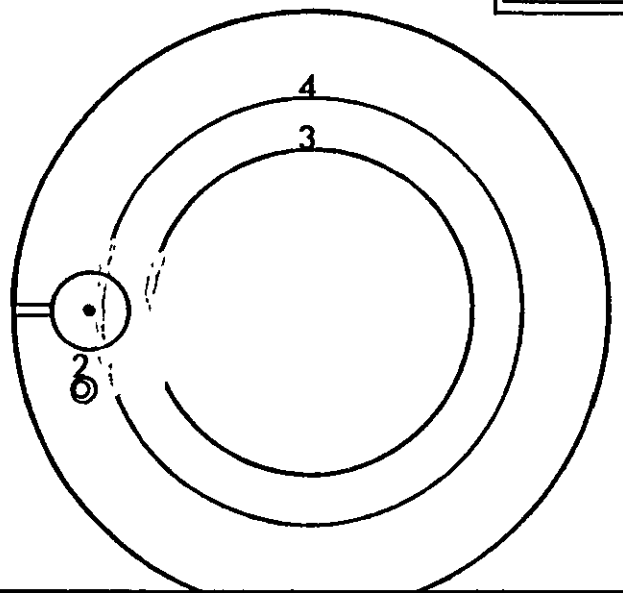


Fig 3.13 The calorimeter system thermostat bath

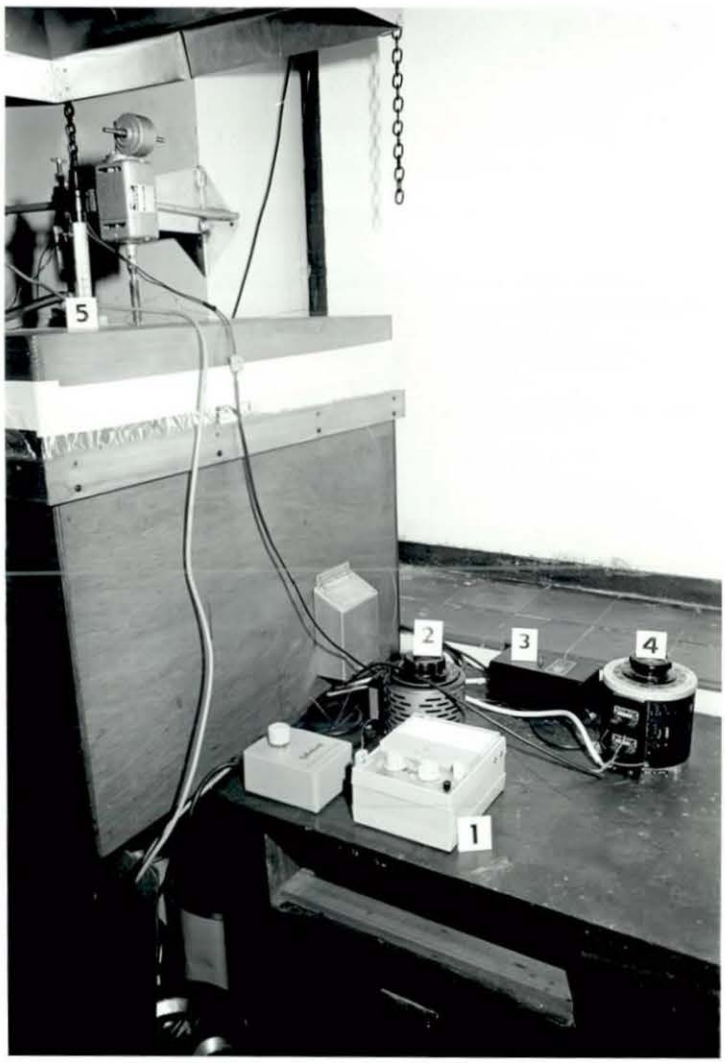


Fig 3.14 Calorimeter thermostat bath controls

1. electronic thermometer
2, 4. variac
3. electric relay
5. mercury contact thermometer

i. Potential leads, to evaluate the potential drop across the calorimeter heater, instead of being attached at the mid points of the two current leads, as in the previous set-up, are fixed to one current lead at the heater, and to the other current lead at the jacket. In this way it is reckoned that it is not necessary to correct for the energy developed in the heater current leads between calorimeter and shield (Stout, 1968).

ii. A dummy resistor, with the same wattage as the calorimeter heater was positioned as alternative to the latter, in order to allow the circuit to stabilise before the power is switched to the calorimeter heater at the start of the heating period.

iii. The resistors that constitute the potential divider were substituted by precision resistors with temperature coefficient as low as -5ppm/°C. The resistances of these resistors were increased, in relation to the previous ones, to lessen the magnitude of the current entering the potential divider.

iv. A high precision battery operated null detector, Cropico type ND4, with a full deflection scale of 0.3uV at maximum sensitivity was substituted for the one used in the previous calorimetric set-up.

The instruments used for energy measurement can be seen in Figure 3.15 which is a photograph of the whole calorimetric set-up.

c. Temperature measurement

Temperature measurement is the most important one in all calorimetry. The most common thermometers used in calorimetry are thermocouples and resistive elements.

Thermocouples

The emf generated by a thermocouple when one of the junctions is maintained at a temperature T_1 while the other is held at a different temperature T_2, is a function of the temperature difference T_1-T_2. The

Fig 3.15 Calorimeter bath, energy and temperature measurement apparatuses

1. Accumulator
2. Vernier potentiometer
3. Null detector
4. Potential divider
5. Dummy resistor switch
6. Standard cell
7. Standard resistor
8. Rheostat
9. D.C supply
10. Cold junction
11. Thermostat bath
12. High vacuum pump

simplest form of a thermoelectric thermometer consists of a thermocouple of two dissimilar metals, and an instrument suitable for measuring the emf produced by the couple. Because of its large emf gradient e.g. 15uV/°C at -200°C to 60uV/°C at 350°C, and, therefore, high sensitivity to temperature change, the copper-constantan thermocouple is in wide use in temperature measurement in calorimetry.

Although copper-constantan thermocouples are not suitable for temperatures above 350°C, because of oxidation of the copper, they are widely used for accurate measurements below 350°C.

The output from a thermocouple is best measured by means of a potentiometer and null detector, since only in this way is its reading entirely independent of lead resistance.

Resistive elements

Resistive elements include platinum resistance thermometers and thermistors. The former consists of a coil of pure platinum wire, wound on a mica support and enclosed in an appropriate metal or glass casing. Usually the resistance change per degree Celsius is about 0.1 Ω hence 0.001 Ω corresponds to 0.01°C, so that the resistance must be measured very accurately in order to obtain high sensitivity. For this purpose a high precision d.c Wheatstone bridge is used. Thermistors are devices, usually in the form of small beads, made by sintering mixtures of metallic oxides such as those of manganese, iron, copper, nickel and cobalt. Thermistors are produced in a variety of shapes and sizes with the two leads embedded permanently in the element. The most striking characteristic of the thermistor is its very high negative temperature coefficient. Comparing a thermistor with a platinum resistance thermometer, a rise of 1°C, from 20 to 21°C, causes a fall of 100 Ω in the resistance of a 2,000 Ω thermistor compared with an increase of 0.1 Ω in the resistance of a platinum coil. The consequence of this is that a comparatively

inexpensive Wheatstone bridge and sensitive galvanometer are quite adequate to detect a change of the order of 0.001°C using a thermistor. The main disadvantage of the platinum resistance thermometer is the expense of the thermometer bridge. Disadvantages of thermistors are their sensitiveness to changes in light intensity, to pressure changes and to shock, a tendency to drift and secular change in resistance, these last two being important in high precision work.

The construction of thermocouples is considerably easier than the construction of a reliable resistance thermometer; thermocouple materials are inexpensive and widely available, so that in cases where thermometers of special form are required, thermocouples are more convenient. So for the sake of simplicity, allied to the availability of a potentiometer and null detector, temperature measurements in these experiments were carried out using copper constantan thermocouples.

The calorimeter temperature is measured by a single copper-constantan, 32 s.w.g thermocouple screw-clamped to the calorimeter body at half height. The bath temperature is monitored by another identical copper-constantan thermocouple placed inside a glass tube filled with vegetable oil and introduced into the bath symmetrically to the contact thermometer. The cold junctions of these thermocouples, placed in oil filled glass tubes, are immersed in a Dewar flask filled with a mixture of distilled water and melting (distilled-water) ice.

The thermocouples were calibrated over the range 30-120°C against a mercury glass thermometer holding a NPL certificate of compliance. Thirty four points were measured, each point being read three times, when the mercury thermometer with the thermocouples attached to its bulb was immersed into the bath at thermal equilibrium.

d. The determination of specific heat of herring as a function of composition and temperature

The influence of composition and temperature on the specific heat of herring muscle was tested by determining the specific heat of muscle of two different fat contents over the temperature range 35-112°C approximately, using the calorimetric system described above. For this purpose the calorimeter was calibrated by carrying out 10 measurements of the specific heat of a gelatinised solution of 0.8% agar over the temperature range 40-70°C. The use of agar had the aim of reducing convection and making conditions inside the calorimeter, as far as possible, similar to those prevailing when it is filled with fish.

Thirty three individual experiments were carried out for low fat content samples, i.e. fish with about 8% fat and 71% moisture, and 27 experiments for high fat content samples having approximately 16% fat and 64% moisture. In each of these experiments temperature increases were kept around 3.5°C so that heat exchange due to radiation and conduction follows Newton's Law.

1. Filling the calorimeter

In a single calorimetric run samples taken from skinless fillets of about ten fish were used. Portions were taken from top, middle and tail parts of the fillets with a stainless steel punch (Fig. 3.10) designed to cut them to a shape that fits the vanes snuggly. The vane assembly without the horizontal circular vane was placed at the bottom inside the calorimeter and the portions, cut as described above, are put into place, some of them being trimmed with a scalpel blade to fit the vane height. Another vane assembly was placed, with its horizontal vane down, on top of this bottom one and the operation was repeated, consecutively until the calorimeter was filled.

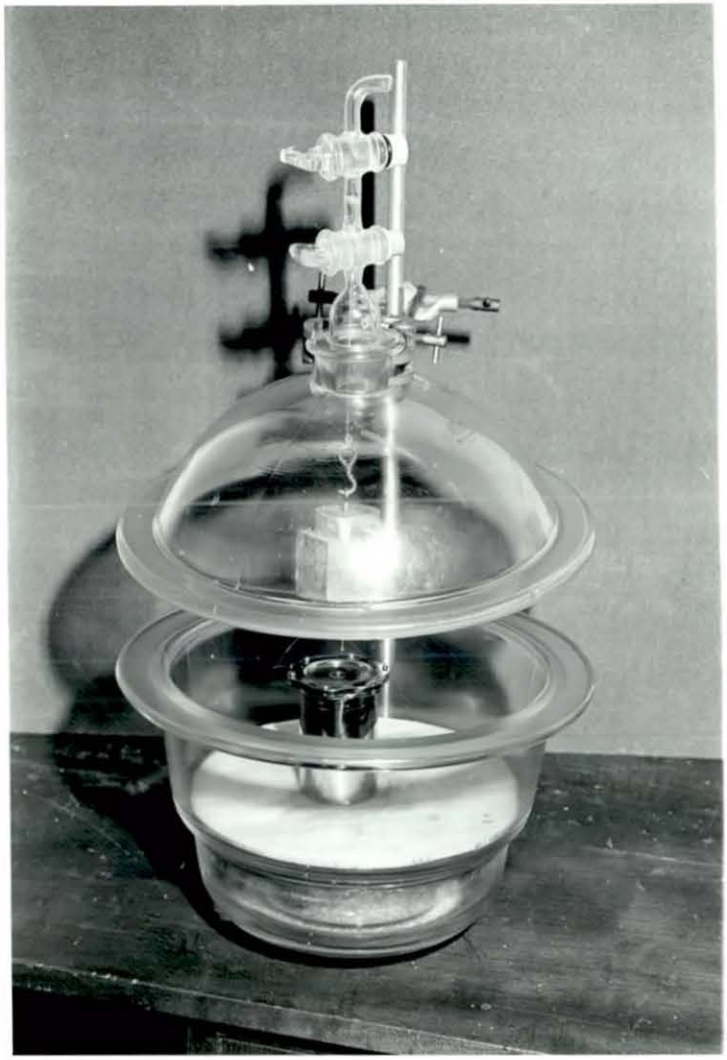


Fig 3.16 Calorimeter vacuum sealing device

In order to reduce pressure inside the calorimeter vessel by eliminating air and thereby diminish the possibility of water vapour leakage during the experiments at higher temperatures, a device was designed which permitted the vacuum sealing of the calorimeter.

The device, Figure 3.16, consisted of a desiccator with two taps, one allowing the connection of a vacuum pump and the other one permitting the lifting and lowering of a heavy lead weight by a hook tied to a nylon thread wound on a groove cut in the barrel of the tap.

For the purpose of vacuum sealing, the calorimeter containing the sample was placed inside the desiccator on top of a thick wooden board with its lid in place but unscrewed. When the vacuum inside the desiccator reached the desired value the lead block was lowered on top of the calorimeter lid, the vacuum pump disconnected, and air allowed to enter the desiccator by fully opening the upper tap. With the lead block still in position the calorimeter lid was then screwed down tight.

2. Moisture and fat determinations

Moisture and fat determinations were carried out using the methodology described in section A.5 after each calorimetric run. The fish inside the calorimeter was minced and thoroughly stirred with a glass rod and 3 samples of 10g each were used for the moisture and fat analysis.

CHAPTER 4

RESULTS AND DISCUSSION

A. Water and Fat Holding Capacity

An exploratory experiment in which herring fillets of moisture content about 77-79% were cooked for 45 minutes inside polyethylene bags in a water bath at several temperatures, indicated that weight losses might not proceed regularly with temperature. These results, which are shown in Figure 4.1, revealed a large increase in the ability of the fish to retain its weight (mostly water) when heated to about 55°C. As the fresh fish utilised in these preliminary measurements were from different catches for the different temperatures studied, and therefore subject to large variations in size and state of freshness, the validity of these results was limited. However, the increase in weight retention at 55°C was unusual enough to justify an extension of these experiments under more controlled conditions, as described in Section A of Chapter 3, examining also the influence of duration of heating.

Chapter 3, Section A described how by cooking a number of fillets, measuring weight losses, sampling for measurement of fat and water content before and after pressing, the weight losses and the amounts of water and fat released by cooking and pressing were measured. These results are tabulated and analysed in this Chapter in the following sequence. First loss of water is described in detail followed by loss of fat, both being related to previous measurements and to the theories on water and fat holding described in the literature review. Then weight losses are discussed because such losses are of economic significance in technological processes and because many studies of heat processing have measured only the weight losses. Finally the amountand composition of the fluids released

Table 4.1 Influence of temperature and holding time on release of water by cooking of LFC muscle

Temperature (°C)	Loss of water in relation to initial water weight (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.1	4.81	6.19	9.19	7.46	6.91
40.6	6.85	8.81	10.82	11.00	9.37
46.2	9.76	9.66	14.72	13.19	11.83
51.5	8.95	11.01	8.83	10.07	9.71
56.1	7.28	7.50	8.68	6.85	7.58
61.5	6.00	6.59	6.17	6.35	6.28
65.8	6.44	8.70	9.35	5.44	7.48
75.6	9.07	11.68	12.28	11.56	11.14
85.8	10.38	13.52	9.76	10.57	11.06
101.4	16.17	17.91	(17.84)	15.67	16.90
112.7	26.43	22.47	(24.67)	21.36	23.73
Mean	10.19	11.28	12.03	10.87	11.09

Statistical significance of the influence of temperature and holding times

Factor	F value	Degrees of freedom for F	Least significant difference at the 5% level
Temperature	41.91***	(10,28)	2.3
Holding times	2.56 (ns)	(3,28)	1.4

*** significant at the 0.1% level

** significant at the 1% level

* significant at the 5% level

Values in brackets have been statistically estimated

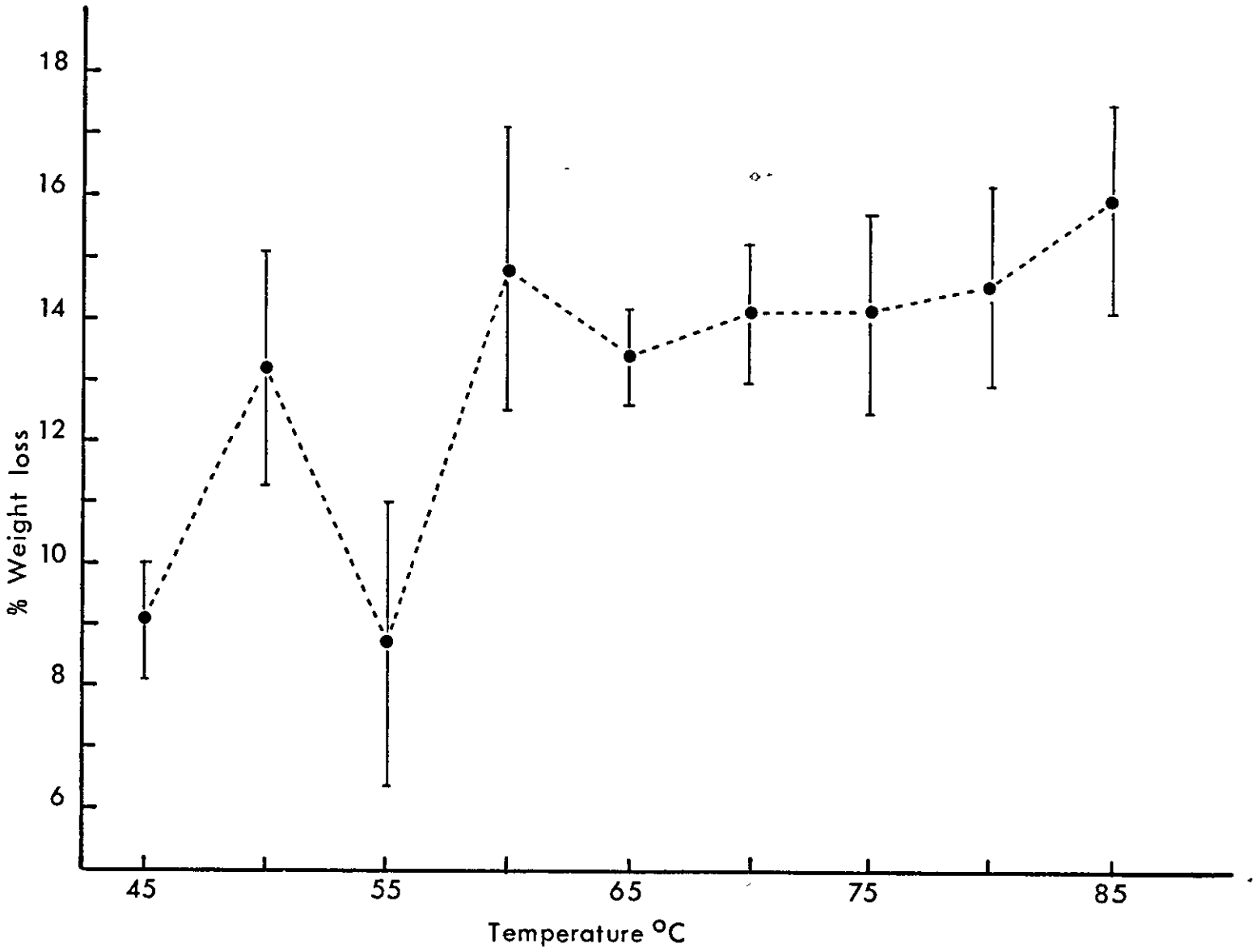


Fig 4.1 Weight losses[a] by unskinned herring fillets when cooked at the plotted temperatures for 45 minutes

a. average for six fillets

on cooking and pressing are dealt with. Generally the results for the two batches of fish, of low and high fat content, are given separately and are then discussed together.

1. Release of water

1.1 Release of water on cooking

In lean fish most of the weight loss caused by physical processes is represented by the loss of water, and both parameters can therefore be used interchangeably to evaluate the effect of heat on the release of water from muscle. Many researchers have tried to correlate water loss, as estimated by weight loss, with protein changes in the muscle. However, when utilising fatty fish muscle, such as the HFC fish used in this study, it is important to use the actual water loss when attempting to relate WHC to protein changes.

1.1.1 Low fat content samples

Analysis of variance of the data presented in Table 4.1, revealed that the influence of temperature on the water losses due to heating only, was statistically significant at the 0.1% level, whereas the influence of the duration of the heat treatment up to 1 hour was not significant. From Figure 4.2 showing data averaged over the 4 holding times studied, it can be observed that water losses increased rather sharply from 35 to 45°C and then decreased with temperature, reaching a minimum around 60°C. Only when the temperature reaches 100°C does the figure for water loss exceed that at 45°C. It must be pointed out that although the statistical analysis has indicated that time had no significant influence on the release of water, the data in Table 4.1 suggest that time might have some bearing on the release of water. For temperatures below 50°C water losses were consistently larger for longer heat treatment.

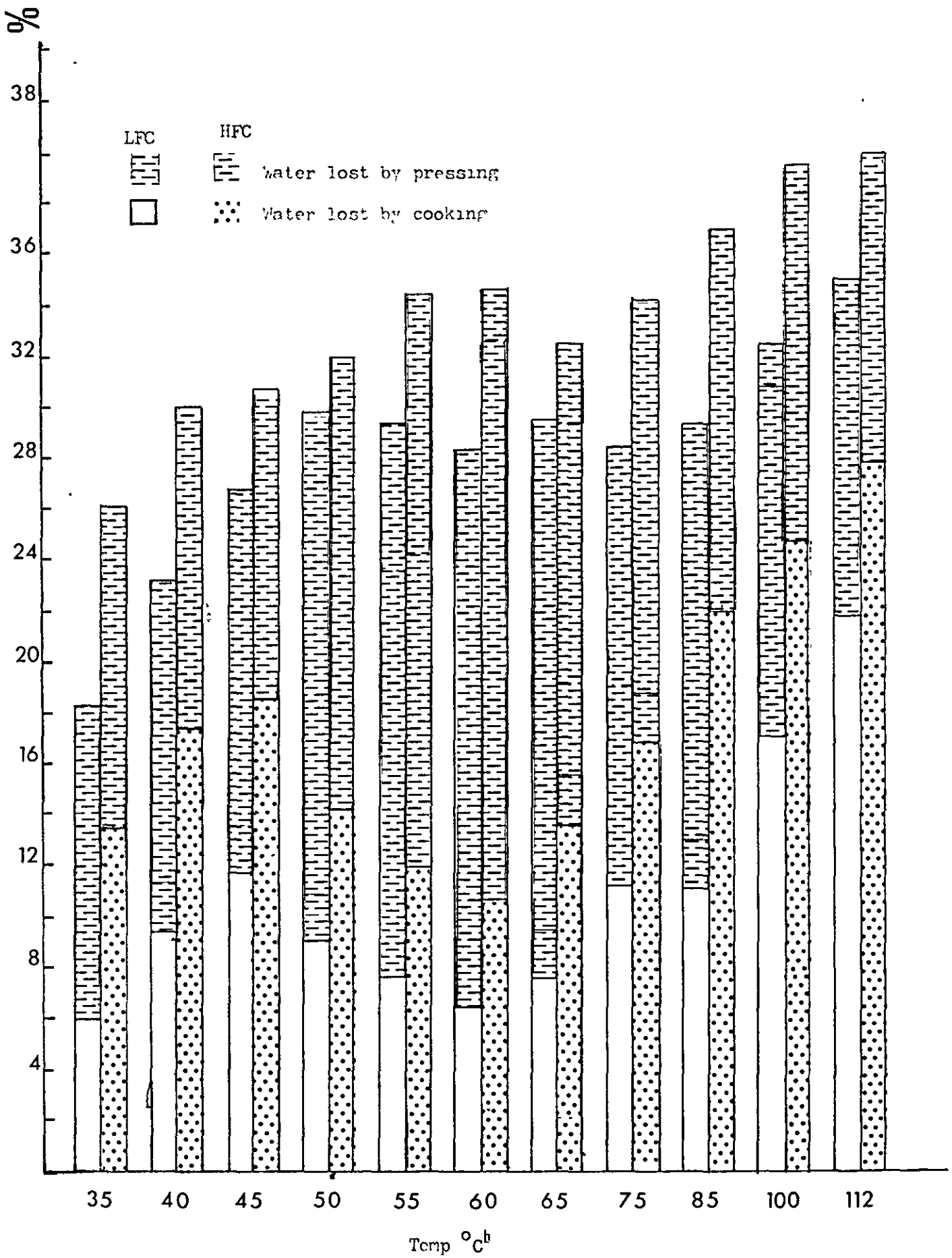


Fig. 4.2 Loss of water[a] in relation to the original amount in the raw muscle, by cooking and by pressing

a - averaged over the four holding times studied

b - rounded temperatures; actual ones as in Fig 4.3

1.1.2 High fat content samples

In the same fashion as for LFC fish, statistical analysis of the data shown in Table 4.2 indicated that temperature influence on water losses for high fat content fish was significant at 0.1% level but the influence of holding times was not significant.

Water losses as a function of temperature, Figure 4.3, were generally distributed in the same manner as for low fat content fish: losses increased with temperature up to 45°C, and then decreased reaching a minimum at about 60°C. The figure for water loss at 45°C was exceeded only when the cooking temperature was as high as 85°C.

Unlike LFC samples there was no obvious indication that time had any influence on the release of water for temperatures below 50°C.

1.1.3 Discussion of water release by cooking

Two remarkable findings emerged from the analysis of these cooking results. One was the fact that HFC muscle had larger losses of water for all temperatures studied than LFC muscle. The other, more important, finding was that 45 and 60°C were the cooking temperatures at which, for both types of muscle, maximum and minimum release of water respectively occurred. These findings are discussed in the sequence given above.

The temperature distributions of water loss during cooking were remarkably similar for both batches of fish. Figures 4.2 and 4.3 gave strong evidence that the observed points of maximum and minimum for water loss as a function of temperature were genuine.

Unexpectedly the fish having a high initial moisture content i.e., high water-to-solids ratio, lost less of the original water during cooking (Fig. 4.2) than the low moisture content batch, and, as shown in Figure 4.3, it also lost less of its water expressed as a percentage of raw material. This may be an indication that the mechanism of water release by heat treatment is not wholly dependent on the amount of water

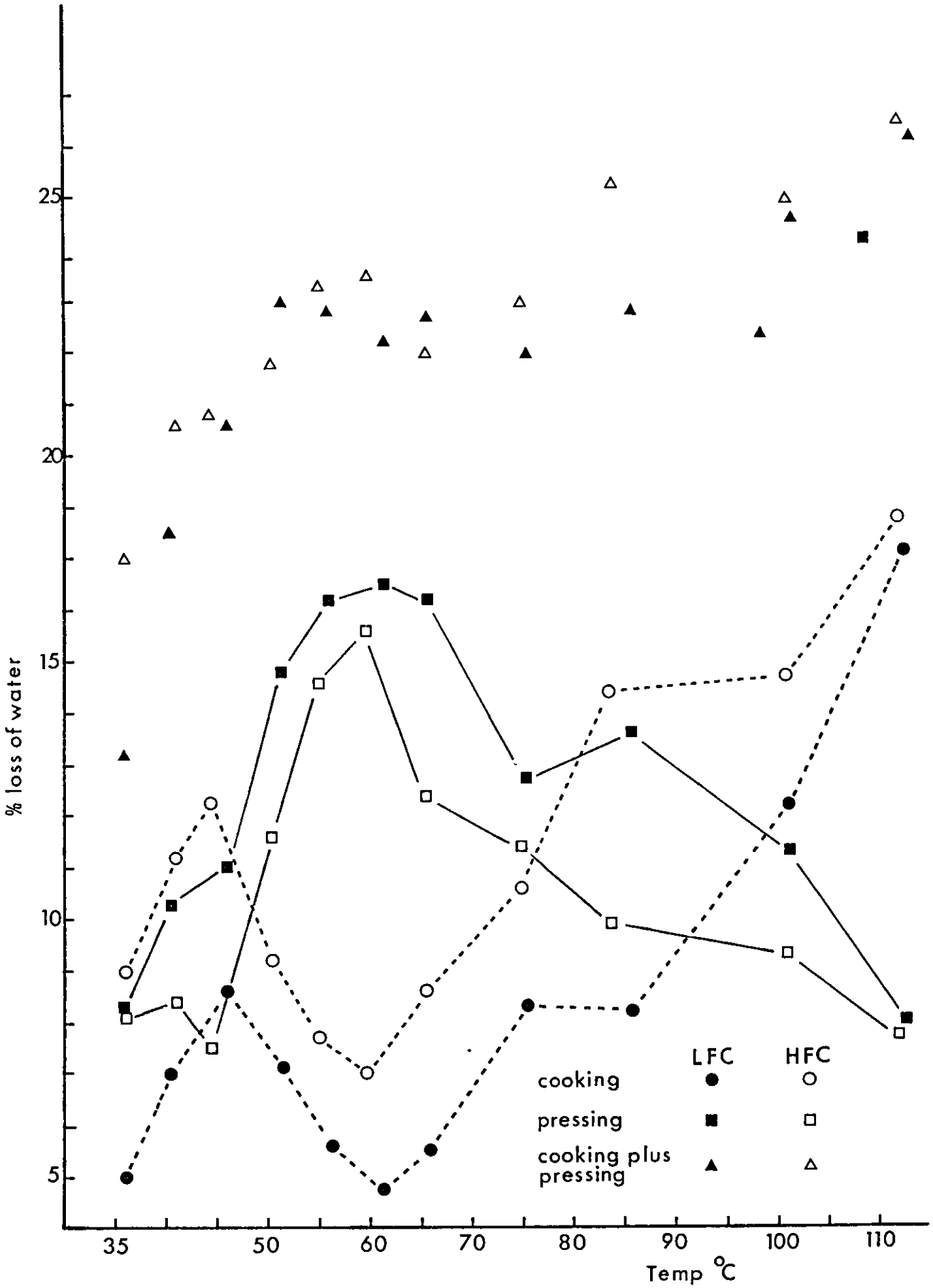


Fig 4.3 Losses of water[a] during cooking and pressing, in relation to the raw muscle weight, as a function of temperature

a- averaged over the four-holding times studied

Table 4.2 Influence of temperature and holding time on release of water by cooking of HFC muscle

Temperature (°C)	Loss of water in relation to initial water weight (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.2	10.42	13.88	17.16	13.60	13.76
41.1	17.27	16.52	16.94	19.11	17.46
44.5	14.57	18.03	18.80	23.27	18.66
50.4	15.13	12.93	12.04	17.02	14.28
55.1	13.24	10.96	10.76	12.76	11.93
59.7	10.94	10.06	11.35	10.77	10.78
65.6	10.27	14.08	13.51	15.48	13.33
75.0	13.95	17.63	17.67	14.82	16.01
83.7	22.93	21.58	22.38	20.60	21.87
101.0	23.53	22.46	23.73	23.58	23.32
112.0	27.25	26.70	28.33	28.40	27.67
Mean	16.31	16.80	17.51	18.12	17.19

Statistical significance of the influence of temperature and holding times

Factor	F value	Degrees of freedom of F	Least significant difference at the 5% level
Temperature	34.36***	(10,30)	2.6
Holding times	2.15 (ns)	(3,30)	1.6

held by the protein component i.e., a higher water-to-protein ratio does not mean that some of this water is more loosely bound to the tissues. This is contrary to the argument (Strentrŏm, 1965) that if the fat free residue is considered to be the water binding component of the fish muscle, it should hold water more strongly when the fat percentage is high.

As already pointed out the distributions of water losses with temperature were remarkably similar for both types of muscle studied, with the exception that a plateau on the increasing losses occurred at 75-85°C for LFC samples and at 85-100°C for HFC ones. This difference in shape of the two water loss curves over the 75-112°C interval seems genuine and may indicate that whatever changes are occurring at these temperatures affect the two samples differently.

Table 4.3 compares values of water losses found in this study with those reported in the literature. Keeping in mind the differences that might arise from the use of different species, the water losses during cooking of HFC fish, expressed as % of the initial water weight, agree with the values reported by Ward et al. (1977), for comparable temperatures and fish of the same approximate composition. They were generally discrepant from the values found by Kushtalov and Saduakasov (1971) in their study. The water losses reported by Meesemacker and Sohier (1959) on steaming sardines with 14% fat at 100°C were reasonably comparable to our results. In agreement with the conclusion reached by the same authors on cooking sardines, the results in this work indicate that lean herring would need a more intensive heat treatment during cooking to lose the same amount of water as fatty ones.

Table 4.3 Comparison between water losses found in this work and those in the literature*

	water losses as percentage of original water								
Temperature (°C) Authors	40	45	50	60	70	75	80	90	100
Kushtalov and Saduakasov (1971)**	6.6	25	15	16.6	18.0	-	26	28	-
Ward et al. (1977)	-	-	-	10.6	-	-	14.5	-	23.1
Cutting et al. (1956)	-	-	-	-	-	-	-	-	34
Meesemacker and Sohier (1959)	-	-	-	-	-	-	-	-	27
This work (LFC)	9.4	11.8	9.7	6.3	-	11.1	-	-	16.9
This work (HFC)	17.4	18.6	14.3	10.8	-	16.0	-	-	23.2

** Minced catfish (22.5% fat(?)) cooked in sealed test tubes immersed in a water bath.

* Fish species used and methods of cooking, as described in Table 4.11.

Several conjectures may be put forward to try to explain this difference in water holding capacity between LFC and HFC herring muscle. First it must be said that since total loss of water (to be discussed later) was similar for both types of muscle only the relative amounts released during cooking and pressing seemed to have been influenced by the fat content. Considering there was little difference in water/solids ratio between both muscles this factor can be excluded as a possible explanation. Possible hypothesis might be given as follows.

a. Change in WHC of muscle proteins

i. It is possible that the thermal stability of the myofibrillar proteins alters with season in such a way that the proteins of low fat content have a higher WHC than those of high fat content. Further support to this view comes from the different organoleptical behaviour, demonstrated by subjective observations, shown by the two muscles and which will be discussed later.

ii. Variation in nature of proteins, e.g. change in connective tissue content with season may mean differences in the extent to which it is hydrolysed.

b. Structural reasons

i. Growth of fish may result in a more open structure so that both fat and water are more easily released.

ii. 'Cooperative' effect - As more fat, which is situated among muscle fibres, is liberated the tissue structure becomes more open and more water can flow out.

The outstanding features of the data presented in Figures 4.2 and 4.3 are the large drop in water holding capacity during cooking at around 45°C and the remarkable increase about 60°C. Kushtalov and Saduakasov (1971) also found that minced flesh of catfish on being cooked showed a minimum of water holding capacity at 45°C and a maximum between 50 and 60°C (See Table 4.3), this latter effect being attributed to swelling of collagen. On the other hand, Tulsner (1976) pointed out that his results for water binding capacity (WBC) during heating of minced cod levelled out as early as 40°C, remaining constant on the whole up to 90°C, the highest temperature studied.

These irregularities at 45 and 60°C assume a more interesting aspect when it is noticed that these temperatures coincide with those reported

by several authors to be ones where the most dramatic changes in muscle proteins occur. Simidu and Takeda (1951) found that fresh fish cooked at various temperatures for 30 minutes and then immersed in a salt solution presented a maximum and minimum of water uptake after cooking at 45 and 60°C respectively. They attributed the drop in hydration at 60°C to the coagulation of the sarcoplasmic proteins. Further evidence that these changes in hydration are associated with changes in proteins is given by Simidu and Takuma (1951), who found that half of the protein of mackerel, horse mackerel and tuna became insoluble at 40-45°C, and all of it became insoluble by 65°C, and by Suyama (1951) whose studies indicated that myosin coagulates at 45°C. Mohr (1971) found that thermal shrinkage of cod connective tissue, the collagen ⟶ gelatin transition, occurs at approximately 40-42°C; this could explain the increase in translucence of cod muscle when heated to 42 ± 2.9°C found by Aitken and Campbell (1969b). In addition, it is worth noting that when actomyosin of carp was heated at various temperatures for 60 minutes in 0.6 M KCl (pH = 6.3) firm gels were formed above 40°C and the gel formed at 60°C was the hardest (Itoh, et al., 1979a). Itoh (1979b) also concluded that reactive SH groups which appeared on the molecular surface by heating, contributed to the gel formation of actomyosin through some bonding between protein molecules.

So a consensus of the work of the authors cited so far might allow one to hypothesise that the drop in water retaining ability of the fish muscle around 45°C might be explained by the coagulation of myosin and consequent shrinkage of the fish tissues expelling water from the cells. The progressive increase in water retention up to 60°C could have been caused by swelling of collagen, as has been suggested elsewhere (Saduakasov and Kushtalov, 1971), since this retained water is easily released by pressing as can be seen in Figure 4.3.

On the other hand, if it is really the case that maximum coagulation of the sarcoplasmic proteins takes place at 60°C, then the increase in WHC around this temperature might have been brought about by the denatured sarcoplasmic proteins forming a layer over the coagulated myofibrillar proteins, and acting as a barrier against water movement to the fish surface. This was the suggestion made by other authors (Bendall and Wismer-Pedersen, 1962) to explain how the denaturation of the sarcoplasmic proteins decreases the solubility of the myofibrillar fraction. In this respect, however, it is advisable to underline that Warrier et al. (1973) reported no precipitation of the sarcoplasmic proteins when protein extracts from Bombay Duck were heated to 100°C; similarly Sadowska and Sikorski (1976) reported that 80% of the sarcoplasmic fraction is still soluble after thermal treatment at 85°C.

Assuming that most of the sarcoplasmic and myofibrillar protein fractions are denatured in the temperature range 40-60°C another hypothesis is that the increase in water holding capacity at 60°C may be caused bygelation of the denatured proteins under suitable conditions in the fish as a result of heating. The ability of the myofibrillar proteins to form an elastic gel upon heating forms the basis for the production of the traditional Kamaboko of Japan . Cooking is usually done at 50°C (Watanabe et al., 1974). Denatured protein gels are formed by a two stage process involving the initial denaturation of native protein into unfolded polypeptides, which then gradually associate to form the gel matrix, if attractive forces and thermodynamic conditions are suitable (Ferry, 1948).

1.2 Release of water by pressing

1.2.1 Low fat content samples

The influence of temperature, as evaluated by statistical analysis of the data in Table 4.4, indicated that, as for cooking, the influence

Table 4.4 Influence of temperature and holding times on the release of water during pressing of cooked LFC muscle

Temperature (°C)	Loss of water in relation to initial water weight (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.1	12.33	14.95	10.30	8.92	11.62
40.6	16.77	11.96	15.39	15.12	14.81
46.2	13.70	14.44	15.37	16.33	14.96
51.5	16.91	19.56	22.47	22.19	20.28
56.1	19.47	22.07	22.41	22.98	21.73
61.5	21.51	21.96	23.39	21.24	22.03
65.8	21.87	20.44	21.02	24.55	21.97
75.6	19.96	17.99	14.93	15.54	17.10
85.8	19.20	18.24	17.35	18.79	18.40
101.4	17.16	14.69	(15.44)	14.25	15.39
112.7	9.43	12.18	(11.03)	11.28	10.98
Mean	17.12	17.13	17.19	17.38	17.21

Statistical significance of the influence of temperature and holding times

Factor	F value	Degrees of freedom of F	Least significant difference at the 5% level
Temperature	17.46***	(10,28)	2.8
Holding times	.04 (ns)	(3,28)	1.7

of temperature was highly significant at 0.1% level whereas the influence of holding time was not significant. As illustrated in Figures 4.2 and 4.3 the distribution of the loss of water during the pressing operation as a function of temperature, was generally inversely related to the distribution of water loss during cooking, except for temperatures between 35-45°C when both pressing and cooking losses are increasing. The temperature range between 50-65°C was that of maximum release of water by pressing, and, from 75°C upwards, as losses during cooking increased those due to pressing decreased. Losses at 100°C and 112°C were smaller than those during cooking at these temperatures.

1.2.2 High fat content samples

As for low fat content samples, the loss of water by high fat content ones during pressing, Table 4.5, was highly significantly influenced by temperature while holding times had no significant effect.

Unlike the low fat samples, the losses due to pressing were lower than the cooking losses up to 45°C (Figs. 4.2 and 4.3). The pressing losses then increased to a maximum around 60°C and fell again, to values much lower than the cooking losses after 85°C was reached.

1.3 The combined effect of cooking and pressing

The effect of temperature on the release of water caused by cooking and pressing combined was statistically highly significant at the 0.1% level for both types of fish (Tables 4.6 and 4.7) but holding times had no significant effect. Using the LSD at the 5% level to test the significance of the difference between the means in Table 4.6, the conclusion is that for LFC muscle the three lower temperatures were different from each other and from all other temperatures studied. From 50°C to 100°C the means were not significantly different from each other, confirming the impression given by Figure 4.3. So the results for the total water losses i.e., caused by both treatments, can be seen to increase up to 50°C, and from there remain constant up to 100°C.

Table 4.5 Influence of temperature and holding times on the release of water during pressing of cooked HFC muscle

Temperature (°C)	Loss of water in relation to initial water weight (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.2	14.23	10.30	11.54	13.60	12.41
41.1	11.89	13.47	12.67	14.21	13.06
44.5	12.16	14.40	11.00	11.08	12.16
50.4	17.70	19.07	18.57	15.77	17.77
55.1	20.10	24.92	22.65	22.28	22.48
59.7	23.89	25.96	23.64	22.90	24.09
65.6	23.05	15.11	21.00	17.51	19.16
75.0	17.85	15.53	19.56	(16.69)	17.40
83.7	15.35	17.95	14.77	12.16	15.05
101.0	14.77	16.30	14.39	13.78	14.81
112.0	11.99	14.33	9.19	12.67	12.04
Mean	16.63	17.03	16.27	15.69	16.40

Statistical significance of the influence of temperature and holding times

Factor	F value	Degrees of freedom of F	Least significant difference at the 5% level
Temperature	17.87***	(10,29)	2.9
Holding times	0.90 (ns)	(3,29)	1.7

For HFC samples the results (Table 4.7) can be considered essentially the same as those for LFC samples, apart from small discrepancies in the distribution of water losses as a function of temperature. Thus, water loss was significantly lower for 35°C than for all other tempuratures studied. From 40 to 50°C values did not differ among each other, nor did they differ from 55 to 85°C. From 85 to 112°C there was no significant differences between the means.

1.4 Discussion of water release by pressing and by cooking plus pressing

Water losses during pressing were much lower than those reported by other authors eg Kushtalov and Saduakasov (1971) and Ward et al. (1977); this operation is responsible for the difference between the total water losses caused by heating and pressing found in this work and those described by the latter authors. Their reported water losses due to pressing were practically constant for the three temperatures they studied whereas in this work, as already indicated, water losses were inversely related to losses during cooking. This last phenomenon was also found in the figures given by Kushtalov and Saduakasov. Although part of the difference between results in this study and the others quoted may presumably be attributed to differences in the way pressing was carried out, the effect of differences in pressures applied may be ruled out since the pressure in this study (30.3×10^6 N/m^2) was much higher than that used by Ward et al. (11.76×10^6 N/m^2). In this respect it is worth mentioning that these authors found that up to 80% of the total liquor was released by applying a pressure as low as 1-2 kg/cm^2.

Two aspects of water release by muscle on pressing arise. First, the amounts of water released by pressing were very similar for the two samples of muscle when expressed as percentages of the original water present (Fig. 4.2). (It was earlier pointed out that the losses during heating were quite different for the two samples, the high fat one

Table 4.6 Influence of temperature and holding times on water release by the combined effect of heating and pressing - LFC muscle

Temperature (°C)	Loss of water in relation to initial water weight (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.1	17.15	21.14	19.49	16.39	18.54
40.6	20.62	20.77	26.22	26.13	23.43
46.2	23.46	24.10	30.10	29. 52	26.79
51.5	25.86	30.57	31.50	32.27	30.00
56.1	26.75	29.58	31.11	29.83	29.32
61.5	27.52	28.55	29.57	27.59	28.31
65.8	28.81	29.15	30.37	30.00	29.58
75.6	29.03	29.85	27.21	27.10	28.30
85.8	29.59	31.77	27.12	29.37	29.46
101.4	33.34	32.61	(33.25)	29.93	32.28
112.7	35.86	34.65	(35.68)	32.65	34.71
Mean	27.09	28.43	29.22	28.25	28.25

Statistical significance of the influence of temperature and holding times

Factor	F value	Degrees of freedom of F	Least significant difference at the 5% level
Temperature	16.59***	(10,28)	3.0
Holding times	2.04 (ns)	(3,28)	1.8

Table 4.7 Influence of temperature and holding times on water release by the combined effect of heating and pressing - HFC muscle

Temperature (°C)	Loss of water in relation to initial water weight (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.2	24.65	24.18	28.70	27.20	26.18
41.1	29.16	29.99	29.61	33.32	30.52
44.5	26.73	32.43	29.80	34.35	30.82
50.4	32.83	32.00	30.61	32.79	32.05
55.1	33.34	35.61	33.41	35.04	34.35
59.7	34.83	36.02	34.99	33.67	34.87
65.6	33.32	29.19	34.51	32.99	32.50
75.0	31.80	33.16	37.23	(34.65)	34.21
83.7	38.28	39.53	37.35	32.76	36.98
101.0	38.31	38.76	38.12	37.36	38.13
112.0	39.24	41.11	37.52	41.07	39.73
Mean	32.95	33.81	33.80	34.10	33.67

Statistical significance of the influence of temperature and holding times

Factor	F value	Degrees of freedom of F	Least significant difference at the 5% level
Temperature	13.48***	(10,29)	3.0
Holding times	0.62 (ns)	(3,29)	1.8

releasing a much higher proportion of its water than the low fat sample.) However, when these pressing losses were expressed as percentages of initial weight, Figure 4.3, they were rather higher for LFC fish, with the consequence that total losses of water as a percentage of initial weight became similar for both types of muscle. Second, the total water loss due to cooking and pressing varied with temperature for both samples to a much lesser extent than the separate losses for cooking and pressing (Fig. 4.3). This fact leads one to suggest that a reasonably constant amount of water is 'loosened' by heat treatment above 60°C but, for some reason not entirely clear, only a part of this water is released by cooking while the remainder is trapped by the muscle fibres only to be released by pressing. It may be speculated that increased heating causes progressive changes in the tissues, like denaturation of the contractile proteins and solubilization of collagen; such changes may reduce whatever physical forces hold water in muscle, with the result that the amount of water 'loosened' inside the tissues increases up to around 60°C. Some of this water is easily freed, the water released during cooking, but the rest is retained in the tissue by swelling of the connective tissue or by gelation of the myofibrillar proteins, this swelling and/or gelation also reaching its peak at about 60°C. At higher temperatures the connective tissue becomes soluble and/or the forces responsible for the 'gel' formation inside the muscle structure are weakened, so that increasingly larger proportions of water are released during cooking than during pressing.

2. Release of fat

2.1 Release of fat on cooking

2.1.1 Low fat content samples

Statistical analysis of the losses of fat during cooking indicated that the influence of temperature was significant at the 5% level. The

means over the 4 times and 11 temperatures studied are presented in Table 4.8 and 4.9 respectively. The complete tables and statistical analysis are shown in Appendix 2. There was considerable scatter in these results when it is considered that the 95% confidence limits for a single value were ± 21.9. Considering that mean values at different temperatures are significantly different at the 5% level only if they differ by at least 15.5, it can be seen from Table 4.8 that only values of fat loss for the 65°C and 112°C treatments were significantly different from the rest, but not significantly different from each other. Similarly for time means a difference between means equal or larger than 9.3 would indicate that they were significantly different at 5% level but as can be deduced from the data in Table 4.9 they did not differ.

2.1.2 High fat content samples

The fat losses of high fat content muscle, Appendix 3 were more consistent than those for low fat muscle as indicated by a much smaller error figure.

The influence of temperature, from the data which are summarised in Table 4.10, was statistically significant at 1% level, and time had no significant influence. The least significant difference at the 5% level for the effect of temperature was 8.7. It appears that the losses at 35, 40 and 45°C were significantly lower than at higher temperatures; the 75°C loss was perhaps also low. From these results it can be inferred that temperature does not influence the release of fat for thermal treatments above 45°C.

2.1.3 Discussion of fat release by cooking

As already pointed out the values of fat loss during cooking of LFC fish were very scattered and in some cases, mainly for low temperatures, there was an apparent increase in the fat content of the cooked

Table 4.8 Influence of temperature on losses of fat during cooking and pressing of LFC muscle in relation to original fat content

Temperature (°C)	Percentage losses*		
	Cooking	Pressing	Total
36.1	0.41	11.87	12.28
40.6	3.18	-4.88	-1.62
46.2	7.94	15.34	23.28
51.5	7.50	11.60	19.11
56.1	6.69	26.55	33.24
61.5	5.53	26.55	32.09
65.8	28.48	13.49	41.98
75.6	4.12	31.10	35.22
85.8	7.96	10.46	18.42
101.4	13.87	22.27	36.15
112.7	21.32	16.38	37.70

* Averaged over the four times studied; complete data and statistical figures given in Appendices 2, 4 and 6

Table 4.9 Influence of holding time on fat losses during cooking and pressing of herring muscle in relation to original fat content

Time (min)		Percentage losses*			
		0	15	30	60
Treatment					
Cooking	1	11.71	8.97	7.50	10.72
	2	14.66	16.10	16.70	14.76
Pressing	1	18.31	15.67	14.42	17.32
	2	20.29	19.79	20.47	21.73
Cooking plus Pressing	1	30.00	24.64	21.93	28.04
	2	34.95	36.01	37.17	35.91

* Mean over the eleven temperatures studied; complete data and statistical analysis is given in Appendices from 2 to 7

1 LFC muscle
2 HFC muscle

fish. This anomaly has been reported by meat workers (Meyer et al., 1969; Woolsey and Paul, 1969) who found that when lean meat is cooked there is an apparent increase of its fat content expressed as a percentage of the dry solids. This increase in fat content of the cooked muscle was attributed to improved extractability of fat because of heat alteration of the muscle proteins. In the present work the following reasons might explain the apparent fat increase in the cooked muscle.

a) a large proportion of the fat of the fish is located under the skin, and the manual filleting operation can introduce substantial differences in the amount of skin removed from the two paired fillets which could influence the fat figures; in other words the heated fillets appeared to gain fat only because they had a higher fat content initially than the comparison fillets;

b) the small amount of fat in lean fish increases the effect of errors in sampling, i.e. finding a 4% figure when the true value is 3% represents 25% error whereas for high fat content samples a 13% figure instead of 14% represents only 7% error;

c) protein bound lipids (triglycerides) may have been released during cooking;

d) phospholipids may become extractable.

Data on fat release during cooking of fish are even more difficult to find than for water release. The figures for fat losses of HFC fish agreed reasonably well with those reported by Ward et al. (1977) when results are expressed as a percentage of the fat present in the raw material and agreed much more closely when results were expressed as a percentage of the raw material weight. The figures for LFC samples were in line with the values for sardines found by Meesemacker and Sohier (1959). The fat loss reported by Cutting et al. (1956) for steamed herring, 4.3% of the original fat, was much lower than in this work. In this work HFC muscle generally lost more of its original fat than LFC muscle;

this could be in part accounted for by the fact that the fat cell in the LFC muscle would have thicker walls (Hughes, 1963) and also the better retention of structure on heating, suggested earlier.

The striking feature of the effect of temperature on fat release lies in the finding that temperatures as low as 55-60°C were as efficient as temperatures as high as 100-112°C in facilitating the removal of fat from the muscle tissues. A similar conclusion was reached by Mohr (1979) using isolated fat cells from herring. He found that 50°C was the point of maximum weakness of the fat cell walls and therefore the temperature suitable for maximum release by mechanical agitation. When it is considered that the fish meal industry relies on the use of high temperatures during cooking to separate fat from the fish tissues, it is surprising that temperature in this study did not have a greater influence on the release of fat. There may be good reasons for this. If it is agreed with Mohr (1976), whose findings are in part substantiated by Yamada (1964) that most of the fat in the muscle is located outside the muscle cells, then it is possible to envisage a mechanism for fat release upon heating. Heat weakens or causes dissolution of the fat cell walls and fat is made free by the collapse of the cell walls and/or by the muscle shrinkage caused by heat. Once this fat is free it is "washed out", coalescing as fat droplets in the water protein solution leaving the tissues when the tightening of the myofibrillar structure (Hamm, 1963) takes place. It can be further assumed that part of the lipids are brought out of the tissues by attaching themselves to polar side chains of amino acids in the proteins coming off with the water. Considering this mechanism, fat release during heating of the muscle would proceed as follows:

1. up to 45°C, although a large amount of liquor is released, heat has not caused a great disruption of the fat cell walls and tightening

of the network of the myofibrillar proteins has not occurred to a large extent;

2. from 50 to 60°C, maximum disruption of the fat cell walls (from 45-50°C collagen is converted to gelatin) occurs, and fat constitutes a large proportion of the liquor released at these temperatures;

3. above 60°C, maximum fat cell disruption has already occurred and as more water is released more fat would be also expected to be released, but this did not happen. The explanation for this may lie in the findings of Shenouda (1974) who reported that heating increases the binding of neutral lipids to myosin and actin. In support of this view, Søbsted (1976) reported that there is an increase in the amount of fat remaining in the fish tissues with increased temperatures of heating, which he attributed to interaction of the fat with the "water soluble proteins" which are present in the fish tissues in large amount after heating at temperatures higher than 60°C.

2.2 Release of fat by pressing

2.2.1 Low fat content samples

The results for fat losses during pressing for LFC fish (Tables 4.8 and 4.9) revealed that the influence of temperature was greater for pressing than for cooking and the analysis of variance revealed that the influence of temperature on fat release was significant at 1% level (Appendix 3). Holding times, as with previous results, did not have any statistically significant effect on fat release. However, as for the release of fat during cooking, there was considerable scatter in the data, the 95% confidence limits for a single value being ± 20.3. The least significance difference for temperature effect was 14.4 and therefore, considering the means in Table 4.8, it is impossible to establish a clear picture of the temperature effect on the distribution of fat losses due to pressing.

2.2.2 High fat content samples

The values of fat losses as a function of temperature and time (Tables 4.9 and 4.10) were less scattered about the overall means than those for LFC fish as indicated by a much smaller residual standard deviation. The influence of temperature was statistically significant at 0.1% level (Appendix 4), hence more significant than the results for low fat content. Holding time had no statistically significant effect on the fat release. Using the least significant value of 7.7 at the 5% level to check the difference between the means shown in Table 4.10, it can be concluded that means for temperatures 55°, 60°, 75°, 85° and 112°C were not statistically different from each other, but different from lower temperatures. So it is not possible to point out any trends on fat release due to pressing brought about by different cooking temperatures. Similarly to the LFC samples the only possible conclusion is that temperatures above 45°C are as efficient as temperatures as high as 112°C in easing the release of fat from the muscle tissue.

2.3 The combined effect of heating and pressing on the release of fat

For both types of fish the influence of temperature was significant at the 0.1% level (Appendices 6 and 7), and as for the fat losses for each treatment separately, figures of fat losses due to the combined effect of both treatments were much more scattered for LFC samples; the 95% confidence limits for a single value were $\pm$ 20.3 against a figure of $\pm$ 11.8 for HFC ones.

For LFC muscle the release of fat Table 4.8, may be considered almost constant for temperatures above 50°C since a LSD of $\pm$ 14.4 indicates that there was no significant difference between the means of the different temperatures with the exception of the 85°C treatment which was a point

Table 4.10 Influence of temperature on losses of fat during cooking and pressing of HFC muscle in relation to original fat content

Temperature (°C)	Percentage losses*		
	Cooking	Pressing	Total
36.2	4.51	4.15	8.66
41.1	8.77	5.24	14.02
44.5	12.15	19.07	31.54
50.4	21.82	18.57	40.39
55.1	21.05	24.09	45.14
59.7	17.01	32.26	49.27
65.6	16.17	21.87	38.04
75.0	11.66	28.14	38.21
83.7	16.87	25.23	42.11
101.0	17.76	18.77	36.53
112.0	23.34	28.87	52.21

* Averaged over the four holding times studied; data and statistical figures given in Appendices 3, 5 and 7

of minimum. The same applies to HFC generally, Table 4.10, although it is necessary to point out that fat losses for the 55-60°C treatments were not statistically different from those at 112°C.

2.4 Discussion

Pressing produced a larger release of fat than cooking and this supports the view that the fat released by physical treatments like cooking and pressing is physically entrapped in the fish muscle.

It can be generalised that heating and pressing released much more of the fat existing in the raw tissues of HFC muscle than for LFC muscle since maximum release of fat represented respectively some 45-50% and 30-35% of the original fat. This higher retention of fat by LFC samples might be connected with the fact of LFC fish having thicker cell walls. Total losses of fat constituted 1-2.5% of the raw material weight for LFC muscle, and those for HFC muscle amounted to some reasonably constant 6-8% of the raw material weight for temperatures above 45°C. In the only work available for comparison, Ward et al. (1977) found much higher values of fat losses during pressing of cooked sprats. This difference can probably be attributed to the extensive breakage of the muscle structure caused by their cooking procedure.

From the above, it may be speculated that the release of fat by pressing depends on the ease with which fat can be squeezed out of interfibrillar spaces, to the surface of the muscle.

3. Weight loss

The weight loss of herring muscle of low and high fat content are given in Figures 4.4 and 4.5, respectively. Values are given separately for the weight losses during heating and during pressing together with

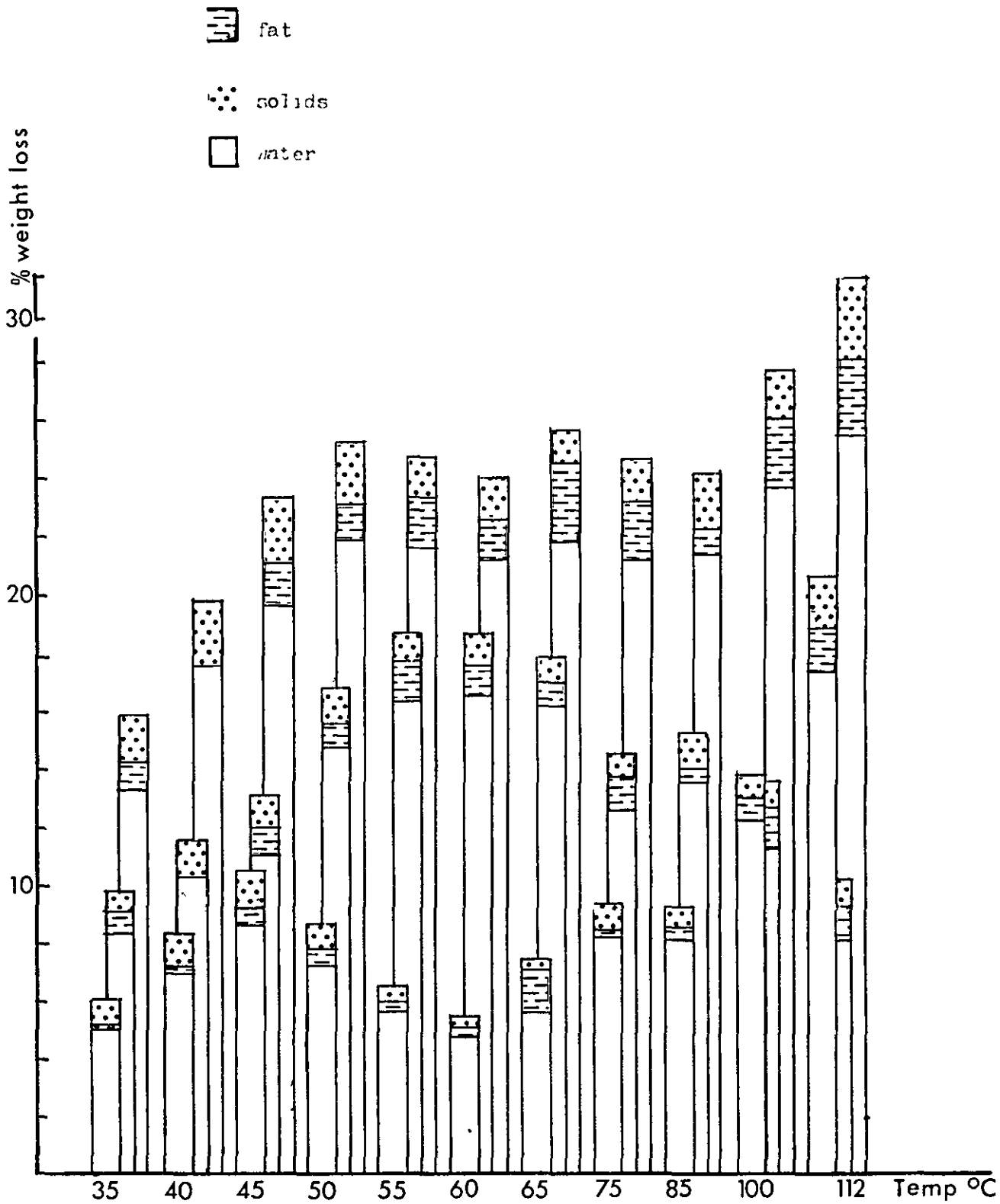


Fig 4.4 Percentage losses of macrocomponents of the muscle, in relation to raw material weight, as a function of temperature[a] (LFC muscle)

a-rounded temperatures; actual temperatures as in Fig 4.3

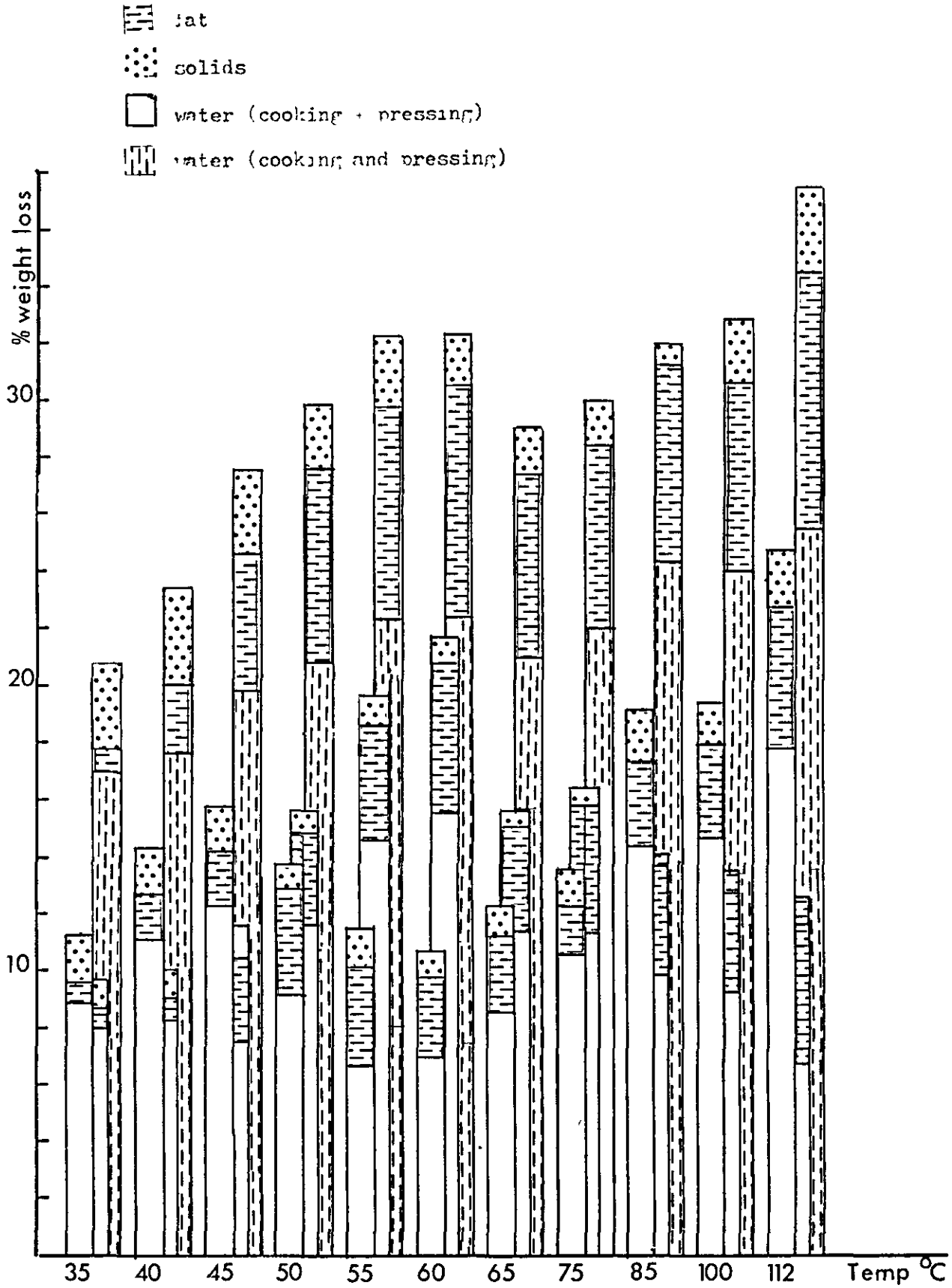


Fig 4.5 Percentage losses of macrocomponents of the muscle, in relation to raw material weight, as a function of temperature[a] (HFC muscle)

a. rounded temperatures; actual ones as in Fig 4.3

the total weight loss by both treatments. The losses are subdivided into moisture, fat and solids losses.

The effects of heating temperatures and holding times on the weight losses due to the cooking and pressing treatments will not be discussed in detail here because they have been dealt with when evaluating their role in the release of water and fat separately. Here it suffices to say that, based on these figures, the distribution of weight loss with temperature was virtually the same for both batches of fish. In addition, it is immediately clear that the total weight loss values were higher for HFC fish for all temperatures studied, this being largely accounted for higher fat losses. However, weight loss is a parameter directly associated with the economics of technological processes and therefore it is interesting to draw a comparison between the figures obtained in this study and those reported by other authors or found in traditional industrial practices.

3.1 Weight loss on cooking

The weight loss on heating only, of LFC muscle at 100°C was about 14%, much lower than values reported by other authors for fatty fish species cooked at about the same temperature, which are summarised in Table 4.11.

The difference between the heating losses for LFC and HFC muscle must have arisen from the different fat contents; other factors such as the use of whole fish rather than fillets, application of different cooking methods, which may permit evporation losses, and the application of physical stress are responsible for the rather higher figures obtained by others. Nevertheless, the agreement is good.

Table 4.11 Literature values of weight losses on heating fatty fish species

Species	Form	Fat content %	Cooking	Weight loss at 100°C %	Authors
Herring	Edible portions	18.1(?)	steaming	21.1	McCance and Shipp (1933)
Sardines	Whole	14	steaming	20	Meesemacker and Sohier (1956)
Sprats	Whole	10	baking*	20.4	Ward et al. (1977)
Herring	Fillets	8	steaming	27	Cutting et al. (1956)
Herring	Skinless fillets	6	baking*	14	This work (LFC)
Herring	Skinless fillets	15	baking*	19	This work (HFC)

*baking = cooking in a closed container

3. 3.2 Weight loss on pressing

From Figures 4.4 and 4.5 it is clear that the difference in total weight loss between HFC and LFC fish was produced mainly during cooking, and that the differences in weight loss between the two types of fish during pressing were very small. As already indicated in the literature review there is very little information available on losses during pressing of cooked fish. Ward et al. (1977) reported a total loss of around 54% from sprats, 20% during cooking at 100°C and 34% by pressing at 11.7×10^6 N/m^2. In the present investigation the total weight loss at 100°C was around 37%, of which 14 to 19% was lost during cooking and 23 to 18% during pressing. The reasons for the quite large difference in the

pressing losses are likely to be the factors already listed above to explain differences in cooking losses together with the influence of the different way pressing was carried out in each case. In commercial processes (manufacture of fish meal) losses due to cooking plus pressing may be as high as 68% (Torry Advisory Note No. 49).

Bearing in mind differences in species used in the various studies, it may be inferred from the above discussion that it is important to define very closely, the fish material and cooking procedures used in cooking experiments. Large differences in results may arise in otherwise similar experiments due to variation in composition, presence or absence of skin, and to fish being cooked whole or in fillets or minced.

4. Composition of the fluid released during cooking and pressing

Knowledge of the composition of the liquors released during cooking and pressing is important in process evaluation. In canning, for instance, knowledge of the composition of the fluid-released by the pre-cooking stage permits one to decide on the economic viability of recovering the oil released. In fish meal manufacture the composition of the liquor squeezed out after cooking and pressing is a parameter used to judge how effective is the process in releasing fat from the fish tissues so that the end product does not have an excessively high fat content.

4.1 Liquor released during cooking

Figure 4.6 illustrates the composition on the liquor released during cooking of the two samples of herring as a function of temperature (error figures in Appendices 8 and 9). For a given temperature, the difference between the values for (fat plus water) losses and water losses represents the percentage of fat present in the cooking liquor at that temperature.

For LFC muscle, water contributed the largest amount to the fluid released, 83-88% (except at 65°C where the low value, 71%, probably resulted from a combination of normal fluctuations). Fat represented another 2-6% (except at 65°C) and therefore solids (proteins and minerals) made up 6-14% of the cooking liquor weight.

For HFC fish, the fluid released by cooking contained a much larger proportion of fat. In this case the distribution of water in the fluid released as a function of temperature closely resembled that for the water loss as a percentage of total raw material weight (Figure 4.3). Water plus fat became almost constant above 50°C, giving some indication of an inverse relationship between the amounts of water and fat composing the released liquor. The fat content of the liquor varied from about 7% at 35°C to a maximum of about 27% at 55°C, falling to about 16% at higher temperatures. Solids made up 16% of the liquor at 35°C falling to about 8% at higher temperatures.

4.2 Liquor released during pressing

The compositions of the liquors released during the pressing operation on the cooked fish as a function of temperature are shown in Figure 4.7 for both types of muscle.

The percentage amounts of water, fat and solids constituting the pressed out liquor in the case of LFC fish, were very close to those during cooking indicating that the relative losses of water, oil and solids were very similar for both treatments. Fat plus water

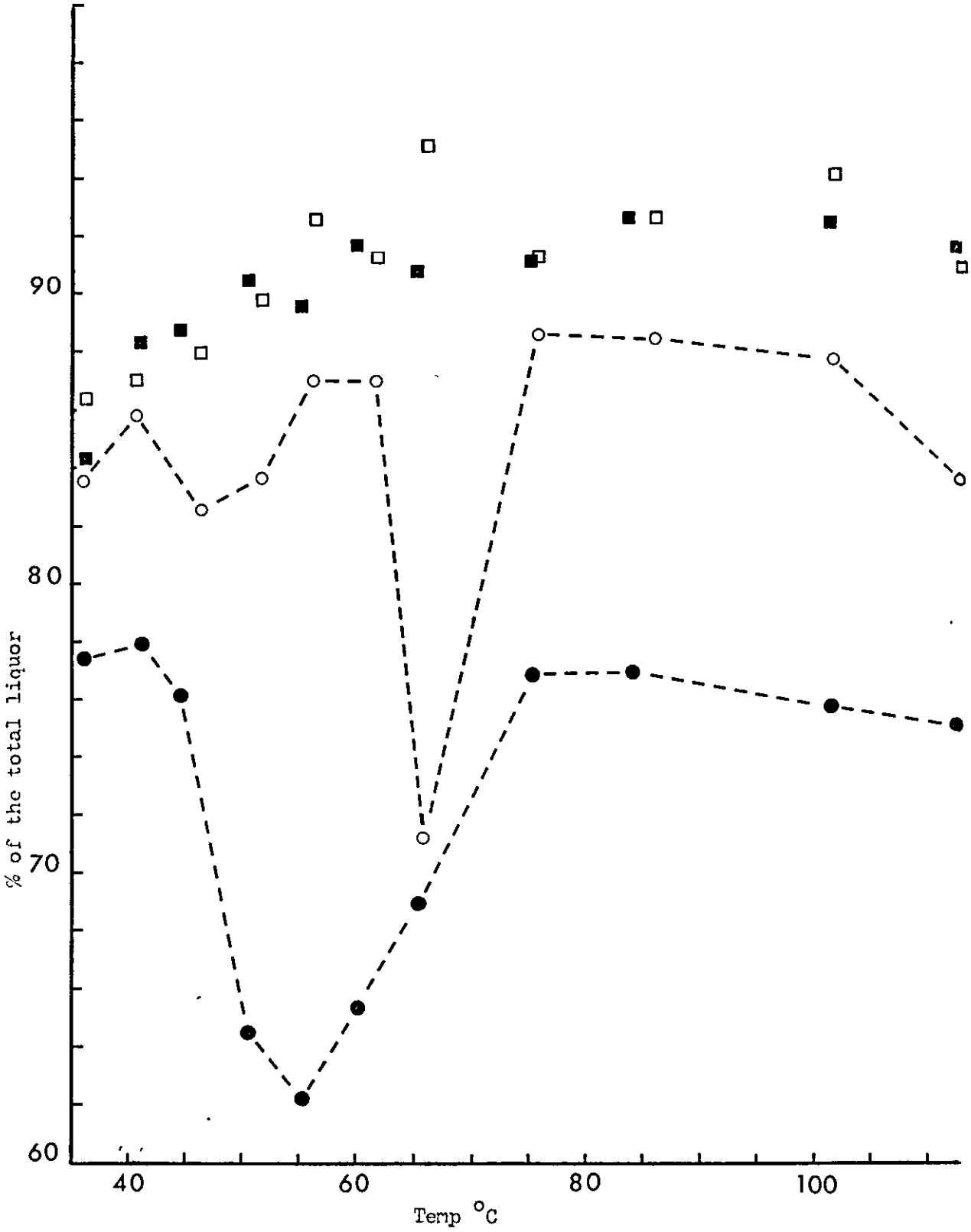


Fig. 4.6 Composition of the liquor released during cooking of the herring muscle

LFC HFC

○ ● % water

□ ■ % water plus fat

For the sake of clarity, error figures are given in Appendices 8 and 9

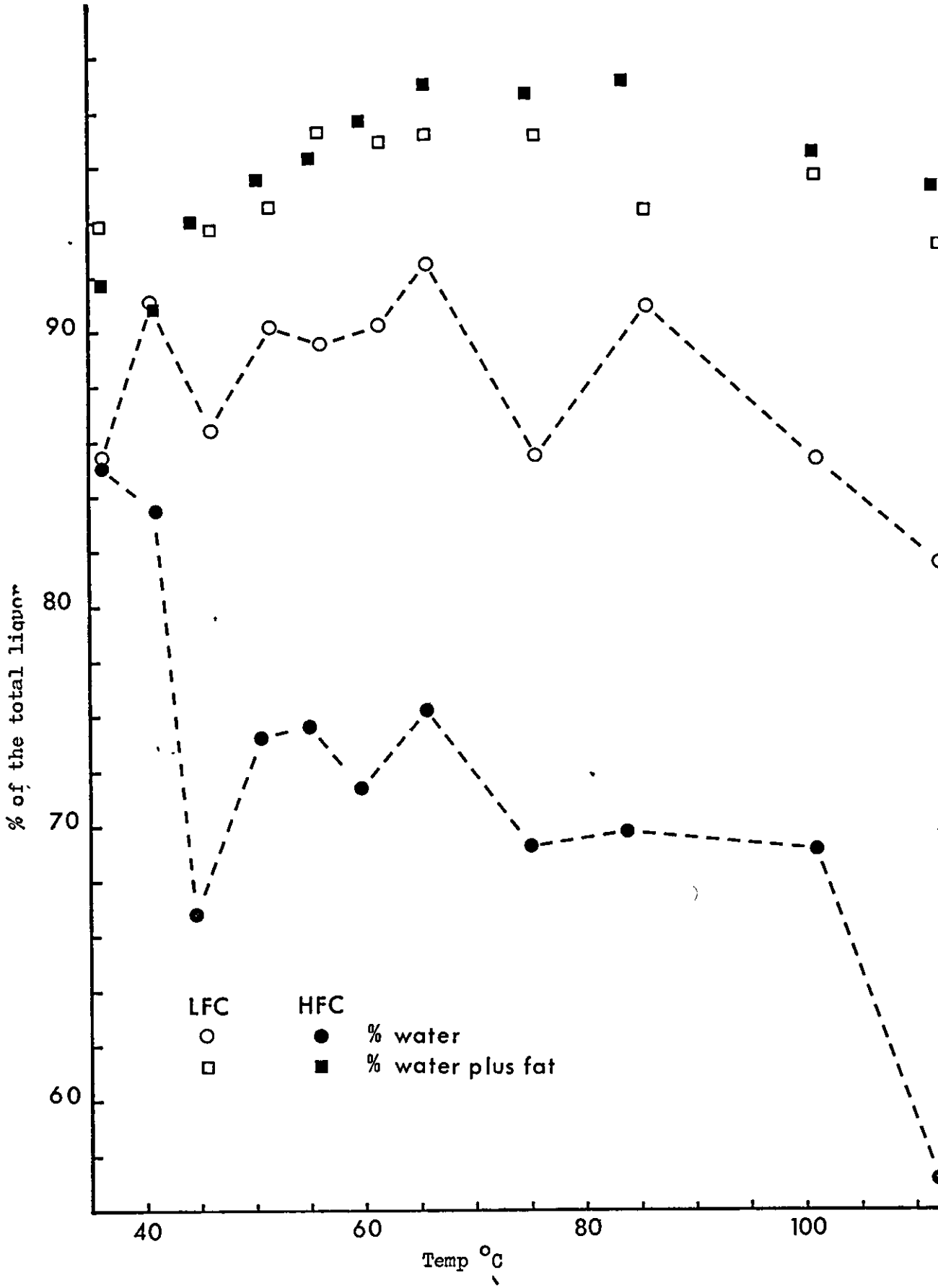


Fig 4.7 Composition of the liquor released during pressing of the herring muscle

For the sake of clarity, error figures are given in Appendices 8 and 9

constituted 92-96% of the weight of the released fluid, water contributing 83-89% with fat making up the rest.

Similarly, for HFC muscle, the contribution of fat and water to the total weight of pressed out liquor was very close to that of the liquor released during cooking. For temperatures between 45° and 100°C water amounted to 67-74% of the weight of the pressed out liquor and as during cooking, fat contributed a significant amount of the released fluid, some 25% of the weight for treatments above 45°C.

The distinctive feature of the composition of the pressed-out liquors was the much larger amount of fat present in the HFC samples, this fat representing only 1% of the raw material weight for LFC fish against 3-5% for HFC fish. Common characteristics were the low amount of solids present in the liquor, and the fact that for both types of fish the amount of solids in the liquor was generally smaller than that found in the cooking liquor.

4.3 The total liquor released by cooking and pressing

The composition of the fluids released by the combined effect of cooking and pressing as a function of temperature is shown in Figure 4.8, for both types of muscle. From this figure it may be noted that the fluid composition is roughly uniform from 55°C upwards, water plus fat constituting 94% of its weight for both types of fish. The fact that water was a very large proportion of the liquor for each treatment separately is obviously reproduced, as is the larger amount of fat in the pressed out liquor of HFC fish. However, the striking characteristic of the liquor released by both types of fish was the low amount of solids which represented, on average, 6-8% of the liquor released by both cooking and pressing.

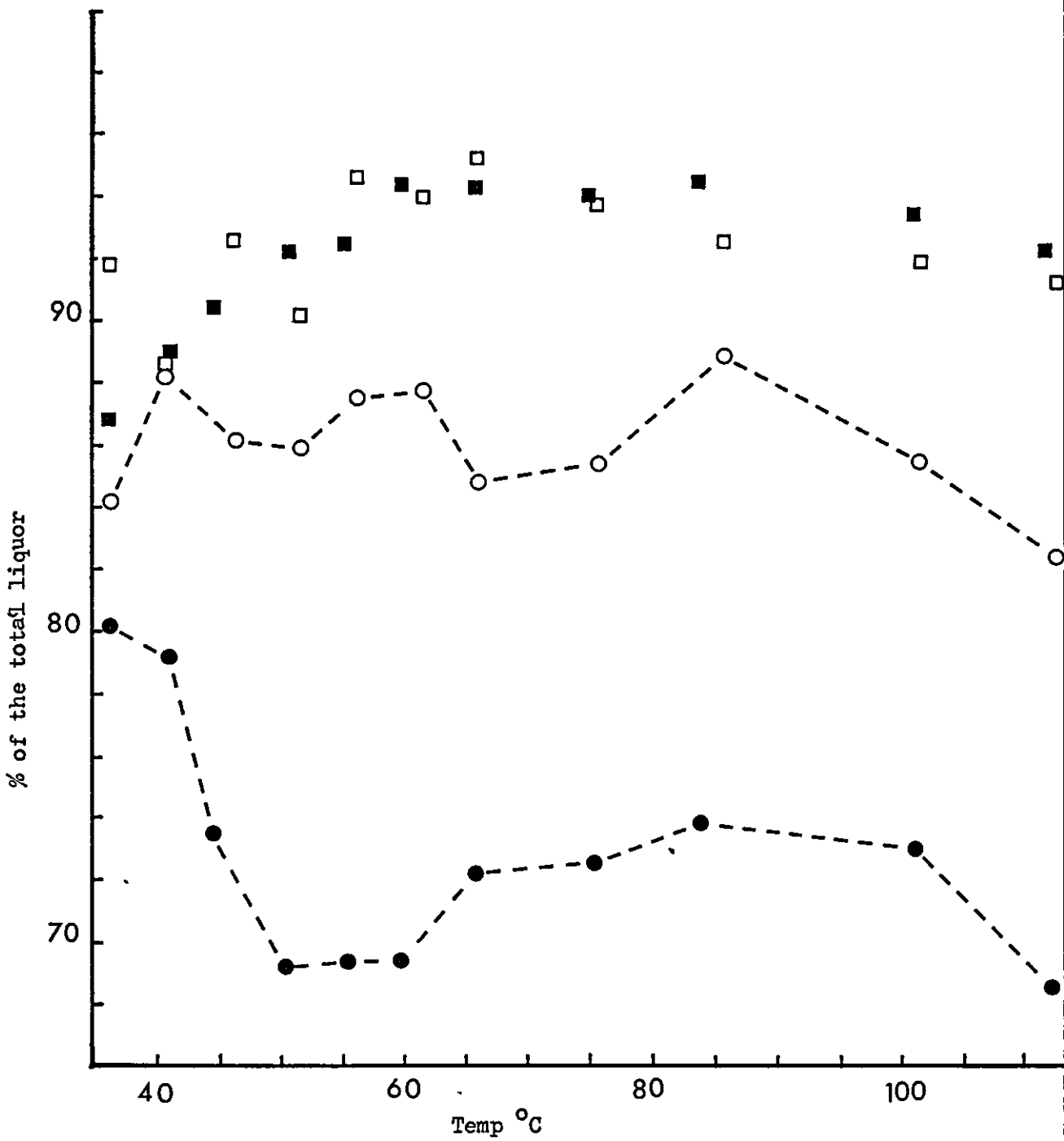


Fig 4.8 Composition of the liquor released by the combined effects of cooking and pressing

LFC	HFC	
○	●	% water
□	■	% water plus fat

For the sake of clarity, error figures are given in Appendices 8 and 9

B. Visual Changes

1. Subjectively observed changes in translucency, texture and palatability

Parallel with the studies on water holding capacity, observations of changes in translucency, texture and degree of palatability of the muscles caused by heating were also made. The integrity of the skin as affected by heating was examined in the control samples.

With the exception of palatability there were striking differences between the two types of muscle in relation to these characteristics as a function of temperature. Thus cooking at 35°C increased the firmness of the LFC muscle but caused the HFC one to become very soft. From 35 to 45°C the flesh of the LFC samples became increasingly firmer whereas those of HFC became softer. From 50°C upwards the texture of both types of muscle grew firmer and more fibrous with increasing temperatures, although it must be pointed out that the cooked LFC muscle tended to be more friable. These heat-induced differences in texture between the two muscles must be due primarily to the differences in the amount of fat present in them. Tulsner (1978) argues that the thermally altered texture in fish muscle is due to denaturation and coagulation of the myofibrillar proteins. He excluded collagen from having any role in textural changes, on the grounds that only a small amount of collagen is present in fish muscle (less than 1% in herring muscle according to his studies) which would be quickly degraded to gelatin. These statements certaintly appear reasonable for heat treatment above 50°C, but there is some evidence that the observed changes in texture between 35 and 45°C could not be entirely due to changes in the contractile proteins. As already stated collagen content in herring varies inversely with fat content (Hughes, 1963), and if this results in thinner membranes more labile to heat, then changes in collagen

fibres might explain to some extent the increased softening of HFC muscle in comparison with the LFC one. It is worth mentioning that LFC muscle between 45 and 50°C was gelatinous and "sticky" whereas at 55-60°C it was still gelatinous (gel like to the touch) but not "sticky". It is interesting to add, from the calorimetric experiments, that at about 108°C the fish muscle inside the calorimeter turned to a jelly-like texture and did not adhere to the calorimeter wall as in experiments at rather lower temperatures. This indicates that hydrolysis occurs at these high temperatures. Further indication of breakdown of the muscle components at high temperatures was given by the blackening of the muscle and the formation of black deposits on the calorimeter lid. This can presumably be attributed to the formation of H_2S.

Heating at 35°C slightly increased the opacity of the LFC muscle but caused a large increase in translucency of the HFC muscle. Generalising from all the samples examined, the maximum increase in translucency in the LFC muscle occurred at 40-45°C whereas for HFC muscle the phenomenon occurred at about 35-40°C. Above these temperatures both muscles became opaque and it was not possible to make any certain subjective distinction between the effects of different temperatures.

Before attempting to explain the increases in translucency and opacity, and the different behaviour of the two muscles in relation to these properties it is worth examining how changes in the fish skin proceeded with heat, since both phenomena can be correlated. At 35°C the skin of HFC samples became loose and could be easily removed intact from the muscle, whereas the skin of LFC samples was still firmly attached to the muscle at this temperature. Only when the cooking temperature reached 45°C could the skin of the LFC muscle be easily removed.

Generally from 50°C upwards the fish skin tended to stick to the bag, a phenomenon similar to that occurring during canning of herring, which is attributed to the formation of gelatin (Hughes, 1963).

All these observed changes taking place in the muscle during heating must be a consequence of the underlying changes in the fibrillar proteins and connective tissue; differences in behaviour of fish of different composition might reflect, as already speculated, differences in the lability of their proteins and connective tissue to heat. To distinguish what is cause and what is effect in these changes is not an easy task. Degradation of the connective tissue to gelatin, at 35°C for HFC muscle and at 40-45°C for LFC muscle, would increase the muscle homogeneity and might be the cause of the increase in translucency at these temperatures. However, it is also possible that an increase in the solubility of myosin, the release of water within the muscle tissues, or even the spread of oil within the muscle can increase the homogeneity of the tissues and therefore increase its translucency. Aitken (1974) studying cooking of cod muscle by observing changes during heating of the tissues under a microscope speculated that the reasons for an increase in translucency of the tissues at around 39.7°C could be the result of increased hydrolysis of the connective tissues to form gelatin or of an increased solubilisation of myosin. This latter explanation would require that the minimum ionic strength necessary to dissolve myosin, around 0.3 at 0°C, would decrease at higher temperatures. The increase in opacity is more easily explained. Until the cooking temperature reached 50°C the liquor released during cooking had a milky appearance suggesting the presence of a considerable amount of soluble protein in it; at this temperature part of this liquorcoagulated and at 60°C all of it coagulated since there was no "free" water (transparent fluid) in the bag. Consequently the increase in opacity at 50°C is fairly certain to be due to the

precipitation of the proteins within the tissues and the consequent increase of light scattering surfaces.

Finally, it is worth pointing out that both types of muscle were completely palatable at temperatures as low as 50°C.

These subjective observations, although useful for indicating areas where more objective approaches can be fruitful, are obviously of limited use. For this reason it was thought that microscopy of the tissues could give some more objective evidence in allowing visual changes to help in elucidating the changes in water and fat holding capacity.

2. Microscopic changes

All microscopy relies on the assumption that changes in the structures caused by sample preparation are reproducible and, therefore, it is possible to attribute any additional differences to any treatments applied to the sample. In the discussion that follows it is assumed that this is the case, and that differences in the slides of raw muscle and those of heated samples are solely due to heating.

a. Optical microscopy

Plates 1 to 3 show a representative series of micrographs of raw muscle. The longitudinal section in Plate 3 shows well compacted fibres and collagen fibres enveloping them can be distinguished. It is possible to discern also that these collagen fibres are linked to the connective tissue between the myotomes. The transverse sections shown in Plates 1 and 2 reveal extensive cracks in the fibres that can be attributed to freezing and cold storage of the muscle. In these plates can also be noted the presence of numerous globules in the connective tissue space around the fibres. By analogy with the plates presented by Ross (1977), these globules may be identified as fat cells.

Plate 1. Raw muscle transverse section (95X)

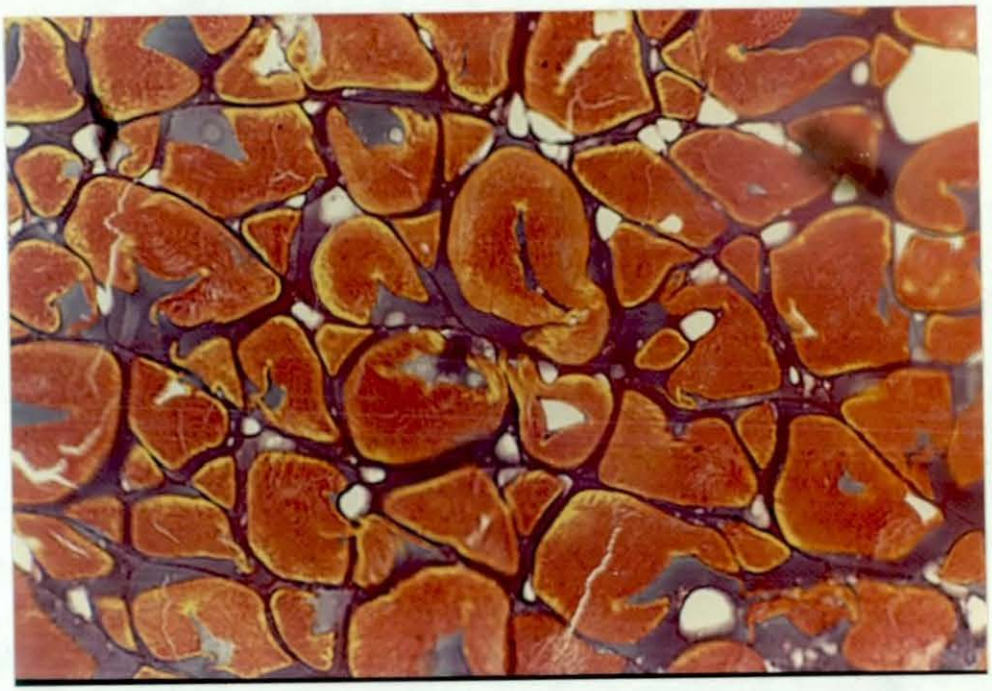


Plate 2. Detail from Plate 1 (240X)

Comparing Plate 4 with the previous ones it may be concluded that cooking the muscle to 35°C did not alter markedly its microscopical appearance. However, holding the muscle at this temperature for 1 hour (Plate 5) resulted in the spread of a bluish coloration in the connective tissue space which could be related to melting of collagen.

When the muscle is heated to 45°C, Plate 7, membranes and structures that contain collagen are still discernible and there is evidence that granulation in the connective tissue spaces and around the fibres has started to take place. This formation of granulated material has been reported by Paul (1965) during heating of rabbit muscle to 80°C and by Charley and Goertz (1958) during the heating of salmon to an internal temperature to 70°C. Fibres still look quite close together although there are signs of shrinkage in length. The colour taken up from the histological stains has not yet altered. Greater changes occurred upon heating the tissue to 55°C (Plate 8). Large quantities of granulated material were deposited in the connective tissue space and among fibres; there was also an apparent increase in extracellular space. Strands of collagen are still visible in the connective tissue space but the colour taken up from the histological stains by the muscle fibres is altered. The connective tissue appears to be gelatinised. For the cooked and pressed muscle (Plate 9) the picture is very much the same but the red staining in the granular material is accentuated. Cooking the muscle at 60°C did.not produce greater changes in its microscopic appearance (Plate 10) than cooking at 55°C. However, there is indication of an increase in extracellular space that may be due in part to the fibres shrinkage in width. Holding the muscle at 60°C for an hour (Plate 11) seems only to have slightly increased the amount of granular material. The higher magnification in Plate 12 is instrumental in showing

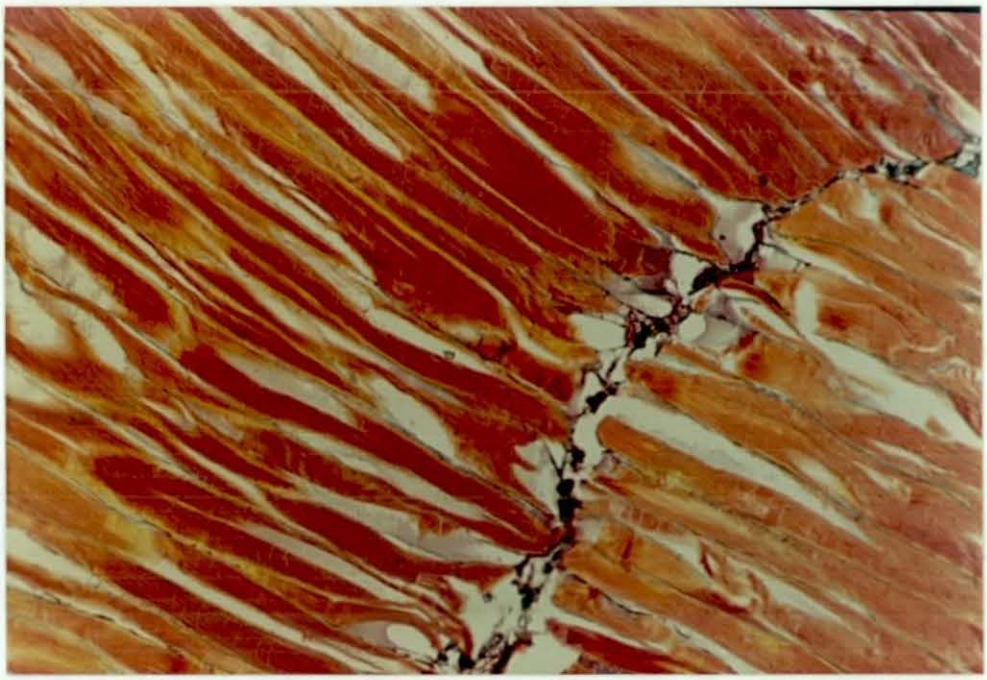


Plate 3. Raw muscle longitudinal section (95X)

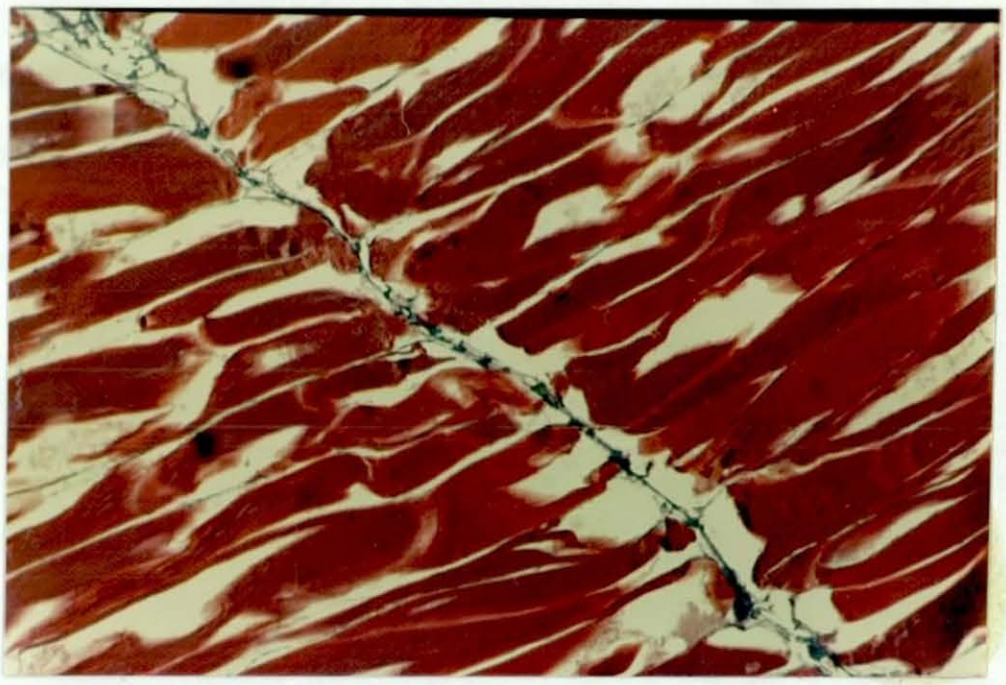


Plate 4. Section of muscle heated to 35°C (95X)

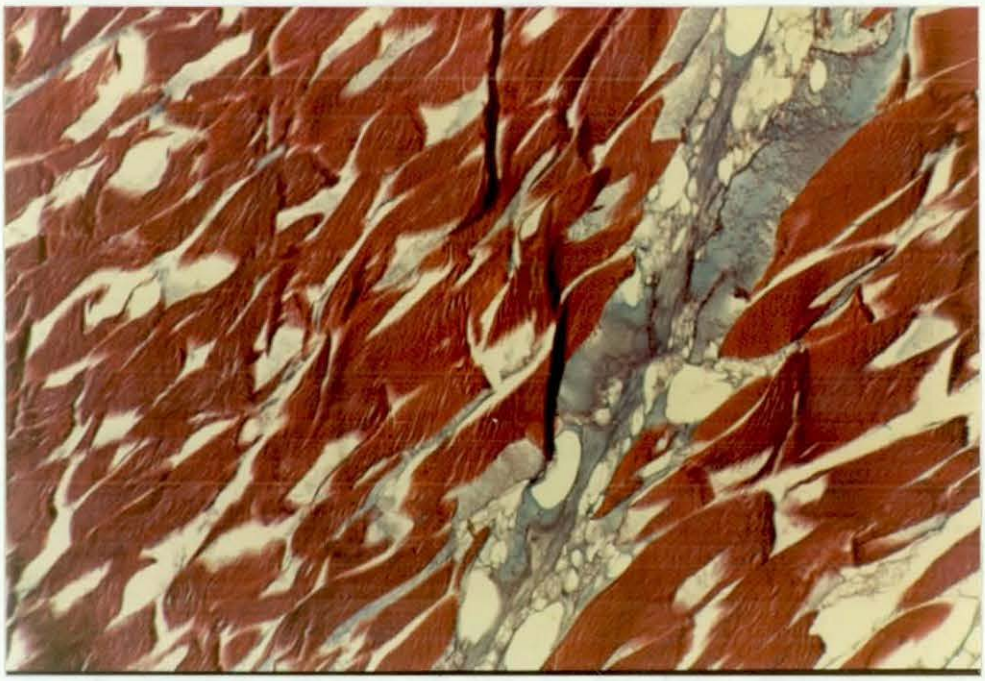


Plate 5. Section of muscle heated to 35°C and kept at this temperature for 1 hour (95X)

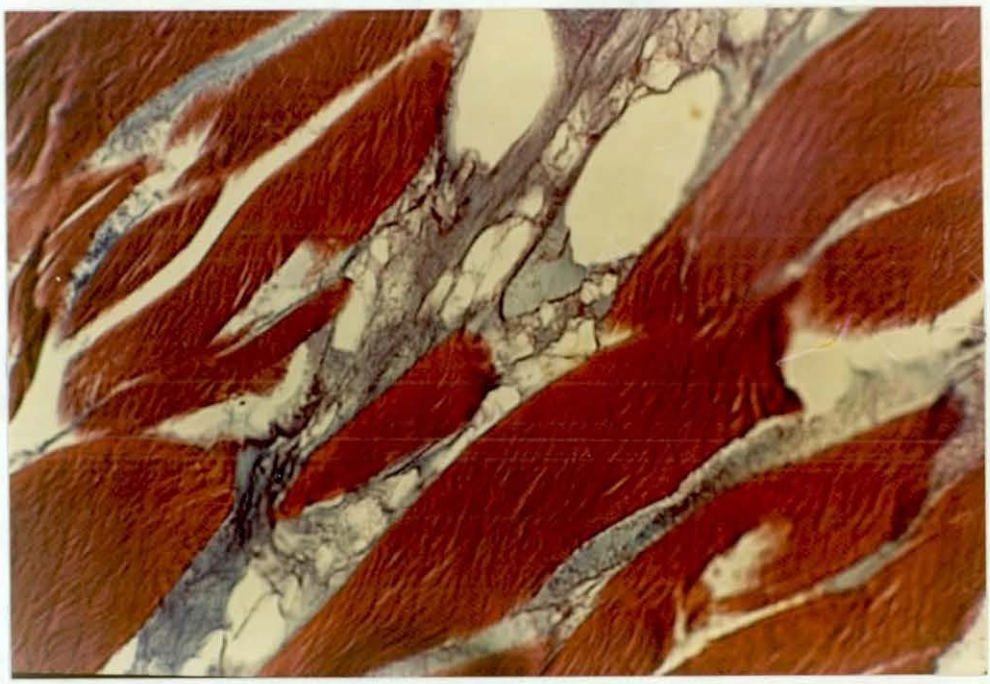


Plate 6. Magnification of detail from Plate 5 (240X)

Plate 7. Section of muscle heated to 45°C (95X)

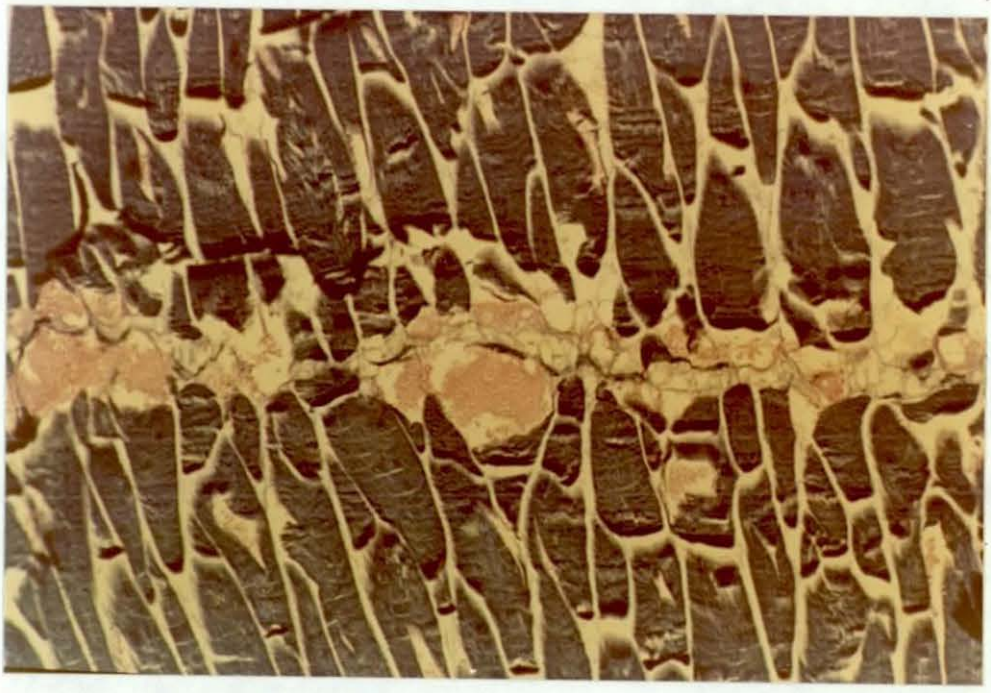


Plate 8. Section of muscle heated to 55°C (95X)

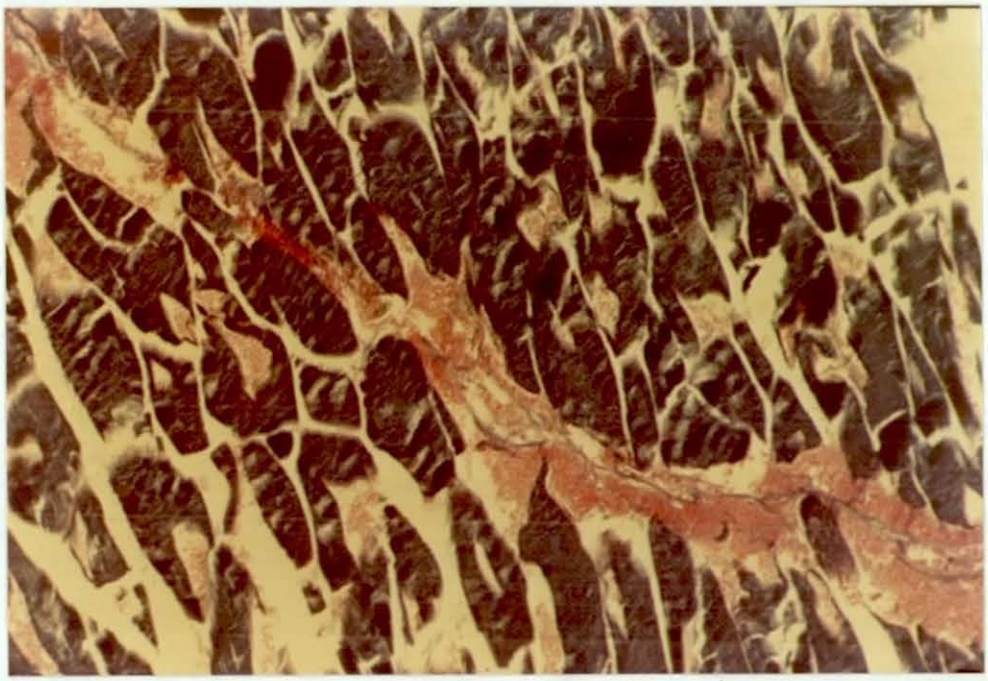


Plate 9. Muscle heated to 55°C; then left to drain and pressed at 30 x 10^6 N/m^2 for 3 minutes (95X)

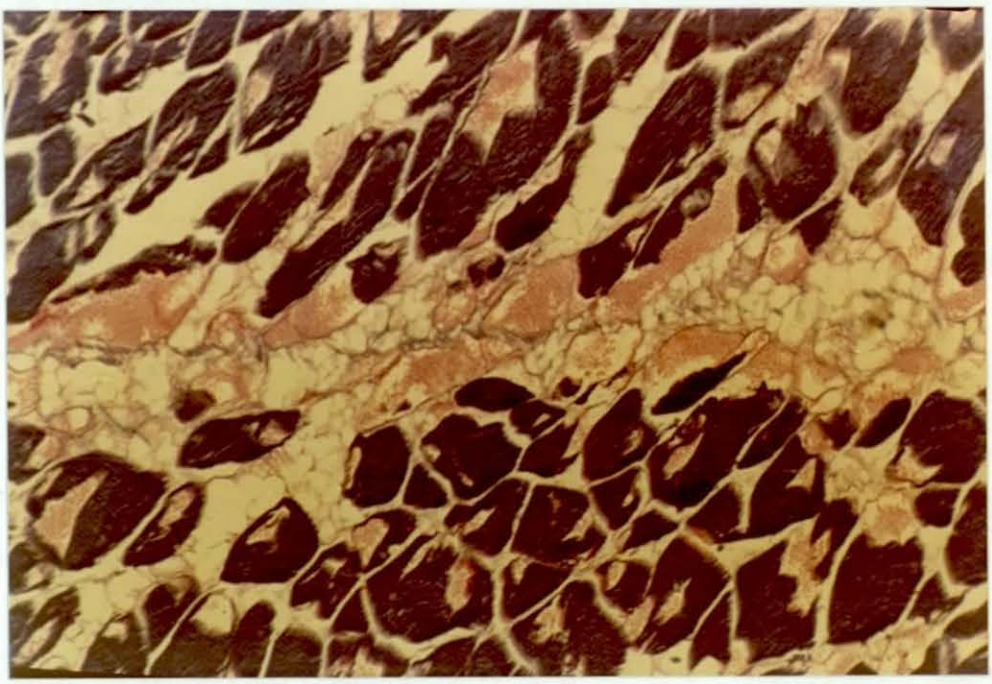


Plate 10. Section of muscle heated to 60°C (95X)

the retraction of the fibres from their cell walls and from the connective tissue space. Plate 13 indicates that the additional removal of water caused by pressing, as in Plate 9, only appears to increase the concentration of the granular material while the connective tissue space is stained a deeper red.

From the above it is interesting to point out that there is no evidence from the micrographs to support the view that the large losses of water at 45°C were due to shrinkage of the myofibrillar structure. Also the microscopic appearance of the muscle indicates extensive shrinkage of the tissues at 60°C although this was the temperature of maximum retention of water during cooking. Considering in addition that the colour taken up by the muscle at 45°C did not change, chemical changes may have been small, and processes affecting water holding capacity may be mainly physical. For example denaturation of myosin and rupture of membranes due to solubilisation of collagen might be factors responsible for the decrease in water holding capacity at 45°C. On the other hand coagulation of sarcoplasmic proteins and the gelatin derived from collagen may have a more important role in retaining water in muscle than has been so far supposed.

b. Electron microscopy

Electron micrographs in Plates 14 and 15 show the myofibrillar structure of the raw muscle. All the typical characteristics of striated muscle cells are present in the sarcomeres: Z-lines are very distinct as are the H-zones. The sarcoplasmic reticulum can be clearly seen in Plate 14 and its membranes and structure are easily discernible. All the original basic structural elements can be recognised after heating the muscle to 55°C (Plate 16), although many details have been lost. Z-lines have disappeared and great disruption of the H-zone has occurred

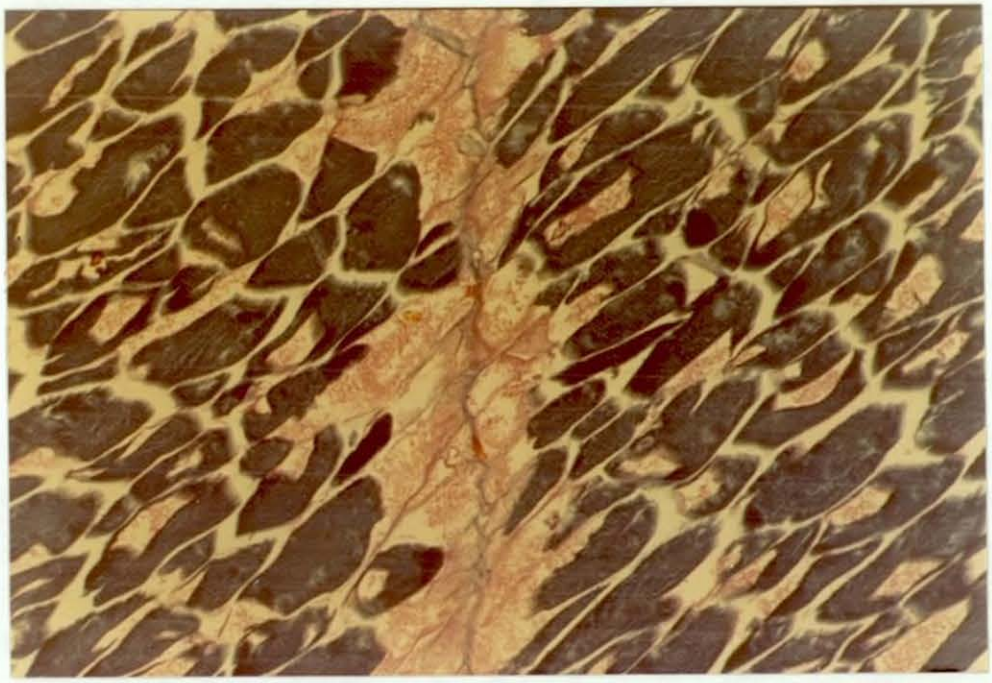


Plate 11. Muscle heated to 60°C and kept at this temperature for 1 hour (95X)

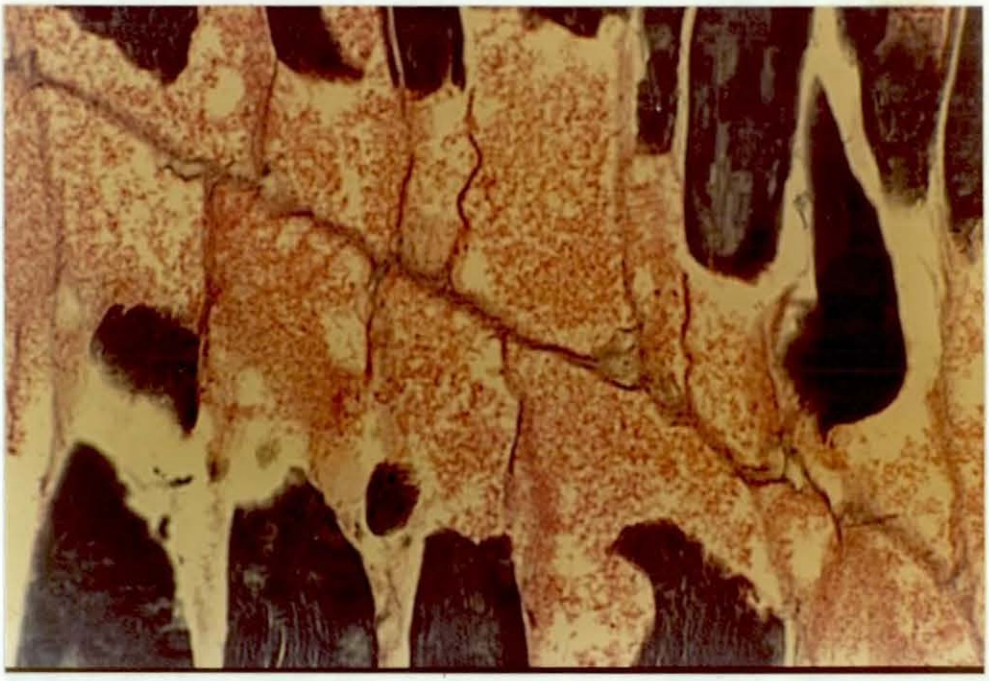


Plate 12. Magnification of detail from Plate 11 (240X)

Plate 13. Muscle heated to 60°C; then left to drain and pressed at 30 x 10^6 N/m^2 for 3 minutes (95X)

Plate 14. Transmission electron micrograph of a longitudinal section of herring raw muscle (24000X)

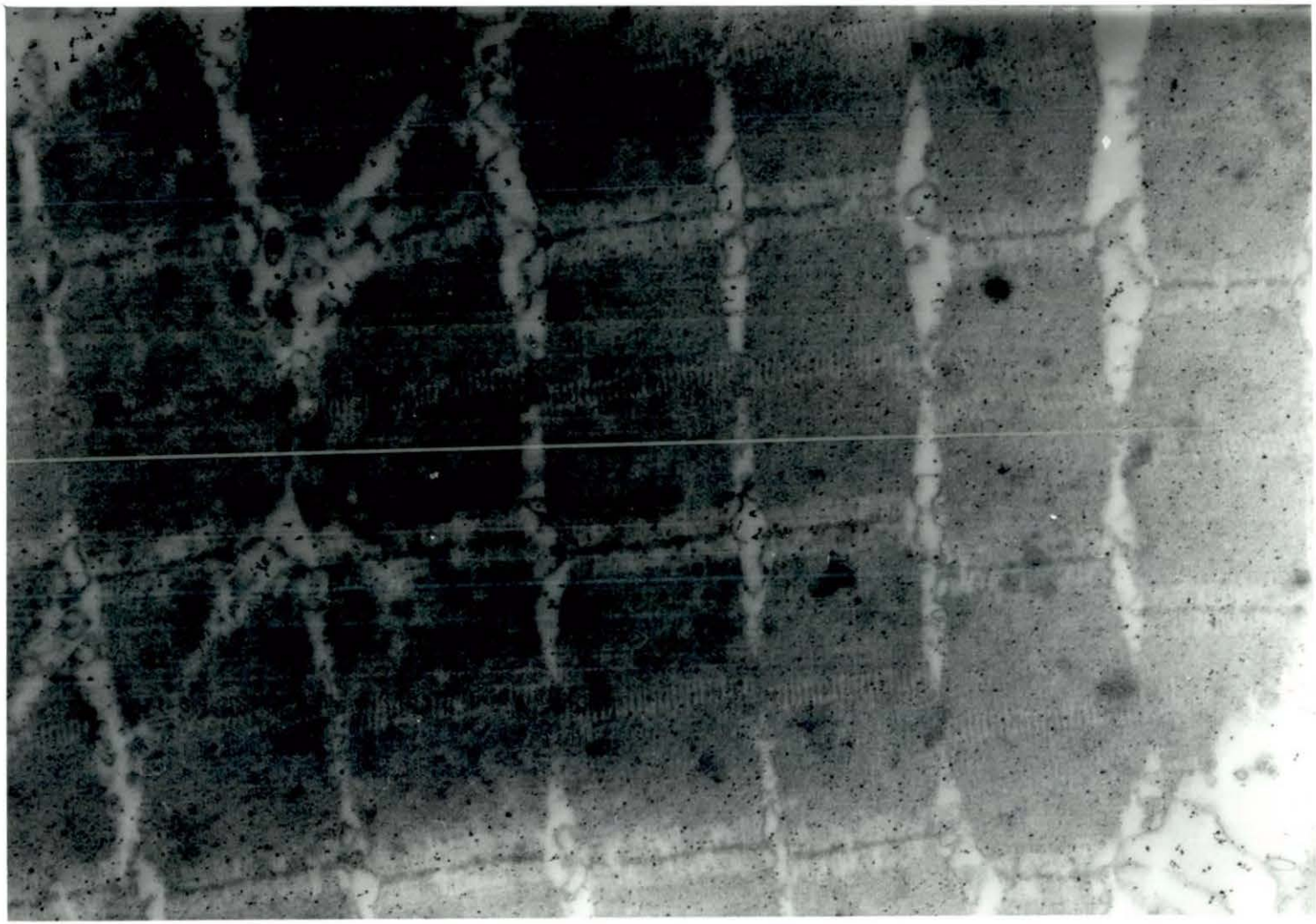


Plate 15. Transmission electron micrograph of a longitudinal section of herring raw muscle (30000X)

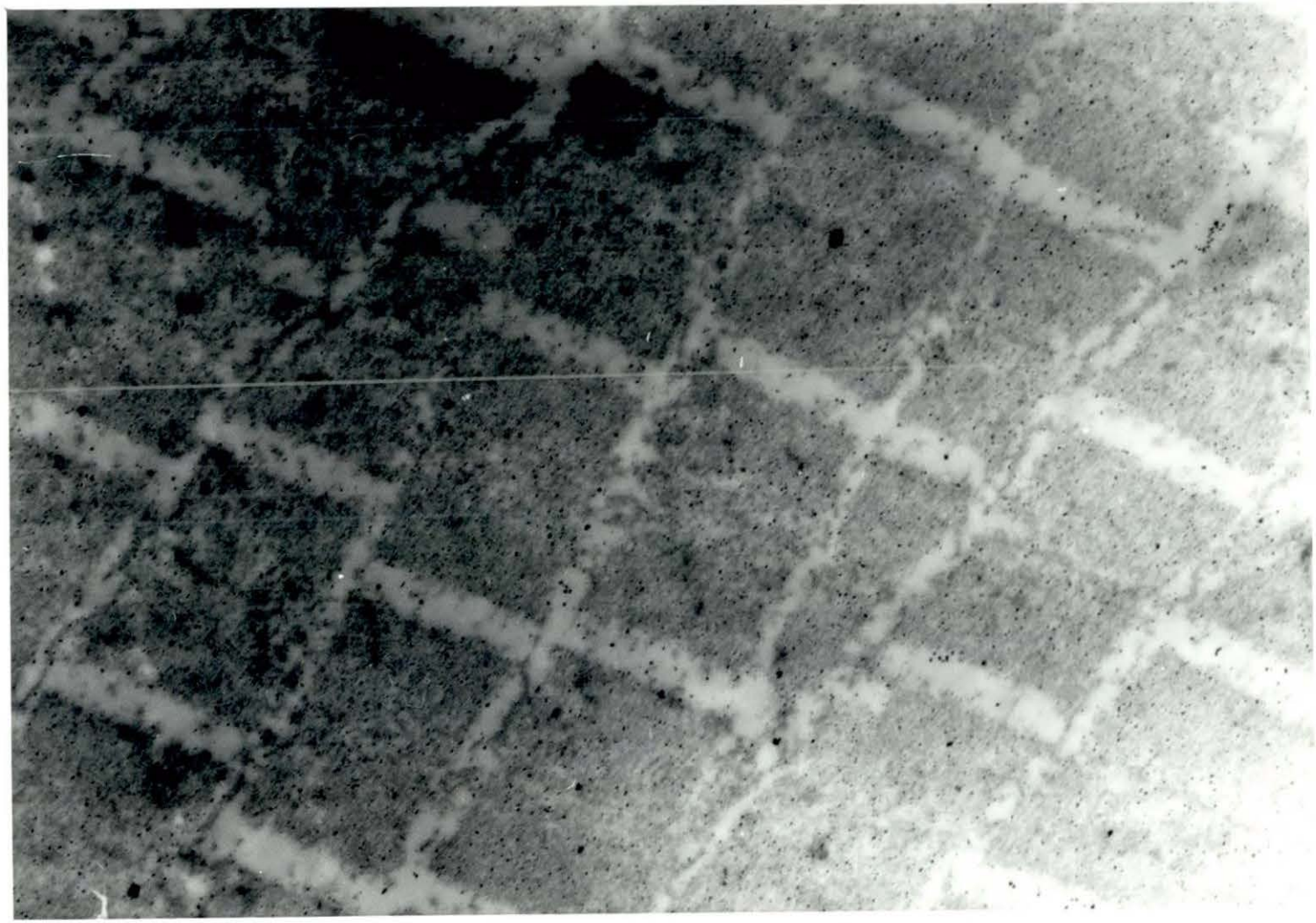


Plate 16. Electron micrograph of longitudinal section of muscle heated to 55°C (30000X)

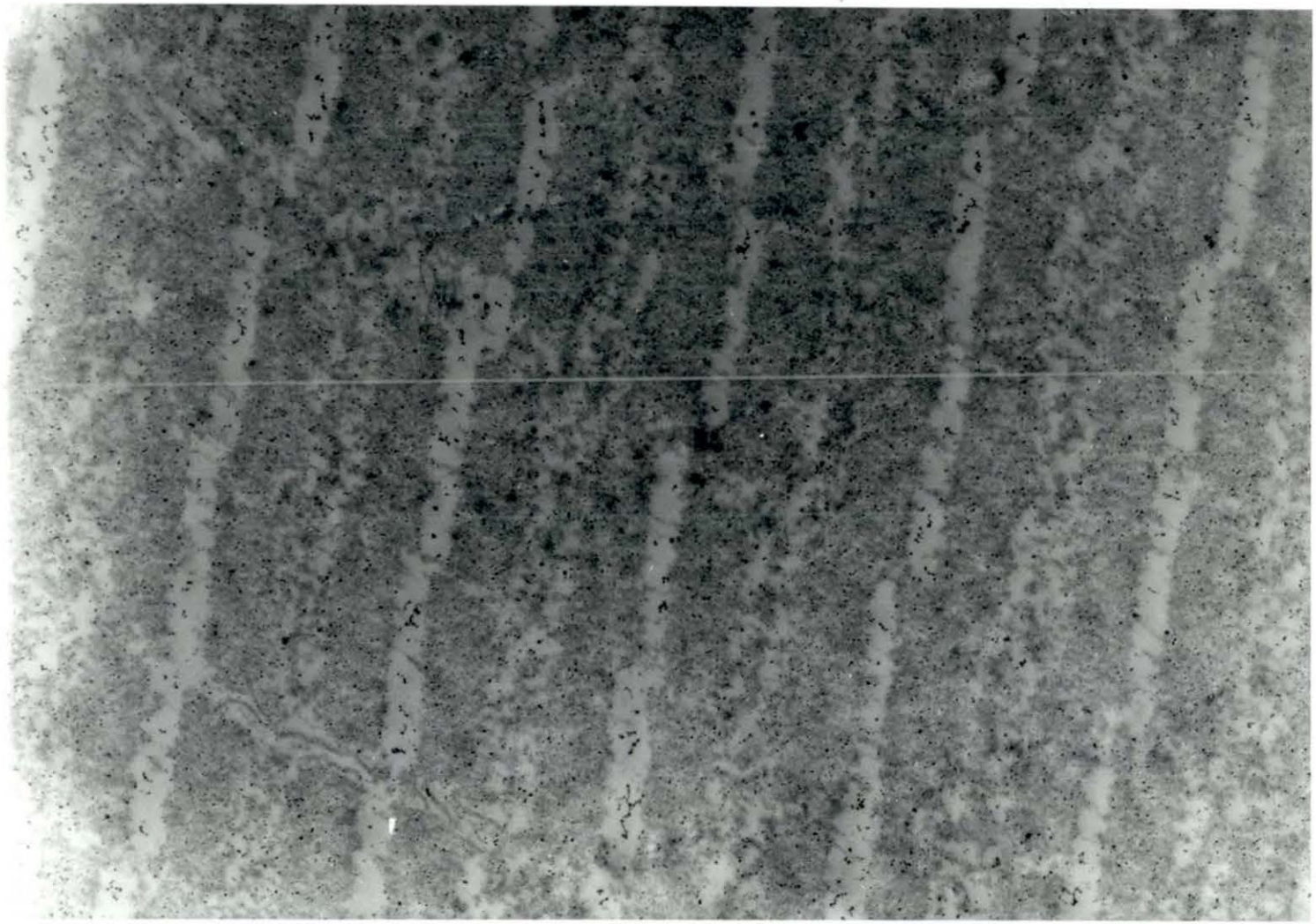


Plate 17. Electron micrograph of muscle heated to 85°C (30000X)

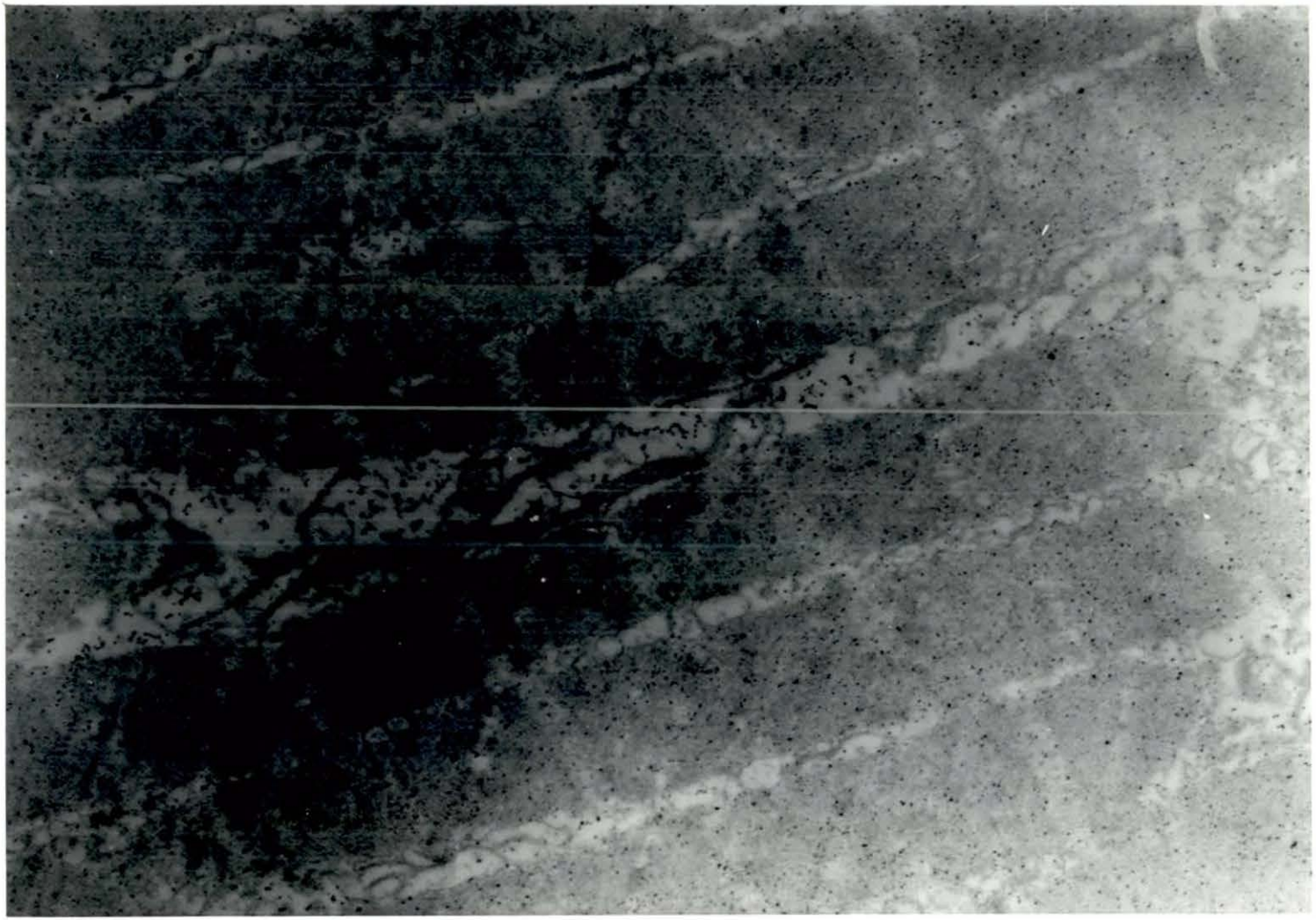


Plate 18. Electron micrograph of muscle heated to 85°C then left to drain and pressed at 30×10^6 N/M^2 for 3 minutes (30000X)

but the remains of what might have been the M-line is still evident. The sarcoplasmic reticulum is no longer discernible. Even with samples heated to 85°C (Plates 17 and 18) the sarcomere structure can still be discerned. The A-band is dense while the H-zone is still perceptible containing some dark precipitated material, presumably the proteins constituting what was originally the M-line. The sarcoplasmic space between myofibrils has disappeared so that the H-zones of different fibrils are fused together. This contraction in sarcoplasmic space may be explained by an increase in the width of the fibrils as the sarcomeres contracted lengthwise upon being heated.

C. The Determination of Specific Heat of Fatty Fish Muscle

1. A typical experiment

As the calorimetric method used in this work is not commonly used in determining heat capacity of foods, a typical experiment will serve to illustrate the measurements and calculations involved. In the example given the sample used was fish muscle but the procedures are the same for other kinds of sample and for calibration experiments.

a. Measurements

Weights	1.	empty calorimeter	231.70 g
	2.	calorimeter plus sample	373.81 g
	3.	fish sample	142.11 g

Thermostat bath temperature (Table 4.12)

Calorimeter time - temperature data (Table 4.12)

Energy measurement data (Table 4.13)

Table 4.12 Typical time-temperature data in a calorimetric run

Time (min)	T_{cal} (μV)	T_{bath} (μV)	Time (min)	T_{cal} (μV)	T_{bath} (μV)	Time (min)	T_{cal} (μV)	T_{bath} (μV)	Time (min)	T_{cal} (μV)	T_{bath} (μV)
1a	2085.2		17	2090.4	2160.7	33	2189.1		49	2231.5	2161.0
2	2085.6		18	2090.7		34	2198.6		50	2231.2	
3	2085.9	2160.8	19	2091.0		35	2208.1		51	2230.9	2161.0
4	2086.2		20	2091.3	2160.7	36c	2217.3		52	2230.6	
5	2086.5		21b	2091.6		37	2224.5	2160.8	53	2230.4	
6	2086.8	2160.7	22	2093.8		38	2228.4		54	2230.0	2160.9
7	2087.1		23	2099.3		39	2230.6		55	2229.5	
8	2087.4	2260.7	24	2106.4		40	2231.8		56	2229.2	2160.9
9	2087.8		25	2114.7		41	2232.4	2160.7	57	2228.9	
10	2088.1	2160.7	26	2123.6		42	2232.6		58	2228.5	2160.7
11	2088.4		27	2132.7		43	2232.7	2160.8	59	2228.3	
12	2088.7	2160.7	28	2242.0		44	2232.6		60	2228.0	2160.8
13	2088.9		29	2151.5		45	2232.5	2160.9	61	2227.7	
14	2089.3	2160.8	30	2161.0		46	2232.3		62	2227.4	2160.7
15	2089.6		31	2170.5		47	2232.1	2160.9	63	2227.1	
16	2089.9	2160.7	32	2179.8		48	2231.8		64	2226.8	2160.6

Table 4.12 (contd.)

Time (min)	T_{cal} (μV)	T_{bath} (μV)	Time (min)	T_{cal} (μV)	T_{bath} (μV)	Time (min)	T_{cal} (μV)	T_{bath} (μV)	Time (min)	T_{cal} (μV)	T_{bath} (μV)
65	2226.5		78	2222.9	2161.0	91	2219.4		104	2216.2	2160.9
66	2226.2	2160.6	79	2222.7		92	2219.1	2161.0	105	2216.0	
67	2225.9		80	2222.4	2161.0	93	2218.9		106	2215.8	2161.1
68	2225.6	2160.7	81	2222.1		94	2218.6	2161.0	107	2215.5	
69	2225.3		82	2221.9	2161.0	95	2218.4		108	2215.3	2161.1
70	2225.0	2160.8	83	2221.6		96e	2218.1	2161.0	109	2215.0	
71	2224.8		84	2221.4	2161.0	97	2217.9		110	2214.8	2161.1
72	2224.5	2160.7	85	2221.1		98	2217.6	2160.9	111	2214.6	
73	2224.2		86	2220.8	2161.0	99	2217.3		112	2214.4	2161.1
74	2224.0	2160.9	87	2220.5		100	2217.1	2160.7	113	2214.1	
75	2223.7		88	2220.2	2161.1	101	2216.9		114	2213.9	2161.1
76	2223.4	2160.9	89	2219.9		102	2216.6	2160.8	115	2213.7	
77	2223.2		90	2219.6		103	2216.5		116	2213.5	

T_{cal}, calorimeter temperature

T_{bath}, bath temperature

a - calorimeter and contents in steady state
b - heater on
c - heater off
e - steady state re-established; observed calorimeter final temperature.

b. Calculations

All equations and terms used in this section have been presented in Chapter III.

i. The corrected temperature rise

The observed temperature rise, equation 3.5, is $T_e - T_b$; from Table 4.12.

T_e = 2218.1 μV (t = 96 min)

T_b = 2091.6 μV (t = 21 min)

So the observed temperature rise is

$T_e - T_b$ = 126.5 μV

The correction in temperature for heat exchange with the jacket is given by equation 3.8. Its determination involves the following steps.

1. Determination of k, the leakage modulus

$k = (g_i - g_f) / (T_f - T_i)$ equation 3.4

T_i = mean temperature of the fore period (0 to 21 min) = 2088.40 μV

T_f = mean temperature of the final period (96 to 116 min) = 2215.75 μV

g_i and g_f are obtained by least squares fit of the temperature data in Table 4.12 for the fore and final periods respectively. So,

g_i = 0.31818 μV/min (= 0.007 °C/min)

g_f = -0.23052 μV/min (= -0.005 °C/min)

and therefore,

$k = 0.00431 \text{ min}^{-1}$

Table 4.13 Typical energy measurement data in a calorimetric run collected in conjunction with data in Table 4.12

Time (min)	V_h (V)	V_s (V)
$22\frac{1}{2}$	0.062223	
$23\frac{1}{2}$		0.016743
$24\frac{1}{2}$	0.062264	
$25\frac{1}{2}$		0.016742
$26\frac{1}{2}$	0.062283	
$27\frac{1}{2}$		0.016742
$28\frac{1}{2}$	0.062294	
$29\frac{1}{2}$		0.016743
$30\frac{1}{2}$	0.062295	
$31\frac{1}{2}$		0.016742
$32\frac{1}{2}$	0.062295	
$33\frac{1}{2}$		0.016742
$34\frac{1}{2}$	0.062303	
$35\frac{1}{2}$		

V_h, potential drop across heater,

V_s, potential drop across standard resistor.

2. Determination of T_m, mean temperature of the calorimeter wall during the main period from equation 3.9

T_m = (2093.8 + 2099.3 + ... 2228.6 + 2218.4) + (2218.1 + 2091.6) /2) 1/(96-21)

$T_m = 2210.36\,\mu V$

ΔT, temperature drop due to heat exchange between calorimeter and jacket, from equation 3.8.

$\Delta T = -\left[-.23052 + (0.00431 \quad (2215.75 - 2210.36))\right]$ (96-21) $= 15.54\,\mu V$

then, the corrected temperature rise, ΔT_{corr}, (equation 3.5)

$= 126.5\,\mu V + 15.54\,\mu V$

$= 142.0\,\mu V$

$= 142.0/42.44\,\mu V/^{\circ}C$

$= 3.346\ ^{\circ}C$

ii. <u>The energy input</u>

E = energy input = $\bar{V}_h\ \bar{I}\ t$ (equation 3.1)

$\bar{V}_h$ = (0.062223 + ... + 0.062303)/ 7 x 153.633 = 9.5682 V

$\bar{I}$ = (0.016733 + ... + 0.16739)/ 7 - (0.062261/131) x 153.633/10

= 0.25674 A

t = 900 s

E = 9.5682 x 0.2567 x 900

= 2210.89 J

Heat capacity of the system = $E/\Delta T_{corr}$ = $2210.89/3.346$

= 660.75 J/°C

Based on these computations, a summary of all calculations leading to the determination of the specific heat of a sample, for a particular set of conditions, can be drawn together as in Table 4.14.

Table 4.14 Calculation data for a typical isoperibol calorimetric experiment

Calorimeter initial temperature	2091.6 μV
Calorimeter final temperature	2218.1 μV
Observed temperature rise	126.5 μV
Heat exchange correction	15.54 μV
Corrected temperature rise	142.0 μV
	= 3.346 °C
Average heating current	0.25673 A
Average potential drop across heater	9.5682 V
Time of heating	900 s
Energy input	2210.89 J
Heat capacity of the calorimeter system	660.75 J/°C
Heat capacity of the calorimeter	146.8 J/°C
Heat capacity of the sample	513.9 J/°C
Fish sample weight	142.11 g
Sample specific heat	3.62 J/g°C

2. Evaluation of an existing calorimeter

In order to estimate the degree of accuracy of the existing calorimeter, it was calibrated through the use of water as a reference substance, and then utilised to determine the heat capacity of diphenyl ether, which is known and obtainable from the literature (Ginnings and Furukawa, 1953). In this manner, comparing the experimentally determined values for the heat capacity of diphenyl ether with those from the literature, it would be possible to verify if the existing calorimeter gave reasonably accurate results. Table 4.15 shows the values obtained for the heat capacity of

Table 4.15 Experimental data and calorimeter heat capacity as a function of temperature

Average exp. temperature (°C)	Corrected temp. rise (μV)	Corrected temp. rise (°C)	Energy input (J)	Total Heat capacity (J/°C)	Calorimeter heat capacity (J/°C)
30.54	152.3	3.57	2276.21	637.59	111.57
32.61	151.2	3.54	2263.41	639.38	113.39
37.01	156.8	3.54	2269.31	641.05	115.04
39.31	154.0	3.56	2271.19	637.97	111.95
42.66	156.1	3.56	2269.93	637.62	111.59
44.93	156.5	3.56	2266.43	636.64	110.51
47.71	157.3	3.53	2267.41	642.33	116.11
48.90	157.8	3.51	2271.22	647.07	120.81
51.88	158.8	3.54	2270.97	641.52	115.15
54.36	159.1	3.53	2270.51	643.20	116.72
56.34	160.4	3.56	2269.83	631.59	111.02
58.86	161.0	3.56	2272.99	638.48	111.78
61.24	161.3	3.57	2272.18	636.46	109.63
64.10	163.8	3.58	2274.60	635.36	108.63
66.45	160.6	3.50	2269.42	648.40	121.24
69.25	164.0	3.54	2271.02	641.53	114.16
71.29	163.8	3.53	2259.76	640.16	112.64
73.76	163.8	3.53	2239.53	634.43	106.77
76.15	166.0	3.47	2258.06	650.74	122.82
78.32	166.5	3.47	2254.69	649.77	121.65
				Mean	114.1
				Standard error of mean	1.0

the calorimeter vessel in relationship to temperature. Values of its heat capacity were obtained by subtracting from the total heat capacity the heat capacity of 125.89 g of water at these temperatures, according to specific heat values of water given by Ginnings and Furukawa (1953). The calorimeter is made mostly of copper and as the specific heat of copper varies very little within the experimental temperature range, from 0.384 J g^{-1} $^{o}C^{-1}$ at 25°C to 0.394 J g^{-1} $^{o}C^{-1}$ at 100°C (Weast, 1979), the variation of its heat capacity between 30 and 100°C would be approximately 2 joules from this source. This figure is much less than the random experimental error, and therefore although it would have been much more reasonable to use temperature increments as large as 10°C, the option was for small temperature increments of 3°C, so that any abnormal behaviour of the calorimeter for a particular temperature range could be detected. Since the observed scatter of values in Table 4.15 showed no tendency to increase or decrease with temperature they were assumed to be random, and an average value for the heat capacity of the calorimeter was calculated over the temperature range studied. This average value for the calorimeter heat capacity was used to determine the diphenyl ether heat capacity.

The values for the heat capacity of diphenyl ether at four different temperatures are shown in Table 4.16 for the sample of 134.78 g and values per mol are compared with the literature values of Ginnings and Furukawa (1953). Since these four single values were obtained under different experimental conditions, different temperatures and different rate of energy input, and all showed the same trend of being smaller than the more highly accurate results reported in the literature, they were considered sufficient to estimate the degree of accuracy of the existing calorimeter. From the comparison of these results, it was concluded that the accuracy of the calorimetric apparatus could be estimated to be around 3%.

Table 4.16 Comparison between experimental and literature values for diphenyl ether heat capacity

Average experiment temperature (°C)	Energy input (J)	Temperature rise (°C)	Total heat capacity (J)	Diphenyl ether heat capacity (J/°C mol)	Literature value* (J/°C mol)	Deviation (%)
40.25	1445.08	4.40	328.43	270.62	274.16	-1.29
47.47	1296.47	3.96	327.39	269.31	277.38	-2.91
55.54	1263.73	3.83	329.96	272.55	281.05	-3.02
67.28	1159.97	3.43	338.18	282.93	286.37	-1.20

* Ginnings and Furukawa (1953)

Karmas and Di Marco (1970) state that enthalpies of endothermic transitions in proteins are of the order of 4.18 J/g protein. Assuming a fish muscle sample of 138 g with a protein content of 20%, an endothermic transition would absorb approximately 115 J. Under the experimental conditions used in this work, it would mean a deviation of approximately 0.15 °C in the calorimeter temperature rise. Thus, if a thermal transition in the fish muscle occurred within a small temperature range, 3-4°C, there was a possibility that the existing calorimetric apparatus could detect it. However, if the transition was spread over a large temperature range then almost certainly the existing apparatus would not register it.

Based on the above considerations it was felt at this stage that the construction of an apparatus definitely able to detect any thermal transition occurring during heating of fish muscle, would demand a knowledge of physics and electronics far beyond the author's field of interest. It was decided therefore, to establish the determination of specific heat of herring as the main purpose of the calorimetric studies. However, in an attempt to obtain better overall precision, thus increasing the chance of any large thermal transition being detected, it was also decided to design and construct a new calorimetric apparatus with improved characteristics to determine the specific heat of fatty fish muscle.

All the subsequent measurements described in this Chapter were obtained with the new calorimeter, whose design was discussed in Chapter 3.

3. The specific heat of herring muscle

a. The calorimeter calibration

Table 4.17 shows the figures used in obtaining a mean value for the heat capacity of the calorimeter vessel through the use of a 0.8% agar

Table 4.17 Improved calorimeter, first calibration

Temperature (°C)	Calorimeter heat capacity (J/°C)
36.42	153.27
42.16	158.56
42.56	153.51
42.77	148.52
44.98	151.62
47.94	154.76
52.25	151.61
64.08	150.52
67.60	158.23
70.92	153.09
Mean	153.3
Std. deviation	3.2
Std. error of the mean	1.0

gel which was taken as having the same specific heat as water. This mean value of 153.3 J/°C was the one used in the calculations for determining the specific heat of the high fat content herring muscle in the first series of runs. The large number of calorimetric runs, necessary to determine the specific heat of these samples, necessitated considerable handling of the calorimeter vessel in filling and cleaning operations, with the consequence that at the end of these runs its internal chronium-plate had been impaired. For the same reason two of the screws sealing the lid had to be replaced. The internal surface of the calorimeter jacket became slightly tarnished as also did the aluminium foil covering its lid. Further use of the calorimetric system accentuated these changes that must have altered the emissivity properties of the surfaces affected and also changed slightly the calorimeter weight. All these changes might have modified the pattern of heat exchange between the calorimeter and its jacket, and therefore it was thought advisable to recalibrate the calorimeter after the calorimetric runs using low fat content fish were completed. As the results with the previous calorimeter, Table 4.15, and also those for the first calibration, Table 4.17, did not show any influence of temperature, this second calibration was carried out essentially at one temperature (Table 4.18). The replication which gave a new mean value of 146.8 J/°C for the calorimeter heat capacity, allowed an additional estimate of the calorimeter repeatability, as will be discussed later. This new calibration value was only about 4% below the first one, and the specific heat of water (Table 4.18) calculated using the heat capacity of the calorimeter from the first calibration would be at most 1.5% below the literature value.

Table 4.18 Improved calorimeter, second calibration

Temp. (°C)	Energy introduced (J)	Temp. increase (uV)	Temp. increase (°C)	Specific heat of water (J/°C)*	Deviation[a] (%)	New calibration value (J/°C)
62.394	2228.82	135.33	3.132	4.12	-1.51	144.81
62.440	2233.57	135.31	3.132	4.13	-1.23	146.36
62.599	2213.67	144.80	3.351	4.12	-1.37	146.29
62.733	2230.53	145.50	3.363	4.15	-0.88	148.84
62.772	2233.93	134.73	3.116	4.16	-0.58	150.08
65.939	2228.85	135.87	3.132	4.12	-1.51	144.53

Mean 146.8

Std. deviation 2.2

Std. error of the mean 0.9

* - determined using previous calorimeter calibration given in Table 4.17

a - deviation from specific heat of water taken as 4.18 J/g°C

b. The influence of composition and temperature on the specific heat of herring muscle

The results shown in Table 4.19 and Table 4.20 clearly indicated that the influence of the small variations in composition within the two batches of fish used in these studies was negligible, and could not be distinguished from the random errors, considering the precision that the experimental method allowed.

The difference between the means for the two samples of fish over the whole temperature ranges studied (Table 4.21) was shown by a t-test to be statistically significant at 0.1% level. The higher specific heat of the LFC fish reflects the overwhelming influence of the high specific heat of water on the specific heat of foods. Based on these two sets of data (Table 4.21) equation relating the specific heat of herring muscle to its composition is:

$$Cp = 0.65\ X_F + 0.46\ X_s + 1.0\ X_w \qquad (4.1)$$

where, X_F, X_s and X_w are respectively the weight fractions of fat, solids and water present in the muscle. The specific heat of the fat fraction obtained in this way is rather higher than values reported for oils in general (Charm, 1971) and in particular for marine oils (I.C.T. (1927), Tidemann, 1977) that are about 0.5 cal/g°C. On the other hand the specific heat of the fat-free solids fraction (mostly protein) agrees rather better with the figures given by Bull and Breese (1968) and Krecheck and Benjamin (1964) of 0.396 and 0.457 respectively for egg albumin. It is however larger than the figure of 0.3 cal/g°C given by Riedel (1956) for the "dry substance" of fish and meat. Thus it seems that the high specific heat of the fat fraction is genuine and it is necessary to reconcile it with the published values. It may be that the published figures were obtained for temperatures near room temperature and

Table 4.19 Specific heat of high fat content herring as a function of composition and temperature

Moisture (%)	Fat (%)	Temp. (°C)	Specific heat J/g°C	Moisture (%)	Fat (%)	Temp. (°C)	Specific heat J/g°C
64.2	17.5	34.61	3.45	64.9	13.9	71.12	3.51
63.0	18.2	36.25	3.45	64.9	13.9	74.34	3.51
64.7	15.8	38.91	3.43	64.4	16.0	78.64	3.53
64.4	15.7	44.06	3.41	63.5	17.1	85.57	3.48
65.0	14.7	46.52	3.42	63.4	15.4	85.32	3.42
64.5	15.7	51.57	3.38	63.4	15.4	89.03	3.45
62.6	18.8	53.00	3.38	63.5	17.1	89.16	3.59
65.0	14.7	55.63	3.51	62.9	17.4	93.66	3.51
62.6	18.8	56.84	3.50	62.9	17.4	97.18	3.53
64.8	15.7	60.02	3.49	64.0	15.9	99.02	3.61
64.8	15.7	63.44	3.54	64.0	15.9	103.04	3.61
65.0	16.0	66.19	3.46	65.1	14.8	106.09	3.54
65.0	16.0	69.64	3.50	65.1	14.8	109.59	3.51
				65.6	14.5	112.69	3.48

Table 4.20 Specific heat of low fat content herring as a function of composition and temperature

Moisture (%)	Fat (%)	Temp. (°C)	Specific heat (J/g°C)	Moisture (%)	Fat (%)	Temp. (°C)	Specific heat (J/g°C)
69.1	9.8	39.15	3.59	71.7	6.7	70.07	3.62
71.2	7.8	42.60	3.51	70.8	7.8	72.84	3.57
71.0	8.9	45.48	3.56	70.0	9.2	76.53	3.56
72.2	7.3	50.46	3.62	70.8	7.8	76.64	3.57
71.6	7.7	53.76	3.62	-	-	78.97	3.54
72.2	7.3	54.42	3.62	69.3	9.6	79.32	3.66
70.9	8.6	56.74	3.56	69.8	8.9	81.24	3.63
71.6	7.4	59.14	3.53	69.8	8.9	84.96	3.59
70.5	8.7	59.22	3.52	69.3	9,6	86.23	3.64
70.7	8.1	60.93	3.60	70.7	8.1	87.07	3.58
71.6	7.4	62.82	3.56	70.7	8.1	91.61	3.59
70.5	8.7	62.84	3.53	70.6	7.9	94.66	3.68
69.1	9.8	63.17	3.57	70.6	7.9	98.37	3.67
70.6	8.9	64.29	3.56	70.5	7.8	101.93	3.53
71.3	8.0	66.56	3.48	70.5	7.8	105.88	3.56
71.3	8.3	66.89	3.58	71.1	7.5	108.75	3.53
				71.0	8.2	108.88	3.44

it has been noted (Meara, 1978) that there is a progressive increase in specific heat of fats with increase in temperature and this could account for part of the high values for the specific heat of the fat component. Meara (1978) also collected evidence suggesting that there is an increase in specific heat with increasing unsaturation of fatty acids and their simple triglycerides. It is well known that herring oil, like fish oils in general, is very unsaturated and this could be another reason to explain the high specific heat of the fat fraction.

Concerning the effect of temperature on the specific heat, Figure 4.10 shows that there was no major heat effect that could be detected by the calorimetric method employed. There are no definite peaks or troughs in Figure 4.10 that can be attributed to reactions taking place during heating and that might be associated with the protein changes or changes in water holding capacity described previously. Any heat effect comparable to the heat denaturation of rabbit muscle of 16.7 J/g of protein reported by Wright et al. (1977) would be close to the scatter of these results. However, a closer look at Figure 4.10 indicates that the specific heat values of the high fat content samples were consistently smaller for the lower range of the temperatures studied than those of the upper range. The correlation coefficient between specific heat and temperature for HFC results is 0.61, fairly low, but very highly significant, and confirms the influence of temperature. As major changes in water holding capacity of the herring muscle, discussed in previous sections, occurred below 60°C this temperature served as reference point to divide the whole population of specific heats in two ranges: one below 60°C inclusive, and the other above 60°C. For LFC samples the mean specific heats of the two populations (Table 4.21) were the same, confirming the impression given by Figure 4.10. On the other hand the mean specific heat for the data collected below 60°C,

Figure 4.9. Time Temperature Plot of Calorimetric Runs with and without Heat being Evolved.

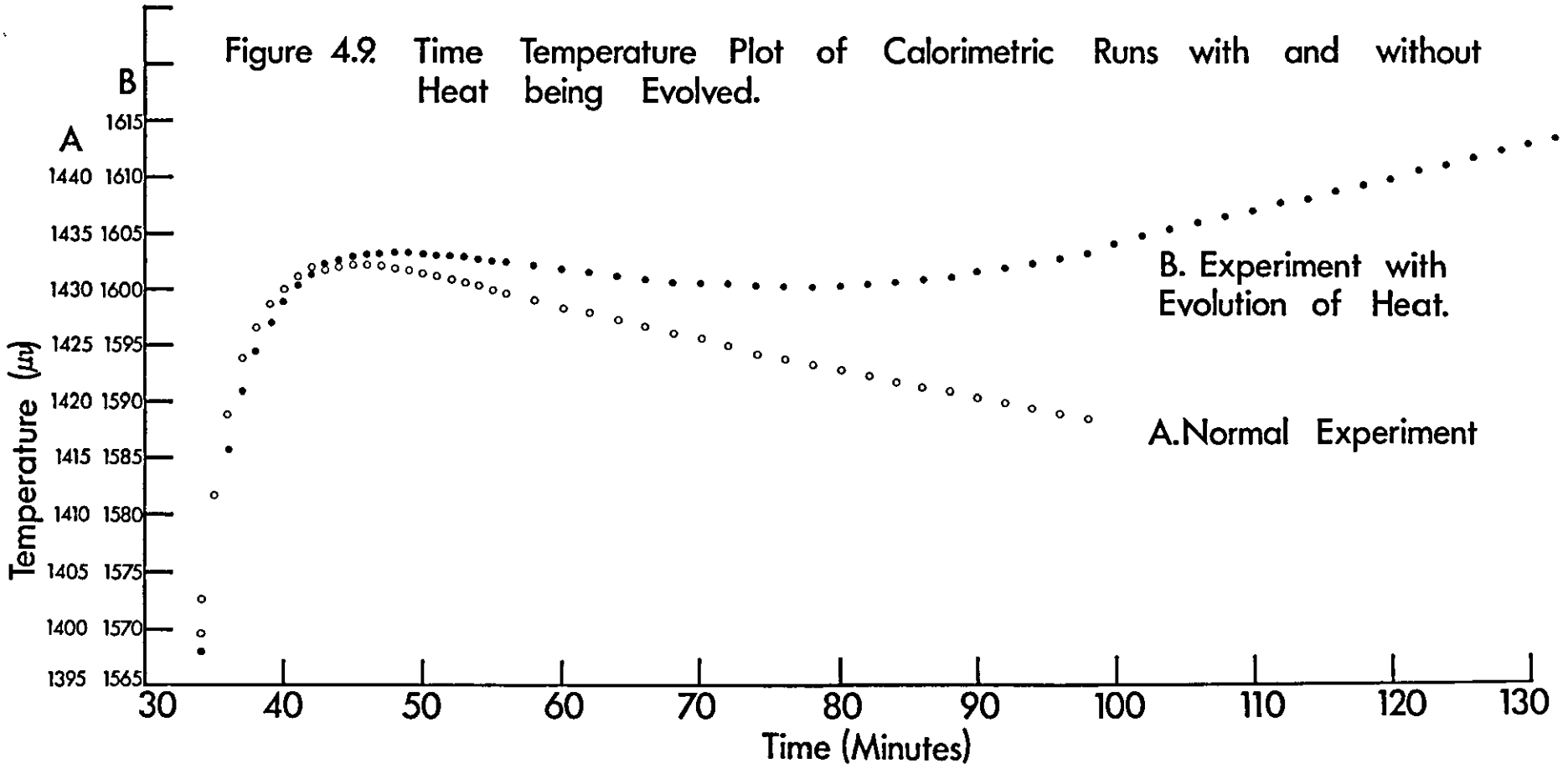


Table 4.21 Influence of composition and temperature on specific heat of herring muscle

Composition[a]		Type of muscle		Temperature range (°C)	Number of experiments	Mean specific heat (J/g°C)	Statistical analysis of means[b]		
Moisture	Fat						SEM	SEMD	t-test
64.1	16.5	HFC	1	34.6 - 60.0	10	3.44	0.01		
64.2	15.7		2	63.4 - 112.7	17	3.52	0.01		
								0.020	4.01***
71.1	8.1	LFC	1	39.1 - 60.9	10	3.57(3)	0.01		
70.5	8.3		2	62.8 - 108.9	23	3.57(5)	0.01		
								0.018	0.11 ns
64.1	16.1	HFC	1	34.6 - 112.7	27	3.49	0.06		
70.7	8.3	LFC	2	39.1 - 108.9	33	3.57	0.05		
								0.014	5.71***

SEM - Standard error of the mean

SEMD - Standard error of the difference between means

a - Average over respective temperature range

b - Comparison between pair of means 1 and 2

for HFC samples, was statistically highly significantly smaller than the mean for the upper temperature range. This different behaviour of the specific heat of HFC fish in relationship to temperature might be correlated with thedifferentway that other changes proceeded with temperature during the cooking of the two batches of fish. The softness exhibited by HFC fish when cooked at lower temperatures, in contrast to the increase in rigidity shown by LFC fish, might have been associated with collagenolytic, proteolytic and lipolytic reactions, as the enzymes present in the muscle went through their optimal temperature range of activity; these reactions, or their subproducts reacting further, could have caused evolution of heat leading to the apparent slightly depressed specific heat.

This suggestion derives from a problem experienced during the determination of the specific heat of HFC samples at lower temperatures. When the running of two consecutive experiments with the same filling was tried, at the end of or during the second run heat began to be evolved inside the calorimeter due to bacterial and/or muscle enzyme action. In the few cases that were followed to investigate if micro-organisms may have been involved, the fish was completely liquefied at the end of the run and displayed strong off-odours. Figure 4.9 shows one of the experiments followed, and from its time-temperature curve, the large amount of heat evolved is noticeable when compared with the curve for a normal calorimetric experiment carried out at a slightly lower temperature. Based on these curves calculations revealed that the rate of temperature increase, probably during the period of exponential growth of the micro-organisms or of maximum enzyme activity, was 0.016°C/min. Taking the average value of specific heat of the fish muscle as 3.49 J/g°C the rate of heat evolved would be circa 0.072 J/min g of muscle. In the particular example given, the temperature inside the calorimeter before heat was supplied was around 37°C, but even at a temperature as high as 45°C, the

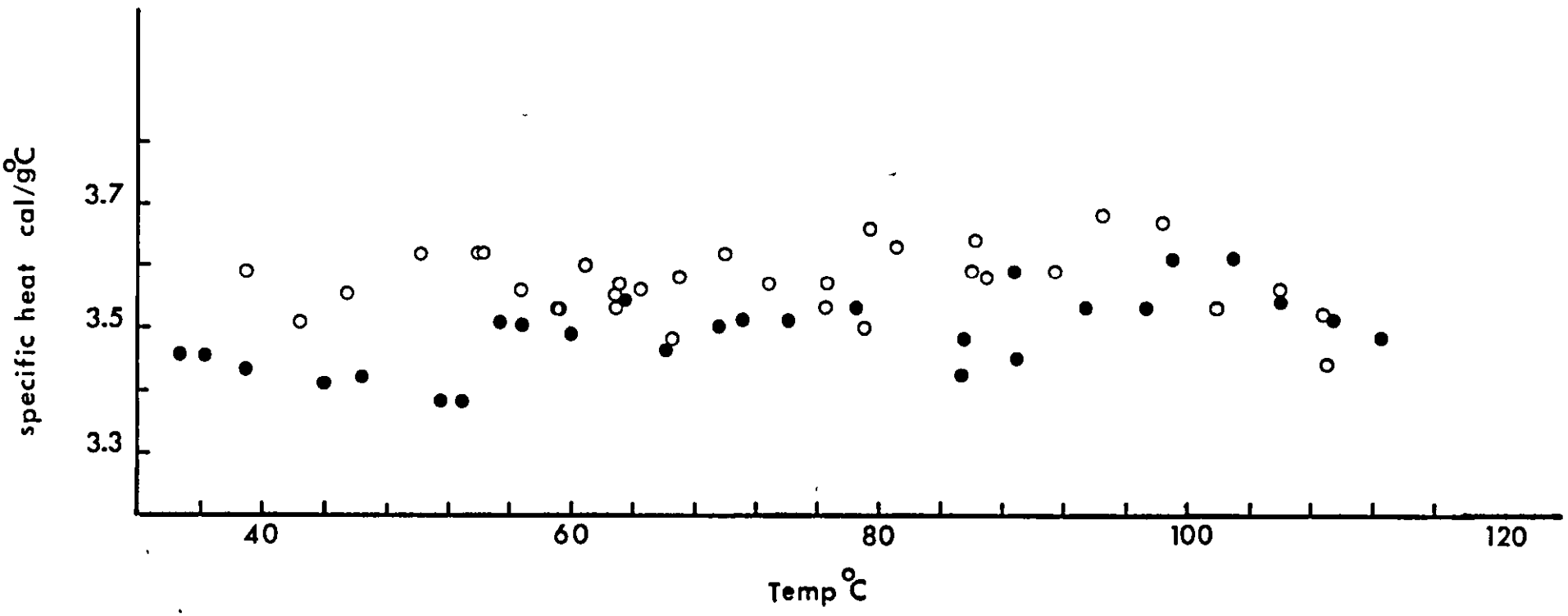


Fig 4.10 The specific heat of herring muscle as a function of temperature
LFC ○
HFC ●

same phenomenon was observed. The fish muscle in those experiments where microbiological growth occurred had been in the calorimeter for about $4\frac{1}{2}$ hours; such a fast and extensive hydrolysis would not be expected in so short a time, by the action of micro-organisms alone, since pure cultures in pure substrates have a lag period extending from 2 to 5 hours before the micro-organisms enter the exponential growth phase and heat is evolved (Forrest, 1969).

Initially it was suspected that there was a sudden explosion of bacterial growth due to favourable conditions of temperature and oxygen tension prevailing inside the calorimeter (the calorimeter was evacuated before sealing, so the medium inside it was at least partially anaerobic). Two factors could have significantly contributed to a sudden growth of bacteria: heavy contamination during the hand filleting operation or growth of dormant bacteria after the thawing operation. In relation to this latter factor, it has been found that very low storage temperatures, like the $-30^{\circ}C$ used in this work, are less effective in reducing the bacterial flora than higher temperatures (Heen and Karsti, 1965). However, in another experiment where evolution of heat and hydrolysis of the muscle occurred, the total viable count of micro-organisms at the time of filling the calorimeter was only 4×10^3 micro-org/g of muscle, indicating that the fish muscle was in good condition as far as microbiological standards are concerned. After the calorimetric run the total viable count had increased to 1.5×10^5 micro-org/g of muscle a figure still within acceptable limits for good quality fish. Bacterial proteinase activity only becomes significant when the spoilage flora reaches 10^7 bacteria/g of muscle, although sufficient enzyme is apparently elaborated by such a population to produce proteolysis in the absence of living bacteria (Liston, 1965). On these considerations bacterial growth alone would

not explain the extensive hydrolysis of the muscle and, therefore, muscle enzymes may have played an important role in degrading the muscle proteins. Mitochondrial and microsomal membranes are disrupted post mortem, leading to a redistribution and liberation of enzymes, including proteolytic and lipolytic enzymes (Granroth and Karvinen, 1978). Considering that fish muscle contains about ten times as much cathepsins as mammalian muscle and that their optimum activity is around 40°C (Siebert and Schmitt, 1965), or even at higher temperatures at a suitable pH (Makinodan and Ikeda, 1969), it can be strongly suggested that in the hydrolysis muscle enzymes might have had a predominant role. It is of interest to mention that traditional fermented liquid fish products are prepared using fatty fish species (Mackie et al., 1971).

The phenomenon just discussed underlines how easily fish protein suffers degradation, and further heightens the need for proper control previous to or during cooking of fish. If, for any reason, the fish is kept at a high ambient temperature before cooking, or kept in low heat during cooking, there is the risk of considerable unsuspected bacterial growth, that, if not followed by a suitable sterilising process, may cause the fish to become spoiled during the storage period preceding its consumption.

In agreement with Mohsenin (1975) and Rha (1975), the specific heat values found in this study were higher than those that could be obtained from knowledge of the fish composition, through the use of formulae available in the literature which are based on the specific heat of the individual components of the food material. Equations that do not make allowance for fat content like those of Siebel (1892), Lamb (1976) and Riedel (1956) obviously give as a result specific heats much lower than the experimentally determined ones as shown in Table 4.22. But even

equations that make allowances for the presence of fat in the foodstuff like that given by Charm (1971) and Elston (1977) still give low specific heat values compared to those found in this work (Table 4.22). For practical purposes the differences between the experimental values and those obtained by equation 2.6 are probably not important and this method of addition of specific heats can be used.

a. Errors in the measurement of specific heat

Errors in determining specific heat can be divided into determinate and indeterminate (Stansbury and Brooks, 1969). Determinate errors are those associated with the measurement of the quantities in equation 2.3, and which can be quantitatively evaluated. These errors put a direct upper limit on the accuracy of the measured specific heat. Indeterminate errors represent those brought about by factors such as uncorrected heat exchange between calorimeter and its surroundings so that the measured power, EIt, is not the correct heat input to the calorimeter system.

In discussing errors in calorimetry, it is necessary to define terms like accuracy, repeatability, precision and reproducibility. Accuracy is used to indicate how close is the measured quantity to its true value which is influenced by both determinate and indeterminate errors. Repeatability can be defined (Stansbury and Brooks, 1969) as the difference in successive values when the measurements are repeated under as nearly the same conditions as possible. Reproducibility, on the other hand, is the difference in successive values when measurements are made under various operating conditions. These two last terms in calorimetry are evaluated by calculating the standard error of the mean of a set of results, which is also adopted as the measurement of precision.

Table 4.22 Calculated specific heats of the fish used in this work according to equations available in the literature

Type of fish		
HFC	LFC	Source
2.96 (-15.1)*	3.17 (-11.2)	Eq 2.4, Siebel (1892)
2.29 (-20.0)	3.38 (-5.3)	Eq 2.5, Lamb (1976)
3.26 (-6.6)	3.42 (-4.2)	Eq 2.7, Riedel (1956)
3.26 (-6.6)	3.38 (-5.3)	Eq 2.6, Elston (1977), Charm (1971)

* Figures in brackets represent percentage deviation from the mean measured values given in Table 4.21

Although the estimation of reproducibility might give an idea of the magnitude of indeterminate errors, a constant, systematic error would not affect the reproducibility. The overall accuracy of the specific heat measurement as mentioned above is determined by the combination of the determinate and indeterminate errors. In what follows, an attempt is made to estimate both types of error in the specific heats obtained in this work.

1. Determinate errors

The error δCp in the specific heat ($Cp = EI\Delta t/m\Delta T$, (eq 3.1 and 2.3) is given by the expression:

$$\delta Cp = \frac{\partial Cp}{\partial E}\delta E + \frac{\partial Cp}{\partial I}\delta I + \frac{\partial Cp}{\partial \Delta t}\delta(\Delta t) + \frac{\partial Cp}{\partial m}\delta m + \frac{\partial Cp}{\partial T}\delta(\Delta T) \qquad (4.2)$$

The detectabilities, δ , of E, I, t and m in equation 4.2, are easily evaluated because they are represented by the accuracy of the instruments used in their measurements. The detectability of T however, is not so straightforward, because it comprises errors in the calibration of the thermocouple measuring the calorimeter temperature and in estimating the temperature correction added to the observed temperature rise. Errors in determining the temperature interval depend on the output signal of the thermocouple relative to the accuracy of the device used to measure the signal. With the copper-constantan thermocouples used in this work, the output signal is larger than $40\,\mu V/^{\circ}C$, the vernier potentiometer used has an accuracyof0.005% and can be read directly to $0.1\,\mu V$. In the range of temperature studied this would correspond to $\pm$ 0.002°C. An estimation of errors arising from the measurements and calculations necessary to determine the temperature interval ΔT, Appendix 10, indicates that the uncertainty appears to be $\pm$ 0.3%. The error in absolute temperature is not significant because the specific heat does not change greatly with temperature. Table 4.23 summarises the determinate errors in

measuring specific heat, from data in Tables 4.12 and 4.13.

Table 4.23 Errors in the terms of equation 2.3

Quantity	Value	Detectability
$\bar{E}$ (V)	9.5682	0.0001
$\bar{I}$ (A)	0.25674	0.00001
Δt (s)	900	0.2
m (g)	375.41	0.05
ΔT (^{o}C)	3.34	0.01

The sum of the contributions to δCp (Appendix 11) from each term of equation 4.2 are respectively.

$$0.000018 + 0.000068 + 0.00038 - 0.00023 - 0.005$$

$$= 0.005$$

so,

$$\delta Cp = 0.005$$

It is clear from the above that the error in ΔT is the predominant one and that for higher accuracies the measurement of the temperature interval should be improved. The calculated value of Cp using the quantitites in Table 4.23 is 1.73/J $g^{o}C$ and therefore the determinate error is about $\pm$ 0.3%.

2. Indeterminate errors

Indeterminate errors are those which are reflected in the repeatability of the measuring technique. Two of these sources of errors are temperature gradients inside the calorimeter and heat conduction from the calorimeter to its surroundings.

i. Temperature gradients inside the calorimeter

Since the temperature of the fish plus calorimeter is taken as that measured on the surface of the calorimeter it is necessary to consider whether this measurement correctly represents the temperature of the system. Calibration should have eliminated most of the errors arising from this source and, based on the error found in the determination of the heat capacity of water when the calorimeter had been calibrated with a 0.8% agar gel, errors from this source would be around ± 0.6%.

ii. Heat conduction from the calorimeter system

Heat conduction along the thermocouple, potential taps and heater lead wires introduces an error since the heat input to the calorimeter is not truly represented by the measured input. Considering that the thermal conductivity of fish and water are of the same order, these errors should have been eliminated by the calibration procedure and by the estimation of the temperature correction, so that they probably are of a magnitude that makes them negligible in the present work.

3. Accuracy of the specific heat measurements

Using the values of the heat capacity of the calorimeter in Table 4.18, the standard error of the mean indicates that the repeatability of the method of measurement is about ± 0.6%. However, results for specific heat of fish in Tables 4.19 and 4.20 (standard error of means given in Table 4.21), indicate that the precision or reproducibility is about ± 0.3%. Considering the estimates for determinate and indeterminate errors it can be estimated that the accuracy of the method is better than ± 1%.

CHAPTER 5

SUMMARY AND TECHNOLOGICAL SIGNIFICANCE OF RESULTS

1. Summary

This work embodied three approaches to studying the physical effects of heating on herring muscle at two fat levels in order to obtain basic information that might be usefully applied to the manufacture of fish meal, the canning of fish and other industrial processes.

The first approach involved the determination of water and fat release during cooking and pressing of the muscle as a function of temperature and holding times. Temperatures studied were in the range of about 35-113°C and holding times were 0, 15, 30 and 60 minutes. Time 0 was taken as the time when the fish muscle reached the desired cooking temperature. Results indicated that temperature had a statistically significant effect on fat and water release, for both cooking and pressing treatments, but holding times had no significant effect. Distribution of water and fat losses with temperature were practically the same for both low fat content (LFC) and high fat content (HFC) muscles; contrary to current knowledge losses of water and fat did not proceed regularly with temperature. Water losses during cooking increased from about 35°C to about 45°C and then decreased, reaching a minimum value around 60°C. From there water losses increased again with temperature. Only when the cooking temperatures were about 100°C for LFC and 85°C for HFC muscle did the figures for water loss exceed those at about 45°C. The distribution of water loss with temperature during pressing was inversely related to that during cooking, for both samples. Thus, the temperature range where maximum release of water by pressing occurred was 50-65°C. Water losses during pressing were generally higher than during cooking, except for temperatures about and above 100°C, in the case of LFC samples, or 85°C, for HFC samples, when losses were smaller than during cooking.

It was not possible to form a clear picture of the distribution of fat losses by cooking and by pressing as a function of temperature because values, especially for LFC samples, were somewhat scattered. However, it was possible to discern that temperatures as low as 55-60°C were as efficient as temperatures around 100°C in easing the liberation of fat from the fish muscle. Fat losses by pressing were generally higher than by cooking only, indicating that the fat loosened by heating of the muscle is physically entrapped within the muscle fibres.

The composition of the fluids released by cooking and pressing is an important parameter in evaluating the efficiency of any particular fish meal process. For LFC muscle water contributed the largest amount (some 83-88%) to the fluid released during cooking, fat was only 2-6% and therefore solids made up 6-14% of the cooking liquor weight. For HFC muscle the fluid released by cooking contained a much large proportion of fat. The fat content of the liquor varied from about 7% at 35°C to a maximum of about 27% at 55°C, falling to about 16% at higher temperatures. The percentage amounts of water, fat and solids constituting the pressed out liquor were very close to those released during cooking for both types of muscle, the amount of solids being generally smaller than that found in the cooking liquor. The striking characteristic of the liquor released by both types of muscle by cooking and by pressing was the low amount of solids, on average only 6-8% of the liquor weight for temperature above 55°C.

The second approach involved the design and construction of a simple isoperibol calorimetric system to determine the influence of composition and temperature, in the range of about 35-112°C, on the apparent specific heat of herring muscle. This study had also the aim, by noting any significant variation in the apparent specific heat of the muscle, of detecting any large energy change occurring during heating that could

be associated with the changes observed during the cooking studies. There was no significant temperature influence on the apparent specific heat of LFC muscle (71% moisture, 8% fat) but for HFC muscle (64% moisture, 16% fat) the average of specific heat values under 60°C was significantly lower, at the 0.1% level, than the average for the upper temperature range. There were no obvious peaks or troughs in the specific heat values as a function of temperature that could unequivocally be attributed to endothermic or exothermic reactions taking place during heating. However, in measurements at temperatures under and around 45°C and only after prolonged residence of the fish inside the calorimeter there developed a considerable evolution of heat (0.07J/min g of muscle); this was attributed to the combined effect of microbial growth and muscle enzyme activity, the latter factor being considered predominant. Concerning the influence of composition on the apparent specific heats, the value of 3.57 $\pm$ 0.01J/g°C found for LFC muscle, over the temperature range 39-109°C, was statistically significantly higher, at the 0.1% level, than the value of 3.49 $\pm$ 0.01J/g°C for HFC muscle, in the temperature range 34-112°C. For practical purposes, these values show good agreement with those derived from formulae that make use of the food material composition.

The third approach encompassed subjective observations on changes in translucency, texture, palatability, integrity of the skin as affected by heating, and optical and electron microscopic examination of the heated muscle.

There were striking differences in the sensory properties of the two types of muscle when heated. From 35 to 45°C the flesh of LFC samples became increasingly firmer whereas those of HFC became softer. From 50°C upwards the texture of both types of muscle grew firmer and more fibrous that of LFC tending to be more friable. It is suggested that in addition

to the role of the denaturation of contractile proteins, changes in collagen must have contributed to these textural differences between the two types of muscle.

Maximum translucency in LFC muscle occurred at 40-45°C whereas for HFC muscle it occurred at 35-40°C. Above these temperatures both muscles became opaque and it was not possible to make any certain subjective distinction between the effects of different temperatures. At 35°C the skin of HFC samples became loose and could be easily removed intact from the muscle whereas it was necessary to heat HFC muscle to about 45°C to repeat the phenomenon. From 50°C upwards the skin of both types of fish would break easily and tended to stick to the cooking bag. Increases in translucency were attributed to increase in the homogeneity of the tissues caused by degradation of connective tissue to gelatin, increase in the solubility of myosin, and release of water and fat within the muscle. The increase in opacity at 50°C was considered to have been brought about by the precipitation of the proteins within the muscle tissues. Finally, concerning palatability, both types of muscle were completely palatable at a temperature as low as 50°C.

Only HFC samples were used in microscopic studies. Significant changes in the microscopic appearance of the muscle, using optical microscopy, occurred when the muscle was heated to 45°C. At this temperature granulation in the connective tissue spaces and around fibres started to take place and there were also signs of shrinkage in the length of the fibres. Greater changes occurred upon heating the tissues to 55°C: larger quantities of granular material were deposited as described previously; there was an apparent increase in extracellular space and the colour taken up from the histological stains by the muscle fibres was altered; the connective tissue appeared to be gelatinized. Heating the muscle to 60°C did not produce further greater changes in

its microscopic appearance. Pressing did not alter greatly the microscopic appearance of the heated muscle apart from concentrating the red stained granular material. From these results coupled with the sensory observations it is suggested that the coagulation of sarcoplasmic proteins and the gelatin derived from collagen may have a more important role in retaining water in muscle then has been so far supposed.

Electron microscopy showed that even after heating the muscle at 85°C the sarcomere structural organisation could be discerned although the Z line had disappeared and the H zone had been disrupted.

2. Technological significance of results

Although, as mentioned earlier, the cooking and pressing procedures used in this study were not designed to conform with processes applied in fish meal and oil manufacture, canning or hot-smoking, the results may nevertheless be relevant to these industrial processes.

Several different processes may be utilised in the manufacture of fish meal and oil but most producers still make use of the traditional method. This means that the fish is heated to about 100°C inside horizontal cylinders provided with a conveyor screw. Heating is carried out directly, by injection of live steam, or indirectly by means of a steam heated jacket. The use of this high temperature for cooking was based on the assumption that the more drastic the heat treatment the greater the release of water and oil on the subsequent pressing operation. The results of this work have shown however that, as far as the effect of heating concerned, cooking at about 45°C is as efficient as the use of temperatures around 85°C in releasing water from herring muscle. However, in fish meal, and oil manufacture, cooking at such a low temperature would not be adequate because, as discussed previously, the release of oil was small, release of solids was high and a substantial

amount of water was retained in the tissues after the pressing treatment. Maximum release of water and oil, with maximum retention of solids, conditions required for efficient production of fish meal, was achieved by cooking the muscle at about 60°C and then pressing.

It is likely that considerable energy savings could be made by cooking at this low temperature. To a first approximation, using the specific heat data obtained in this investigation, only half the heat is required to raise fish from room temperature to 60°C as is required to heat it to 100°C. Exernal heat losses would also be less. Full advantage could be taken of this heat saving only if the subsequent drying were also carried out at lower temperatures than at present. Drying is the critical step in influencing the nutritional quality of the meal. In some driers the cooked pressed fish, called press cake, flows parallel to air heated to about 500°C; if properly controlled, the fish meal temperature should never exceed 100°C but there is clearly a risk of the meal being scorched, impairing its quality attributes like digestibility and availability of essential amino acids. Considering that there is a trend to upgrade fish meal for human consumption by improving its functional properties, and taking also into consideration that the effects of heat on foodstuffs may be cumulative it could be advantageous to cook fish at a low temperature as 60°C. In respect of heat changes, as well as in respect of energy saving, there would be advantage in drying at the lowest practical temperature. It is worth adding that proteins are more susceptible to heat damage when the moisture content is high, as in the case of the fish being cooked.

Presumably the quality of the oil obtained could also be improved by cooking the fish at lower temperatures since boiling the fish causes

hydrolysis and polymerization of its extracted oil (Quaglia et al., 1974).

The fact that the amount of fluid released by pressing was inversely related to the amount released by cooking indicates that this latter operation controls the process and that factors like thickness of press cake and degree of disintegration of the cooked muscle may be more important than the actual pressure exerted. Also the fact that release of water and oil was independent of residence time suggests there is no need to extend the heating process beyond the time necessary for the fish to attain the desired temperature.

For canning of fish, it is important that in the precooking operation the fish should be heated sufficiently to ensure that the water exuded during sterilisation is not excessive. The results of this work for HFC fillets indicate that if the precooking is carried out at about 45°C the additional water released upon heating to 112°C would be about 5% (in relation to the fish weight) which in certain cases can be acceptable. For LFC fish however, this figure would rise to 9% and, therefore, if precooking was done at 45°C it should be over a longer period of time (in industrial processes cooking time will have an influence on the amount of liquor released because of losses by evaporation of water), or cooking should be carried out at higher temperatures, say over 65°C. The retention of fat in the muscle at about 45°C would have a beneficial effect on the texture of LFC fish, although probably the greater losses of solids (soluble protein) could be detrimental. Considering the possibility of increased enzyme activity and bacterial growth in the muscle at this temperature, it is as yet inadvisable to recommend this temperature for precooking, without trials under industrial conditions. The fact that at this temperature the skin could be easily removed from the flesh suggests a new procedure for producing a speciality

product like skinless canned fillets. Heating the fillets to temperatures around 40°C to remove the skin could be followed by a more intensive heat treatment to reduce the water content. In this direction further studies would be necessary to establish a relationship between fat content and skin fragility.

In relation to the hot-smoking process, the sensory observations in this study confirmed that the initial stages of smoking should be carried out at temperatures close to 30°C, since considerable softening of the high fat muscle occurred upon heating to 35°C. For maximum retention of water and greater yield of end product, after the initial low-temperature stage, heating should perhaps proceed as fast as possible to temperatures around 60°C, with minimum residence times around 45°C.

The almost negligible influence of temperature on the specific heat of the muscle showed that during heating of fatty fish muscle there is no great uptake or liberation of heat to be taken into account in heat processing. It was also demonstrated that some equations available in the literature are sufficiently accurate for most practical uses when dealing with muscle of natural moisture content. The values in this study however, are more accurate and of immediate use when the specific heat of herring or, probably, of other fatty species, is needed for process design purposes.

CHAPTER 6

SUGGESTIONS FOR FURTHER WORK

Considering that one of the outstanding findings in this work was the irregular relationship between water release from muscle and heating temperature, design of new experiments in this field of water holding capacity of fatty fish muscle should be primarily directed to further elucidating the causes for the points of maximum and minimum of water retention as a function of temperature. Another obvious need would be to determine whether the results of this work are valid for other fatty fish species, submitted to the same conditions of thermal treatment. Consequently it is expected that experimental work on the following topics might contribute to shedding more light on the basic mechanisms of water retention by fish muscle upon heating.

a. Since many of the more fundamental work carried out to study effects of heat on fish muscle assume that the water and salt soluble proteins remain the same as those present in the raw muscle it may be of interest to confirm this by actually identifying the proteins present in the liquor released by cooking using say, gel electrophoresis.

b. Repeat the experiments of this study using as raw material whole fish, minced muscle and fillets in parallel experiments. This could give a clear picture of the role of state of disintegration on the water holding properties of fish.

c. To confirm the hypothesis that collagen has an important role in water retention by the muscle between 50 and 60°C it would be profitable to determine at what temperature collagen of herring muscle is transformed to gelatin, and possibly determine the gel forming

properties of this gelatin. As it is known that the behaviour of extracted collagen is different from that in situ, it would be necessary to devise experiments where the extracted collagen is studied under conditions as close as possible to those of intact muscle.

To confirm the conjecture that release of fluid by pressing cooked muscle may be controlled by the state of disintegration of the muscle and thickness of the cake the following studies should be developed. Raw fish minced to different degrees of disintegration would be cooked under the same conditions and pressed for a given time under a constant pressure. In this respect it could also be fruitful to determine the effects of disintegrating the fish before or after cooking on the effectiveness of the pressing operation.

Finally, in this field of fat and water holding capacity it is still necessary to clarify the relationship between fat release from muscle and heat treatment. For this, systematic work should be conducted to verify the influence of heating on the interactions of lipids and crude extracts of muscle proteins, over as large a temperature range as the one used in the present study.

Concerning thermal transitions in muscle and measurement of specific heat, it is clear that the present set up is adequate for the latter purpose, and, in this respect, it is recommended that the work be extended to other species like mackerel and sprats. However, more fundamental work in detecting thermal changes would require further improvements in the calorimetric system such as:

a. a circulating pump, replacing the stirrer in the calorimeter bath, and better thermostatic control, through the use of a thermistor thermostat, which would make it possible to control the bath temperature to perhaps $\pm$ 0.001°C;

b. higher sensitivity in the measurement of the calorimeter temperature by use of more precise devices like thermistors or through the use of thermopiles instead of a single thermocouple junction.

It would also be of interest to obtain similar data, and possibly at the same time detect transient heat effects in the muscle, by use of a differential scanning calorimeter (DSC).

Finally, systematic microscopic examination of heated muscle, in conjunction with biochemical analysis, may reveal the nature of the granular material deposited among muscle fibres and in the connective tissue space when herring muscle is heated to about 45°C and higher temperatures. Improved techniques e.g. cooking the sample on a microscopic slide, using frozen sections, may give more reliable results by avoiding the inevitable handling during sample preparation.

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APPENDICES

Appendix 1 A computer program to calculate the total heat capacity of calorimeter plus sample as illustrated in Chapter 4, Section C.1

LIST OF VARIABLES

AV1:	Mean temperature of the initial period;
AV2:	Mean temperature of the final period;
G1:	Heating rate of the initial period;
G2:	Cooling rate of the final period;
RK:	Leakage modulus;
TETAB:	Calorimeter initial temperature;
TE TAE:	Calorimeter final temperature;
TOT:	Sum of temperatures during main heating period;
TAVG:	Mean temperature of the calorimeter wall during the heating period;
DTETA:	Temperature drop due to heat exchange between calorimeter and jacket;
TINCR:	Increase in temperature without considering heat losses;
TEMP:	Corrected temperature rise;
TETBC:	TETAB in degree Celsius;
TEMPC:	TEMP in degree Celsius;
TEXP:	Mean temperature of experiment in degree Celsius;
VOLTM:	Average potential drop across heater;
CURRM:	Average potential drop across standard resistor;
ENERG:	Energy introduced to the calorimeter system;
DELTA:	Increase in temperature during individual experiment in degrees Celsius;
THC:	Heat capacity of system calorimeter plus sample;
S2LRS and S2LR:	Are sub-programs used to find the least squares linear regression equation of temperature on time.

```
// FOR
*LIST ALL
*ONE WORD INTEGERS
*IOCS(PAPER TAPE,1132 PRINTER)
*NAME B1PR1
      REAL MICRO(44)
      DIMENSION T(150),TIME(150),SUM(5),REG(7),THER(44),SLOP(44),
     1VOLT(10),CURR(10)
   15 FORMAT(3F20.4)
   17 FORMAT(2I3,(6F12.7))
  111 FORMAT(I3)
   20 FORMAT(5(F6.0,F7.1))
   30 FORMAT(1X,'TIME',6X,'TEMPERATURE'///(F6.0,F7.1))
   31 FORMAT(1X,'I1=',I3,1X,'I2=',I3,1X,'I3=',I3)
  100 FORMAT(1X,'AV1=',F8.2,3X,'AV2=',F8.2,3X,'G1=',F8.3,3X'G2=',F8.3
     1 3X,'RK=',F8.5)
  101 FORMAT(1X,'TETAB=',F8.2,3X,'TETAE=',F8.2,3X,'TOT=',F12.2//
     11X,'TAVG=',F8.2,3X'DTETA=',F7.2,3X'TINCR=',F7.2,3X,'TEMP=',F8.2
  103 FORMAT(1X,'TETBC=',F8.3,3X,'TEMPC=',F8.3,3X,'TEXP=',F8.3//
     21X,'VOLTM=',F9.6,3X,'CURRM=',F9.5,3X,'ENERG=',F10.4//
     11X,'DELTA=',F6.3,3X,'THC=',F8.3)
   10 PAUSE 111
      READ(4,111)NUMB
      IF(NUMB)99,99,105
  105 READ(4,20)(TIME(I),T(I),I=1,NUMB)
      DO 1 I=1,NUMB
      IF(T(I)-10000.)2,3,2
    3 I1=I-1
    2 IF(T(I)-20000.)4,5,4
    5 I2=I-1
    4 IF(T(I)-30000.)1,7,1
    7 I3=I-1
      GO TO 8
    1 CONTINUE
    8 CONTINUE
      WRITE(3,30)(TIME(I),T(I),I=1,I3)
      WRITE(3,31)I1,I2,I3
      DO 50 J=1,5
   50 SUM(J)=0.
      DO 70 K=1,7
   70 REG(K)=0.
      CALL S2LRS(TIME,T,1,I1,SUM)
      AV1=SUM(5)
      CALL S2LRE(I1,SUM,REG)
      G1=REG(1)
      DO 80 M=1,5
   80 SUM(M)=0.
      DO 90 N=1,7
   90 REG(N)=0.
      I2=I2+2
      NOB=I3-I2+1
      CALL S2LRS(TIME(I2),T(I2),1,NOB,SUM)
```

```
      AV2=SUM(5)
      CALL S2LRL(NOB,SUM,REG)
      G2=REG(1)
      RK=(G1-G2)/(AV2-AV1)
      WRITE(3,100)AV1,AV2,G1,G2,RK
      TETAB=T(I1)
      TETAE=T(I2)
      TOT=0.
      K=I1+2
      L=I2-2
      DO 13 J=K,L
      TOT=TOT+T(J)
   13 CONTINUE
      TAVG=(TOT+((TETAE+TETAB)/2))/(L-K+2)
      DTETA=-((RK*(AV2-TAVG))+G2)*(L-K+2)
      TINCR=TETAE-TETAB+DTETA
      TEMP=TETAB+TINCR
      WRITE(3,101)TETAB,TETAE,TOT,TAVG,DTETA,TINCR,TEMP
      PAUSE 2222
      READ(4,15)(THER(I),MICRO(I),SLOP(I),I=1,44)
      DO 24 I=1,44
      IF(TETAB-MICRO(I)) 23,24,24
   23 K=I-1
      TETBC=THER(K)+((TETAB-MICRO(K))/SLOP(K))
      GO TO 22
   24 CONTINUE
   22 DO 42 I=K,44
      IF(TEMP-MICRO(I)) 25,42,42
   25 J=I-1
      TEMPC=THER(J)+((TEMP-MICRO(J))/SLOP(J))
      GO TO 43
   42 CONTINUE
   43 TEXP=(TEMPC+TETBC)/2.
      PAUSE 4444

      READ(4,17)N,M,(VOLT(I),I=1,N),(CURR(J),J=1,M)
      VSUM=0.
      CURRS=0.
      DO 46 I=1,N
      VSUM=VSUM+VOLT(I)
   46 CONTINUE
      DO 47 J=1,M
      CURRS=CURRS+CURR(J)
   47 CONTINUE
      VOLTM=VSUM/N
      CURRM=CURRS/M
      AMPVD=VOLTM/131
      HTCUR=(CURRM*15.3633)-AMPVD
      DELTA=TEMPC-TETBC
      ENERG=VOLTM*153.633*HTCUR*900
      THC=ENERG/DELTA
      WRITE(3,103)TETBC,TEMPC,TEXP,VOLTM,CURRM,ENERG,DELTA,THC
      GOTO 10
   99 CALL EXIT
      END
```

Appendix 2 Influence of temperature and holding time on the release of fat during cooking of LFC muscle

Temperature (°C)	Loss of fat in relation to original fat content (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.1	10.88	9.43	-8.69	-9.95	.41
40.6	16.20	-4.48	-16.01	17.02	3.18
46.2	8.45	15.37	7.48	.45	7.94
51.5	9.69	-13.78	24.78	9.32	7.50
56.1	6.43	9.30	.98	10.03	6.69
61.5	9.06	14.70	-3.42	1.80	5.53
65.8	25.13	23.34	32.25	33.21	28.48
75.6	-.69	14.25	3.11	-.19	4.12
85.8	14.23	-14.37	11.33	20.66	7.96
101.4	12.19	16.83	(11.64)	14.81	13.87
112.7	17.30	28.11	(19.10)	20.79	21.32
Mean	11.71	8.97	7.50	10.72	9.73

Anova	DF	SS	MS	F	LSD
Temperature	10	2809.99	280.99	2.47 (*)	15.5
Time	3	115.05	38.35	0.33 (ns)	9.3
Error	28	3183.36	113.69		
Total	41	6108.41			

Appendix 3 Influence of temperature and holding times on the release of fat during cooking of HFC muscle

Temperature (°C)	Loss of fat in relation to original fat content (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.2	9.25	-.56	.86	8.50	4.51
41.1	4.91	5.75	14.75	9.70	8.77
44.5	17.27	11.97	19.97	-.60	12.15
50.4	22.08	28.14	19.04	18.02	21.82
55.1	15.81	23.31	28.27	16.81	21.05
59.7	11.89	21.42	17.18	17.56	17.01
65.6	20.53	10.30	20.79	13.08	16.17
75.0	12.61	14.32	4.56	15.15	11.66
83.7	18.93	23.95	13.74	10.89	16.87
101.0	14.37	15.94	20.14	20.59	17.76
112.0	13.68	22.61	24.41	32.66	23.34
Mean	14.66	16.10	16.70	14.76	15.55

Anova	DF	SS	MS	F	LSD
Temperature	10	1335.16	133.51	3.88 (**)	8.5
Time	3	33.40	11.13	.32 (ns)	5.1
Error	30	1030.64	34.35		
Total	43	2399.21			

Appendix 4 Influence of temperature and holding time on the release of fat during pressing of cooked LFC muscle

Temperature (°C)	Loss of fat in relation to original fat content (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.1	6.86	-5.98	23.35	23.25	11.87
40.6	-7.92	16.73	-8.36	-19.97	-4.88
46.2	7.32	11.90	17.82	24.33	15.34
51.5	19.26	25.43	-5.29	7.01	11.60
56.1	20.05	29.00	26.34	30.79	26.55
61.5	41.62	10.19	27.49	26.90	26.55
65.8	15.66	10.72	11.50	16.09	13.49
75.6	38.42	32.41	21.33	32.25	31.10
85.8	12.75	7.63	9.84	11.61	10.46
101.4	$27\frac{1}{2}43$	18.44	(20.26)	22.97	22.27
112.7	19.98	15.88	(14.36)	15.29	16.38
Mean	18.31	15.67	14.42	17.32	16.43

Anova	DF	SS	MS	F	LSD
Temperature	10	3993.15	399.31	4.05 (**)	14.4
Time	3	98.50	32.83	0.33 (ns)	8.7
Error	28	2759.54	98.55		
Total	41	6851.21			

Appendix 5 Influence of temperature and holding times on the release of fat during pressing of cooked HFC muscle

Temperature (°C)	Loss of fat in relation to original fat content (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.2	7.38	5.44	.46	3.33	4.15
41.1	5.00	5.28	2.09	8.60	5.24
44.5	17.70	(18.29)	17.00	23.30	19.07
50.4	16.87	12.41	20.22	24.81	18.57
55.1	23.71	25.12	24.26	23.30	24.09
59.7	30.68	24.77	39.79	33.82	32.26
65.6	9.31	27.42	22.12	28.63	21.87
75.0	24.04	23.21	36.01	(29.30)	28.14
83.7	33.46	22.00	20.83	24.64	25.23
101.0	21.84	20.15	16.82	16.29	18.77
112.0	33.23	33.62	25.61	23.04	28.87
Mean	20.29	19.79	20.47	21.73	20.57

Anova	DF	SS	MS	F	LSD
Temperature	10	3251.29	325.12	11.43 (**)	7.7
Time	3	22.48	7.49	0.26 (ns)	4.7
Error	28	795.982	28.42		
Total	41	4069.75	28.42		

Appendix 6 Influence of temperature and holding time on the release of fat by the combined effect of heating and pressing LFC muscle

Temperature (°C)	Loss of fat in relation to original fat content (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.1	17.74	3.45	14.65	13.30	12.28
40.6	8.58	12.24	-24.37	-2.94	-1.62
46.2	15.77	27.27	25.30	24.79	23.28
51.5	28.95	11.65	19.49	16.33	19.11
56.1	26.49	38.31	27.33	40.82	33.24
61.5	50.68	24.90	24.07	28.71	32.09
65.8	40.80	34.07	43.75	49.30	41.98
75.6	37.73	46.65	24.45	32.05	35.22
85.8	26.98	-6.73	21.17	32.28	18.42
101.4	39.62	35.28	(31.91)	37.78	36.15
112.7	37.29	43.99	(33.46)	36.08	37.70
Mean	30.06	24.64	21.93	28.04	26.17

Anova	DF	SS	MS	F	LSD
Temperature	10	6932.94	693.29	7.02 (***)	14.4
Time	3	428.66	142.88	1.44 (ns)	8.7
Error	28	2761.45	98.62		
Total	41	10123.05			

Appendix 7 Influence of temperature and holding times on the release of fat by the combined effect of heating and pressing HFC muscle

Temperature (°C)	Loss of fat in relation to original fat content (%)				
	Holding Time (min)				
	0	15	30	60	Mean
36.2	16.63	4.87	1.32	11.83	8.66
41.1	9.91	11.03	16.84	18.30	14.02
44.5	34.97	(31.54)	36.97	22.70	31.54
50.4	38.95	40.55	39.26	42.83	40.39
55.1	39.52	48.43	52.53	40.11	45.14
59.7	42.57	46.19	56.97	51.38	49.27
65.6	29.84	37.72	42.91	41.71	38.04
75.0	36.65	37.53	40.57	(38.12)	38.21
83.7	52.39	45.95	34.57	35.53	42.11
101.0	36.21	36.09	36.96	36.88	36.53
112.0	46.91	56.23	50.02	55.70	52.21
Mean	34.95	36.01	37.17	35.91	36.015

Anova	DF	SS	MS	F	LSD
Temperature	10	7357.01	735.70	22.11 (***)	5.0
Time	3	27.15	9.05	0.27 (ns)	8.4
Error	28	931.32	33.26		
Total	41	8315.49			

Appendix 8 - Percentage fractions of water and fat present in the liquors released by cooking and by pressing LFC muscle

	Cooking						Pressing						Cooking plus Pressing					
	water		fat		water + fat		water		fat		water + fat		water		fat		water + fat	
Temp (°C)	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S
36.1	83.4	10.7	2.8	13.6	86.2	3.7	83.5	8.3	8.5	8.9	92.0	4.0	84.2	4.0	5.4	3.5	89.7	2.1
40.6	84.9	9.0	2.2	8.3	87.1	2.1	90.9	4.6	-1.7	6.8	89.2	2.9	88.3	2.7	0.4	3.2	88.3	1.0
46.2	82.6	5.4	5.4	5.2	87.8	1.3	84.5	3.0	7.3	2.9	91.8	1.2	83.7	2.5	6.4	2.3	90.2	0.4
51.5	83.7	11.1	6.1	13.3	89.8	3.6	88.2	3.8	4.4	5.2	92.6	1.7	85.9	2.0	5.0	2.0	90.9	1.1
56.1	87.1	4.5	5.6	3.4	92.7	3.0	87.6	1.3	7.7	0.9	95.3	2.0	87.5	1.4	7.2	1.2	94.6	2.1
61.5	87.1	8.5	4.2	6.4	91.3	2.5	88.2	1.1	6.6	3.3	94.8	2.5	87.8	1.3	6.2	2.6	94.0	2.3
65.8	71.2	3.0	24.0	5.7	95.2	3.0	89.5	1.3	4.7	1.0	95.2	0.9	84.8	1.5	10.3	1.4	95.2	1.4
75.6	88.6	3.8	2.7	4.8	91.3	3.4	83.5	1.9	11.9	2.0	95.4	1.0	85.3	2.1	8.3	2.8	93.7	1.8
85.8	88.5	7.7	4.3	8.5	92.8	1.9	88.9	1.6	3.4	0.4	92.3	1.3	88.8	4.0	3.7	3.6	92.5	0.6
01.4	87.8	3.6	6.5	1.6	94.3	2.0	83.3	1.5	10.3	1.5	93.6	0.9	85.4	1.9	8.4	1.3	93.8	0.5
12.7	83.7	0.9	7.3	2.1	91.0	1.2	79.5	1.1	11.4	2.8	90.9	1.7	82.3	0.5	8.8	0.5	91.1	0.2

S, Standard deviation

a), means averaged over the four times studied

Appendix 9 Percentage fraction of water and fat present in the liquors released by cooking and by pressing of HFC muscle

	Cooking						Pressing						Cooking plus Pressing					
	water		fat		water + fat		water		fat		water + fat		water		fat		water + fat	
Temp (°C)	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S	a)Mean	S
36.2	77.5	6.8	6.9	8.0	84.4	1.5	83.0	1.9	6.7	4.6	89.7	2.8	80.2	3.8	6.6	5.2	86.8	2.1
41.1	77.9	5.1	10.2	5.3	88.1	2.1	81.4	2.6	7.3	2.4	88.7	2.2	79.2	2.1	9.8	2.2	89.0	0.2
44.5	76.2	7.6	12.6	8.8	88.8	1.7	66.8	4.2	25.3	1.1	92.1	3.5	72.4	3.9	17.9	6.3	90.3	2.3
50.4	64.5	3.6	26.0	5.6	90.5	2.0	73.2	5.6	20.4	7.0	93.6	1.5	69.2	2.0	23.2	1.6	92.4	0.8
55.1	62.2	5.0	27.4	8.6	89.7	3.7	73.7	1.8	20.7	1.3	94.4	0.7	69.4	0.7	23.1	2.4	92.5	1.7
59.7	65.4	4.4	26.3	6.3	91.7	1.9	71.4	3.2	24.4	4.5	95.8	1.3	69.4	2.1	25.0	3.0	94.4	0.9
65.6	69.0	6.5	21.9	7.7	90.9	1.2	74.2	8.8	22.8	10.9	97.0	2.2	72.3	2.8	22.0	3.6	94.3	0.9
75.0	76.9	5.2	14.4	6.3	91.3	1.1	69.3	2.3	27.4	1.4	96.7	1.3	72.6	1.6	21.4	1.8	94.0	0.6
83.7	77.0	2.8	15.7	2.9	92.7	0.6	69.8	6.2	27.2	6.1	97.0	1.9	73.9	2.8	20.6	3.4	94.5	0.9
101.0	75.8	1.8	16.7	2.1	92.5	0.3	69.1	1.6	25.4	3.0	94.5	1.5	73.1	0.6	20.3	0.7	93.4	0.6
112.0	75.1	3.9	16.4	4.4	91.5	0.8	57.0	4.6	36.2	5.1	93.2	1.5	68.5	2.5	23.7	1.9	92.2	0.7

S, Standard deviation

a), means averaged over the four times studied

Appendix 10 Estimation of the error in determining the temperature interval ΔT of equation 2.3

The calculations that follow have been based on data presented in Table 4.12.

Equation 3.8 gives the correction to be added to the observed temperature rise,

$$\Delta T_1 = -\left[gf + k\,(Tf - Tm)\right]\ (te - tb)$$

Standard errors of gi, gf and T_i and T_f were obtained from the linear regression fit to the fore and after period time-temperature data. They were:

$g_i = \pm 0.00211$ $\qquad$ $g_f = \pm 0.00155$

$T_i = \pm 0.0128$ $\qquad$ $T_f = \pm 0.00959$

From these, the standard error of $k = (gi - gf) / (T_f - T_i)$ can be calculated. It was found to be $\pm$ 0.00002.

Using the formulas for sum and products of errors it was possible to estimate that the error in calculating ΔT would be $\pm$ 0.1 μV. The error for the total temperature interval, assuming an error of $\pm$ 0.1 μV in the observed temperature rise, appears then to be $\pm$ 0.2 μV or $\pm$ 0.006°C.

Appendix 11 Partial derivatives and error contribution from each term in eq. 4.2, from data in Table 4.23

The partial derivatives in Equation 4.2 are:

$$\partial Cp/\partial \bar{E} = \bar{I}\,\Delta t/m\,\Delta T = .1842$$

therefore the error contribution from the potential drop across the heater is:

$$\frac{\partial Cp}{\partial \bar{E}}\,\delta\bar{E} = 0.1842 \times 0.0001$$
$$= 0.000018$$

$$\partial Cp/\partial \bar{I} = E\,\Delta t/m\Delta T = 6.8678$$

and the error contribution from the measurement of current is:

$$\frac{\partial Cp}{\partial \bar{I}}\,\delta\bar{I} = 6.8678 \times 0.00001$$
$$= 0.000068$$

$$\partial Cp/\partial \Delta t = \bar{E}\bar{I}/m\,\Delta T = 0.0019$$

the error from the measurement of time is

$$\frac{\partial Cp}{\partial \Delta t}\,\delta\Delta t = 0.0019 \times 0.2$$
$$= 0.0038$$

similarly, error contribution from mass and temperature measurements are respectively:

$$\partial Cp/\partial m = -\bar{E}\bar{I}\Delta t/\Delta Tm^2 = -0.00469$$

$$\frac{\partial Cp}{\partial m}\,\delta m = -0.00469 \times 0.05$$
$$= 0.00023$$

$$\partial Cp/\partial \Delta T = -(\bar{E}\bar{I}\Delta t)/m\,\Delta T^2 = -0.5279$$

$$\frac{\partial Cp}{\partial \Delta T}\,\delta \Delta T = -0.5279 \times 0.01$$
$$= -0.005$$