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Contributions and editorial correspondence should be sent to Dr E. C. Bate-Smith, A.R.C. Institute of Animal Physiology, Babraham, Cambs.

General correspondence (including items for the News Letter) should be sent to Mr S. H. Cakebread, Florasynth Ltd, 327 Oldfield Lane, Greenford, Middlesex.

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Progress in the chilling and freezing of foods

E. C. BATE-SMITH

This review is based on an advanced study institute held at the University of Strathclyde in September 1966 on the subject of Low Temperature Biology of Foodstuffs. It deals especially with the more technological aspects of the papers read, i.e. about half the actual content of the course. The Organizing Committee (see p. 205) recognized the need to lay down a really substantial foundation of basic science not only in subject areas altogether unfamiliar to most of those taking part, but also in the introduction to subjects of a more immediately practical character. It was quite evident that the striking advances in knowledge and understanding of such subjects as the molecular structure of water and aqueous solutions, ice crystal nucleation and the formation of ice, protective mechanisms in frost-hardy plants, and physico-chemical changes in foods arising from freezing, should be more widely known to food scientists generally. Only a brief outline of the contents of these papers can be given here, but the full proceedings are to be published at an early date.

Basically, the subject concerns the effects of lowering the temperature of biological systems due: (a) to the slowing down of chemical and enzymic reactions, and (b) additional effects, at temperatures below the freezing point, of the removal of water as ice. Even the lowering of the temperature of water to the freezing point is not so simple a process as it might seem, as evidenced by the fact that there is a point of maximum density in pure water at 4°C. The state of the molecules in liquid water and in ice, and the transition between melting ice and water needed first to be explained in terms of modern molecular theory (Némerthy, 1968). Apparently in liquid water there is a tendency for the individual molecules to aggregate in clusters by hydrogen bonding of the oxygen atom of one molecule with a hydrogen atom of another, forming loose and transitory clusters in which the bond angle (about 120°) of the free molecule is increased to near linearity, thus increasing the average molecular volume. The numbers of molecules so associated increase rapidly as the temperature is reduced to the freezing point, and as 4°C is approached, their numbers increase at such a rate that the increase in volume from this cause first equals, and then exceeds, thermal shrinkage. In ice, the configuration of the hydrogen and oxygen atoms is strictly colinear, the molecular volume in the crystal lattice being even higher than in the clusters, hence the additional expansion which takes place on freezing. Any substance dissolved in the water (especially a substance which itself forms hydrogen bonds with water), disturbs the configuration of the clusters and, therefore, affects the 'structure' of liquid water.

Author's address: Institute of Animal Physiology, Babraham, Cambridgeshire.

The existence of these ice-like associations in liquid water is responsible for the relatively low latent heat of fusion of ice and their gradual decomposition with rise in temperature explains the high specific heat of water between its freezing and boiling points. Furthermore, the effect of the clusters is extended beyond the limits of their actual structure in a boundary zone in which the freedom of movement of the individual molecules is limited. The properties of water as a solvent are necessarily influenced by the existence of these clusters and increasingly so as their numbers increase with falling temperature.

When water reaches a temperature at which freezing becomes possible, whether it does or does not do so depends on whether there are solid surfaces (nuclei) to initiate crystallization. The structures most efficient for this purpose are the surfaces of pre-existing ice crystals, but other surfaces may act as nuclei, the more competently the more nearly their crystal structure approaches that of ice.

If no such structures are present, the water will not freeze even though its temperature may be very much below the freezing point. Very small droplets where chance occurrence of a nucleating structure is reduced to a minimum can, in fact be supercooled as low as $-50°C$, but in practice 'pure' water still contains traces of impurities in particulate form so that it is difficult to supercool it below $-15°C$. Both water and quite complex biological systems such as eggs can be supercooled quite easily to $-7°C$, frequently to $-10°C$, and even lower.

Water specially purified (e.g. by ion-exchange processes) to a point where, in small droplets, not a single nucleus is present undergoes, at a very low temperature, 'homogeneous', spontaneous nucleation (Hallett, 1968). When reduction of temperature is extremely rapid, nucleation by this process can be added to crystallization on pre-existing nuclei, and in this way ice may be formed *within* intact cells where, otherwise, it will only form outside the cells by growth inwards from nuclei on the cold external surface.

The rate of growth and the form of ice crystals are dominated by the requirement to dissipate the latent heat released when water changes state from liquid to solid. This heat must be lost by conduction away from the advancing ice front, and loss of heat will be greater the greater is the exposed surface of that front. It is for this reason that snowflakes and the pattern of ice on windows assume a leaflike or 'dendritic' pattern—the crystals grow in such a way as, at all times to provide the greatest possible surface from which heat will be lost into the still unfrozen aqueous phase, and also down the gradient of temperature towards the surface from which cooling is proceeding. The consequent *limited* rate of advance of the ice front enables water to diffuse towards the front from cells or other formed elements in its path, and this, if for no other reason, is why water is lost from cells and is added to ice forming in the intercellular spaces, rather than freeze within the cells themselves. It is only when the rate of fall of temperature is so rapid that it 'beats' the rate of passage of water through cell membranes that the front will reach the cell wall, and possibly, pass through it and into the cell. Such rates of freezing are

seldom encountered in commercial practice, and as a rule it is only the contents of damaged cells which will be frozen. In all other circumstances the cells will be dehydrated but unfrozen.

If supercooling could, in fact, be safely achieved in practice, there are many foodstuffs which would benefit from the lower temperature of storage which could then be utilized. There are, however, very few foods which do not present a moist surface and, therefore, a vulnerable one to nucleation in a freezing environment. Eggs, for instance, might safely, and with considerable advantage, be stored at temperatures below the freezing point even down to −5°C (Moran, 1924), but they require delicate handling to prevent deposition of frost when removed to a warmer, moist atmosphere. Seeding then takes place and the eggs crack as ice forms within them. Experiments have shown that the rate of deterioration in eggs stored at −1·5°C is only about one-half that at 0°C.

Behaviour of foodstuffs at chill temperatures above the freezing point

The reduction of temperature of foodstuffs below that of the normal environment is mainly with a view to inhibiting the multiplication and activity of micro-organisms. The first thing to consider, therefore, is the effect of cold on these organisms.

It is customary to divide microbes into three categories: thermophiles, mesophiles and psychrophiles, depending upon their optimum temperatures of growth. Definitions of these categories are not yet agreed, but for food spoilage organisms psychrophiles can be regarded as having a temperature range of below freezing to about 30°C, with an optimum at about 25°C; mesophiles 10°C to about 45°C with an optimum at body temperature; and thermophiles from 30°C to no specified temperature with optima at about 50–60°C. The *rates* of growth within these ranges are not included in the definition, and it is important to realize that mesophiles growing at temperatures well below their optimum may still be multiplying at a greater rate than a psychrophile which has its optimum at the same temperature. Mesophiles whose range of temperature extends deeply into the psychrophilic zone are of particular importance in food preservation. The term psychrotrophic has recently been proposed for all organisms capable of growing at temperatures near 0°C.

The physiology of psychrophiles, discussed by Stokes (1968), was the subject of a symposium at the *VIIIth International Congress of Microbiology*, 1962, to which the reader is referred for information in greater detail.

Fruits and vegetables tend to suffer less from the attack of micro-organisms than from the continuation of their own life processes after harvesting. These life processes can be slowed down by reduction of temperature, but there is a limit to survival imposed either by the impossibility of halting these processes completely (without actually freezing the tissues) or by unbalancing them to such an extent that adverse changes occur. The circumstances in which the latter situation arises vary for each and every commodity, and even for varieties of any particular commodity, so that the limiting treatments to be

respected and observed represent an enormous body of specialized and detailed knowledge.

In principle, however, the causes of low temperature injury are fairly general over a wide range of commercial fruits and vegetables. 'If a given cultivar of a given species of fruit or vegetable is susceptible to injury, then there is a more or less definite minimum temperature below which injury occurs. Storage at a temperature just above this critical value results in a maximum storage life. The critical temperature can be quite high. Thus the highest yet recorded is for bananas, for the Lacatan 14·4°C, for the Gros Michel 11·6°C (Fidler, 1968, quoting Furlong, 1962–63).

The critical temperature may vary with cultural conditions. Thus Cox's Orange Pippin apples grown in mainland Australia may be stored at 1·5°C, while the minimum temperatures for this variety grown elsewhere are: Tasmania 2°C, New Zealand 2·5–3°C and the United Kingdom 3–4°C.

The actual nature of the injury, and therefore its probable cause, varies from one commodity to another. Some light is thrown on the cause in different species by ringing the changes on the many and various parameters that affect their behaviour in storage. From the technical point of view, one of the most valuable discoveries is an alternation in temperature between a low, and a relatively high, storage temperature, seen especially well in plums. Commercial varieties imported from South Africa tend to develop woolliness when held at the low temperature necessary for the voyage, but after a transient rise in temperature during or after the storage period, the plums ripen in a normal way when brought on to the market.

The multiplicity of commercial varieties and their obstinately individual way of behaving has made it very difficult for a sustained, concentrated attack to be made on the cause of low temperature injury. That basic disturbances in the metabolism of the fruit precede and accompany the injury is both axiomatic and demonstrable. Hulme and his co-workers, for instance, found that when apples began to develop low temperature breakdown, there was an increase in the concentration of oxaloacetic acid (one of the acids concerned in the Krebs cycle of sugar utilization), the increase beginning before visible signs of injury. When the temperature was raised as described above, the oxaloacetic acid concentration rapidly fell. It is not implied that this acid is, of itself, responsible for the injury; it probably indicates a dislocation of parts of the mechanism involved in normal living processes. The production of a volatile toxin has been invoked by Plank (1941) as the agent responsible for injury, but Smith (1962) found no evidence of any increase in the production of volatiles either during warming or after return to low temperature. It seems rather unlikely that specific toxic substances will be responsible for the symptoms of injury in so many different species and circumstances. Rather, it is suggested, a general pattern of metabolic disturbances is created by lowering the temperature from the optimum for the particular plant and/or organ. In some ('susceptible') species this has lethal consequences, in others not. Might not the same considerations apply to the hardening, or absence of hardening, of plants in the field?

Especially, in this respect, comes to mind the conversion of starch to soluble sugars which is almost a universal feature of the chilling of vegetable tissues. This phenomenon will be discussed again in the section dealing with freezing injury. Nevertheless Fidler (1968) is right in drawing attention to the poverty of effort put into the study of the effects of chilling temperatures on these commodities relative to the economic return which would undoubtedly reward such studies.

In the case of foodstuffs of animal origin there is little recent progress to report. The transport of meat, for instance, has returned unchanged to its pre-war practices of chilling from South America and freezing from the Antipodes. (An interesting condition of 'cold rigor' has, however, been reported by New Zealand workers, and this will be mentioned again below.) Interest in fish and poultry is also centred on frozen, rather than chilled, storage. However, some interesting work on the cold (both chilled and frozen) storage of milk, especially from the point of view of its subsequent use for cheese-making, has been reported (Mocquot, 1968). This mainly concerns the production of off-flavours by virtue of the selective growth of psychrophilic bacteria (see earlier, p. 193). 'Cold storage of milk (also) slightly diminishes its ability to clot with rennet and its growth medium for lactic acid starter, but by suitable adaptation of technological processes it should be possible, in most cases, to overcome these difficulties.'

A chilling stage must, of necessity, always precede freezing, and the precise pathway by which the fall in temperature is achieved—the time taken to reduce the temperature in life, to that at which freezing is initiated—may be very important. This falls more appropriately for consideration, however, under the heading of freezing of the different commodities.

The freezing of foods

1. *The freezing of aqueous solutions, suspensions and complex systems*

It will be unnecessary here to enter at all deeply into the principles underlying the differences in behaviour of solutions from that of pure water, beyond the general statement that the freezing point of water is lowered to an extent determined by the mole concentration of the dissolved species. Freezing only begins to be affected in a significant way when constituents are present in different phases, such as solid particles, immiscible droplets, or formed elements. This of course is always the case with biological systems, which foodstuffs almost always are.

Knowledge of the patterns which ice may adopt in such systems owes much to the painstaking observations of Father B. J. Luyet (1968, and in Meryman, 1966). In concentrated solutions, at varying rates of freezing, to different ultimate low temperatures, and with the progress of freezing impeded by the different kinds of structures present in organized tissues, the form and habit of the crystals of ice may differ enormously, and it is a formidable task to relate these differences to the changes often found to have taken place when the system is thawed. The most conspicuous of these changes are the liberation of free fluid (fluid previously held within the structure of the system

before it was frozen) and alterations in texture, consistency and appearance. In foods it is not usually material whether living tissues recover their living properties when thawed, but much useful information can be gained by ascertaining the extent to which, if at all, those properties are regained when such bodies as red blood cells, spermatozoa and bacteria are frozen and thawed, and the means by which living functions can be protected from damage by the use of protective substances. This is the great contribution that medical science has made to cryobiology in recent years (see, e.g. Smith, 1961; Meryman, 1966).

Several key observations afford a starting point for an appreciation of the situation:

(1) Many types of single cells can be frozen and thawed without suffering in any way from this treatment.

(2) Most (if not all) single cells will be irreversibly injured if ice forms *within* the cell.

(3) Many types of cells which are irreversibly injured by relatively slow freezing and thawing can be protected against injury by the presence of particular substances in the aqueous phase.

(4) Rapid freezing and thawing often enables cells otherwise susceptible to injury to survive the treatment more or less unimpaired.

(5) Freezing at a temperature only slightly below freezing point often causes more severe injury than more drastic freezing treatments. Since such temperatures necessarily have to be passed through on the way to lower temperatures, damage even at rapid rates of freezing has to be taken into consideration.

2. *Freezing of bacteria*

The last observation is particularly relevant to the freezing of bacteria. 'In the range of from -4 to $-10°$ far more micro-organisms are inactivated than at $-15°$, and at $-30°$ the frost killing is even less pronounced' (Christopherson, 1968). (This is not the case with yeasts, where killing is greater the lower the temperature of freezing). Gram-negative bacteria, e.g. coliforms, pseudomonads, achromobacteria and salmonellae, are susceptible, food poisoning streptococci and enterococci are more resistant, and spore-forming organisms are most resistant—in fact many spores are quite resistant to freezing.

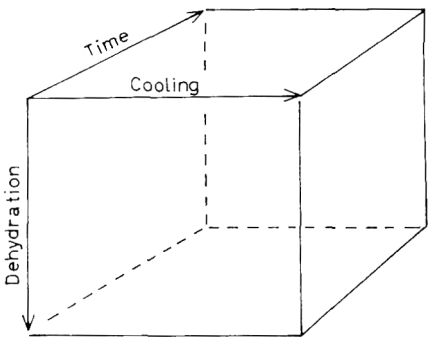
While the question of survival is obviously of paramount importance, the retention of enzymic activity, even though cells are dead, may be significant in particular circumstances. Thus Arpai (1961) has observed that the peptidase activity of certain bacteria has actually been stimulated by freezing and thawing.

The risks from the survival of food poisoning organisms are precisely indicated by the above considerations. While the risks from salmonellae are likely to be reduced, those from clostridia (e.g. botulism) still remain, and frozen foods are, therefore, no less a hazard from this point of view than they would have been before freezing.

As regards the mechanism of injury, the reader is especially referred to P. Mazur in Meryman (1966). Intracellular ice does not form under normal conditions of freezing

and probably occurs only with 'homogeneous nucleation (see p. 192) at very low temperatures. Apparently an explanation of injury must be sought which excludes internal freezing. All such explanations recognize that it is the removal of water from the cells—dehydration—that is the immediate cause of injury, leading in susceptible organisms to death. It is, further, becoming more and more generally accepted that protein denaturation is concerned in the injury. This is supported especially by the progressively increasing killing during frozen storage, and by the kinds of substances which afford protection from injury, about which more will be said later.

This situation can be represented diagrammatically as a system of three co-ordinates:



The time axis can provide both for rates of cooling (of orders between hours and milliseconds per degree) or periods of storage (of the order of hours or years). All situations in the frozen state must fall within the boundaries of such a cube. This scheme represents equally well the situation in multicellular systems such as meat, fish, fruits and vegetables.

3. *Freezing of fruits and vegetables*

Much insight into the behaviour on freezing of fruit and vegetable tissues can be gained from the study of frost susceptibility and resistance in living plants. This again is comprehensively dealt with in Meryman (1966) and in particular aspects by Modlibowska (1968), Hudson & Idle (1968) and Santarius (1968). Many plants, as we have seen (p. 195), respond to a reduction in temperature by an increase in their soluble sugars and the ability to do this is associated with, and in many cases can be regarded as the reason for, resistance to frost damage. Most, if not all, plant cells are irreversibly injured by intracellular ice formation, so that protection from freezing damage must be due either to the effect of the protective agents on the freezing of the intercellular fluid or, if they are formed within or are able to penetrate into the cells, to their effect on the dehydrated cell contents. It has been shown in many cases that merely the lowering of the freezing point of the aqueous phase by increased concentration of solutes cannot account for the difference between hardy and non-hardy plants. Increase of viscosity, affecting the rate of dehydration of the cells, or the presence of the agent *within* the

cells affording protection from the effects of dehydration, must be presumed. From what we have seen of the action of protective agents in the case of bacteria the latter seems most likely. It is indeed significant that sugars are involved in this protective effect, since these are also among the most efficient protective agents in the case of bacteria.

Irreversible injury to plant tissues by freezing is manifested by a loss of turgor and the copious liberation of free fluid on thawing. It is towards the mitigation of these defects that most of the early research, and much of present-day research, was and is directed. Secondary consequences of this increased freedom of movement of water throughout the tissues are an increase, always undesired, of enzymic activity and susceptibility to the attack of micro-organisms. Fruits and vegetables are always, therefore, 'blanched' before freezing, i.e. subjected to a short, sharp scalding treatment in order to inactivate enzymes. The range of frozen fruits and vegetables on offer is extremely small because only those which find favour (at the price attached to them) by comparison with the fresh produce are saleable in a reasonable time. Others, however attractively they may be presented, are 'cabinet cloggers': they occupy valuable and costly cabinet space on the retailer's premises.

Some insight into the mechanism of freezing injury has been gained from studies of isolated chloroplasts during freezing and thawing in the laboratory of U. Huber (Santarius, 1968). This has clearly shown that irreversible injury is due to the uncoupling of phosphorylation (the biochemical step by means of which light energy is converted into chemical energy in photosynthesis). It is later suggested (p. 199) that the protective action of sugars may be specifically exercised on lipoprotein systems; the grana of the chloroplast, where these phosphorylating processes are carried out, is conspicuously one such system. Addition of sugars prior to freezing prevents this uncoupling (Jagendorff & Avron, 1958; Duane & Krogmann, 1963). The mitochondria, whose membranes are similarly composed of lipoprotein-laminated systems, may be protected in the same way. Sugars, therefore, may be protective against freezing damage both in frost-hardy plants and when added as syrups to frozen fruits, by their action on living, or dead as the case may be, lipoprotein systems.

4. *Freezing of eggs*

Eggs are particularly interesting because they can be supercooled to quite low temperatures (p. 193). It is conceivable that this property might be utilized in commercial practice but for the fact that the shells crack when their liquid contents are frozen, as is likely to happen when they are removed from the cold store.

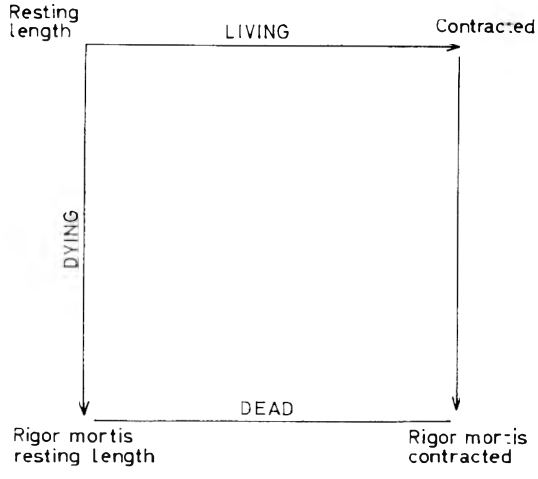
Egg yolks and liquid whole egg suffer a change in consistency when frozen to $-7°C$ or below and thawed. The fluid, previously viscous, becomes thickly gelatinous and technically unusable. Sodium chloride, at a concentration of 10% acts as a protective agent against this gelation, and the thawed yolks can be used for such industrial purposes as emulsification and stabilization of emulsions, but the foaming property of liquid

whole egg is lost. However, this property also can be protected by the addition of sugars (sucrose or glucose but not lactose or maltose) before freezing (Powrie, 1968). These observations recall the similar behaviour of liquid whole egg on spray drying (Brooks & Hawthorn, 1943).

Powrie describes recent research on the microstructure of yolk and the distribution of the lipoproteins phosvitin and lipovitellin (Joubert & Cook, 1958; Bernardi & Cook, 1964) (which prove to be the most important emulsion-stabilizing factors) between the granules, droplets and plasma. The protective action of sucrose can be explained by its ability to prevent the insolubilization of the lipoproteins. This was demonstrated by the present author (Bate-Smith, 1935) and reported in a publication now almost unprocurable, as follows: 'The greatest difficulty in handling this protein—lecitho-vitellin—is the denaturation which follows any attempt to separate the fat of the yolk from its association with the protein. A method of treatment which promises to be of considerable value consists in adding 20–30% of cane-sugar to the yolk before extracting with ether. By this means the protein: fatty acid ratio of the vitellin complex (originally 0·14) has been increased to 5·1 without the slightest loss of solubility in salt solution, and to 7·5 with only partial loss of solubility.'

5. *Freezing of meat, fish and poultry*

While in the main the effects of freezing on these essentially muscular tissues are similar, there are important differences in detail between them. Recent work has emphasized the importance of the post-mortem changes in all kinds of flesh, and the extent to which these changes have progressed before they are frozen. It has proved helpful to the reviewer to consider the state of muscle at any time after death as falling within a square bounded as shown:



The pathway followed by the muscle, and the point reached at the bottom of the square, may differ enormously depending on the time–temperature sequence of events.

For all practical purposes it can be regarded as desirable for rigor mortis to be established before the flesh is frozen, and at as low a temperature as possible without actually freezing, because in so doing the muscle will have the least tendency to shorten. It has recently been found (Locker & Hagyard, 1963; Marsh & Leet, 1966; Davey & Gilbert, 1967; Davey, Kuttel & Gilbert, 1967) that muscle which shortens during rigor is tougher than that which remain at its resting length; this being thought to be due to the overlapping of actin and myosin filaments, with consequently tighter bonding between them. Since toughness, or texture generally, is the most important single factor affecting quality in flesh foods (Connell, 1967), the expenditure of effort along these lines is well worth while.

In all types of flesh 'dying' is associated with the running down of the energy reserves of the muscles. Rigor mortis is itself due to the depletion of adenosine triphosphate (ATP), the immediate source of energy for contraction. The ultimate source of energy is glycogen, and in most flesh foods the formation of lactic acid as this is broken down is a critically important event in their post-mortem history. The level reached when this breakdown comes to a standstill is usually determined by the amount of glycogen originally present in the muscles of the animal. There is some evidence that the actual value of the ultimate pH influences the amount of damage to texture suffered when the flesh is frozen; it certainly affects the changes that take place during storage. In either case, however, there are marked differences between various kinds of flesh, and the behaviour of each is a subject for extensive and intensive independent study. Only the briefest possible statement of the facts can be undertaken in the present review, and it is proposed to deal with the effects of freezing as such, and of subsequent storage in the frozen state together under the different commodity headings, after first describing their behaviour during storage in general terms.

6. *Storage in the frozen state*

For many years 14°F (−10°C) was specified as the 'safe' temperature for storage of frozen foods. It was recognized, but regarded as unavoidable, that the quality of the produce would gradually go downhill at this temperature. When, however, the advantages of quick freezing and the preservation of quick-frozen character came to be appreciated, lower temperatures of storage were advocated, and 0°F (−18°C) was selected [more because it was a round number than for any particular technological reason (Symons, 1968)] as the temperature that should be maintained.

'Quick-frozen character' is identified by the small size of the ice crystals in the frozen tissue. During storage, especially with fluctuating and not very low temperature, the size of the ice crystals gradually increases, and at the same time (but not necessarily for that reason) the textural properties of the flesh when thawed deteriorate. Progressively better results are almost always obtained when both the rate of freezing is increased and the temperature of storage is reduced. The fluid (drip) liberated on thawing is a fair indication of the textural quality of the product: the less the drip, the better the texture

—and incidentally, but of great practical importance, the less the loss in weight and the greater the margin of profit to the processer.

In a situation like this it is always a balance between extra cost and extra care, and the extra return gained from the sale of a product of better quality.

In fruits and vegetables, collapse of the cells is an inescapable result of freezing and thawing, and preservation of quality in the frozen state is, therefore, mainly concerned with the preservation of colour and flavour. The principles of freezing and low temperature storage were discussed by Gutschmidt (1968). More information concerning the frozen products is given by M. A. Joslyn in Meryman (1966).

In animal tissues, the change in texture during frozen storage is almost certainly due to the slow denaturation of proteins. This statement, has, however, little significance unless the meaning of denaturation is more closely defined. This, in the case of fish, is discussed in great detail by Connell (1968).

The situation is still at present complicated by the uncertainty as to the part played by the skeletal, as distinct from the contractile, elements of muscle in the texture and toughness of flesh. It can be expected, however, that rapid strides will be made in this respect now that it is possible to determine the relative amounts of gross connective tissue (and specifically of collagen, which softens on cooking, and elastin, which does not) and of muscle proper by chemical means (see e.g. Lawrie, 1966). There is an additional difficulty in the case of poultry from the incidence of toughness in a percentage of birds for which no reason has at present been found.

This brief general statement must suffice as an introduction to the description of the particular cases of meat, fish and poultry. The only additional statement it is necessary to make is that anything which may be observed in one of these commodities does not necessarily hold for the others, although they are all physiologically so similar.

7. *The freezing and storage of meat*

Beef, mutton and pork, the three main types of meat, are different both technologically and biologically. As between the first and the second, the difference is mainly a matter of scale; between the first two and the last of susceptibility to oxidation of the fat.

Scale affects the behaviour on freezing because a large carcase such as beef is cut into joints for retail purposes from which 'drip' will flow and give rise to unfavourable comment; while the meat on a carcase of lamb will always be sold with so much bone that the drip from it will be unobtrusive, the resultant loss in weight will be negligible, and the conditions of freezing and storage, therefore, relatively unimportant.

There is one respect, however, in which beef differs from mutton (and also from pork), and that is, that the glycogen reserves of beef are usually so high that there is an *excess* over what is required to reach the pH 5·3–5·4 at which the formation of lactic acid is arrested.

Most attention is being directed at the moment to the question whether the overlap of the myofilaments in contracted muscle is, or is not, a cause of toughness. Here it must be

emphasized that there is a world of difference between excised muscles which, being under no stress, can shorten freely, and muscles *in situ* on a carcase, which cannot. We have referred to contraction as the function of muscles in the living animal, but strictly speaking it is their ability to *develop tension*—to do work—which is their function. It is not only the physiologist in the academic laboratory who should respect this distinction, but the technologist in the meat works. There is, in fact, relatively little tension developed in muscles during rigor mortis, compared with the behaviour of living muscle when stimulated. Meat on an intact carcase, prevented from shortening by its bony scaffolding, suffers less increase in toughness even in thaw rigor than do cut joints.

Mutton and lamb, which are built on a smaller scale, present few problems either in freezing or storage, as evidenced by the long and profitable history of the New Zealand industry. Nor do they suffer, as does pork, from susceptibility to oxidation of the fat.

This susceptibility of pork fat to oxidation is not reduced, in fact it may be increased, in the frozen state, so that the handling of pork carcases and quick-frozen cuts of both cured (bacon) and uncured pork needs to have its own special technology. For instance, the use of ultraviolet irradiation to control surface growth of micro-organisms during the conditioning of the carcase prior to freezing is precluded in the case of pork (Jul, 1968).

Pork also suffers more than red meats from a tendency to high pH in the flesh. This may or may not, however, be a disadvantage in frozen pork, where, except for the initial conditioning period and the later handling of the thawed meat, there is no hazard from the growth of bacteria, otherwise favoured by the high pH. In fact, the higher pH may result in an increase in tenderness and water-holding capacity, the latter being especially important in chopped meats.

8. *The freezing and storage of fish*

Fish is much more susceptible to changes in texture during storage in the frozen state than other flesh foods. These changes are represented by toughness and dryness in the mouth when the thawed fish is cooked and eaten. They are 'the direct result of changes in the proteins constituting the fish and, in fact, the main incentive for the study of fish muscle proteins has come from a need to understand and control these textural changes' (Connell, 1968).

Pre-freezing history is especially important in the case of fish. While there are marked differences in the storage behaviour of different species of fish, there are equally marked differences within any one species depending on where and how it was caught, how then treated (whether frozen before, during or after the onset of rigor), the rate of freezing, the time and temperature of storage, and finally, but of equal importance, how the textural changes are evaluated and reported by different workers. Thus the *facts* themselves are at present open to question.

The most significant, and perhaps the most reliable indication of textural changes is given by the determination of the fall in protein extractability in saline solutions of moderately high (about 0·5) ionic strength. Judged in this way, the gadoid species

whiting and cod are the most susceptible of those so far examined, and flat fish, e.g. plaice, the least. While there are some indications of species differences in ultimate pH being correlated with extractability data (flat fishes with pH 6·0 having lower values than gadoid fish with pH 6·3–6·5) this does not account satisfactorily for many intermediate species. One case which might be especially worth while investigating is that of another gadoid fish, the haddock, which closely resembles the whiting, has only a slightly lower ultimate pH, but is much less susceptible to textural changes and fall in protein extractability (Connell, 1968).

The physico-chemical changes responsible for the toughening and fall in protein extractability are giving rise to much speculation and fruitful investigation. Dyer (1968) describes his own and his colleagues' work on the possible implication of free fatty acids in the insolubilization of the proteins in cod muscle. There is no doubt that in this species the FFA do increase *pari passu* with the decrease in extractability, but the work of Olley and co-workers (quoted by Connell, 1968) on a wide range of species suggests that the two events have no simple cause and effect relationship.

Evidence that it is not only the extractable proteins that are involved in textural changes is derived from the work of Love (1962) and of Love & Mackay (1962), using the former's cell fragility method, which consists in measuring the optical density of a sample of muscle in presence of formaldehyde. The opacity of the suspension decreases with increasing time in storage. It is not, however, correlated with decreasing extractability of protein, and the most recent results of Kelly *et al.* (1966) indicate that there is a strong influence of pH, as such, on the values obtained.

9. *The freezing and storage of poultry*

This subject was not specifically provided for in the programme of the Institute; few problems arise which are not general to the freezing and storage of flesh foods. In fact, Shrimpton (1965) can go so far as to say that 'with young chickens it has not been found necessary to impose a holding period between evisceration and freezing; consequently considerable effort, both technically and organizationally, has been devoted to developing a continuous flow procedure from slaughter through plucking, evisceration and chilling to the freezing tunnels'.

This reference provides an opportunity for drawing attention to the activities and publications of Commission 4 of the International Institute of Refrigeration. This Commission is responsible for the application of refrigeration to foodstuffs and agricultural products. It meets not only at the International Congresses of Refrigeration, but also between congresses to discuss specific topics.

The freezing and storage of eggs and poultry were also dealt with by Shrimpton in the *Annual Reports on Applied Chemistry*, 1965.

Technology: the interdigitation of science and industry

Important though it is, technology occupies a relatively minor position in the structure

of the frozen food industry (Symons, 1968). Foods are frozen to be sold and are bought to be eaten, so it is important that the retail pack shall be visually attractive, and this may be more a matter for the packaging expert than the food technologist. The latter does come into the picture, though, because frost inside the pack while it is frozen, and drip when it is thawed, may have an adverse effect on the potential purchaser. Of much greater importance, however, is the extent of deterioration in quality which can be tolerated without adversely affecting purchase, and that means, at what length of time after processing must the goods be withdrawn from sale because the deterioration which all refrigerated foods must suffer has fallen below the level of 'consumer acceptance'.

A great step forward was made when the Western Regional Laboratory of the United States Department of Agriculture introduced the concept of High Quality Life (HQL). The difficulty about 'consumer acceptance' is that it is so completely subjective and circumstantial. HQL, on the other hand, is as nearly objective as a test based on subjective assessment can ever be. It sets out to determine the moment when *any perceptible difference* can be detected between the stored and the original produce; the 'original' having been kept under such conditions that deteriorative changes in it can be completely ruled out. The test (Guadagni, 1968) is carried out either as a triangular or as a duo trio test with panels of six or more subjects, the criterion for a definite quality change being the point in time when 70–80% of the judgments correctly identify the control sample in the test. As of itself, it has no immediate commercial implications, but it represents a significant point in the shelf life of the goods which can be related to any concept of consumer acceptance which the manufacturer may like to adopt. Various evaluations of 'consumer acceptance' level reveal a three- to six-fold time in store beyond that of the HQL before the CA level is reached, varying of course with the storage behaviour of the particular product.

Olsen (1968) describes the relationships which have been found between HQL and deterioration in such measurable quantities as the conversion of chlorophyll to phaeophytin and of ascorbic acid to dehydroascorbic acid plus diketoglutaric acid. This work formed part of a massive co-operative programme undertaken on behalf of the frozen food packers and described as the 'time–temperature–tolerance' programme. Guadagni (1968) deals with the quality changes expressed in terms of HQL with corresponding evaluation of consumer acceptance obtained either by questioning representative consumers or by experts drawn from the industry. The HQL has been introduced routinely as a measuring device into commercial quality control in this country (Symons) and into food research in Denmark (Jul).

Guadagni also describes an ingenious way of integrating the fluctuations in temperature during a sequence of operations from freezing to retail sale, so as to express the history in terms of equivalent time at a chosen steady temperature, and gives practical examples of the application of this calculation.

Jul (1968) deals especially with meat products. He stresses the importance of losses in

weight due to evaporation and drip, a 1% loss from these causes absorbing the whole of the profit margin on which the frozen meat trade operates.

Other examples of the bearing of technology on industrial practice have been mentioned as they arose incidentally in the course of this review. In conclusion it may be interesting to recall the questions that appeared to a scientist in the early days of research in this field to be of outstanding importance (Moran, 1924), to consider how important they now seem, and to what extent they have been answered:

1. Why does water freeze outside and not inside cells?
2. Can this external ice separation be prevented?
3. If a certain amount of external ice separation is inevitable, what conditions favour its reabsorption?
4. What is the particular factor in freezing responsible for the death of living tissues?

The reader is invited to frame for himself the answers to these questions in the light of the information made available to him in this review; and to consider how relevant they still are to the problems of low temperature technology in the food industries.

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I would like to thank my fellow-members of this Committee, Mr Rolfe, the authors and the Editors of the publication for their help in the preparation of the review.

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A critical look at two objective tests for cold storage deterioration

J. OLLEY, E. STEPHEN, J. FARMER AND I. ROBERTSON

Summary. Protein solubility in salt solutions and cell fragility are two possible measures of textural changes in cold stored fish. These measurements are discussed for a wide range of species. The discrepancies between the methods, the artefacts in measurement caused by proteolysis at higher temperatures and possibly by free fatty acid are considered. The effect of pH, horizontal and vertical strength of the myofibrils, and initial protein solubility on taste panel assessment are discussed.

Introduction

Protein solubility and cell fragility measurements have been considered as two of the most likely objective tests for the determination of textural changes during the cold storage of fish. During the course of several years work on the effect of lipid breakdown in cold stored fish several phenomena have become apparent which are reported here and which show that the interpretation of the 'numbers' obtained by these tests should not be attempted without some basic understanding of the kind of phenomena which may be occurring.

The protein solubility test and free fatty acids

Dyer & Dingle (1961) and Hanson & Olley (1965) have pointed out that protein solubility falls incompletely and to a different level for different species of fish. The latter workers attributed the phenomenon to the presence of neutral lipids present in sufficient quantities to protect the proteins from denaturation by free fatty acids (FFA). The cessation of protein change was related to the cessation of further production of FFA. The development of more micro methods of lipid analysis (Hanson & Olley, 1963a, b) enabled the determinations of soluble protein, total lipid, FFA, phospholipid and neutral lipid to be determined on the same few myotomes of fish. Neutral lipid was calculated as total lipid $-[(P \times 25) + \text{FFA}]$ and was consequently subject to a certain amount of error. Six fish of each species, air-blast frozen and thawed immediately, were used to obtain the initial value of insoluble protein (Ironside & Love, 1958) and results after any given period of cold storage were obtained on a

Authors' address: Torry Research Station (Ministry of Technology), Aberdeen.

pooled sample of three fish. The *increase* in insoluble protein was plotted against the ratio FFA : neutral lipid and the results are shown in Fig. 1. The scatter was large and the results are most graphically expressed in a block diagram. However it can be roughly said that a FFA : neutral lipid ratio of 2 represents the insolubilization of about 4·5 g of actomyosin/100 g muscle. The data in the cross-hatched area of the

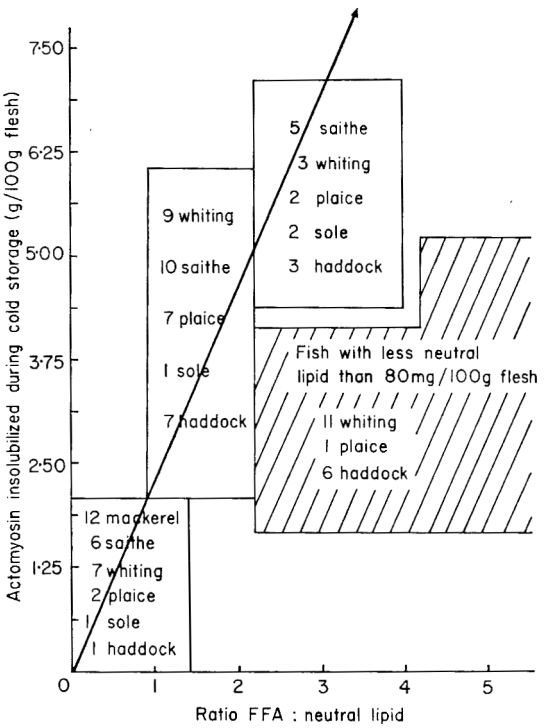

FIG. 1. The possible relationship of protein insolubilization during cold-storage, with free fatty acid formation and protection by neutral lipids. To emphasize the possible validity of these relationships it should be noted that the saithe, for example, in the top block had been stored 4–5 months at −14°C while the lemon sole had been stored for 6 years at the same temperature.

diagram shows the fish which did not fit into the general pattern. They were all characterized by having an extremely low neutral lipid content and the lower the neutral lipid content the more they deviated from the relationship. It has been pointed out that the neutral lipid determination is subject to error. It is also extremely difficult to measure the increase in insoluble protein in the whiting (*Gadus merlangus*) as the initial 'blank' value may be as high as 6 g/100 g flesh whereas the initial value for the lemon sole (*Microstomus kitt*) would be one-tenth of this. It would, however, be unwise to exclude these results as they may represent fish of a different nutritional status or some other phenomenon.

Anderson & Steinberg (1964) in model experiments added FFA to muscle homogenates. In cold-storage the FFA is actually derived mostly from the phospholipids of the sarcoplasmic reticulum which are in close proximity to the myofibrillar proteins. The remaining phospholipids could also possibly protect the proteins from FFA. Replotting the data to equate insolubilized protein against the ratio FFA : total lipid–FFA showed an extremely wide scatter of results and the protection by neutral lipid would appear to be the more tenable hypothesis.

Does the protein solubility test have any organoleptic significance?

Could the test itself be an artefact?

Anderson, King & Steinberg (1963) thought that the protein solubility measurement might be an artefact because the addition of fatty acids to an homogenate of fresh muscle or to the extractant salt solution resulted in reduced protein solubility, the effect in the slightly fatty species being mitigated by neutral lipid (Anderson & Steinberg, 1964). Hanson & Olley (1965) pointed out that several species of frankly fatty fish were not protected by their neutral lipid and denatured quite rapidly. They argued that the deterioration must have taken place during cold storage. Their conclusions were based on results for three Indian species (Sawant & Magar, 1961) and the fatty elasmobranch, *Squalus acanthias* (Love & Olley, 1965). On re-examination of the Indian paper it was noted that the homogenization had been done at room temperature. Cold stored fish are particularly sensitive to deterioration during extraction at elevated temperatures (Dyer, French & Snow, 1950) and no conclusions should be drawn from the Indian work. Continuing work at Torry has shown that two other non-oily elasmobranchs, skate (*Raja maculata*) and nursehound (*Scyllium canicula*), denature rapidly during cold storage at $-7°C$ despite a negligible production of FFA (cf. Olley, Pirie & Watson, 1962). The percentage soluble protein in the former species dropped from an initial value of 60 to 40% in 6 weeks. Plate 1 shows that myofibrillar proteins also aggregate in these species without FFA production. On the other hand the moderately fatty mackerel (3% lipid) denatured only very slowly at $-7°C$ by the criterion of protein solubility. There is thus no longer any reason to conclude that neutral lipid in the fattier species does not protect them from denaturation by FFA. Although in the method of Ironside & Love (1958) the homogenization with salt solution is done at a pH which does not cause insolubilization of actomyosin by added fatty acid (Hanson & Olley, 1965); it is possible that the more naturally dispersed FFA and neutral lipid may have some effect on the protein solubility at the homogenization stage.

Insoluble protein unrelated to lipid changes

The work with the non-oily elasmobranchs has shown that there is no cause to equate protein insolubilization with FFA production in these species. In Fig. 2 the amount

of insoluble protein found after merely air-blast freezing and thawing various species has been plotted against their FFA content. Whiting (*Gadus merlangus*) and skate for example can have almost half their myofibrillar proteins insoluble in salt solution before FFA production has barely commenced. Twelve to 20 mg FFA/100 g flesh

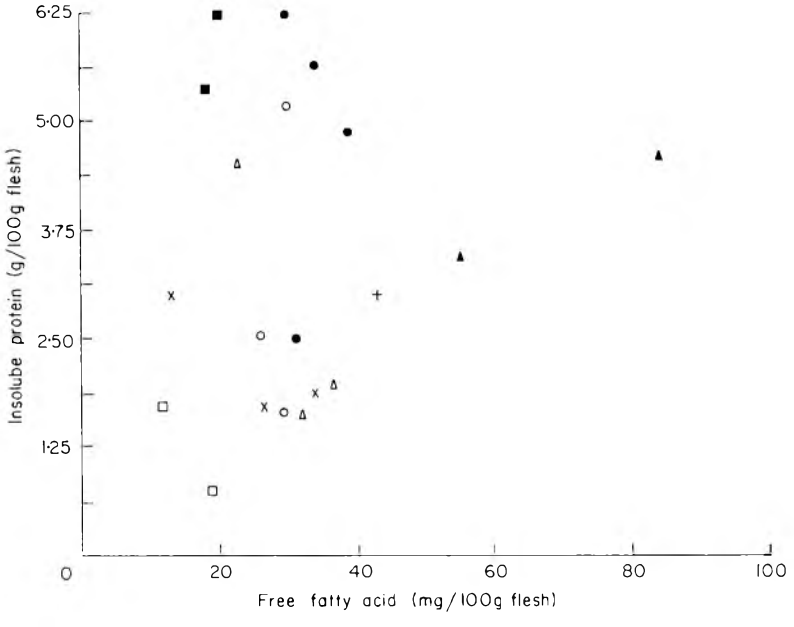

FIG. 2. Lack of relationship between the initial FFA values of fish frozen and thawed without cold storage and the initial amount of protein insoluble in 5% salt solutions. ■, Skate; □, sole; ●, whiting; ○, saithe; ▲, mackerel; Δ, haddock; ×, plaice; +, gurnard.

is quite a normal figure for fresh unfrozen fish. If FFA is involved at all in protein insolubilization, does it produce the same kind of textural change as the insoluble protein which is found in some species prior to cold storage?

pH and organoleptic rating of fish

It has recently been found that the firmness of fresh and freshly frozen cod (*Gadus callarias*) is inversely related to pH (Kelly *et al.*, 1966). In cod the effect of pH predominates over cold storage changes to such an extent at −29°C that Cowie & Little (1966) found no correlation between toughness and insoluble protein. A striking correlation was however found between toughness and muscle pH. At −14°C Connell & Howgate (1966 unpublished) found that all the myofibrillar proteins could become

insolubilized with approximately 1·5 units change in firmness score as detected by taste panel. One unit change in pH also caused a change in firmness of this order of magnitude.

The cell fragility test

Love & Mackay (1962) found that the cell fragility of a fish muscle homogenate as measured by optical density $E_{\frac{1}{2}cm}$ always fell on cold storage, but that they had to take a large number of samples to get a statistical mean value which then fell on a reasonable first order curve (Love & Olley, 1965). This meant that one could not equate the taste panel assessment of an individual fish to an $E_{\frac{1}{2}cm}$ reading. In actual fact, when the present authors attempted to do so on unfrozen or freshly frozen and thawed cod they found that they could, and that the cell fragility measurement was directly related to firmness and inversely related to the pH of the flesh (cf. Kelly *et al.*, 1966). Some of the previous scatter was obviously related to fish of varying pH. Unfortunately while $E_{\frac{1}{2}cm}$ values of fresh or freshly frozen fish are directly related to firmness, the effect of cold storage leads to aggregation of myofibrils and *increase* in firmness but a *decrease* in $E_{\frac{1}{2}cm}$. At the present time, the only recommendation that can be made, is to measure the pH of the muscle and also to examine the cell fragility homogenate under the microscope.

On cold storage of any species of fish the cell fragility measurement $E_{\frac{1}{2}cm}$ always falls. This is due in most cases to a side to side cohesiveness of the individual myofibrils. However, phase contrast microscopy has shown that the same phenomena are not always occurring in different species or for that matter in the same species at different temperatures. Plate 2(a) and (b) shows phase contrast photographs of haddock (*Gadus aeglefinus*) and whiting both stored for 3 weeks at $-14°C$. In the haddock the homogenate from the cell fragility determination shows side to side cohesion of the myofibrils with a teasing out or fraying longitudinally. The whiting on the other hand stored for the same period at the same temperature shows side to side aggregation in the same way, but with a tendency for the weakness in the fibril to be in the opposite direction, that is horizontally across the fibril. This should not be taken to indicate a fundamental difference in the haddock and whiting. Plate 3(a–c) shows that both phenomena can occur in the cod. Cod either frozen in the air-blast freezer and then brought back to $-2°C$ or $-3°C$ or frozen directly down to these temperatures tended to break horizontally after storage for two weeks, while the same batch of cod frozen directly to $-4°C$ or air-blast frozen and brought back to $-4°C$ tended to show the more usual long myofibrils with some side to side aggregation.

These observations are more in the nature of warnings than explanations; they must, however, make a difference to readings of $E_{\frac{1}{2}cm}$ and would, one suspects, be related to chewing properties. Although homogenization of a tissue in cold formalin is not comparable to eating it after cooking, there has been found to be a very real correlation between the degree of fragmentation of the tissue in weak formalin as

measured by $E_{\frac{1}{2}cm}$ and its texture. As stated above, this correlation may be positive (Kelly *et al.*, 1966) or negative (Love & Mackay, 1962; Love, 1966).

Comparison of protein solubility and cell fragility measurements

Love and Olley (1965) pointed out that the rates of denaturation of frozen fish as measured by change in soluble protein and cell fragility were not the same. Although the two measurements rated the species in the same order, the discrepancy between the ratios became greater, the slower was the rate of denaturation as measured by protein solubility: the latter measurement in turn with the exception of the elasmobranchs was related to the neutral lipid content of the species.

It is interesting to speculate in species where protein solubility shows little change and cell fragility rapid decrease whether the fish is toughening or not. The training of an efficient taste panel, for even one species, takes several months and at present at Torry only cod and haddock have been critically examined. Connell & Howgate (1966 unpublished) have found that the cell fragility measurement changes more rapidly than the taste panel assessment, and that the firmness scores continued to decrease long after the cell fragility measurement had reached an asymptote. Love has of course always claimed that his method was more sensitive than a taste panel in the early stages of cold storage.

PLATE 1. Phase contrast micrographs of elasmobranch muscle after cell fragility test. Skate (a–c) and Nursehound (d–f) frozen at −29 C.

	Storage at −7°C (weeks)	Cell fragility ($E_{\frac{1}{2}cm}$)	Free fatty acid (mg/100 g flesh)
(a)	0	1·2	8·0
(b)	1	0·54	9·0
(c)	3	0·35	27·0
(d)	0	1·6	10·5
(e)	2a*	1·1	10·5
(f)	2b*	0·5	11·0

The values for FFA were all within the normal range for teleost muscle.

* 2a and 2b represent two different fish; the large bundles in (f) were typical of the whole field.

The slight production of FFA in the skate after 3 weeks storage was negligible compared to the 150–300 mg which would be found in teleost muscle. The FFA in the skate remained below 50 mg/100 g flesh for 10 weeks; after this period there was a strong smell of ammonia the pH rose above 7·0 and phospholipase activity commenced. The interaction between protein and FFA is far more rapid above pH 7 (Hanson & Olley, 1965; Anderson *et al.*, 1965) but the protein denaturation had occurred long before the pH rise.

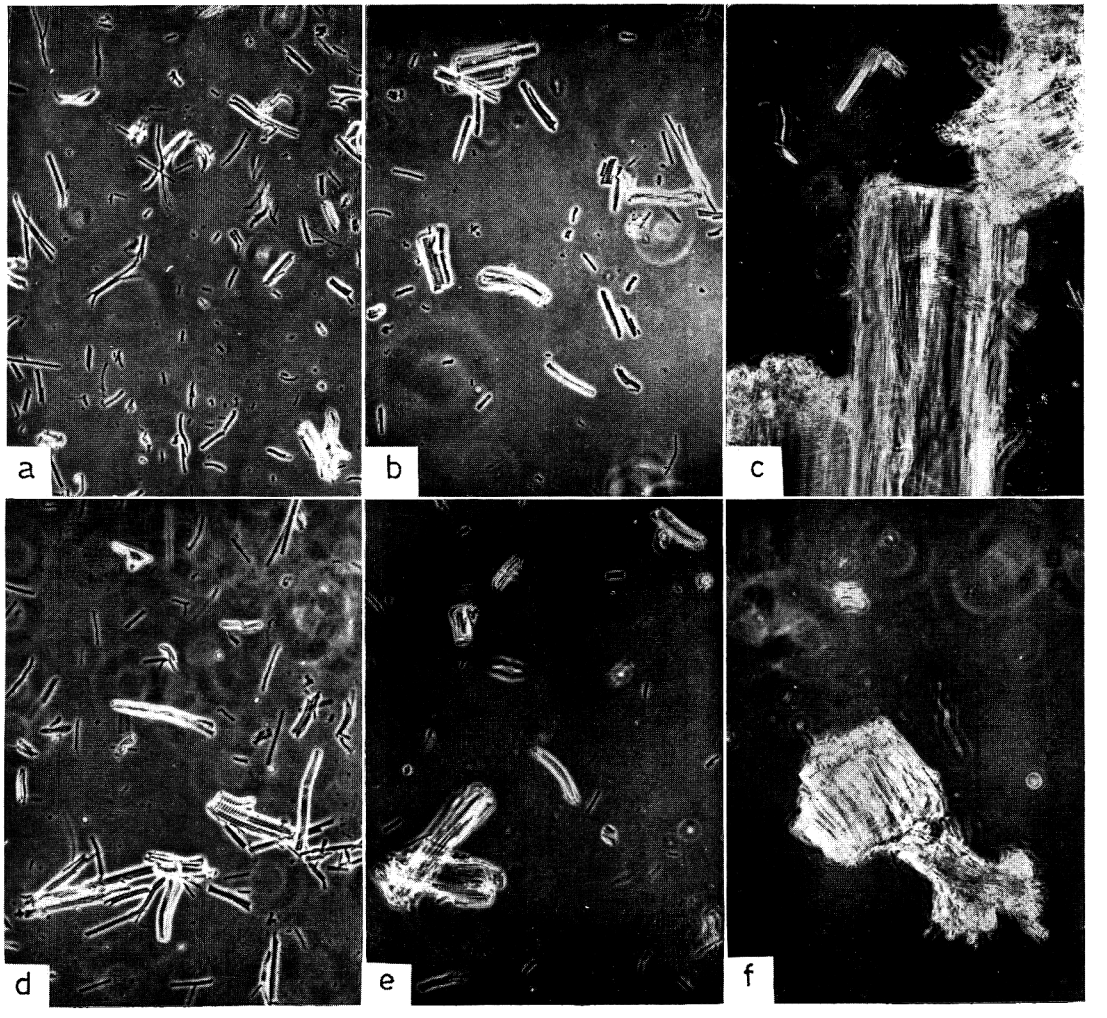

PLATE 1. (For legend see facing page.)

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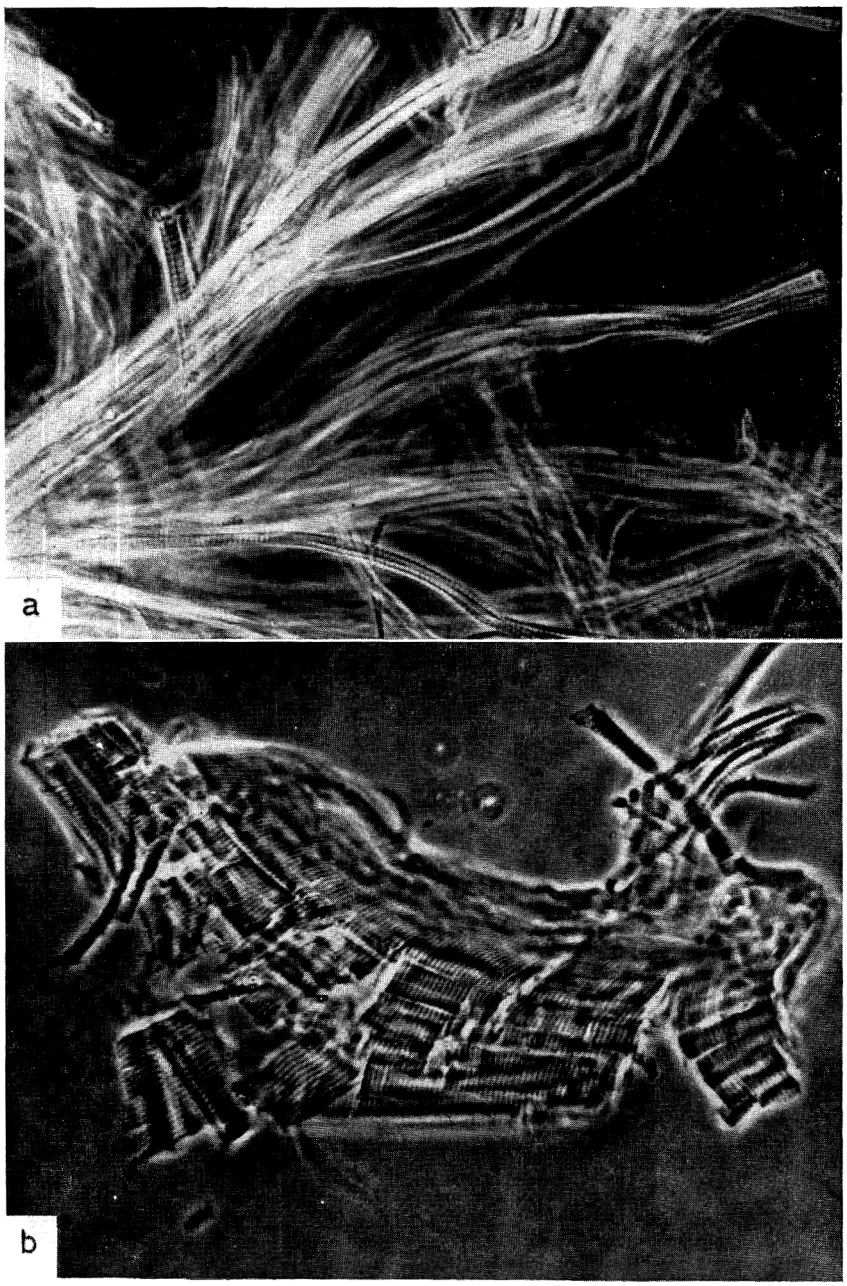

PLATE 2. Phase contrast micrographs of cell fragility homogenates from haddock (a) and whiting (b) stored 3 weeks at −14°C.

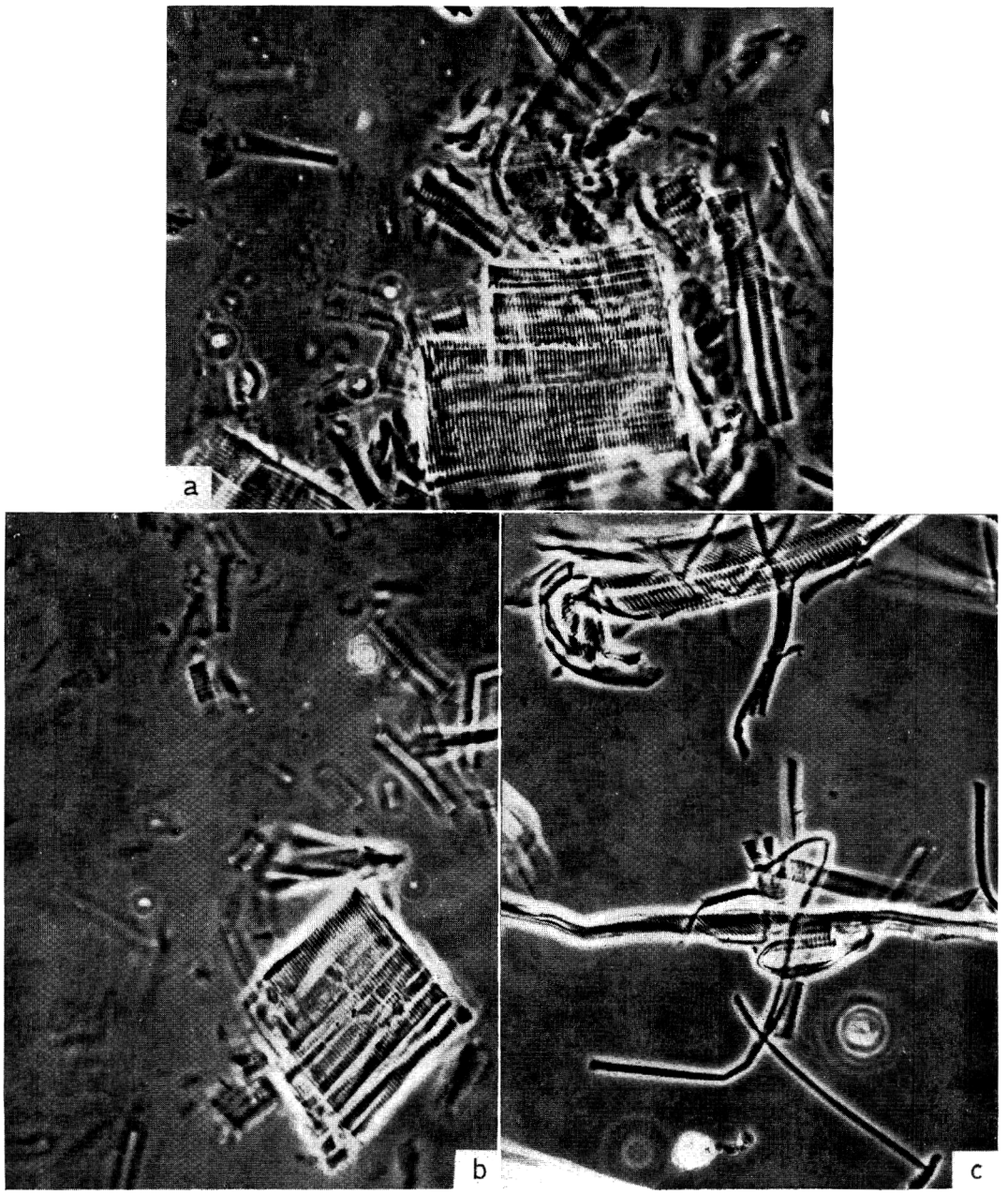

PLATE 3. Phase contrast micrographs of cell fragility homogenates from cod stored for 2 weeks at −2°C (a), −3°C (b) and −4°C (c). These samples were taken from an experiment by M. K. Elerian and R. M. Love.

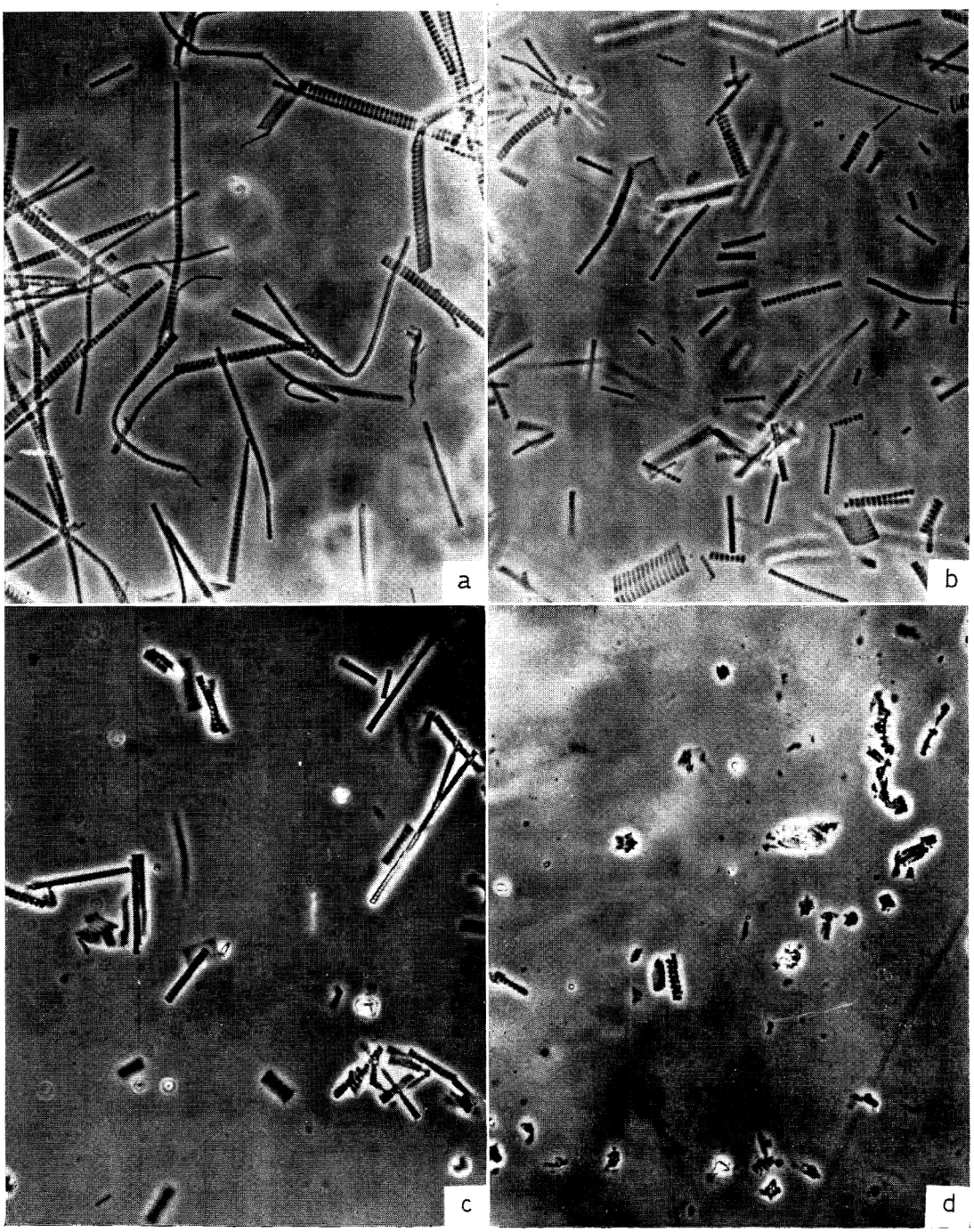

PLATE 4. Phase contrast micrograph of cell fragility homogenates from lemon sole stored at −7°C for 0 weeks (a), 1½ weeks (b), 3 weeks (c) and 22 weeks (d).

Artefacts in soluble protein and cell fragility determinations

The slow rate at which reactions occur at temperatures below 0°C makes the study of cold storage changes extremely slow and tedious and workers are therefore tempted to try and correlate effects at temperatures such as −7°C, which is low enough to prevent bacterial spoilage but which would not be used in commercial practice. The advent of superchilling has, however, brought temperatures just below the freezing point into the commercial range. Fig. 3 shows the soluble protein and cell fragility

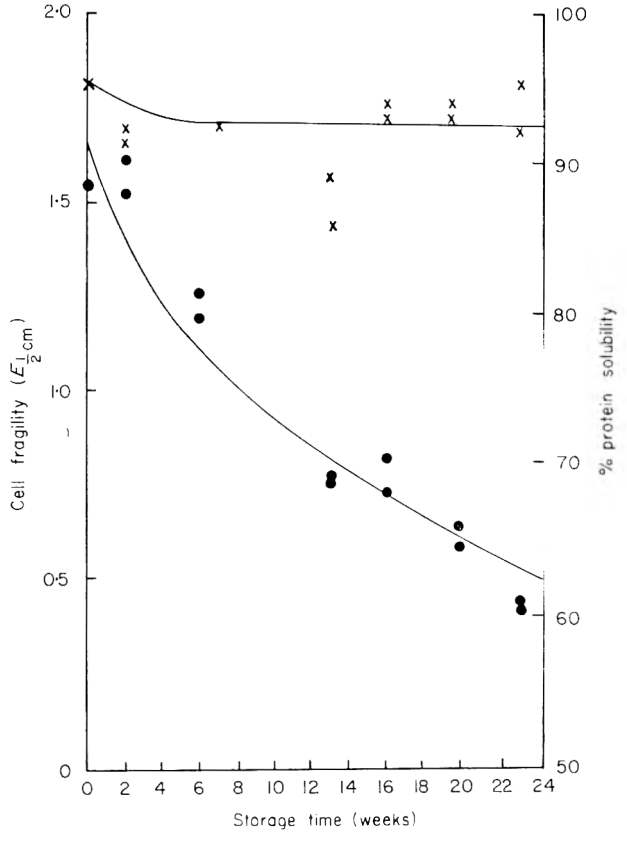

FIG. 3. Protein solubility (×) and cell fragility (●) of lemon sole stored at −7°C.

results for lemon sole stored at −7°C. The protein solubility data would not have fitted the lipid relationship shown in Fig. 1 as much more denaturation would have been expected. However, phase contrast microscopy of the cell fragility homogenate showed that the expected aggregation of myofibrils was not occurring, but that there was a progressive break-up of the myofibrils into fragments of a few sarcomeres (Plate 4 a–d). Instead of the expected toughening a marked sloppiness occurred in the flesh,

the total protein content of the flesh decreased and the remaining protein or peptides of sufficient chain length to be precipitated by trichloroacetic acid showed an increasing tendency to be soluble in salt solutions of low ionic strength (Fig. 4). This effect with

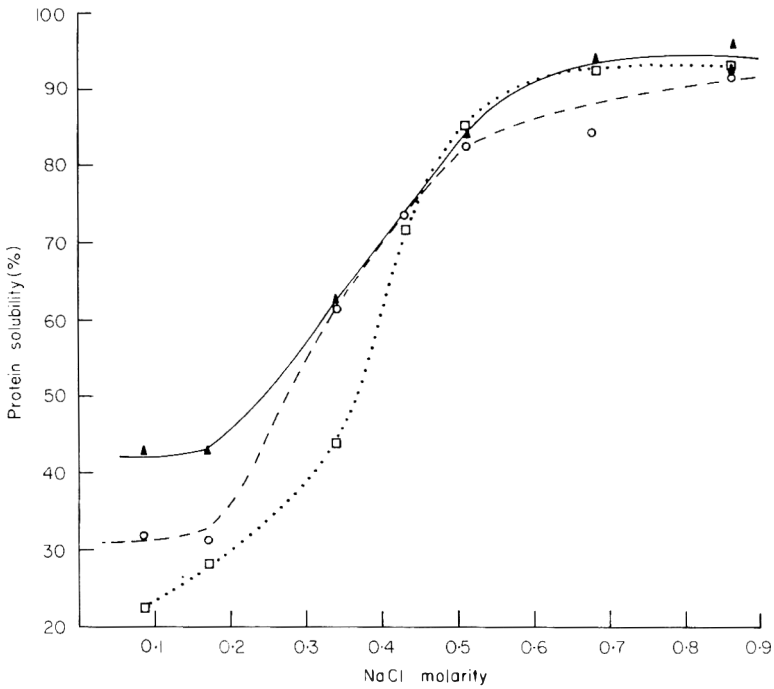

FIG. 4. Increase with time in the solubility at low ionic strengths of the proteins of lemon sole stored at −7°C. □, Fresh; ○, 14 weeks; ▲, 22 weeks.

lemon sole was noted in three different experiments with fish caught in January, May and October. The fish were gutted, air-blast frozen in threes and wrapped in aluminium foil for storage at −7°C. A similar experiment in May of the following year showed that the breakdown of the flesh was not always uniform and appeared to encroach inwards from the fins. In this batch of lemon soles some had almost completely turned to a slime after 5 months' storage at −7°C, others showed the effect at the outer edges, while others appeared virtually unchanged. Although the fish were found to be sterile a penetration of the flesh by bacterial enzymes could not be ruled out. Experiments on pre-rigor fish which had been held in fish tanks showed no breakdown of the muscle protein, whether the surface of the fillet was sterilized or not. The effect would appear to be a *post-rigor* autolytic phenomenon. This is in contrast to Love's (1967 unpublished) observation with superchilled cod where the retardation of fall in protein solubility appears to be due to solution of the proteins in tissue salts *in situ* rather than to proteolytic enzyme action.

These observations have been gone into in some detail in order to show the importance, if soluble protein or cell fragility determinations are to be used at all, of the experimenter keeping his or her eyes open for unexpected effects. A fall in cell fragility can represent fragmentation of myofibrils and not aggregation, cf. also the work of Love *et al.* (1965) on iced fish. A lack of fall in soluble protein may represent proteolysis and not stability of actomyosin.

Conclusions

Protein solubility of fish muscle in various species may be related to the free fatty acid and neutral lipid content of the flesh. These lipids may produce some artefacts at the homogenization stage of the determination.

Protein solubility can decrease in elasmobranchs during cold storage without FFA production and in some other species considerable quantities of protein may be initially insoluble in salt solution after freezing and thawing before FFA production commences.

As protein solubility determinations may be the resultant of several factors they are unlikely to correlate well with organoleptic scores, especially as firmness after cooking is also related to the pH of the flesh.

A high cell fragility reading of $E_{\frac{1}{2}cm}$ may represent a tough fish of low pH or a freshly cold-stored fish of higher pH which is highly acceptable to the palate and not denatured by cold storage. The tester should always do pH measurements. Phase contrast microscopy of the cell fragility homogenate is of considerable interest and may throw light on the nature of the toughening phenomena.

Protein solubility and cell fragility do not measure the same changes and both may be subject to artefacts caused by proteolysis in fish stored at high temperatures.

Neither method could be considered a panacea for the fish processer or manager in charge of quality control.

Acknowledgments

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The relation between the toughness of cod stored at −7°C and −14°C, its muscle protein solubility and muscle pH

W. P. COWIE AND W. T. LITTLE

Summary. Protein extractability, muscle pH and organoleptic toughness have been measured in cod fillets stored for up to 34 weeks at −7°C and −14°C. The decrease in protein extractability and the increase in toughness of fillets stored at −7°C proceeded at a faster rate than in fillets stored at −14°C. In order to assess if a fillet stored at −7°C or −14°C had an acceptable or a tough texture it was necessary to measure both protein extractability and muscle pH.

Introduction

In a previous paper (Cowie & Little, 1966) it was shown that the toughness of cod fillets stored at −29°C was more closely related to muscle pH value than it was to protein solubility.

Unfortunately however not all frozen fish is stored at −29°C throughout its distribution chain. Several workers (Dyer, 1951; Love, 1962) have shown that the higher the temperature of frozen storage the faster the rate of protein denaturation. We have, therefore, extended our previous work to investigate the relationship between the toughness of cod fillets stored at −7°C and −14°C and their protein solubility taking the muscle pH into account.

Experimental

Materials

All the cod (*Gadus morrhua*) used in this work were 26–30 in. long and were bought from the Aberdeen Fish Market. They had been caught in the North Sea and had been stored 1–2 days in ice. They were filleted in the laboratory, and the fillets were placed in sealed polythene pouches and blast frozen to −30°C. One of the fillets from each fish was transferred to a cabinet at −14°C and the other fillet to a cabinet at −7°C. At about weekly intervals a pair of fillets belonging to the same fish were removed from the cabinets for examination. At the longer periods of storage more than two fillets were examined in a day and these were not necessarily from the same fish.

Authors' address: Unilever Research Laboratory (Colworth), Greyhope Road, Torry, Aberdeen.

Methods

The muscle protein extractability and the muscle pH of each raw fillet were measured as before (Cowie & Little, 1966). The toughness of the cooked fillets was assessed by a taste panel. The method of cooking the fillets and of scoring for texture have already been described (Cowie & Little, 1966).

Results and discussion

The changes in the extractability of the cod muscle proteins in 5% sodium chloride with time of frozen storage at −7°C and −14°C are shown in Fig. 1. As expected, the

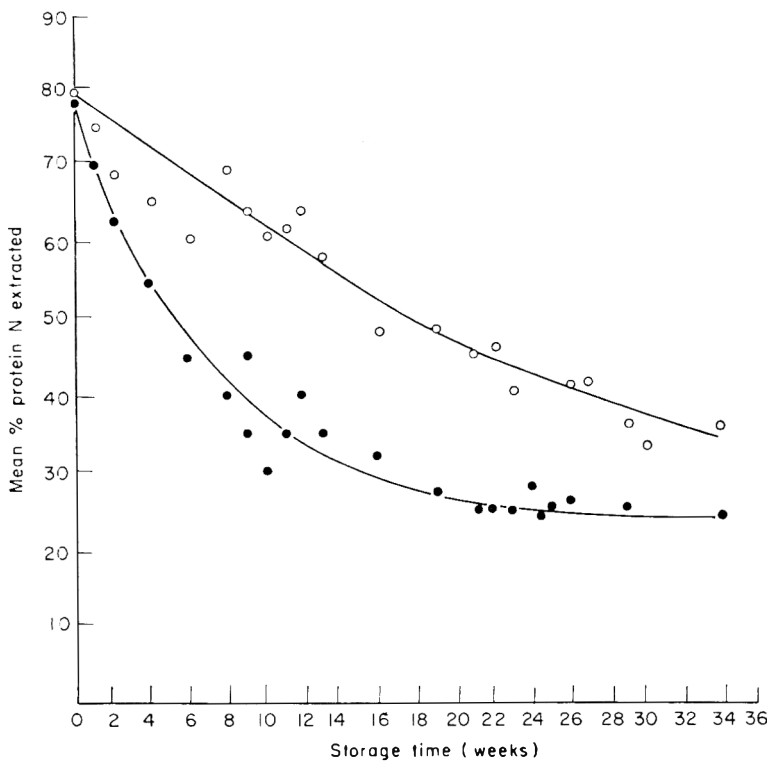

FIG. 1. Relationship between mean per cent protein N extracted and storage time at −7°C (●) and −14°C (○).

extractability of the proteins from cod stored at −7°C decreased more rapidly than the extractability of the proteins from cod stored at −14°C. The minimum level of extractability, 25%, that is when only sarcoplasmic proteins were being extracted was reached after about 20 weeks of storage at −7°C, whereas for fillets stored at −14°C for the same period the protein extractability was 50%.

In Fig. 2, the toughness ($\bar{Y}$), of the fillets stored at $-7°C$ and $-14°C$ was plotted against time of storage. No definite relationship was found to exist between these two parameters. It was found, as was expected, that of two fillets from any one cod, the one stored at $-7°C$ was always tougher than that stored at $-14°C$ for the same period

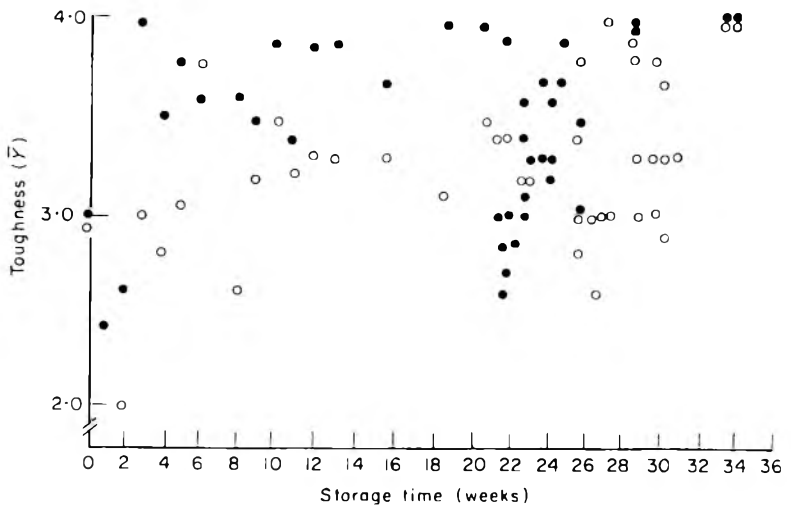

FIG. 2. Relationship between toughness ($\bar{Y}$) and time of storage at $-7°C$ (●) and $-14°C$ (○).

of storage. It was also observed that some fillets stored for only 4 weeks at $-14°C$ were tougher than fillets from other cod stored for 22 weeks at $-7°C$. This indicates that the eating toughness of cold stored cod cannot be explained simply in terms of time/temperature relationships. The fact that there was no correlation between toughness and protein extractability (Fig. 3) supports this view. As was found for fillets stored at $-29°C$ (Cowie & Little, 1966), the texture of fillets stored at $-7°C$ or $-14°C$ depended to a large extent on the muscle pH. A plot of toughness against muscle pH (Fig. 4), revealed that fish with a pH of 6·8 or greater had acceptable textures (i.e. $\bar{Y} < 3·0$) irrespective of their storage history at $-7°C$ or $-14°C$. However, the relationship between toughness and pH was not so distinct for fish stored at $-7°C$ and $-14°C$ as it was for those stored at $-29°C$.

When protein extractability was plotted against the muscle pH of fillets stored at $-7°C$ and $-14°C$ with each point indicating the toughness of each fish examined, it was possible to draw by inspection a diagonal line that separated the tough from the acceptable fish (Fig. 5). This means that the toughness of frozen cod muscle is dependent on two factors, the muscle pH and the amount of cold storage protein denaturation. Under $-29°C$ storage conditions, the amount of protein denaturation is small even

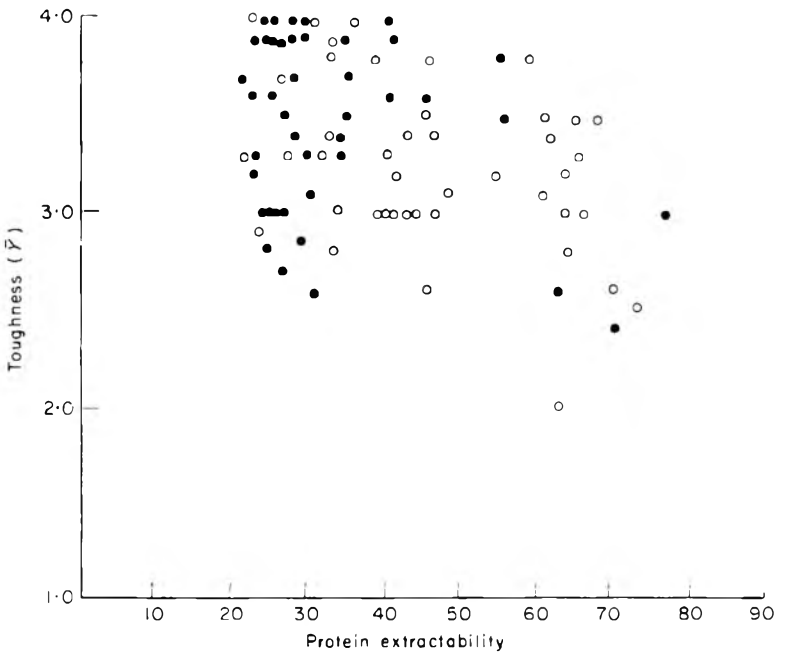

FIG. 3. Relationship between toughness ($\bar{Y}$) and protein extractability. ●, −7°C storage; ○, −14°C storage.

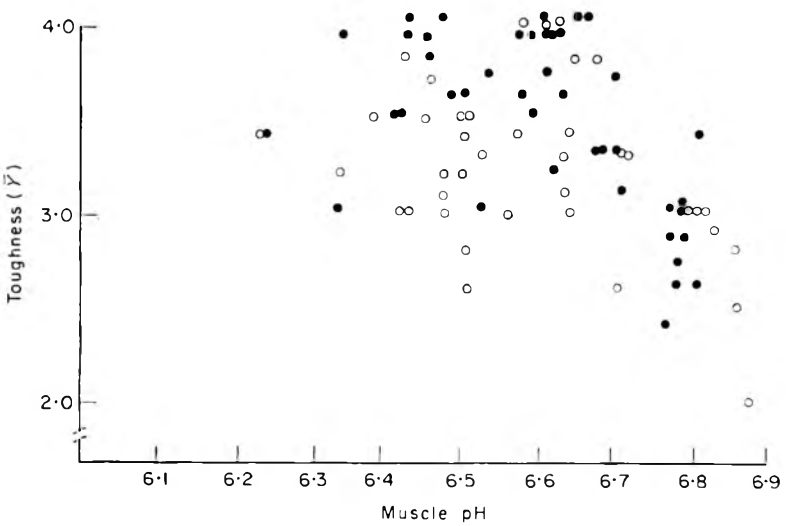

FIG. 4. Relationship between toughness ($\bar{Y}$) and muscle pH for fillets stored at −7°C (●) and −14°C (○).

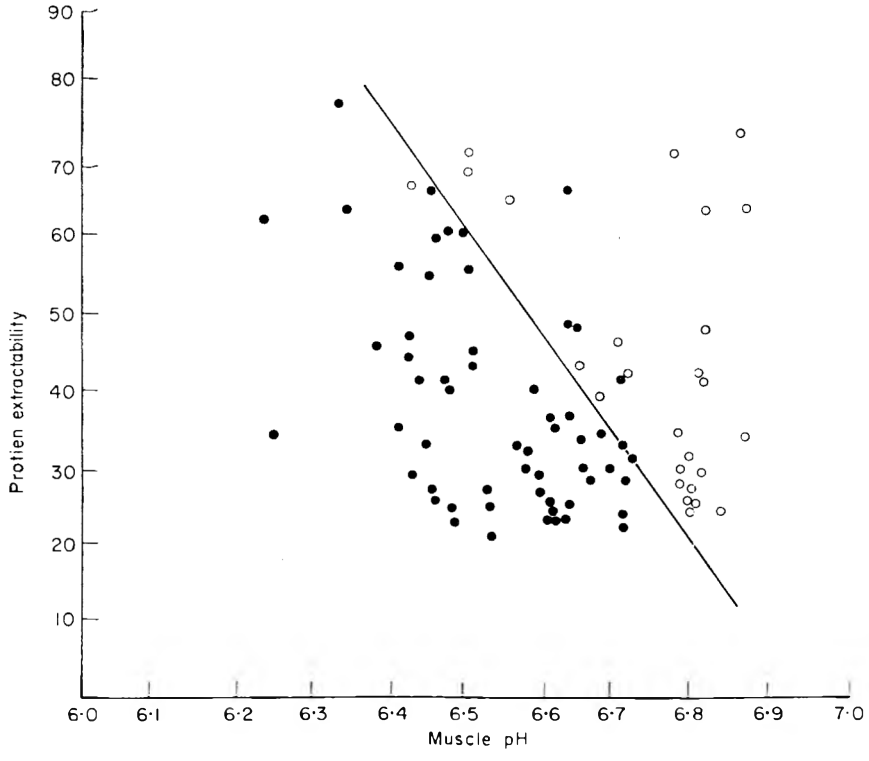

FIG. 5. Relationship between protein extractability and muscle pH including toughness (Υ) for fillets stored at -7°C and -14°C. ●, Tough $\Upsilon > 3{\cdot}0$; ○, acceptable $\Upsilon < 3{\cdot}0$.

after lengthy storage periods (Cowie & Little, 1966), and toughness depends mainly on the muscle pH. However, at higher storage temperatures toughness due to protein denaturation becomes more significant. It is obvious, therefore, that the toughness of frozen cod cannot be accurately described by the use of protein extractability alone. Any objective test for the quality of frozen cod must include measurement of muscle pH. The observation of Kelly *et al.* (1966) on the cell fragility test reported by Love & Mackay (1962) would confirm this.

This paper, therefore, shows that by measuring both the protein extractability and the muscle pH of a cold-stored cod fillet, it is possible by using Fig. 5, to decide if the texture of the fillet will be tough or acceptable. It is also possible to predict from Fig. 5, the amount of protein denaturation that can be tolerated for a fillet of any pH value. Thus fillets with a pH of less than 6·5 are not a good prospect for frozen storage at -7°C or -14°C whereas fillets with a pH of 6·6 or higher would have acceptable textures after normal storage periods at these temperatures.

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An assessment of the potential application of the method of attenuated total reflectance (ATR) infrared qualitative analysis to food materials

A. G. CAMERON

Summary. The ATR method is described and its application to food materials in the form of solids, powders, solutions and colloidal mixtures is considered. It is shown that the ATR method has several advantages over the conventional infrared technique. In particular the ATR technique eliminates the need to measure sample thickness and also simplifies the experimental procedure. It enables the spectra of aqueous, colloidal, viscous and complex multicomponent materials to be obtained more easily than in transmission measurements and in such cases often yields more useful spectra. ATR spectra generally show less detail than corresponding transmission spectra, and it is this lack of sensitivity that is the main limitation of the technique.

Introduction

The attenuated total reflectance (ATR) technique using infrared radiation was first developed by Harrick (1960) and Fahrenfort (1961), and since then it has proved its usefulness in extending the application of the infrared technique.

If infrared radiation is incident on a transparent prism of high refractive index which is in contact with a second material (the sample) of lower refractive index, total internal reflection is expected if the angle of incidence is greater than the critical angle. Such an arrangement is shown in Fig. 1. The sample is applied to one face of the prism and held in position by the sample holder. On reflection at the prism–sample interface, radiation penetrates the outer layer of the sample to the extent of a few (less than five) microns. If, during penetration, there is interaction between the infrared radiation and molecules in the sample layer, energy is dissipated and reflection is no longer total, i.e. the reflected energy is attenuated. Under such circumstances a reflection spectrum is obtained and has similar characteristics to the corresponding transmission spectrum.

Author's address: National College of Food Technology (University of Reading), St George's Avenue, Weybridge.

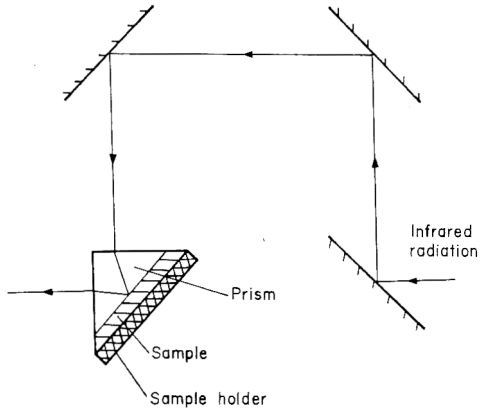

FIG. 1. The optical arrangement of an ATR unit.

The ATR technique can be used successfully with a variety of materials that cannot be investigated easily using transmission measurements. This is particularly true of food materials many of which, because of their physical nature (e.g. high viscosity, colloidal character, high water content), do not give satisfactory transmission spectra. Even where such spectra can be obtained they may be difficult to interpret because of the chemical complexity of the sample. Sample preparation and cell handling may also be difficult. The following work was undertaken to assess more precisely the contribution that the ATR technique can make to the analysis of food materials.

Materials and methods

Spectra were obtained using a Unicam SP200 infrared spectrophotometer and an ATR unit TR3 incorporating a KRS5 (thallium bromide iodide) prism (Research and Industrial Instruments Ltd – R.I.I.C.). This unit was mounted directly in the sample beam of the SP200 without further modification, an R.I.I.C. attenuator AT-02 being placed in the reference beam. The light path through the ATR unit is indicated in Fig. 1.

The TR3 unit, shown dismantled in Fig. 2, contains a sample holder (a) which can accommodate both solid and liquid samples, solid samples being pressed against the prism (b) face and held in position by the backing plate (c) and the sample clamp (d). Liquid samples were introduced through the Luer ports of the sample holder after sealing the holder with the backing plate (e). The ATR unit could be set to any angle of incidence between 25° and 65° by adjusting the position of the mirrors on the stand (f).

In order to obtain the best possible ATR spectrum certain conditions must be fulfilled. The intensity of an ATR spectrum varies according to the angle of incidence

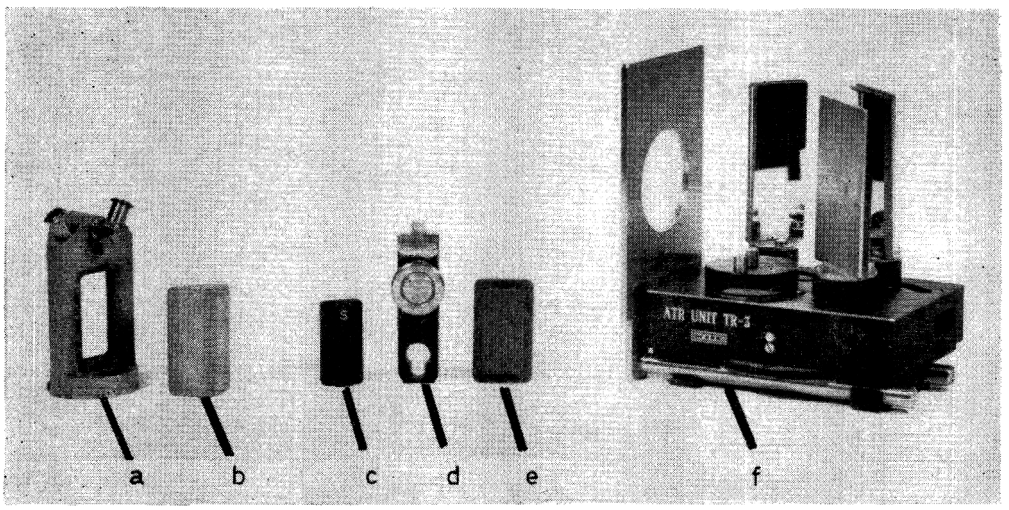

FIG. 2. An ATR unit TR3 dismantled to show: (a) sample holder, (b) prism, (c) backing plate for solid samples, (d) sample clamp, (e) backing plate for liquid samples, and (f) stand for sample holder with tracking mirrors.

used, because reflection attenuation is greatest near the critical angle of the sample. The optimum angle of incidence was, therefore, found empirically for each sample. Reflectance spectra tend to be weak because the intensity of the radiation incident on the sample is reduced by scattering. In order to obtain ATR spectra of the highest possible intensity, the energy control of the SP200 was set at the optimum value; usually as high as possible compatible with the maintenance of stable pen response. Also the reference beam was attenuated so as to give high reflectance.

The most important requirement for obtaining satisfactory ATR spectra is that there should be good contact between the prism face and the sample. With liquid samples this presents no difficulty but with powders it was found best to grind the sample and make a mull with methylene iodide saturated with sulphur as described by Ingram (1964). The mull was then applied directly to the prism face, and although the methylene iodide gave strong absorption bonds at 715 and 1100 cm^{-1}, the spectra obtained were much improved compared with those of dry powders. With bulk solids (e.g. cheese) it was only necessary to cut the sample so that it filled the sample holder and apply pressure sufficient to obtain good contact with the prism face.

Viscous samples were treated in a similar way to aqueous solutions. The sample was introduced into the sample holder via the Luer ports using a hypodermic syringe, and this technique presented no difficulty.

Dilute aqueous colloidal samples (e.g. milk) gave very poor spectra and such samples were first subjected to membrane filtration as suggested by Hannah & Dwyer (1964). 'Oxoid' membrane filters, having a pore diameter of 0·5–1·0 μ in the

upper layers of the filter, were used. Sufficient sample was used to ensure that after filtration the membrane surface was completely covered with a film of the residue. The membrane was dried in an oven at 50°C and a portion of membrane was cut to fit the sample holder of the ATR unit. The membrane was then treated in the same way as a solid sample.

The KRS5 prism, though reasonably hard and only slightly soluble in aqueous media, was handled with care and polished lightly at frequent intervals. The surface of the prism became covered with a whitish 'bloom' after prolonged contact with aqueous samples, and this was removed by polishing the prism on a polishing lap after adding a drop of isopropanol to the prism.

Results and discussion

ATR frequencies may be assigned to structural features of a molecule by correlation with transmission measurements. However, this must be done with caution, partly because ATR frequencies vary very slightly with the angle of incidence used and partly because the correspondence between ATR and transmission frequencies is not exact. For example, the peak wavenumbers of the ATR spectrum of butter at 40° are shown in Table 1 (cf. Fig. 3b), which also shows the corresponding wavenumbers for the transmission spectrum found by Goulden (1956).

TABLE 1. A comparison of peak wavenumbers in transmission and ATR spectra of butter

Transmission value (cm^{-1})	3300	2900	2850	2100	1740	1600	1465	—	1245	1170	1110	965
ATR value (cm^{-1})	3500	2870	2800	—	1735	1640	1460	1380	1230	1150	1100	960

It is, therefore, of advantage to build up a library of ATR spectra, so that ATR frequencies may be related to structural features by empirical comparison with other ATR spectra. In the present work a series of some 200 spectra have been obtained.

Water

The ATR spectrum of water is shown in Fig. 3(a) in which the intense band around 3400 cm^{-1} due to OH stretching and the strong band at 1640 cm^{-1} due to HOH deformation, are apparent. As most food materials contain an appreciable proportion of water, it is important that these strong bands, due to water, do not obliterate the spectral features due to other components. The intensity of the 3400 cm^{-1} band does swamp any other spectral features that are present in this region, but this is not a great disadvantage in ATR work because resolution in this region is poor in

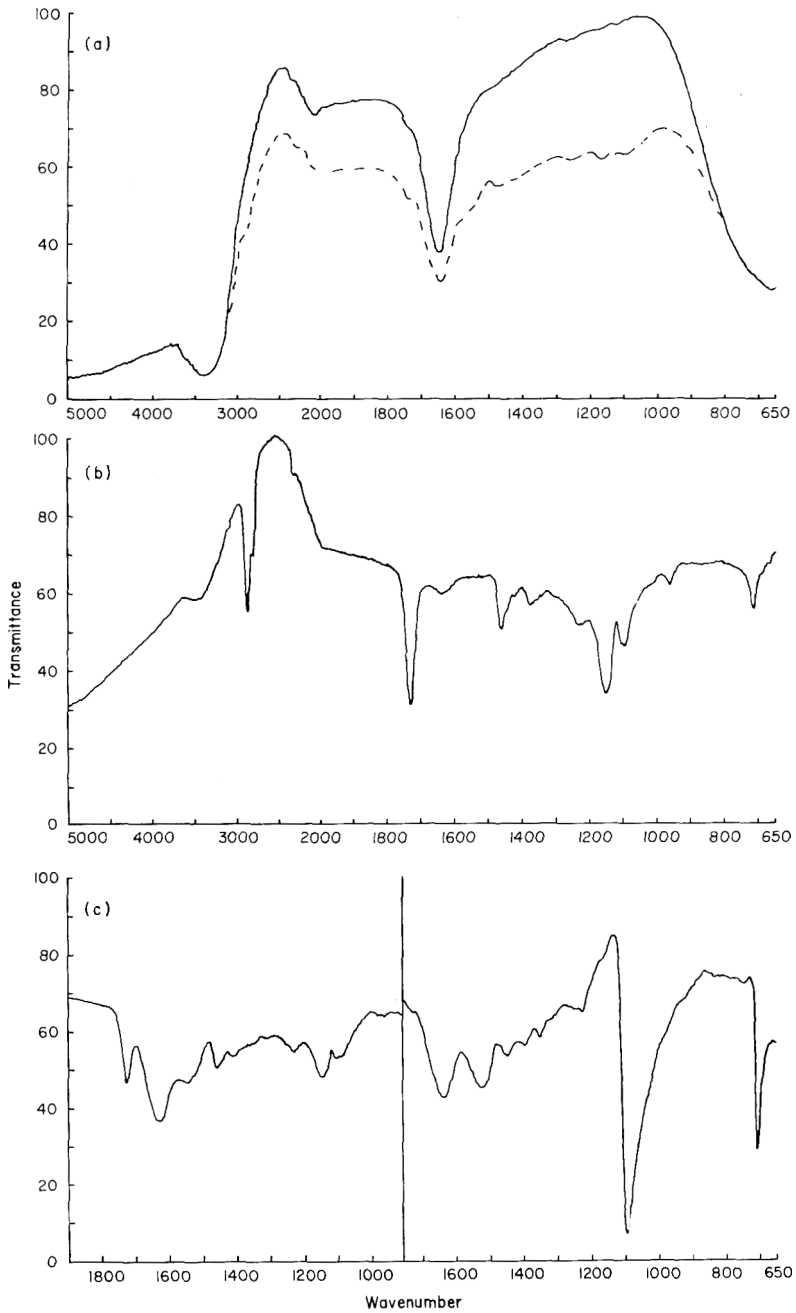

FIG. 3. ATR spectra of: (a) water (—) and milk (- - -) at 40°C, (b) butter at 40°C, and (c) cheddar cheese (left) and casein as a methylene iodide mull (right) both at 45°C.

any case. Useful ATR spectra can be obtained throughout the remainder of the 650–5000 cm^{-1} range in the presence of water.

Infrared transmission spectra of water due to Goulden (1959) show intense absorption bands around 3300 and 1640 cm^{-1}, and a strong band around 2000 cm^{-1}, and even if very thin films are used the spectrum is opaque except for the regions 3000–1700 cm^{-1} and to a small extent 1500–950 cm^{-1}. In order to obtain a transmission spectrum using samples containing water, the path length must be kept small, 75 μ being the maximum possible. No such restriction operates with ATR, however, because the spectrum results from reflection at the surface of the sample.

The fact that transmission measurements on aqueous samples involve the use of thin films means that filling, emptying and cleaning of cells is difficult for viscous materials (e.g. salad cream and condensed milk). ATR cells, on the other hand, can be of any desired volume so that these difficulties do not arise.

Oils and fats

A comparison of four oils and fats—margarine, butter, lard and olive oil—was made, and Fig. 3(b) shows the ATR spectrum of butter. The common features due to triglyceride are as follows. Methylene stretching vibrations appear at 2870 cm^{-1} and 2800 cm^{-1}, though the resolution of the latter is often not good and with the energy control set at high values only a shoulder appears. The main peak at 1735 cm^{-1} is due to the carbonyl group whilst the characteristic triple peak at 1230, 1150 and 1100 cm^{-1} is given by C–O stretching of triglycerides. A CH_2 bending vibration appears at 1460 cm^{-1} and a CH_2 rocking vibration due to long chain hydrocarbons at 720 cm^{-1}.

Apart from the spectral features mentioned above which are common to all four fats, there are other features which enable them to be distinguished from each other. Thus a peak at 960 cm^{-1} is caused by *trans* unsaturation and this peak is strong in margarine, weak in butter, very weak in lard and absent in olive oil. The presence of water in butter and margarine is shown by the presence of a band at 1640 cm^{-1} and an ill-defined band at 3500 cm^{-1}. These bands are absent in lard and olive oil. A small double peak around 1100 cm^{-1} is present in margarine, lard and olive oil, but is not resolved in butter.

Proteins

Proteins as a class show two prominent bands in transmission measurements. The amide I band around 1660 or 1630 cm^{-1} corresponds to carbonyl stretching and the amide II band around 1550 or 1530 cm^{-1} corresponds to NH bending and CN stretching. In addition Beer *et al.* (1959) have shown that there is a somewhat less strong band at 3290 cm^{-1} corresponding to NH stretching. Individual proteins have additional characteristic absorption peaks. The amide I and amide II peaks can be used to identify protein using ATR and Fig. 3(c) shows the ATR spectrum of casein in the

form of a methylene iodide mull. The amide I band appears at 1640 cm^{-1} and the amide II band at 1520 cm^{-1}. There is also a broad ill-defined shoulder around 3400 cm^{-1} (not shown). Additional small peaks occur at 1450 and 1400 cm^{-1}, the other peaks being due to methylene iodide.

Sugars

Crystalline sugars may be investigated by using either finely ground crystals or a methylene iodide mull, but the latter gives much improved resolution. In solution the fine structure of crystalline sugars is lost, and the spectra appear in the form of a limited number of bands as shown in Fig. 4(a). This loss of resolution is similar to that found in transmission spectra by Goulden (1959).

A comparison of the ATR spectra of solutions of fructose, glucose and sucrose (Fig. 4a) shows that although the spectra can be distinguished from each other, they all show strong bands in the 900–1200 cm^{-1} region. The differences in this region make it possible to identify individual sugars in foods and also components in sugar mixtures. Thus the presence of sucrose in condensed sweetened full cream milk (Fig. 4b) is shown by the triple peak at 1050, 990 and 925 cm^{-1}.

Multi-component foods

The ATR technique not only shows well defined spectra for single food components but—more important—it gives equally clear spectra for foods containing several major components. The ATR spectrum for cheddar cheese containing approximately 20% protein, 35% fat and 40% water is shown in Fig. 3(c). The triple peak at 1240, 1150 and 1100 cm^{-1} and the peak at 1730 cm^{-1} are due to fat, the broad shoulder at 1550 cm^{-1} and the small peak around 1410 cm^{-1} are due to protein, a strong peak at 3300 cm^{-1} (not shown) is due to water. Both fat and protein contribute to the band around 1450 cm^{-1}, and both water and protein contribute to that around 1640 cm^{-1}. Thus the main components are easily identified.

A study of milk and milk products using ATR enables a good assessment of the technique to be made. Liquid milk gives a poor ATR spectrum (Fig. 3a) because components, other than water, are present in such small amounts that peaks are poorly defined. Neverthless fat at 1730 cm^{-1} and the triple peak around 1200 cm^{-1}, and protein as a shoulder at 1520 cm^{-1}, can just be detected. On the other hand carbohydrate (lactose) cannot be detected. These results are similar to those obtained by Goulden (1956) for transmission measurements using a thin film of milk between barium fluoride plates. The ATR spectrum of milk emphasizes a main limitation of the technique; namely that constituents present in small amounts cannot be detected. Also the limit of detection varies for different constituents.

Samples, such as liquid milk, which are colloidal in nature and which gave a poor ATR spectrum because of the large proportion of water present, can be treated effectively by membrane filtration. In the absence of water protein shows up clearly at

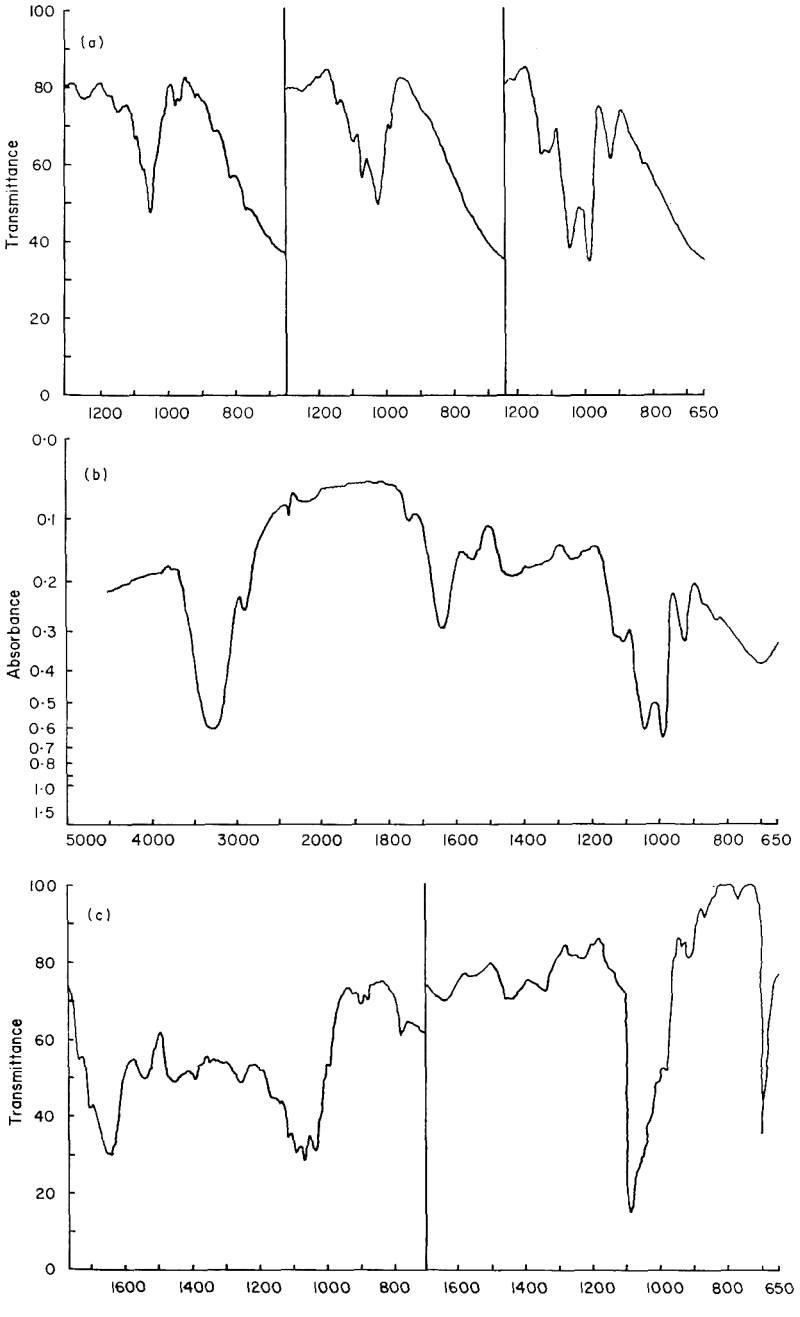

FIG. 4. (a) ATR spectra of 50% solutions of fructose (left), glucose (centre) and sucrose (right) at 40°C; (b) ATR spectrum of condensed sweetened full cream milk at 40°C; and (c) IR transmission spectrum (KBr disc) of full cream dried milk (left) and corresponding ATR (right) spectrum at 40°C.

1640 and 1540 cm^{-1} and fat at the expected wavenumbers, though soluble components, such as lactose, are missing from the spectrum having been removed during filtration.

The spectrum of milk obtained from a membrane is similar to that of full cream dried milk prepared as a methylene iodide mull (Fig. 4c). Apart from the peaks due to methylene iodide, the main difference between them is a series of peaks due to lactose below 1100 cm^{-1} in the dried milk spectrum. Lactose is identified by a weak triple peak around 900 cm^{-1}. The peaks due to casein and butter fat appear at the expected frequencies, and are of similar intensity, in both spectra. When the ATR spectrum of dried milk is compared with the transmission spectrum produced from a potassium bromide disc (Fig. 4c) the similarities between them are obvious, except that in the case of the mull the region below 1100 cm^{-1} is dominated by the methylene iodide spectrum.

TABLE 2. Comparison of ATR spectra of canned milks

	Wavenumber of peak (cm^{-1})					
	3300	1740	1640	1550	1430	1050, 990, 925
Assignment	Water (+ NH of protein)	Fat	Water + amide I	Amide II	Sucrose	Sucrose
Sweetened skimmed milk	VS	Absent	S	W	W	S
Skimmed milk	VS	Absent	S	Absent	Absent	Absent
Sweetened full cream milk (Fig. 4b)	VS	W	S	W	W	S

W = Weak, S = strong, VS = very strong.

The main features of ATR spectra of canned milks are summarized in Table 2. In the case of the two sweetened milks the sucrose triple peak around 1000 cm^{-1} is so strong that lactose cannot be detected, but in the case of evaporated milk a typical lactose profile is obtained (peaks at 1040 and 1070 cm^{-1}). The results show that these three samples can be distinguished from each other using ATR.

A study of a number of other foods has been made. For example, a study of eggs, has indicated that the ATR technique shows up clearly the differences in composition of egg white and egg yolk. As expected egg powder (in the form of a methylene iodide mull) shows improved resolution, particularly of protein. ATR spectra of cocoa products indicates that the main components are easily distinguished. In drinking chocolate and plain and milk chocolate the fine structure of sucrose shows up clearly, but the differences between the spectra are considerable and enable them to be clearly distinguished from each other and from cocoa.

Conclusions

The ATR technique has certain advantages over transmission infrared measurements for the analysis of many food materials. The ATR technique is simple to carry out and is easily adaptable to samples in different physical forms. Solids and solutions may be used directly, and powders mulled with methylene iodide. Aqueous colloidal materials, which give poor results in transmission measurements, may be examined directly using ATR but are preferably subjected to prior membrane filtration. Difficulties of cell filling, emptying and cleaning encountered in transmission work of aqueous viscous materials required as thin films are eliminated using ATR. A single ATR cell can be used for all types of sample, and the measurement of sample thickness is unnecessary.

ATR spectra show well developed fine structure for crystalline materials but, as with transmission spectra, this disappears when the material is in solution. In general the detail obtained in ATR spectra is less good than in transmission spectra because of the large proportion of radiation that is lost by scattering. This lack of sensitivity means that components present in small amount, in the range 1–5%, are often not detected. This is the main limitation of the ATR technique using a single reflection, but this can be partially overcome by the use of multi-reflection cells which have recently become available commercially.

The main advantage of the ATR technique is that it makes possible the rapid detection of major components in foodstuffs. The presence of water, protein, fat and carbohydrate in the form of sugars as major components is easily established. The detection of differences in composition of a particular component may also be done in many cases. Thus the spectral profiles of sugars are well differentiated and unsaturation in fats can be detected. On the other hand different proteins are poorly differentiated.

It may be concluded that though the ATR technique does not replace the transmission technique, it is a useful adjunct to it. The simplicity of the ATR technique for samples in many physical forms and its ability to give simple well-defined spectra from complex samples makes it well suited to the analysis of foodstuffs.

Acknowledgment

Thanks are due to Mr C. P. Wright for his assistance in recording ATR spectra.

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The determination of the nitrogen contents of various edible hydrogenated fats

A. C. ROBERTS AND D. J. McWEENY

Summary. Conditions are set out for the satisfactory measurement of nitrogen contents of edible, refined, hydrogenated fats at levels of 100 μg N/g fat, by the wet oxidation of the fat with acid–hydrogen peroxide and subsequent Kjeldahl micro-distillation.

The Nessler method has been evaluated and found to be unsatisfactory when used in conjunction with acid–hydrogen peroxide digestion. Examples indicatting the range of nitrogen contents, *ca.* 100–200 μg N/g fat, which can be expected in edible, refined, hydrogenated fats, from different sources are reported.

Introduction

In the course of studies on the stability of hydrogenated fats a number of measurements of the nitrogen contents of various fats from a number of refineries have been performed. Existing information on the nitrogen content of fats is very limited (Thornton & Kraybill, 1942; McGuire, Earle & Dutton, 1947; Govind Rao & Raghavendar Rao, 1963). The present work describes:

(a) The application of a rapid method for the wet oxidation of fats (Whalley, 1963; Taubinger & Wilson, 1965) (originally devised for trace metal determination) to the measurement of the nitrogen content of various types of fats.

(b) Comparison of the two main end-methods which are used to determine nitrogen in the digest: (1) the Nessler method, which is specific for NH_4^+ in the digest, and (2) the Micro-Kjeldahl distillation method, which will measure ammonia and any other steam volatile basic compounds.

The suitability of these two methods of determining the nitrogen content of fats, digested by the chosen wet oxidation method, has been investigated by experiments with model compounds.

Experimental methods and materials

The conventional catalysed wet oxidation of fats by sulphuric acid was found to be

Authors' address: Ministry of Agriculture, Fisheries and Food, Food Standards, Science and Safety Division, Bishopgate, Norwich, Norfolk.

both tedious and time-consuming so the wet oxidation method of Whalley was used to digest fat samples for subsequent measurement of nitrogen content. This method involves treatment of the fat with 50% w/w hydrogen peroxide, after it has been well charred by heating with concentrated sulphuric acid. This treatment enabled the fat samples to be digested in a much shorter time than would have been possible using conventional catalysed methods; for example, samples weighing 2 and 10 g required approximate times of 1½ and 5 hr, respectively, for the digestion to be completed. This compares very favourably with the times required to perform a catalysed digestion, this being approximately 10 hr to completely digest a 2-g sample of fat.

The Nessler method of Middleton (1960) was investigated to determine its usefulness in measuring the nitrogen contents of fats after they had been digested by the acid–hydrogen peroxide system. This method has a lower limit of measurement of 20 μg and should allow the digestion to be carried out on smaller samples of fat. The published method was modified slightly to allow a larger aliquot of the neutralized digest to be taken for colour development and the volumes of the reagents used were:

10 ml of the neutralized digest,
5 ml of 0·6 N sodium tartrate,
5 ml of gum arabic solution (Middleton, 1960),
5 ml of Nessler reagent (Middleton, 1960), and
5 ml of 2·0 N sodium hydroxide.

The mixture was then treated according to the published method, the optical density being measured in a 1-cm cuvette at 430 mμ.

The Kjeldahl micro-distillation method of Ma & Zuazaga (1942) was used to determine the nitrogen content of the model compounds and of the digested fats. It has a lower limit of measurement of 140 μg and thus requires at least a 3-g sample of fat if nitrogen contents of the order of 100 μg/g are to be accurately measured.

Throughout the determination it was essential to use materials of the lowest nitrogen content, so wherever possible either 'nitrogen-free' or A.R. quality materials were used. The use of water distilled from acid solution in an all-glass still appreciably reduced blank values.

Results

Fig. 1 shows the response of the Nessler end-method to ammonia standards (with and without sulphuric acid–hydrogen peroxide digestion) and to a number of model compounds after such digestion. The measurements of optical density were performed in a 1-cm cuvette at 430 mμ using a Unicam SP-500 spectrophotometer.

The Kjeldahl micro-distillation end-method was employed to obtain the results set out in Tables 1, 2 and 3. The nitrogen values recorded have been corrected for the amount of nitrogen in the blank determination. The blank value was reproducible and was about 100 μg N as compared to the approximate 450 μg N from a 3-g sample of fat.

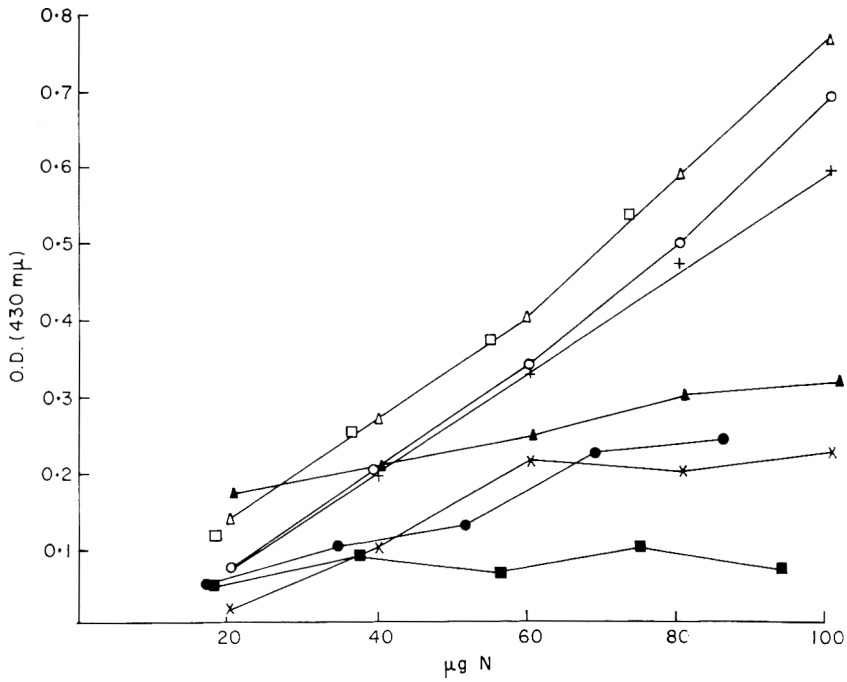

FIG. 1. The response of the Nessler reagent to various solutions of ammonium salts and digests of organic nitrogenous compounds. +, (NH_4^+) standard; ○, (NH_4^+) standard in 1·8 N Na_2SO_4; △, (NH_4^+) standard digested by acid–peroxide; ×, choline standard; □, hexa-decylamine standard; ■, cetyl tri-methyl ammonium bromide standard; ● and ▲, lecithin standards.

Table 1 shows the results obtained from four separate determinations of the nitrogen content of six different fats. In this experiment a sulphuric acid–fat ratio of 9 : 10 w/w was used in the wet oxidation.

TABLE 1. The measurement of nitrogen contents by the micro-Kjeldahl method after wet oxidation using an acid–fat ratio of 9:10 w/w

Type of hydrogenated fat	μg N/g fat			
	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Coconut	29·1	108	108	—
Soyabean	45·2	192	—	137
Whale	45·7	199	158	120
Palm kernel	38·1	131	119	—
Fish	47·5	199	178	128
Herring	47·0	201	164	—

TABLE 2. The measurement of nitrogen contents by micro-Kjeldahl after wet oxidation, using an acid/fat ratio of 9:3 w/w

Experiment	Type of hydrogenated fat	μg N/g fat	Experiment average	Deviation from grand average (%)
1	Herring	192·5 180·7	186·6	− 2·5
	Palm kernel	129·6 137·4	133·5	− 1·5
2	Herring	195·0 189·5	192·2	− 0·4
	Palm kernel	126·3 130·3	128·3	− 5·4
3	Herring	201·0 195·5	198·2	+ 3·5
	Palm kernel	148·0 147·1	147·5	+ 8·8
4	Herring	188·0 187·5	187·7	− 1·9
	Palm kernel	131·8 134·9	133·3	− 1·7

Grand average: Herring 191·4, palm kernel 135·6.

Table 2 shows the results obtained from four separate determinations, done in duplicate, of the nitrogen contents of two fats having widely differing digestion characteristics. The sulphuric acid–fat ratio used in their wet oxidation was 9 : 3 w/w.

TABLE 3. The levels of nitrogen content found in refined, hydrogenated edible fats derived from different sources

Source of fat	No. of samples	μg N/g fat	
		Average value	Range
Whale	16	161·2	101–194
Fish	5	166·0	153–185
Herring	8	181·8	153–207
Soyabean	6	183·7	155–216
Ground-nut	8	158·2	135–170
Cotton-seed	2	196·0	177 and 215
Coconut	8	128·8	99–143
Palm	12	162·4	141–212
Palm kernel	12	131·3	117–147

Table 3 summarizes the values found for the nitrogen contents of seventy-seven different refined, hydrogenated, edible fats from nine animal, marine or vegetable sources, processed in nine different refineries. These results were obtained using an acid–fat ratio of 13 : 3 w/w.

Discussion

The Nessler method

The Nessler method of Middleton (1960) has a lower limit of measurement of 20 μg N as compared to 140 μg N for the micro-Kjeldahl distillation. In an attempt to keep sample sizes small and digestion times correspondingly short, the Nessler method was investigated to determine whether it was suitable for use as an end-method in conjunction with the acid–hydrogen peroxide digestion. This method depends upon the formation of a negatively charged colloidal solution of the complex, which is stabilized by the addition of a protective lyophilic colloid. It has been shown (Middleton, 1960) that the Nessler reaction is [OH^-] sensitive and is best carried out at concentrations of 0·33 N with respect to [OH^-]. This requires dilution and neutralization of the digestion mixture and a subsequent quantitative transfer of an aliquot to the colour developing reagents.

The response of the Nessler method towards solutions of increasing nitrogen concentration is shown in Fig. 1. These solutions were:

(a) NH_4^+ standards,
(b) NH_4^+ standards in 1·8 N Na_2SO_4,
(c) NH_4^+ standards digested by acid H_2O_2,
(d) choline standard,
(e) hexadecylamine standards,
(f) cetyl tri-methyl ammonium bromide standard, and
(g) lecithin standard.

The precise nitrogen contents of solutions (d–g) were found by the micro-Kjeldahl technique. The solutions (a–c) were prepared quantitatively from dried A.R. ammonium sulphate. Solutions (c–g) were digested by the acid–hydrogen peroxide technique, diluted and neutralized before Nessler colour development.

The curves presented in Fig. 1 show great deviations from the standard ammonia curves (a). It will be seen that the presence of sodium sulphate, at the concentration which could be expected from a neutralized digestion mixture, causes a difference in the response of the method. This is attributable to the effect of the sodium ion on the colloid. The reason for the difference between the curves for the ammonia standard with sodium sulphate and the digested ammonia standard is not clear, since both contain the same concentration of sodium sulphate, one added and the other from the neutralization of the digesting acid with sodium hydroxide.

Curve (e) shows a very good agreement with curve (c), the digested ammonia curve, and this agreement indicates that the long-chain primary amine, hexadecylamine, is being quantitatively digested to ammonia by the acid–peroxide method.

The curves arising from organic compounds (d), (f) and (g) demonstrate that digestion with acid–peroxide does not usefully modify the well-known resistance of quaternary amine salts towards oxidation in mineral acids. This precludes the measurement of nitrogen from tertiary amines after digestion with acid–peroxide since tertiary amines form amine oxides whose hydrated forms have salt-like structures which are not detected by the Nessler reagent.

Attention was directed at achieving complete oxidation to ammonia, but the only really effective catalysts (such as mercury compounds or selenium) either interfere with the Nessler colour development (Middleton, 1960) or tend to catalyse the decomposition of the hydrogen peroxide, thus reducing the efficiency of the digestion.

It was because of the difficulties associated with combining the Nessler end-method with the acid–hydrogen peroxide digestion that attention was turned to the micro-Kjeldahl method. This is a perfectly straightforward, standard method (Ma & Zuazaga, 1942) and the only difficulty is the upper limit which it imposes on the volume of the digestion mixture when it has been neutralized.

Wet oxidation method

Because of the large amount of fat which had to be digested and the considerations involved in using a micro-distillation end-method it was necessary to determine the smallest ratio of sulphuric acid–fat which would ensure the complete digestion of the fat, without allowing the escape of volatile nitrogenous compounds. The ratios tried were 9 : 10 w/w and 9 : 3 w/w, this finally being increased to 13 : 3 w/w in order to digest a sample of hardened coconut oil which was highly reactive towards hydrogen peroxide. The sample was somewhat more saturated than the other fat samples and this higher reactivity in a more saturated fat agrees with the work of Taubinger & Wilson (1965) who report liquid paraffin to be very reactive.

The Kjeldahl micro-distillation method was used as an end-method in these comparisons after the effectiveness of the sulphuric acid–hydrogen peroxide digestion method on a model compound had been established by comparing the digestion of lecithin by the acid–peroxide method with the digestion by conventional catalysts. The results obtained showed a very good agreement between the two digestion methods, so the acid–peroxide treatment was deemed to be effective when used in conjunction with a Kjeldahl finish.

It can be observed from Table 1 that digestion with an acid–fat ratio of 9 : 10 w/w does not give reproducible results and as a consequence a higher ratio was investigated.

An inspection of Table 2 shows a good agreement between four separate experiments, each containing two different fats having dissimilar digestion characteristics. The

deviation of the palm kernel samples in run 3 is quite big, but the normal run of deviations can be seen to be quite acceptable. This occasional departure from reasonable duplication has been observed infrequently during the course of the work, but the practice of obtaining a second measurement of the nitrogen content of a fat during another experimental run has largely ensured the rejection of any rogue result emanating from this particular source.

As has been previously stated, the acid–fat ratio during digestion was finally raised to 13 : 3 w/w in order to deal satisfactorily with one highly reactive coconut fat. This ratio, although small in comparison to acid–sample ratios normally encountered in Kjeldahl digestions, or in the original Taubinger & Wilson method, is sufficient to give a quantitative digestion. Any attempt to raise it leads to difficulties associated with the subsequent distillation, i.e. the volumes become larger than the normal micro-Kjeldahl still can handle satisfactorily.

The blank values obtained, as stated previously, are of the order of 100 μg N as compared to 450 μg N for a 3-g sample of fat. This 100 μg N blank value is repeatable to within ± 5 μg so the fact that the blank value is relatively large when compared with the sample value does not introduce any serious error in the determination.

Survey of nitrogen content of fats

In the course of the work the nitrogen contents of a total of seventy-seven different refined hydrogenated edible fats from nine different sources and nine different processors have been determined. In Table 3 is set out a summary of average values and the range of values found. These values are of the same order as the two published values (Govind Rao & Raghavendar Rao, 1963) for refined cotton-seed and linseed oils.

On inspection, Table 3 shows a variation in the nitrogen contents of fats from different sources. Whilst refinery procedure may affect the final nitrogen content, it is evident that the coconut and palm kernel fats tend to have lower nitrogen contents after refining and hydrogenation than do the fats derived from marine sources or from soybean.

Conclusion

The wet oxidation of hydrogenated fats with sulphuric acid–hydrogen peroxide, can be applied, under the appropriate conditions, to the preparation of these fats for total nitrogen determination.

The use of the Nessler end-method is unsatisfactory, because of the incomplete digestion to ammonia of polar and tertiary amine nitrogen-containing compounds. The specificity of the Nessler method for ammonia shows itself in its response to the digested solutions of polar nitrogenous compounds, where under the digestion conditions only a fraction of the nitrogen present is in the form of ammonia, the rest exists as stable quaternary ammonium salts.

The combination of acid–peroxide digestion and Kjeldahl micro-distillation has shown itself suitable for the measurement of nitrogen contents of the order of 100 ppm in hydrogenated fats. The results indicate the nitrogen contents which can be expected in edible, hydrogenated fats.

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β-Glucosidase activity in canned plums

D. R. HAISMAN AND D. J. KNIGHT

Summary. The reasons for enzyme survival in a heat processed pack have been investigated with reference to the β-glucosidase system in the kernels of plums. This enzyme breaks down the diglucoside amygdalin to glucose, benzaldehyde and hydrocyanic acid. The variation of enzyme activity with the pH of the system and its inactivation by heat have been examined. At 60°C, in aqueous solution, the enzyme was 90% inactivated in 32 min at pH 5·0, and in 0·6 min at pH 3·0. In the presence of its substrate, amygdalin, and in the solid phase the enzyme was much more heat stable. Because the enzyme system in the kernels is at its optimum pH, in contact with its substrate, and in the solid phase, it is not wholly inactivated by the normal heat process given to canned plums. The variation in residual enzyme activity in the stone according to the heat treatment given has been demonstrated, and the subsequent breakdown of amygdalin to benzaldehyde and cyanide measured.

Introduction

The corrosivity of plums from certain orchards in this country, after canning, has been shown to be dependent on the heat process used; it appears that short heat treatments, such as holding the hot-filled can for 6 min in boiling water, do not completely inactivate the enzymes in the plum kernel, and it has been suggested that an enzyme system in the kernels continues to produce some corrosion accelerator during storage (Dickinson, 1957). The 'emulsin' (β-glucosidase) enzyme system is particularly active in the kernels of stone fruit, where it breaks down amygdalin [mandelonitrile β-(D)-gentiobioside] to glucose, benzaldehyde and hydrocyanic acid. The presence of hydrocyanic acid could possibly affect the corrosive properties of the pack.

The 'emulsin' system is a mixture of at least three enzymes, amygdalin hydrolase, prunasin hydrolase and hydroxynitrile lyase (Haisman & Knight, 1967), each of which displays some activity towards alien substrates such as salicin (*o*-hydroxybenzyl-β-D-glucoside) and *p*-nitrophenyl-β-D-glucoside. For convenience, and following other investigators, the hydrolysis of salicin has been used for most of the activity

Author's address: The Fruit and Vegetable Preservation Research Association, Chipping Campden, Gloucestershire.

measurements in this investigation. It is probable that this technique adequately reflects the properties of the various enzymes involved.

Materials and methods

A salt-free lyophilized preparation of β-glucosidase from almonds was obtained from Koch-Light Laboratories Ltd. Plum kernel enzymes were obtained in aqueous solution by homogenisation and centrifugation of the chopped kernels. Plums (var. Victoria) were obtained locally.

Citrate and acetate buffers (0·2 M) were prepared using analytical reagent grade chemicals.

Enzyme activity measurements were performed in a shaking incubator tank in glass-stoppered conical flasks. Activities towards amygdalin, prunasin and mandelonitrile were estimated by determination of the cyanide, mandelonitrile and glucose produced during a fixed reaction time (Haisman & Knight, 1967). The activity towards salicin was estimated by the method of Baruah & Swain (1957).

Results

The effect of pH and temperature on the enzyme activity

The activity of four of the enzymes in a β-glucosidase preparation from almonds over the pH range 3·0–6·0 is shown in Fig. 1. The activity is maximal between pH 5·0 and 6·0. The effect of temperature on the activity of almond β-glucosidase and

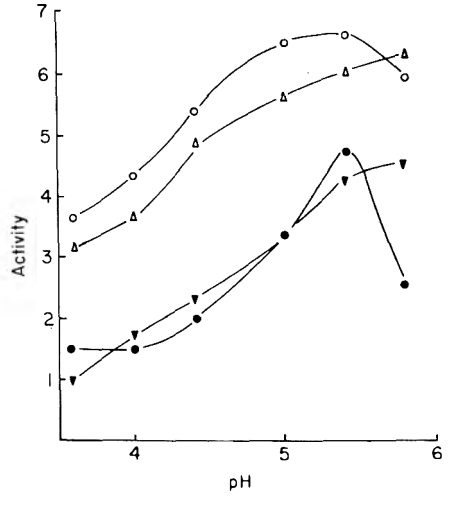

FIG. 1. The variation of β-glucosidase activity with pH. Substrates: salicin (○), amygdalin (△), prunasin (▼) and mandelonitrile (●).

the plum kernel enzyme is shown in Table 1. The activity reaches a maximum at about 50°C, and then falls sharply.

TABLE 1. The effect of temperature on the activity of β-glucosidase towards salicin

Temperature (°C)	% activity at 30°C	
	Almond β-glucosidase	Plum kernel extract
30	100	100
40	122	111
50	141	163
60	29	112

The inactivation of the enzymes by heat

The activities of solutions of β-glucosidase and plum kernel extracts towards salicin were measured before and after heating for various times at 60°C in the presence of buffers. Plots of the logarithm of the activity (expressed as a percentage of the activity before heating) against time of heating were linear, indicating that the inactivation was a first order reaction. The pH of the buffer solution had a marked effect on the rate of inactivation (Fig. 2). Solutions of the enzyme were very unstable at pH 3·0, and showed maximum heat resistance at pH 5·0. The rate of inactivation of the enzyme from plum kernels at pH 5·0 ($3{\cdot}5 \times 10^{-2}$/min) was in good agreement

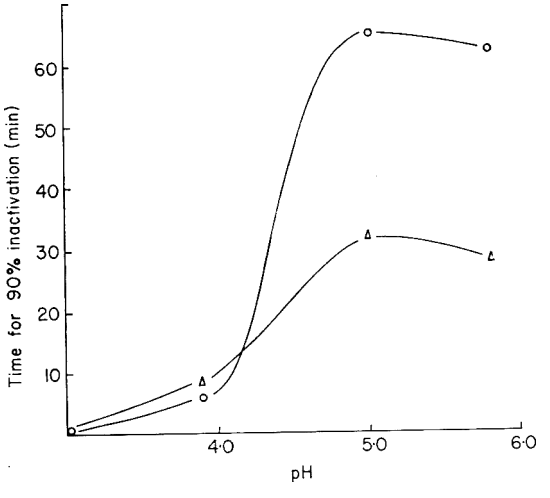

FIG. 2. The effect of pH on the rate of inactivation of β-glucosidase at 60°C. Enzymes from plum kernels (○) and almonds (△).

with the value of 3×10^{-2}/min obtained by Tammann (1895) with almond emulsin.

The temperature coefficients of the heat inactivation rates for solutions of β-glucosidase were determined at pH 5·0 and 3·0 (Fig. 3). Temperature changes neces-

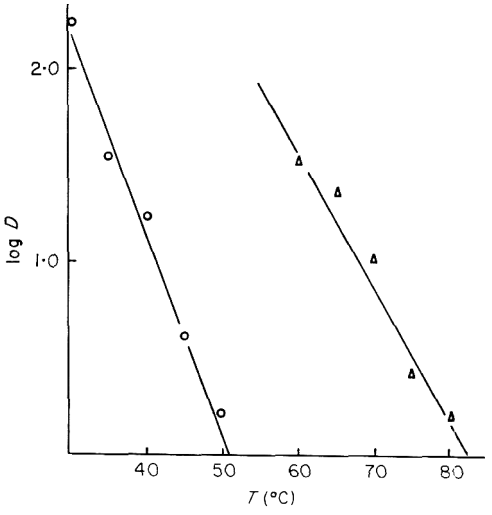

FIG. 3. The inactivation of β-glucosidase at different temperatures. pH 3·0 (○) and pH 5·0 (△).

sary to cause a ten-fold increase or decrease in the rate inactivation were 14·5°C at pH 5·0 and 10°C at pH 3·0.

It is well known that enzymes and many other proteins can be protected from heat inactivation by adsorption in a solid phase or by combination with other substances in colloidal solution. Tonita & Kim (1965) showed that the heat resistance of Taka-amylase A was increased in the presence of starch or its hydrolysis products. The protective action of amygdalin in the heat inactivation of β-glucosidase is shown in Table 2. The time to reduce the enzyme activity by 90% is doubled when 0·2% or more amygdalin is added to the enzyme solution.

TABLE 2. The effect of amygdalin on the heat inactivation of β-glucosidase

Concentration of amygdalin (%)	Time for 90% inactivation at 70°C (D_{70}) (min)
0	11·1
0·05	11·7
0·20	22·8
0·50	27·5
1·00	27·7
2·00	26·4

Enzymes are markedly more stable in the solid phase, particularly at low moisture levels. Thus β-glucosidase is 300 times more stable in the dry kernel than in solution at 80°C (Table 3). Raising the moisture content to 25% reduces the protective action to only fifty-fold. A similar protective effect is observed at 100°C.

TABLE 3. The heat inactivation of β-glucosidase in plum kernels

Material	Moisture content (%)	Treatment temperature (°C)	Time for 90% inactivation (min)
β-Glucosidase in buffer, pH 5·0	—	80	1·6
Plum kernels (powdered)	0	80	318·0
Plum kernels (powdered)	25	80	53·4
β-Glucosidase in buffer, pH 5·0	—	100	0·06
Plum kernels (quartered)	0	100	15·3

The inactivation of the enzymes in the kernels of canned plums during processing

The approximate temperature of the kernels of the plums during processing could be obtained from heat penetration measurements using thermocouples sealed into the plum stones. The time taken to inactivate 99% of the enzyme at each temperature could be estimated from the data obtained by heating solutions of β-glucosidase at pH 5·0 at various temperatures (Fig. 3). The reciprocal of the time for 99% inactivation was taken as the lethal rate and, using the product of the lethal rate and the time of process (the lethality), the inactivation of the enzyme during the heat treatment could be estimated (Fig. 4).

This calculation made no allowance for the protective action of the amygdalin in the kernels, nor for the fact that the enzyme exists in a solid phase at a moisture level of about 60%, and thus probably overestimates the lethality of the process. Nevertheless, it is obvious that even on this optimistic estimate, at least 9 min at 100°C is required to totally inactivate the enzyme.

The effect of some processes, designed to ensure adequate heat sterilization of the canned plums, on the activity of the enzymes in the plum kernels is shown in Table 4. In both short processes there is still significant enzymic activity after processing.

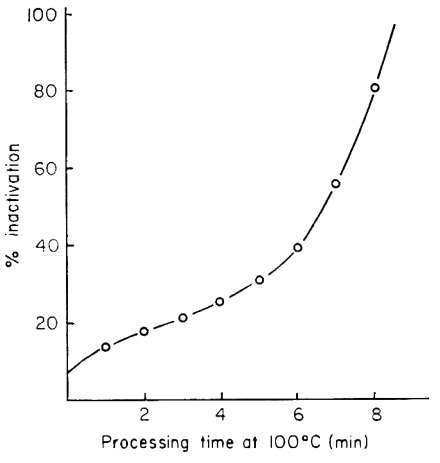

FIG. 4. The heat inactivation of β-glucosidase in plum kernels during processing.

TABLE 4. Enzyme activity in canned plum kernels after processing

Process	Enzyme activity (enzyme units/mg dry kernel)
None	580
Exhausted 6 min at 85°C, flame heated for 50 sec	24
Exhausted 6 min at 85°C, flame heated for 50 sec, held hot for 2 min	62
Exhausted 6 min at 85°C, heated for 12 min at 100°C	0

The effect of enzyme activity on distribution of cyanide in canned plums

The amounts of free cyanide and amygdalin in the kernels and in the syrup of canned plums after heat treatments varying from 6 to 12 min at 100°C and then storage at room temperature are shown in Fig. 5. During storage the syrup penetrates some of the plum stones, so that a certain amount of amygdalin diffuses from the kernels into the syrup. Where the kernel contains active enzymes, most of the amygdalin is broken down during storage to form glucose, benzaldehyde and hydrocyanic acid. Benzaldehyde and hydrocyanic acid are volatile; they diffuse through the stone and into the syrup. It can be seen that the amount of amygdalin decomposed is proportional to the length of the process and, likewise, plums given the shortest processes

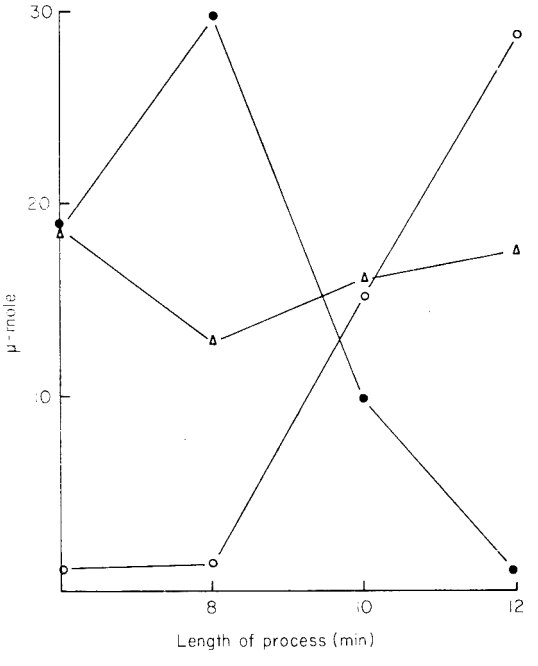

FIG. 5. The effect of processing time on the distribution of cyanide and amygdalin in canned plums after storage for 12 months. Amygdalin in kernels (○), amygdalin in syrup (△) and cyanide in syrup (●).

contain the higher amounts of cyanide. Some cyanide is lost during storage, conceivably by reaction with corrosion products.

Discussion

The β-glucosidase system in plum kernels provides an interesting example of the survival of enzyme activity after treatments which, superficially, would be thought adequate for the complete inactivation of most enzymic activity.

β-Glucosidase is not a particularly heat-resistant enzyme. The time for 90% inactivation of the enzyme at pH 5·0 and 80°C is 1·6 min, compared with estimated values of 0·02 min for catalase, 0·09 min for lipoxygenase and 0·82 min for *o*-diphenol oxidase. Peroxidase, a notably heat-resistant enzyme requires times of 30–230 min for inactivation under similar conditions.

The main reasons for the heat-stability of the β-glucosidase in plums arise from the situation of the enzyme in the kernel, which provides an unusually favourable environment. The kernel, which lies in an air pocket inside the stone of the plum is, to some extent, thermally insulated and is isolated from the acid constituents of the plum flesh. The kernel is a relatively dry medium (50–60% water), and contains

appreciable amounts of the enzyme substrate amygdalin, both factors helping to stabilize the enzyme.

Of equal importance, the pH of the kernel is between 6·0 and 6·5 where the enzyme has the greatest stability. The pH of the plum flesh, which has a high content of malic acid, is about 3·0. β-Glucosidase is rapidly inactivated at this pH, even at low temperatures (in about 6 hr at 30°C). However, only very few of the plum stones are penetrated by the syrup during processing.

Thus, under normal commercial conditions, it is to be expected that when canned plums are given a minimum process, the kernels will contain active β-glucosidase after processing. As hydrocyanic acid and benzaldehyde, two of the products of the enzymic reaction, are volatile, and can diffuse through the stone and into the syrup, the composition of the pack changes during storage. It is found that when plums are given a process of 6 min at 100°C, most of the amygdalin in the kernels is decomposed during storage. The cyanide content of the syrup rises to about 2 ppm and then declines. At this level it does not present a health hazard. Its significance in corrosion phenomena has yet to be assessed.

Acknowledgment

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Technical Note

Salt as a preservative for food

T. McLACHLAN

In their excellent review of the scientific literature on the use of salt as a preservative for food, Ingram & Kitchell (1967) make one or two comments, which, in the interests of history, require clarification.

In the opening paragraph they state 'It will be appreciated, upon reflection, that salt is not applied to foods which do not putrefy, like fruits and cereals'. One would have thought that the use of salt brines in the preparation of fruit and vegetables for pickling was sufficiently well known, but salt is also used for the preservation of fruits, especially when those fruits are employed to tickle the throat and stimulate drinking as with olives and gherkins. During the last war housewives were encouraged officially to preserve cut string beans with salt and salted beans are still sold on the London market. Salt is employed as a preservative for peeled sliced apples for catering purposes, though in these cases, as with vegetables for pickling, the salt is removed as far as possible before cooking or processing. Only recently I have myself examined salted small lemons used as a pickle and there may be other fruits and vegetables which are preserved with salt.

In case it may be suggested that fruits and vegetables do not putrefy it must be remembered that the word 'ret' is the same as 'rot' and that a retting process is commonly employed for the production of white pepper from black pepper or of fibres such as flax, hemp, jute and ramie, and the difficulties attending the addition of pepper and string to meat products are too well known to require elaboration. On the other hand, while salt is often employed as the sole preservative for fruits and vegetables it is seldom employed, as such, for fish or meat, because as Ingram & Kitchell point out even 10% of salt may not stop bacterial growth in these foods. For this reason Germany is pressing the Common market to approve the addition of preservatives to semi-preserves. When reviewing the history of food technology (McLachlan, 1961), I commented on the fact that from earliest times salt had been employed in conjunction with drying, smoking or the development of acidity. Until comparatively recent times the salted meat given to troops and to sailors was putrefying, but large quantities of salt would not be tolerated and could not be added because of the difficulties encountered with water supplies. Hams, however, were better prepared by the dry salting process although the need for care in the production of bacon has been recogni-

Author's address: 4 Hanway Place, London, W.1.

zed over many years and A Lady (1726) recommends that the meat 'be laid on cold bricks for a few hours, and when you salt it, lay it up on an inclining board, to drain off the blood, then salt it afresh', and again 'If you find the pickle begins to spoil, boil it again, and skim it; when it is cold, pour it on your pork again.'

At one time the brine used in the tank curing of bacon contained an antiseptic, as described by Nicholls (1924), who writes: 'The pickle, as the brine is called, is composed of a mixture of salt, saltpetre, antiseptic, brown sugar and water, which is boiled and then allowed to cool, impurities being strained off'. He then mentions that borax must not be employed in the curing brine, but only for preserving it in transit, especially when coming green in boxes from America. The first edition of *Law's Grocer's Manual* (about 1895) recommends that bacon should be stored in dry wood ashes. The use of borax or boric acid was prohibited with the introduction of the Public Health (Preservatives, etc. in Food) Regulations, 1925, but was re-introduced as a war measure by Regulation 60 CAA of the Defence (General) Regulations, 1939 for imported bacon.

Modern bacon production requires a mild cure and is carried out very much on the lines of operating theatre procedure, the meat being injected with sterile fresh brine and covered with old brine, sterilized by filtration; great care being taken with temperature control. In some factories the legs are placed in boiling water for about a minute immediately before curing. For canning the Americans introduced the custom about 1937 of injecting the hams immediately before canning. This process was condemned in this country by the Ministry of Health as contravening the Public Health (Preservatives, etc. in Food) Regulations, but I have written letters for the trade press pointing out that the nitrite was added as a colouring matter and not as a preservative with the result that the Regulations were amended in 1940, providing a maximum quantity of sodium or potassium nitrite in bacon, ham, or cooked meat. It is, therefore, suggested that salt at the present time is used to a larger extent in the true sense for the preservation of fruit and vegetables than for meat or fish.

Ingram & Kitchell (1967) further suggest that the traditional use of potassium nitrate instead of sodium nitrate in curing might be due to the difference between substitutive and non-substitutive salt tolerance, but it is more probable that its use was accidental and resulted simply from the natural occurrence of potassium nitrate and its simplicity in purification as distinct from that of sodium nitrate. Sodium nitrate is approximately three times as soluble in water as potassium nitrate and is, therefore, not so naturally accessible under primitive conditions. Even when the mixed nitrates are found and recrystallized it is the potassium nitrate, which crystallizes out first. The origins of saltpetre are lost in history because, as Mellor (1922) points out, the original word 'neter' (from which both nitre or potassium nitrate, and natrium, or sodium are derived) was the term given to the residue on evaporation of water as mentioned by the prophet Jeremiah (about 700 B.C.). It was only gradually that the differences between sodium carbonate and potassium nitrate became recognized and the study

of the latter was concentrated on its use in the manufacture of gunpowder, which is believed to have been discovered by the Hindus, rather than on that in curing meat. The differences between sodium and potassium nitrates was probably not fully recognized until about the eighteenth century and the study of the latter was increased by the Napoleonic wars, two very interesting articles being published by Chaptal (1797) and Longchamp (1826). It is probable, therefore, that originally the use of nitrate in curing was accidental and the result of impurities in salt, though it was later added as potassium nitrate when the use of this was discovered. Since the discovery of the nitrogen fixation process sodium nitrate has generally replaced potassium nitrate and is commonly employed in curing. It must be remembered, moreover, that while the term 'saltpetre' has been employed over many years two varieties of saltpetre 'Chile saltpetre' and 'sodium saltpetre' have been recognized so that any early literature must be read in this light.

With regard to the use of alkali in curing this is still used as sodium phosphate, sodium citrate, or sodium acetate, but seldom as sodium sesquicarbonate, formerly known as natron. Their uses are described fairly fully by Andersen, Jul & Riemann (1965).

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PROCEEDINGS OF THE INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

Man and his food

SIR WILLIAM K. SLATER

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Hardly a day passes without reference in the press, on radio and on television to the so-called 'population explosion', to famine and to malnutrition. Yet in Britain with our ample food supply it is difficult to grasp the true significance of the crisis with which the world is faced. To do so it is essential to examine the extent and the cause of the sudden acceleration in the growth of population and the possibility of matching this growth in food production.

Estimates of population are notoriously difficult and subject to many unpredictable factors, but there is sufficient broad agreement amongst the experts to enable the size of the problem to be gauged. The world population at the present time is stated to be around 3000 million. By the end of the century it is variously estimated that it will be between 5300 and 6800 million. It is a reasonable assumption, therefore, that it will have doubled to around 6000 million. The average annual rate of growth is given by FAO as 2%, but some in of the less developed countries with the largest populations it is as high as 3%. These percentages, may seem relatively small but following the compound interest law, as they do, they result in a rapid and ever accelerating growth rate. What is more alarming—for reasons given later—these percentage rates are likely to increase rather than diminish.

This phenomenon of ever quickening pace is clearly demonstrated by the growth of world population since A.D. 1600. The numbers at that early date are largely a matter of guess-work, but they have been put at 350 million. By A.D. 1800, that is in 200 years, they had doubled to between 700 and 800 million. It took only another 100 years, to A.D. 1900, for the world population to double again to 1500 million, and 60 more years to reach the present figure of 3000 million. During the first 38 years of this century the average annual increase was 18·3 millions, during the next 12 years this rose to 24 millions and in the last decade to 53 millions, which is roughly equal to the population of the British Isles. If we are first to damp down and ulti-

mately control this explosive force, whilst we try to match the demand for food with the supply, we must try to understand its cause.

Why has the population suddenly begun to rise in this alarming way? We have generally accepted that when an organism begins to multiply at a rapid rate it is due to one of two causes, either it has adapted itself better to its environment, or the environment has changed so that it suits its particular make-up better. The first of these changes is brought about by mutation and selection. It can be rapid in organisms with a high reproductive rate and a short life such as plants and insects, but in the mammal with their lower reproductive rate and longer life it is slow and in man it is very slow. The second, where the environment becomes better suited to the organism, can occur in two ways, the one acting quickly, the other over a long period of time. When climatic conditions provide a specially suitable environment over a number of seasons there can be a rapid build up of, for example, an insect, which may have several generations in a single year. Man clearly cannot be affected by such rapid changes in environment. The slow changes in environment, which lead over a long period to marked changes of population in an ecosphere are such as those following the recession of a polar icecap, or a major change in the course of a large river. The time scale of such changes is much greater than that associated with the present explosion in the human population.

As the rapid growth of the human population cannot be accounted for on the basis of the natural forces known to control populations, a new explanation must be found. This lies in the power which man has found to change his environment to suit his own needs. His first step was to destroy his enemies, the competitors for food and living space. The bear was destroyed in Britain in the Middle Ages, the wolf followed 200–300 years later, the last to be largely eliminated is the rabbit. If any animal stands in his way man will find means to destroy it. Other animals he has tamed and made into his servants, to supply him with much of his food, to provide him with power to work the land and with a means of transport. Plants too he has controlled, growing them in blocks, which we call fields, instead of trusting to collecting the wild seeds and berries where they grew at random.

At the same time he learned to clothe himself against the cold and then to build houses to protect himself against the elements. Later he began to heat his houses to keep a more uniform temperature throughout the year. These were the factors which resulted in the earlier rises in population. These would have been greater had they not been partially offset by disease, famine, and almost continuous warfare.

In the middle of the last century man gained new powers to control his environment through the advance of scientific knowledge which have led to the present population problems. He began to learn how to deal with his most deadly enemies the micro-organisms, bacteria, protozoa, fungi and viruses. From the time of Pasteur onwards the world has changed. Diseases can now be controlled by vaccines, by chemotherapeutic agents, by antibiotics and by hygiene. Surgery from being crude

butchery has passed through antisepsis to asepsis. Many killing diseases, like pneumonia, are no longer feared, and operations which were almost always fatal have now become commonplace events with little risk.

Later, with the rise of biochemistry, man's knowledge of the chemistry of his body enabled him to overcome metabolic diseases such as diabetes and pernicious anaemia. Another important factor in prolonging life, particularly in the tropical countries, has been the knowledge gained from the study of the insect vectors, which carry disease. By the use of pesticides and other means, they can be destroyed and the spread of the disease first checked and then ultimately eliminated.

Whilst life was being prolonged and infant mortality reduced by these advances in medicine, the reproductive rate remained unaffected. In a steady animal population, the births must no more than make up for the deaths. It is possible for a woman to bear a child each year and to go on doing so for 10, 12 or even more years. Before man began to control his environment, this was no more than sufficient to maintain the population. Many of the children born died in infancy, more before they reached puberty and those surviving rarely lived beyond what we consider as middle age.

Man having found ways to defeat many of the enemies which threaten his life, has still retained the same power to reproduce. If this power remains unchecked it must clearly lead to a rapid rise in population. Thus, if on the average every woman had four children surviving to the age when they in turn had children, the population would double in each generation. This point has not yet been reached throughout the world, but in some of the less developed countries the increase is approaching this level. An annual increase of 3% will result in the doubling of the population in just under 24 years.

Modern medicine came first to what is generally known as the Western World, but it did not come alone. With it came a rise in the standard of living and the spread of education. As a result the increased survival rate was partly offset by a fall in the number of births. Men and women wishing to provide the best future for their children and to enjoy the amenities of the better life for themselves, placed a voluntary limitation on the number, a limitation only made possible by knowledge springing from a higher educational level. Even so the population in Britain and in other more developed countries is still rising too rapidly. In the less developed countries medicine has come to populations largely illiterate and with a deplorably low standard of living. To a people which has long been accustomed to having many children in the hope that some would survive to help on the family farm and to support their old age, the idea of a limited family is difficult to accept. Even when they have the desire to reduce the number of pregnancies, they have rarely the knowledge or the means to do so.

This problem is seen in the most acute form in India, where a population of some 480 millions is increasing at the rate of 12 millions each year. It is estimated that there are at least 100 million women married at a child-bearing age. This number

will soon be increasing by up to 6 millions a year. A large percentage, probably over 80%, of these women are illiterate so that any method of contraception must be simple, easy to learn and to remember. Under these conditions the contraceptive 'pill' has not proved reliable; even if it were the cost would be prohibitive. The alternative method known as 'the coil' offers better possibilities. It is much cheaper and once it is fitted it calls for no further action on the part of the woman. The disadvantages are that it requires skill in fitting and that it may not prove 100% effective owing to the possibility of displacement. An army of doctors or trained nurses will be required for its application, if any progress is to be made, particularly if the women who have been fitted return to the clinic for regular examination as they should. The Indian government is making a major effort to solve this problem, but alone it cannot do so; it will need all the help it can get from the more prosperous countries if it is to succeed. What is true of India is generally true of all the less developed countries although in no other is the problem so large and hence so difficult as in India.

With these facts about the growth of population in mind, it is possible to begin to consider how far food production has kept pace with the growing numbers and how far it can be expected to continue to do so in the future. Fortunately whilst science has been responsible for the mounting population, it has also given us the means of increasing the production of food and of preserving in storage what is harvested. Food supplies have been increased in many ways. More land has been brought into cultivation, arid areas by irrigation and heavy land by replacing primitive hand implements by machines. The ways in which the yield from each acre of land can be increased are well known. New heavy yielding varieties are available, the response to fertilizers is often dramatic and the losses from damage by pests and diseases in the field and in store can largely be eliminated by the use of pesticides. Just as human medicine has protected man against disease, so veterinary medicine has reduced diseases in our domestic animals, thereby increasing their productivity. The chemist can produce the necessary supplies of nitrogenous fertilizer from the air and all the pesticides required, provided he can draw on the money, skilled men and materials he needs. In all these ways food production has been increased, but what is important is how far this increase has kept pace with population growth.

As might be anticipated this varies from one country to another. In North America production has so far outstripped the needs of the people for food that it had to be deliberately restricted by keeping land out of cultivation. Even so large stocks of basic foodstuffs were built up, which have been of great value in alleviating hunger in the famine stricken countries. This period of over production is now rapidly coming to an end as the population rises. In the United States the land which has been out of production is gradually being cropped again and yet it is estimated by the Department of Agriculture that there will be no exportable surplus of grain after some date between 1975 and 1980. From then onwards the United States will have to intensify further its agricultural production to meet the needs of its own people. Canada will

have grain to export well into the future, but the amount will depend on how rapidly its own population rises, and there will be many buyers for the wheat there is to sell. The output, moreover, is not indefinitely elastic depending as it does on the limited rainfall in the wheat growing areas.

In Western Europe there has been a steady rise in production which has been greater than that in population, with the result that there is available about 25% more food per head than before the war. This increase has been absorbed by a general improvement in the standard of nutrition and particularly by the spread of higher standards amongst the poorer sections of the populations.

In Eastern Europe and the U.S.S.R. there was a steady rise in the amount of food available per head following the war and lasting until 1958. From then onwards the progress slowed until it appeared to have reached a plateau, where the demand and supply are nicely balanced. In years of poor harvests the U.S.S.R. is buying grain on the world market, in good years there may well be a surplus. The new ideas which have recently been introduced into Soviet agriculture allowing greater freedom to the local management and rewards for individual effort may once more start production moving upwards faster than population. If this proves to be so it is possible that we may once more see Russia as an exporter of grain.

In the South Pacific, in Australasia, there has been a steady rise since the war of food production so that it is 10% higher than in 1939. These countries continue to be major food exporters and they could provide even more if there were customers willing to buy. How great the export surplus may be depends on the interplay between the physical problems which will grow with increased output and the economic factors governing the price the world is prepared to pay.

The food situation is very different in the less developed countries where the great mass of the population live. In Latin America there was a rise after the war in the food available per head, but after a time it began to fall until now it is less than that before the year 1939. In the Far East excluding mainland China, there was an improvement in the food *per capita* up to 1959, then the amount began to fall until it is now below the pre-war level. In the Near East the position is somewhat better, in that the rise up to 1958 was marked so that although it was followed by a fall the food available *per capita* is still 5% above the pre-war level. Unfortunately there has been no further improvement and the general indications are that the position is worsening. In the other great continent, Africa, there has been little change in the relationship between food and population, although now there are signs that the position is slightly worse than it was pre-war.

It is alarming to realize that in spite of all the efforts made to increase agricultural production in the last 20 years, we seem to be losing the race against population growth. The reason for this failure is easily explained, the results from a given effort in medical care are far greater than those obtained from a similar effort in teaching farmers how to improve their yields, providing them with the means to put these lessons into

practice and the outlets which will make the additional effort financially worthwhile. Thus it is easier to eliminate malaria than to feed the people who survive as a result.

The farmers in the less developed countries are largely illiterate. This means that they cannot read a simple pamphlet or take a note of anything they are told; they must rely entirely on memory. They cannot read the label on a bag of fertilizer or the instructions on a packet of insecticide. As a result they require continuous help and supervision from the advisory officers. Fortunately the qualifications of the instructors in the field need not be high provided they have the backing of a limited number of well trained officers who can deal with any special problems which may arise. The advisory officers working with the farmers must, however, have a sound practical training so that they can win the confidence of the farmers and where necessary demonstrate in practice, what they are attempting to teach. In most developing countries there is a great shortage of advisory officers, and many of these who are available have not been rightly trained. The university graduate is all too often well versed in all the latest scientific knowledge without any practical experience. He can discourse learnedly on the latest theories in genetics or soil science, but is quite incapable of demonstrating the simple principles of dairy hygiene or of how to use a new type of plough. He, not unnaturally, wants to spend his time in a university department or a research institute. If he fails in obtaining such a post he will take a relatively lowly civil service appointment rather than go to live in a village and work amongst farmers, whom he despises and who regard him in return with suspicion and contempt.

Of the practical advisory officers with training below graduate level, there are far too few and the facilities for their training too limited. As a result the first requisite in any drive to grow more food, the education of the farmers, is missing. Moreover the agriculture advisory officer from the developed countries can help little to remedy this shortage. Race, language and local conditions all make his task impossible. The only solution is an intensive course of training for the practical advisory officers in each country, with the guarantee of a suitable post at the end of the training and reasonable future prospects.

However well trained and efficient an advisory officer may be he will make little progress unless he can convince the farmer that it will be to his personal advantage to grow more food. Most of the farmers in the less developed countries are very poor and have no funds with which to buy better seed, fertilizers, pesticides or irrigation equipment; if they are to adopt new techniques they must be given either grants or loans. If help is in the latter form as it most often is they must have assurance that the additional expenditure will result in a better crop for which there will be a market at a price high enough to repay the loan and give some return for the extra work and risk. Even the prospect of a monetary return is not enough, the farmer having received cash, will wish to spend it. It is important, therefore, to have available a range of goods which will appeal to him and his wife.

The rapidly rising population has brought about a land hunger. In countries where the family farm is normally divided amongst the sons, the fragmentation has become so acute that no longer is there enough in each portion to provide a living for a man and his family. As a result some of the men are drifting into the towns and cities in the hope of finding work. There they often join the already large number of unemployed. They need the extra food their brothers grow but they have no money with which to buy it. The answer would seem to be industries, producing goods attractive to the farming community, using as little capital as possible and the maximum of labour. As this is diametrically opposite to the trend in the more developed countries with their higher standard of living, it is difficult to convince those concerned with policy and planning in the developing countries that it is the right course to follow. The choice is between the many having a low standard of living, which can be gradually raised, and the few enjoying a relatively high standard whilst the rest of the people are workless and go hungry. The political strains in many of the developing countries, where often 80% of the people live on the land, can be traced to the contrast between the standard of living of the workers in the cities and that of the peasant subsistence farmer and those of his family who have gone hopefully to the city only to find there conditions even worse than on the land. Yet the developing countries continue to copy the industrial pattern of the developed. This results from the wrong question being asked. If instead of enquiring 'Why are the developed countries wealthy', when the answer must be 'Because they are highly industrialized', they should ask 'How did the developed countries become wealthy' when the answer would be 'By building up a sound agriculture, which provided a basic market for their early industries'.

Turning now from the problems of how food production may be increased, to the amounts which will be needed to provide adequate diet throughout the world in A.D. 2000, it is immediately obvious that to maintain even the present level, the food supply must be doubled to meet the growth of population. At the same time it is equally well known that roughly one in seven of the World's population now goes hungry and half are suffering from malnutrition. The words hunger and malnutrition often have different meanings given to them. Hunger in the sense it is used here refers to those men and women whose intake of calories is insufficient to enable them to lead a normally active life; a condition where they are lethargic and tend to be dubbed as idle. This is like the motor car with only enough petrol for part of the day's journey, so that the journey must either be cut short or spread over into another day. Malnutrition is the condition where the diet is deficient in the quantity and quality of its proteins, and in its content of vitamins, and minerals. Each of these constituents of the diet can cause disease showing clinical symptoms, such for example as kwashiokor and pelagra, but much more often they result in a sub-clinical condition where their effect only appears, when the sufferer is placed under some abnormal stress. This is the motor car with worn parts, which breaks down when driven fast.

A personal experience of this type of sub-clinical condition was described to me by the Head of the Billings Hospital in Chicago. After the explosion of the first nuclear bomb at Hiroshima there were many suffering from burns which were refusing to heal and there was speculation as to whether this might be due to their special nature. The patients were put into hospital where the diet changed from one seriously lacking in high quality protein, to one with ample protein of good quality, and within a month their wounds were all healing normally, showing that the real cause was malnutrition.

If hunger and malnutrition are to be banished from the world in A.D. 2000 a rough calculation made by FAO suggests that food supplies must be increased not twice but somewhere between three and four times. This is an enormous but not an impossible task if all those responsible for its fulfilment work willingly together using every modern scientific technique available to them. More land can still be brought into cultivation, but this becomes increasingly difficult as each year passes. It is to be expected that in selecting areas for irrigation, which is by far the most important means of providing agricultural land, the planners and engineers will start with those which present least difficulty. Thus as one scheme after another is completed, the remaining possibilities will be more expensive and generally less rewarding. Even so the schemes which have been completed in the past decade have no more than kept up with the increase in population. In the Far East 25% more land was brought into cultivation between 1950 and 1960; during the same period the population of the area also rose by 25%.

There is another possible approach to the provision of an adequate water supply for crops in the semi-arid regions. So far the emphasis has been on large dams to supply water over a large area and often at the same time to provide a major source of power. Such large schemes appeal both to national planners and equally to international sources of finance. Moreover they are the only possible schemes for regions with very low and unpredictable rainfall. Where, however, there is a rainfall which would be adequate if it were not seasonal, it may be possible to conserve sufficient water locally to grow satisfactory crops. This may be done by building relatively small dams to prevent the water being lost in a series of flash floods when heavy rain falls on hard baked dry earth. The siting and building of such dams calls for hard and unspectacular work in the field, but if they are built with local labour they are cheap and effective. It is difficult, however, to prepare a scheme for a series of such dams which will appeal to those supplying the necessary funds, and much of the possible success must depend on the sympathetic consideration of any applications made to national and international sources of finance.

Another possible approach to this problem is to conserve the water which falls on the land by preventing evaporation which, in hot climates, is responsible for a considerable loss. It has been suggested that, where the land is cultivated in small patches by peasants, it might be practical to form sloping paths between the rows of the crops, by using the local sand mixed with a small quantity of cement. The rain would thus be concentrated on to the cropping area and evaporation greatly reduced.

The major source of additional food must, however, come from heavier crops on the already cultivated land. At the present time the yields obtained in most of the developing countries are pitiably small when compared to those in the developed countries. They could be greatly increased by the application of fertilizers and the use of insecticides to protect them in the field and in store. Apart from the difficulties in getting the individual farmer to fertilize and protect his crops, there is the major international problem of the supply of the necessary materials. Every developing country which is planning to expand its agriculture is calculating on the use of large quantities of fertilizers and agricultural chemicals. To meet the potential demand will call for a major expansion of chemical plant and for large sources of power. To fix 1 ton of nitrogen per annum requires a capital expenditure on plant of about £100. India alone is considering the use of an additional 2 million tons of nitrogen in the next 4 years which will call for a capital expenditure of £200 million. The power required for a nitrogen plant varies with the process, but it is always heavy and may amount to the equivalent of 10 tons of coal for each ton of nitrogen fixed. The pesticides and other chemicals will make equally heavy demands on industry.

The need to take stock of what will be wanted to increase yields to the level required to provide an adequate diet has been recognized by FAO which has undertaken the preparation of a World Indicative Plan. The first volumes of this are already beginning to appear, confirming the magnitude of the task of supplying the basic materials for food production. In addition to these materials, there will be need for a wide range of equipment, machines for cultivation and pumps for irrigation and spraying. It is on these material requirements and on educational schemes that the wealthy countries will have to concentrate their future aid, if food supplies are to match the rising population. Such a new orientation of aid will undoubtedly cause difficulties and dissension between the donor and recipient nations. It will almost certainly be argued by the latter that this is an attempt to hold them in a subservient state and that they have the right to decide on what schemes the aid provided should be spent. The resulting negotiations will call for patience and an understanding of the problems facing the governments of the developing countries. To satisfy the demands of the better educated but more vocative minority and at the same time to hold out prospects of rapidly improving conditions for the illiterate masses of their people, it is tempting to adopt large scale schemes which can be easily represented as bringing the country rapidly up to the level of those already wealthy. It is far more difficult to popularize a plan to produce a slow general rise in living standards, which has no magic quality leading to immediate prosperity but on the contrary calls for changes in traditional methods and much hard work.

If we examine the position of Britain in this changing world, we may get a better understanding of the attitudes of the governments of the developing countries. By A.D. 2000 it is estimated that the population will have risen by one-third. The possible effect of our own population growth and that of the world on food production in

Britain has been described by a working party set up by the Agricultural Research Council in these terms.

'It is fair to assume that both the absolute and *per capita* demand for food in the world will increase. Not only many countries from which we now import have a smaller surplus for export but they may also find new markets, particularly for meat and other protein foods, which may become increasingly scarce and dear. With these possibilities in mind the Working Party considers that agricultural research policy should be based on the assumption that Great Britain may require in 30 years time *at least* twice the present net output of home grown food'.

What will this mean in the disruption of our way of living and how far shall we have to abandon many of our cherished ideals? We are already getting very high yields of arable crops, but there is still room for some improvement. Our grasslands could produce more if we managed them better and our livestock could be made to yield more. To double our production, however, will involve bringing every possible piece of land into use, hedgerows will have to go and the small copse which lends variety and beauty to the countryside. We shall have to control pests and diseases in our crops with the best means available to us, even if this involves some risk where insecticides are used. Our stock will have to be kept under increasingly intensive conditions. Cattle will not graze the pastures as they do now, but be kept in yards with food brought to them from the fields. So called factory farming will have to be extended so that it becomes the rule, rather than the exception. Water will be needed for irrigation and this will have to compete with increased demands for domestic and industrial use. This will mean extensive water schemes such as those which have caused so much controversy in the past. From all these changes and the steady growth of towns wild life will be changed and partially destroyed. None of these results from our rising population are good to contemplate. We should wish to leave to our children and grandchildren a Britain which was beautiful and good to live in, but if we are to do so we must limit the numbers, who will enjoy it.

Yet the choice between limiting our numbers and changing the face of Britain is never fairly presented to the people by those responsible for our destiny. The early symptoms of this change are continual causes of agitation. The uprooting of hedgerows, the use of pesticides, the methods of housing and managing livestock, the use of lakes and mountain valleys for the supply of water are attacked and successive governments meet the agitation with palliatives, without mention of the root cause. At the present time there is much concern about the poverty in large families. No one would wish to deny these unfortunate children, who are already with us, a minimum standard of living and something must surely be done to help them, but if funds are made available to help the larger families it will only tend to make matters worse for future generations, if steps are not taken at the same time to limit them in the future. It is difficult to know how this can be brought about and even its mention and study will be avoided by politicians.

If we recognize this dilemma in our own country it will enable us the better to understand the difficulties of the government in countries such as India. We have a literate population and every form of mass communication, whilst they have to struggle with uneducated masses with little or no means of communication; with a people deeply steeped in tradition and fearful and suspicious of change. Our aid must be directed in the right channels if their peoples are to be fed in the next 30 years, but any pressure on our part must be applied with the greatest tact so that it does not appear to be dictating to their governments a policy, designed to destroy all they wish to preserve of their national heritage and to keep them forever in a state of poverty and subservience.

You may ask 'What have these political and sociological questions to do with the scientist?' In my view the scientist has much to contribute. The relationship between the scientist in the developing and the developed countries is much closer than between their politicians. The scientists speak a common language and are trained to measure and accept facts. They can do much to act as a bridge between two peoples and to make governments aware of the real nature of the problems with which they have to deal.

Summary by P. G. Hart of discussion of Sir William K. Slater's paper, 18 May 1967

During discussion the problems of changing the diets of people was considered. Because of the limited facilities of preparing food in areas where food was in short supply, this was often very difficult. A good example of this is where wheat is supplied in place of rice—rice can be boiled in a pot whereas wheat requires greater knowledge and more refined utensils to prepare a meal. It has been tried in parts of Africa where the farmer was taught new farming methods while his wife was taught how to use the new crop.

There is also the problem of getting people to eat strange food. If a source of high protein is mixed with a foodstuff in the staple diet, i.e. an expansion of the existing diet, it will be acceptable. This has been carried out successfully in South America.

The production of wheat averages 30 cwt/ac rising to a maximum of 2 tons/ac. This yield can be increased by the use of fertilizer and irrigation, but the theoretical yield has still to be achieved.

Very little research has been carried out on how many people the United Kingdom could support without importing food. The diet would be very uninteresting as there are a number of foods which cannot be grown (citrus fruits) as well as a number of raw materials (oil for margarine manufacture). It has been estimated that with a 30% increase in the population the diet would be nearly vegetarian with a minimum of protein.

One possibility of overcoming the problem of food shortage in the under-developed countries is either to introduce domesticated animals or to kill the natural game in such a manner as to have a continuous supply.

The other main source of hope is the use of synthetic foods obtained by using the 'biological engineering systems' with oil by-products as the raw material. Three oil companies are working on this problem but at present the results are uneconomic and unpalatable. A point will be reached when the cost of producing synthetic foods from oil (or by chemical synthesis) will be the same.

J. Fd Technol. (1967) **2**, 253–254.

Book Reviews

Introductory Food Science. By D. B. SMITH & A. H. WALTERS.
London; Classic Publications, 1967. Pp. 164. 25*s*.

This little book attempts, possibly for the first time, to provide both a background textbook for university studies and a link between the Sixth Form and the University. These two objectives differ and inevitably the final result is open to some criticism from both points of view. Written in semi-popular style, the book is a welcome attempt to publicize the emergence of food science as an independent discipline and to stress the essential role of the food scientist in promoting human welfare. It draws attention to the interesting and intellectually rewarding careers now open and is aimed primarily at school leavers and careers advisers; indeed it would probably have been better to avoid the temptation to produce a textbook at the same time.

The opening third of the book comprises an outline of the chemistry and biochemistry of food constituents, illustrated by selected structural formulae to emphasize their complexity. This section assumes a knowledge of chemistry which is quite beyond the scope of a sixth-former, or of a 1st year university student who has still to complete a systematic course of organic chemistry, and would be unintelligible to a plant operative or non-scientific industrialist. This may well discourage the potential student who is not yet ready for it, but at the same time it is inadequate for textbook purposes.

In contrast, microbiology and food spoilage receive only a brief, inadequate treatment in seven pages and the subject of nutrition is compressed into nine pages. To balance the book, both of these essential aspects could usefully be extended to capture the interest of the general reader.

Sections dealing with food raw materials and with methods of food processing, while brief and largely descriptive, contain selections of material and photographic illustrations which are much better suited to the central theme of the book and should be readily intelligible to the interested reader. The student who has embarked upon a food science degree course will require a more advanced textbook treatment, but others following sub-degree courses, particularly in related fields such as domestic science, dietetics, and agriculture, may find an adequate background of general information.

A final section deals with the educational facilities available in food science, the types of courses offered by various institutions, and the conditions of entry to them. This presents a most useful collected assembly of widely scattered and often confusing information. It should be particularly helpful to potential students faced with making a choice and in enabling them to take the right decisions while still at school. Unfortunately it will be difficult to keep this material up-to-date in a rapidly changing educational world.

E. L. CROSSLEY

Proceedings of the 2nd International Congress of Food Science and Technology. Ed. by D. J. TILGNER & A. BORYS.

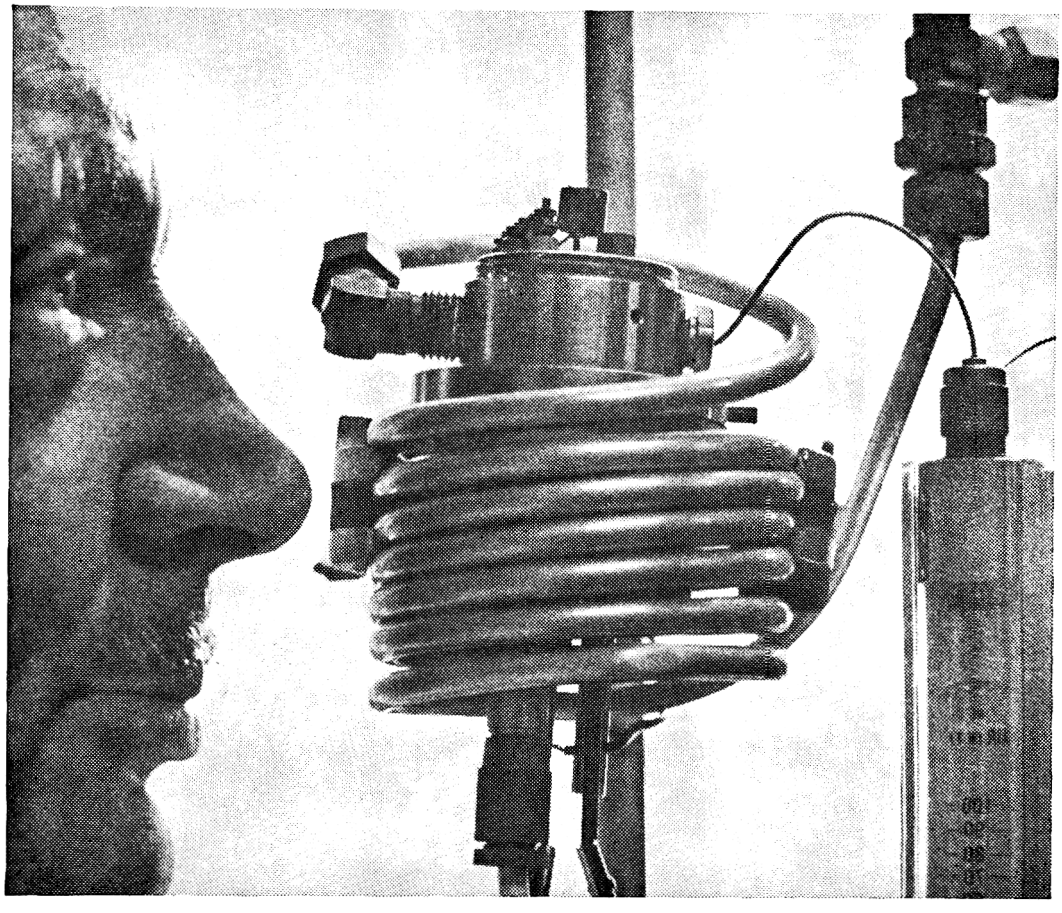
Warsaw, 1967. Pp. 511. $12·60.

In planning an International Congress covering such a wide field as Food Science and Technology, an organizing committee is faced with a difficult decision: to allow a 'free-for-all' results in a programme so unmanageable that all the resources of organization are swamped; but to be selective means forfeiting the interest *and attendance* of those whose subject areas are neglected.

It was especially important in the *2nd International Congress* not to discourage the attendance of every food scientist and food technologist who could summon up the wherewithal to enable him or her to be present, because decisions would be taken and presented at the closing Plenary Session which would be fateful for the international future of our science. The compromise adopted by the Polish Organizing Committee seems to us to have been a successful one. While specific and limited objectives were attained by inviting two or more speakers on selected topics for each of the eight main subject areas, contributions were accepted virtually without restriction on the understanding that though they would not necessarily be called upon for delivery, they would be published as abstracts which would be available during the congress.

The Proceedings, therefore, comprise the thirty-three invited papers, ranging from novel protein foods to documentation and information in food science; the formal addresses and lectures at the opening and closing sessions; and also the organization agenda of the International Committee of Food Science and Technology. The lectures by D. J. Tilgner (Flavour—the challenge to food science and technology), Charles T. Townsend (The role of the food lecturologist in the food industries of the U.S.A.), A. J. Oparin (Biochemistry as a theoretical basis for food technology) and J. Hawthorn (The future of food science and technology) make important reading; especially, for members of the Institute, the last. With the dry humour we expect from him, he gives an optimistic—but nevertheless a realistic—evaluation of the future of food science which expresses very accurately the function we envisage for this Institute and the justification for its coming into being.

E. C. BATE-SMITH



Nose...meets...automatic nose

As any dog will tell you, the human nose is a sensitive but imprecise instrument. In other words your nose can pick up a 'scent' but not discriminate between scents, as a bloodhound can.

But even a prize bloodhound would not be able to equal the degree of discrimination of the apparatus in the picture. Called a gas chromatograph, it is used by Metal Box Packaging Research to determine the characteristics of aromas. Gas chromatography is just one of many research tools used by Metal Box to control the quality of packaging.

Physicists, chemists, biologists, and food technologists are also engaged in developing new forms of Metal Box packaging—not just in metal but in plastics and paper too.

Metal Box — brief facts

Leading manufacturers of packaging in metal, paper and plastics.

Light engineering and marketing organisation with 38 factories in Britain, 32 overseas.

Research team of over 300 continuously engaged in projects involving food technology, physics, chemistry and microbiology.

A youthful and vigorous industry with tremendous potential for the future.

MB *There's more to Metal Box than metal boxes*

– they're leaders in research into metal, paper and plastic packaging

MB 10-105/D/1

INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

TENTATIVE PROGRAMME, 1967

Date	*Place and Time*	*Subject*	*Speakers*
20 September	I.F.S.T. invited School of Pharmacy, 29/30 Brunswick Square, London, W.C.1.	Methods of Assessing Toxicity Colours Their use in the Food Industry Screening Tests Chemical Changes due to Processing of Meat Products Toxicological Aspects of Chemical Changes due to Processing Newer Methods of Assessing Toxicological Assays	R. F. Crampton Opener M. Brook I. F. Gaunt R. A. Lawrie P. Grasso B. Gilbert
28 September	Safeway Stores Ltd 159-165 Edgware Road, W.2. 18.30 for 19.00	Ladies' Evening Open House	
3 October	Manchester	The Role of the Food Consultant	J. G. Davis
3 and 4 October	Agricultural Building Queen's University, Belfast	Symposium: Quality Control of Food Processing	A. J. Howard E. J. Dyett M. Kimber Miss M. Longstaffe T. R. Hayes W. J. Webb B. F. Bowyer L. W. Bass and R. H. Colcott
11 October	Chem. Eng. Dept. University of Birmingham	A.G.M. and Effluent Problems in the Food Industry	S. H. Jenkins
12 October	School of Pharmacy 29/30 Brunswick Square, W.C.1.	SPECIAL MEETING Discussion on Food Products Orders	A. J. Kidney to Open
13 October	Belfast	A.G.M. and Address	A. J. F. O'Reilly
17 October	National College of Food Technology, Weybridge (with Students' Society) 19.00	Heat Processing	A. Hersom
28 October	New Salford Technical College	Symposium: Baking Technology	N. Chamberlain R. S. D. Geary D. E. Baker or J. C. Reid
1 November	College of Heating and Ventilation (adj. Borough Poly.)	Function of a Development Department	J. Sheldon
8 November	14 Belgrave Square, S.W.1. (with Food Group) 17.45 for 18.15	Codex Alimentarius	J. H. V. Davies
8 November	School of Agriculture, Sutton Bonington, Loughborough	Some Aspects of Meat Technology	E. F. Williams
23 November	Belfast	Programme and Films by Oxoid	J. Elstub
27 November	University of Strathclyde (with Students' Soc.)		
29 November	University of Leeds	A.G.M. and Chairman's Retiring Address	W. Cunliffe
5 December	Royal Society, 6 Carlton House Terrace, London, S.W.1. 17.00	Annual General Meeting of I.F.S.T. World Food Technology Problems (*see special notice*)	J. H. Hulse
6 December	School of Agriculture, Sutton Bonington, Loughborough	Ladies Evening: Eating as a Hobby	J. G. Davis
14 December	Queen's University, Belfast	Slides and Films of National Canners' Association	J. Scholfield

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Typescripts (two complete copies) should be sent to the Editor, Dr E. C. Bate-Smith, A.R.C. Institute of Animal Physiology, Babraham, Cambs. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be double-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References.

References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s); (b) year of publication in parentheses; (c) title of journal, underlined; (d) volume number; number of first page of article. References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Verbs which contain the suffix ize (ise) and their derivatives should be spelt with the z. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is greater than one hundred.

Abbreviations. Abbreviations for some of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	μg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Letters and numbers must be written lightly in pencil. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to Figures'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in capitals with an Arabic number, e.g. TABLE 2. Each table must have a caption in small letters. Vertical lines should not be used.

Page proofs will be submitted to the contributors for minor corrections and should be returned to the Editor within 3 days. Major alterations to the text cannot be accepted.

Offprints. Fifty offprints will be issued free with each paper but additional copies may be purchased if ordered on the printed card which will be sent to the senior author with the proofs.

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