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The control and evaluation of spoilage*

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Summary

Methods currently used in the control and evaluation of spoilage of meat are reviewed and their value discussed. In many instances scientific explanations of the effectiveness of the control methods are lacking, e.g. the mechanism of action of carbon dioxide or sodium nitrite in controlling microbial growth. A lack of knowledge regarding the chemical and biochemical processes involved in spoilage hinders the development of completely satisfactory methods of spoilage evaluation. Research directed towards rectifying this could lead to the development of new and perhaps more effective methods of spoilage evaluation and control.

Control

Ignoring the possibility of microbial growth at the expense of meat proteins, the presence in meat of substantial amounts of low molecular weight compounds such as peptides and amino acids, of glycogen, and of a variety of metal ions and soluble phosphorus, together with a plentiful supply of water, provides an ideal environment for the growth of micro-organisms. Accordingly, organisms finding access to meat are able to grow rapidly, being limited only by such factors as temperature, redox potential and pH, factors which also influence the type of organism which will grow. The results of superficial bacterial growth are the appearance of slime on the meat usually accompanied by unpleasant odours, taints and discoloration; these last also characterize deep muscle tissue or formulated meat products in which bacteria have proliferated. It is such phenomena which constitute spoilage. The chemical nature of the compounds producing the unpleasant smells and taints is very poorly understood and what little information there is has been inferred from bacteria growing under conditions quite unlike those found in meat spoilage.

The control of spoilage and hence the increase in storage-life of meat is effected by a combination of the following procedures:

1. Prevention of microbial contamination.

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2. Destruction of microbes which, nevertheless, gain access to the product.
3. Establishment of environmental conditions which prevent or retard development of contaminating micro-organisms.

The conversion of the musculature of animals into meat after slaughter is the result of a complex series of chemical reactions leading ultimately to the state of rigor mortis. During this process oxygen stored in the muscle is depleted and the redox potential gradually falls from above + 250 mV to − 150 mV. Such a low redox value, together with the initial muscle temperature of around 38°C, provides ideal growth conditions for such anaerobic organisms as the clostridia-bacteria associated with the breakdown of animal tissues under natural conditions, i.e. putrefaction. Thus it is usual to cool the meat rapidly before the redox potential falls sufficiently to allow growth of these organisms. However, if immediately prior to slaughter the animal has been excited and has struggled, thus using up a large part of its muscle oxygen store, it is possible for the redox potential to fall rapidly, allowing proliferation of these mesophilic organisms before the carcass is cooled (Ingram, 1962a). The same bacteria have been implicated in 'bone taints'—spoilage associated with sites deep in the muscle near to the bones (Ingram, 1952; Cosnett *et al.*, 1956)—presumably because it is at these deep sites that the temperature falls more slowly.

The subjection of animals, and in particular pigs, to such pre-slaughter stress has also been claimed to result in the passage of organisms from the gut into the tissues of the body (Haines, 1937). There is also evidence that amongst such organisms are those that would benefit from the rapid development of a low redox potential and a slow rate of cooling, e.g. facultative anaerobes such as streptococci and clostridia (Medrek & Barnes, 1962). Furthermore, some streptococci are able to grow at low temperatures, e.g. *Streptococcus faecalis* can grow at 0°C (Foter & Rahn, 1936) while *Streptococcus lactis*, although not able to grow at this temperature, can still produce lactic acid from glucose (Borgstrom, 1962). Thus invasion of the musculature may result in the wide distribution of cells able to grow and/or metabolize in the meat during refrigerated storage. Clearly, ante-mortem conditions offer the first opportunities to exert some control over the subsequent course of spoilage of carcass meat. Pre-slaughter conditions are important for a second reason. Concurrent with the fall in redox potential, the pH falls from an initial value of about 7 to a stable value around 5·5, the so-called ultimate pH. This is due to the breakdown of glycogen stored in the muscles to lactic acid, a process which is irreversible after death, and which occurs as a result of oxygen depletion. The attainment of this pH plays an important role in the stability of meat to microbial attack, pH being an important factor limiting the rate of bacterial growth (Ingram, 1948; 1962a). In general, the nearer the pH is to neutrality the faster the growth. Since various types of stress (e.g. starvation, exercise) are known to deplete an animal's glycogen reserves, and consequently reduce the amount of lactic acid produced, an animal slaughtered in such a condition is likely to produce meat with

a relatively high ultimate pH. Callow (1937) showed this to be true for pigs and it was subsequently shown that the resting of pigs before slaughter, together with the feeding of sugar, produced meat with a low ultimate pH (Gibbons & Rose, 1950) and delayed the time to slime formation on bacon (Madsen, 1943). The attainment of a low pH is a contributory factor in the stability of cured and heat-processed meats due to the effect of pH on the resistance of bacteria to salt and nitrite, on the one hand, and to heat on the other (Ingram, 1962a).

Animals brought to slaughter invariably carry large numbers of micro-organisms on their hides and, together with the gut contents, these appear to be the main sources of the organisms which ultimately contaminate carcasses. Any of the slaughtering and butchering operations that are performed unhygienically, as they frequently are, e.g. the use of heavily contaminated knives for sticking, flaying or splitting the carcass, will spread contamination. Bacterial loads of the order of 10^6/sq cm or more can be obtained during these operations. Thus the second point of attack in the control of spoilage is on the slaughter floor.

Traditionally, a wiping cloth was used to produce visually clean carcasses, and very successfully too. Unfortunately, the same cloth was smearing large numbers of micro-organisms from dirty areas to clean ones (Empey & Scott, 1939) and possibly shortening the subsequent storage life of the carcass. More serious still, if the same cloth was used for more than one carcass, was the risk that food poisoning organisms were being widely distributed throughout the 'kill', for which reason the use of wiping cloths was banned in October 1968. Now, spraying with water (hot or cold), with or without the addition of chlorine, is advocated. The process, especially with water in the temperature range 75–90°C has been shown to reduce the numbers of organisms by as much as 80% (*Ann. Rep. Meat Res. Inst.* 1969–70) and somewhat smaller reductions are produced by 20 ppm chlorine (Patterson, 1968).

Haines (1937) showed that the time taken for slime formation on carcass meat was dependent upon the initial number of contaminating bacteria, and Ayres (1955), that the storage life of ground beef was dependent upon the initial composition of the microbial flora; meat having a low proportion of typical slime-producing organisms, e.g. pseudomonads, keeping longer than meat with a higher proportion. The expectation, in adopting spray treatments, is that the initial numbers of bacteria will be reduced and that, further, if hot water is used, the micro-organisms able to grow at refrigeration temperatures might well be specially susceptible. However, it should be understood (a) that such treatments may alter the meat surface so as to favour subsequent bacterial growth, especially if it remains wet, and (b) that, though the water may start at a temperature near boiling point, at impact it may be down to 60°C and thus unlikely to have the desired effect on the viability of contaminating organisms. So, although spraying may produce a visually clean appearance, its role in control of spoilage should not be over-estimated, the use of hygienic conditions during butchering being of far more importance.

At this stage almost any combination of micro-organisms may be present on the carcass including both mesophilic and cold-tolerant forms, although anaerobes would be expected only in the deep tissues whilst anaerobic forms would predominate at the surface. If the meat is now cooled, as in recommended commercial practice, to 5°C or below, there is a slowing down of microbial multiplication and metabolism. The effect of cooling is to prolong the lag period—that part of the growth cycle before the cells divide—the effect being greatest for the mesophiles. Thus there is a selection of cold-tolerant species which themselves grow more slowly at low temperatures (Elliott & Michener, 1965)—the so-called *Pseudomonas–Achromobacter* association. This name is no longer wholly appropriate because many of the strains previously named *Achromobacter* are now regarded as non-pigmented *Pseudomonas* sp. and many of the remainder have been assigned to the genera *Alcaligenes* and *Acinetobacter*.

Superimposed on these effects of temperature is the effect of desiccation. Drying occurs during cooling because the equilibrium relative humidity (ERH) of meat is normally greater than that of the atmosphere. Scott & Vickery (1939) showed that, if surface water evaporation was prevented by covering the carcass, spoilage occurred more rapidly. In the case of meat cut to expose large areas of naked muscle the effect is not so great (Ingram, 1962) because of easy replacement by water from the deeper tissues—a process hindered in carcass meat by fatty and connective tissues (Haines & Smith, 1933). Desirable though desiccation is for the control of spoilage, drying must be limited in practice, otherwise there is uneconomic weight loss.

Refrigeration effects control by manipulation of environmental conditions which slow down, but do not stop, the process of bacterial growth and spoilage. Equally important, it prevents growth of most of the mesophilic organisms pathogenic for man. In fact, the basis of the selective effect of cool temperatures is not understood and the mechanism may well differ from organism to organism. One of the most popular hypotheses is that low temperatures affect the chemical and physical integrity of cell membranes and thus interfere with the transport of essential metabolites into the cell (Rose, 1968). Damage to cell membranes also appears to be the major cause of the death of micro-organisms as a result of sudden chilling, this phenomenon of 'cold shock' being well documented for Gram negative bacteria (e.g. Strange & Dark, 1962).

Refrigeration, therefore, neither kills nor prevents growth of all of the organisms commonly contaminating meat. Consequently, spoilage eventually ensues, perhaps as the joint result of the growth of cold-tolerant bacteria and of enzymic activity of mesophiles rendered non-viable by the cold. Also, it is known that some cold-tolerant organisms exhibit increased extracellular enzymic activity at lower temperatures, e.g. protease activity (Peterson & Gunderson, 1960; Jay, 1967) and lipase activity (Alford & Elliott, 1960). A logical extension of the process, to restrict this residual spoilage activity, is to reduce the temperature until the meat freezes.

Freezing, like cooling, has selective and lethal effects. Some species of psychrophilic bacteria are able to grow down to around − 7°C though at much lower rates than at higher temperatures. However, many more yeasts and moulds are able to grow at and below this temperature and may become more of a problem than bacteria in the frozen storage of meat, This is because they grow at lower water-activity levels and withstand greater solute concentrations (electrolytes being of particular interest) than bacteria.

The freezing of bacteria results in an initially rapid death rate followed by a slower rate during subsequent frozen storage (Haines, 1938). The extent of the kill varies, certain substrates exhibiting a protective effect. In fact, in studies with pork, Sulzbacher (1950) found no evidence for any effect of freezing to − 18°C on the viability of cold-tolerant bacteria. Where death does occur, the mechanism is unknown, though two theories invoke physical damage due to intra- or extracellular ice formation and protein denaturation (Borgstrom, 1962). Whatever the mechanism, sterile meat does not result and thus strict control must be exercised over the preparation of meat for freezing and over the storage temperature. It is essential to prevent even partial thawing and hence the possibility of microbial growth, in particular of moulds (Gunderson, 1962).

If meat is neither to be frozen nor processed but offered as fresh meat to the consumer, it is more stable under refrigeration in carcass form than cut into smaller units. Cutting distributes bacteria from the dry, fatty, natural surfaces of the meat to freshly exposed muscle surfaces where they proliferate more rapidly. Traditionally, therefore, cutting into retail joints was delayed until the last possible moment in the butcher's shop.

Nowadays, centralized slaughter, buying and preparation offer economic advantages and eliminate the need for skilled butchery at the point of retail sale. Therefore, packaging techniques (see Cavett, 1968 for review) have had to be developed to make possible the breakdown of the carcass at an early stage in its life without the risk of correspondingly early spoilage. However, the retention of moisture is a hindrance in the control of microbial growth (Haines, 1937) but other factors such as permeability of the packaging materials to gases, and in particular to oxygen and carbon dioxide, must be taken into account in assessing the effectiveness of the process.

The microflora of freshly chilled meat consists predominantly of strictly aerobic organisms and if the access of oxygen is controlled a retarding effect upon their growth is to be expected. Nevertheless, it appears that the level of oxygen required to support growth of aerobes is very low, reductions to less than 5 per cent being necessary to affect the growth rate of *Ps. aeruginosa* (King & Nagel, 1967) and c. 1% being capable of supporting aerobic growth on meat (see Ingram, 1962b). In addition, it has long been known that carbon dioxide exerts an inhibitory effect on the rate of growth of many micro-organisms, especially aerobes kept at refrigeration temperatures (Coyne, 1933; Haines, 1937; Kraft & Ayres, 1952), and Ingram (1962b) predicted that the bacterial flora of packed meats was more likely to be influenced by the accumulation of this gas than the restricted access of oxygen. Since the combined respiration of the

meat tissues and the micro-organisms present will reduce the level of oxygen and increase that of carbon dioxide in packaged meats (to an extent dependent upon the permeability of the packaging film to the gases), an effect upon both the rate of growth of the microflora and its composition is to be expected. Several workers have demonstrated the replacement of the *Pseudomonas/Achromobacter* association by facultatively anaerobic types (Jaye, Kittaka & Ordal, 1962; Halleck, Ball & Stier, 1958a, b; Gardner, Carson & Patton, 1967), the precise composition varying both qualitatively and quantitatively. For example, Gardner's group showed that a gas permeable covering had little effect upon the flora of pork stored at 2°C, with the exception of small increases in the proportion of *Microbacterium thermosphactum* and lactobacilli, while an impermeable covering resulted in a large decrease in the proportion of the strict aerobes and large increases in *Microbacterium thermosphactum* and lactobacilli. Incubation at 16°C further modified the picture with larger decreases in the *Pseudomonas/Achromobacter* association and the appearance of *Kurthia spp.* and members of the *Enterobacter-Hafnia* group. Besides studying these flora changes they also analysed the contents of the packages for oxygen and carbon dioxide. With both types of packaging material there was a marked increase in the level of carbon dioxide, the increase being greater for the impermeable membrane. Since there was at least 1% of oxygen left in all cases the microbial changes were attributed to the carbon dioxide concentration. More significant perhaps than these qualitative changes was the increase in storage life of the meat stored at 2°C in the impermeable package, in which microbial counts were lower than in the permeable packs at 2°C or in either type of pack stored at 16°C. The mechanism by which carbon dioxide exerts these effects has not been explained, though one suggestion put forward is that it interferes with dehydrogenase enzyme systems.

Control of spoilage is not the only consideration when fresh meat is packaged, a prime factor being the effect of the process on the colour of the meat. Broadly, fresh meat requires free access of oxygen to maintain its bright red colour whereas cured meats discolour unless oxygen is excluded. One of the earliest reports on the vacuum packaging of fresh meat (Halleck *et al.*, 1958a, b) noted that though the meat darkened in colour, on removal from the pack the normal colour soon returned. Nevertheless, it is assumed that there would be sufficient consumer prejudice to make the vacuum-packaging of fresh meat a doubtfully viable proposition for the retail trade. At wholesale level, the production of prime cuts in vacuo is increasing and, recently, two patent applications have been filed for packing in mixtures of carbon dioxide and oxygen. Here the object is to supply enough carbon dioxide to inhibit sensitive bacteria and sufficient oxygen to maintain the bright red colour of the meat.

The role played in the control of microbial growth by the degree of permeability of the packaging films to water vapour has not been studied for technical reasons, but control of moisture loss is another prime factor in their use. There is at least one report

(Bomar, 1966 cited by Cavett, 1968) that the gas permeable cellulose-based films used for covering small retail cuts of meat can be penetrated by micro-organisms though it is doubtful that this represents a serious contamination problem. Experience with packaged meat, particularly that of Gardner, indicates that the storage life is extended only when strictly hygienic methods of preparation are used and storage is at low temperatures.

The processes described so far have dealt with the storage of carcasses and retail joints of meat in the fresh state. Many meat products are produced by processes involving curing and/or heating, both of which produce flavour and texture changes while at the same time exerting appreciable effects on the types and numbers of micro-organisms.

The use of salt as a means of preserving meats has been practised for thousands of years. Modern curing is an extension of this principle involving the use of sodium chloride, sodium nitrite and potassium nitrate. The process prevents the 'putrefactive' type of spoilage associated with fresh meat, no doubt because of the changes in the microflora caused by the curing salts. The aerobic *Pseudomonas/Achromobacter* group is replaced by a flora consisting mainly of aerobic micrococci with smaller proportions of Gram negative rods and sometimes lactobacilli in small numbers (Kitchell, 1958; Ingram, 1960; Gardner & Patton, 1969). Extensive microbial growth may produce a surface slime on sides of bacon (cf. fresh meat), but without the obvious organoleptic deterioration associated with fresh meat. There may also be an internal development of many thousands per g of similar organisms (Eddy, Gatherum & Kitchell, 1960) depending upon the type of curing procedure used, again without detriment to flavour.

The ultimate flora of bacon reflects the sensitivity of the various bacteria initially present to high concentrations of solutes, micrococci being relatively resistant. Sodium chloride is the main agent, though it may also have another effect by inhibiting microbial metabolism of amino acids. There are indications of this being true for some Gram positive cocci (Tonge, Baird-Parker & Cavett, 1964), and this may be a possible explanation of the relative stability of cured meat in the presence of large numbers of such organisms, even though, as a group, they exhibit strong proteolytic and lipolytic activity when grown at lower salt concentrations (Ingram & Kitchell, 1967). pH influences the action of salt and it has been known for a long time that salt-tolerant organisms from cured meats tend to be unusually sensitive to acidity and those tolerant of acidity are sensitive to salt. Hence the importance of pre-slaughter treatment of pigs intended for bacon production.

The role, if any, of nitrate in spoilage control is not clear, but nitrite at low pH values shows marked antibacterial activity especially against micrococci, undissociated nitrous acid being the active chemical species. This agent is, of course, also a powerful mutagen and an inhibitor of the outgrowth of germinated spores (e.g. Duncan & Foster, 1968; Labbe & Duncan, 1970).

Vacuum packaging of cured meats is often used as a means of preserving the characteristic colour. Under such conditions, as described for fresh meats, oxygen levels are depleted and carbon dioxide levels rise. These factors result in a change in the microflora of the bacon, CO_2-tolerant, facultatively anaerobic lactobacilli increasing rapidly in numbers until they dominate the rest of the flora (Cavett, 1962; Kitchell & Ingram, 1963). Yeasts, when present, follow a similar pattern. Both salt concentration and temperature modify the proportion of each particular group present, high salt concentration favouring the micrococci more than the lactobacilli, and high temperatures resulting in coagulase-negative staphylococci dominating the micrococci (Cavett, 1962; Tonge, Baird-Parker & Cavett, 1964). Similar changes, to a flora dominated by lactobacilli, have been reported for other cured meats stored in vacuum packs, e.g. cooked hams and frankfurters (Alm, Erichsen & Molin, 1961; Shank & Lundquist, 1963) and the overall effect of the process is to extend the storage life of the meat in comparison to the unpacked product. The reasons for the extension of shelf-life are not altogether clear, particularly since the products still contain relatively large numbers of micrococci, but must reflect some change in the metabolic pattern of these organisms, which are presumably growing anaerobically at the expense of nitrate.

Frequently, bacon undergoes a smoking process, which reduces the concentration of available water, thus accentuating the effects of sodium chloride. It also introduces chemical inhibitors to the meat, e.g. volatile acids, phenols and aldehydes. The antimicrobial properties of the latter two (Jensen, 1954) obviously exert an effect, but the reduction of pH by the volatile acids, systematically observed in smoked bacon, in conjunction with the nitrite normally present is thought to be an important influence in vacuum packed bacon (Kitchell, personal communication). For whatever reason, smoking results in a sharp decrease in the numbers of micrococci and Gram negative bacteria (Handford & Gibbs, 1964; Eddy & Ingram, 1962).

High temperatures, as well as refrigeration, are effective in controlling microbial spoilage of meat. In general, the higher the temperature the more effective the treatment. In practice, a compromise has to be accepted because of the deterioration of the organoleptic properties of the meat at high temperatures. Death of the microbes follows a logarithmic course, and thus the effectiveness of the process depends upon the initial load of organisms, i.e. upon the efficiency of any previous treatments given to the meat. The mechanism of heat destruction is widely believed to be a general denaturation of proteins though more specific effects having a lethal effect are more likely, e.g. on DNA (Brannen, 1970). The degree of heat treatment necessary to produce a product both safe from spoilage and from health hazard may vary with environmental conditions. For example, the presence of curing salts allows a shorter heat treatment to be applied without detracting from the safety of the product, the heat in some way sensitizing bacterial spores to the inhibitory effects of the curing salts (Riemann, 1963; Roberts, Gilbert & Ingram, 1966). Nitrite heated in laboratory culture media produces

an extremely potent inhibitor of spores, though the evidence for its production in meat products is not conclusive. If use is made of lower heat processing times, cold storage should be used as an adjunct to delay the growth of surviving bacteria, especially of food poisoning organisms (Ingram, 1964).

Gamma irradiation, like heat, has the ability to destroy both vegetative cells and spores of many micro-organisms and hence is potentially useful for spoilage control. The use of sterilizing doses of radiation (radappertization), unless carried out at freezing temperatures which is extremely costly, produces unacceptable organoleptic changes. However, less severe doses (radurization) resulting in a reduction in numbers of the normal spoilage flora have been shown to extend the shelf-life of fresh and cured meats (Wolin, Evans & Niven, 1957). Again, as with reduced heat treatment, it would be necessary to use conditions such as refrigeration to prevent rapid proliferation of surviving micro-organisms. Moderate doses of radiation make spores of some organisms more susceptible to subsequent heating (see Ingram & Roberts, 1968) and such a process would, in theory, minimize organoleptic changes—though see Hansen (1966). Irradiation, like heat, has been shown to sensitize spores of some clostridial strains to curing salts, e.g. sodium chloride (Roberts, Ditchett & Ingram, 1965), though a report on the effects of irradiation on pork luncheon meat suggested that the treatment reduced the effectiveness of the salts present in preventing growth of *Clostridium botulinum* when the latter was inoculated into the meat (Kempe & Graikoski, 1964).

Evaluation

In the preceding section spoilage has been taken to be the processes leading to the formation of undesirable organoleptic characteristics in or on meat. Obviously it is easy to recognize meat in an advanced stage of spoilage but implicit in the idea of spoilage evaluation is the ability to recognize the incipient stages of the process and hence be able to predict the shelf-life, i.e. time to spoilage. Also implied is the ability to decide upon a value for the property being estimated as the spoilage index, which can be regarded as a cut-off value between acceptable and non-acceptable meat. To satisfy these criteria the property should be measurable with rapidity, reproducibility and preferably without the aid of elaborate, expensive equipment. Correlation with subjective organoleptic properties is also of importance since the latter determine the saleability of the product to the public.

Since micro-organisms are generally regarded as the main causative agents of spoilage the obvious method of evaluation is the enumeration of the total numbers of microbes present. The process requires the use of several different growth media to meet the growth requirements of the various microbial genera and, more important, requires 24–48 hr incubation at the least, to provide meaningful answers. In practice, it is usual to estimate the groups of organisms known to be commonly dominant on the particular type of meat under investigation, e.g. *Pseudomonas/Achromobacter* for refrigerated meats

aerobic micrococci for unpacked cured meats, the assumption being that these are responsible for producing spoilage. That this may not necessarily be true is shown in the work on fish spoilage (e.g. Lerke, Farber & Adams, 1967) where only a small percentage of the total number of pseudomonads present was found to be capable of producing the effects recognized as spoilage in this food. Indirect evidence that a similar situation exists for meat is gained from early workers (see Jensen, 1945) and more recently, for example, from Saffle *et al.* (1961), whose results indicated little correlation between total bacterial numbers and organoleptic properties. Thus, the method has serious disadvantages but is, nevertheless, still much used.

An indirect method of estimating total bacterial numbers is based on an estimation of their overall metabolic activity, e.g. by dye reduction. This depends upon the ability of the microbes present to utilize synthetic dyes, which show colour changes on an alteration in their redox state, as electron and/or hydrogen ion acceptors in place of the physiological acceptors, i.e. oxygen, nitrate, etc. The time taken to effect this change is used as an index of cell numbers and consequently as an estimate of the quality of meat. One would expect such times to vary for different organisms and, moreover, with the physiological state of the cells. There appears to be little information available regarding these questions, so one does not know if the time taken reflects a small number of highly active organisms or a large number of less active ones. In spite of these objections, various workers have reported good correlation between meat quality and the rate of dye reduction—Rogers & McCleskey (1957) using ground meat and methylene blue, Saffle *et al.* (1961) using ground beef and resazurin, and Bradshaw, Dyett & Herschdoerfer (1961) using cooked and cured meats and tetrazolium salts. Although the first and last groups obtained good correlation with bacterial numbers, Saffle *et al.* found that the reduction time correlated better with odour than with total bacterial numbers. The relative rapidity of the methods (a few hours), coupled with ease of performance, makes their use attractive as an indication of the presence of large numbers of micro-organisms. However, what value one assigns as a cut-off, distinguishing between spoiled and unspoiled meats, requires further investigation, as does the use of such methods as a means of predicting shelf-life. Whether the methods are of use for evaluating the spoilage of packaged meats does not appear to have been reported, and in view of the completely different flora from that on carcass meat, it cannot be predicted from published data.

For many years attempts have been made to measure chemical changes in meat and its juices and to correlate these with the degree of bacterial growth and organoleptic properties. Thus, changes in the concentrations of various low molecular weight nitrogen-containing compounds have been studied by a variety of methods, e.g. total non-protein nitrogen (e.g. Ockerman *et al.* 1969), total volatile nitrogen (e.g. Turner, 1960; Pearson, 1968a,c), total α-amino groups (e.g. Saffle *et al.*, 1961; Jay & Kontou, 1964), various individual amino acids (e.g. Gardner & Stewart, 1966a,b), nucleotides

(Jay & Kontou, 1967) and urea (Gardner & Stewart, 1966b). In general, significant changes only occurred at very high microbial populations of the order of $10^8 - 10^9$ organisms/g, or when the meat was already judged to be spoiled, sensorily. Additionally, the changes reported were not always consistent from sample to sample, both overall increases (Turner, 1960; Jay & Kontou, 1967) and decreases (Saffle *et al.*, 1961) having been demonstrated for amino acids for instance. An explanation of these differences is that the meat contained microbial floras with differing biochemical potentialities for metabolizing amino acids. The experiments of Jay & Kontou (1967) with beef, and Adamcic, Clark & Yaguchi (1970) with chicken skin, showing either increases or decreases in amino acids during spoilage, depending upon the type of inoculum used to artificially infect the substrate, support this idea. In view of these discrepancies it is apparent that such measurements do not constitute a basis for a reliable method of predicting spoilage. However, Pearson (1967, 1968a,c) has shown that total volatile nitrogen estimations, which in the case of meat stored at low temperatures is essentially an estimation of ammonia produced, do correlate well with spoilage of beef as judged by odour. He suggests a cut-off value of 20 mgN/100g meat to be used in conjunction with values for free fatty acids and extract-release volume (see below) to assess acceptability. No microbiological data were obtained in this work and it is difficult to reconcile the reported success of the method with other accounts which conclude that it is only an indicator of advanced spoilage, whether judged organoleptically (Turner, 1960) or by bacterial count (Rogers & McCleskey, 1961; Gardner & Stewart, 1966b). There is an obvious need for more work correlating chemical changes of this type with both organoleptic quality and bacterial condition.

Changes in other muscle constituents which could serve as substrates for bacterial growth, e.g. lactic acid, glycogen and glucose, have been reported (Gardner, 1965) for beef but were so haphazard as to be of no use in spoilage evaluation.

The use of pH was proposed many years ago because in meat undergoing aerobic spoilage the pH almost invariably increases to c. 6·5, or above, from its normal post-rigor value of c. 5·5. Again, the correlation between this parameter and bacterial numbers does not appear to be consistent, some authors reporting good agreement (e.g. Rogers & McCleskey, 1961) others, less good agreement (e.g. Gardner, 1965). However, measurement of pH is used as an adjunct to sensory examination in estimating the keeping quality of meat in several European countries, e.g. West Germany and Denmark (see Turner, 1960), values of around 6·5 being regarded as indicative of poor or doubtful quality. Pearson (1968a) suggests a value of 6·0 to be used as a supplement to the other criteria he suggests (see above). With this method, it is obviously essential to know that the meat is in the post-rigor state. Its usefulness lies in the rapidity and ease of performance, plus the fact that it is non-destructive. More recently a titrimetric method to evaluate the spoilage of fresh beef has been described (Shelef & Jay, 1970). The amount of acid required to bring the pH of an homogenate of the meat to 5·0 was

accurately determined and found to increase as spoilage advanced, good correlation being obtained with both bacterial numbers and extract-release volume (see below). One observes that the majority of samples giving titres close to the suggested cut-off value were also characterized by a relatively high pH, and it would appear that the titration was therefore superfluous. Nevertheless, it is possible that the titration method would detect the appearance in meat of substances exhibiting high buffering capacity and produced as a result of protein denaturation or proteolysis. Such compounds would, of course, prevent, or greatly reduce, pH changes and hence their existence might not be detected using the pH value method. However, it is generally accepted that proteolytic changes in meat are not very significant until high bacterial numbers are reached. Again, as in all of these methods mentioned previously, the predictive value of the test as opposed to the diagnostic value is negligible.

A somewhat different suggestion has been the measurement of 'extract-release volume' (ERV) by Jay (1964a). The basis of this procedure is not altogether clear, but it appears to reflect the water-binding capacity of the meat. The test measures the volume of fluid released on filtration by an homogenate prepared under standard conditions. Meat of good organoleptic quality with relatively low numbers of bacteria is said to release large volumes of liquid but spoiled beef only small volumes. Jay found a highly significant correlation between bacterial numbers and ERV. He suggested that meat (ground beef specifically) failing to produce an ERV between 25–30 ml should be regarded as spoiled, and he showed that this value corresponded to a bacterial load of c. $10^{8.5}$/g (Jay, 1964b). The ERV was compared with various organoleptic properties (colour, odour, tackiness) by a taste panel and with a ninhydrin test for α-amino acids (Jay & Kontou, 1964; Kontou, Huyck & Jay, 1966). The results showed that correlation was best between bacterial numbers and organoleptic properties, especially tackiness, but was also sufficiently high between ERV and bacterial numbers to warrant the use of ERV as a rapid test. Borton, Webb & Bratzler (1968) obtained similar results with pork. Later, Jay (1965) studied the relationship between the water-holding capacity of meat (WHC) (e.g. Hamm, 1960) and microbial quality. With both beef and chicken there was a linear decrease in WHC with increase in bacterial numbers, and the author suggested a combination of WHC and ERV as a rapid indicator of meat quality. However, no WHC value was suggested as distinguishing between acceptable and spoiled meat. The exact relation between ERV and WHC is not established, though they may well represent different methods of measuring the same thing. The related phenomenon of meat swelling (SW) which was described by Wierbicki, Tiede & Burrel (1962, 1964) as a method for investigating the water-binding capacity of muscle proteins has also now been shown to correlate closely with ERV and WHC and to be reliable in determining the microbial quality of fresh meat (Shelef & Jay, 1969b). Both ERV and WHC have been shown to be due not to autolysis but to bacterial action by comparing minced beef wrapped loosely in foil either with meat

treated with chlortetracycline or with meat packed in gas-impermeable sterile bags (Jay, 1964b, 1965, 1966b).

Both Jay (1964b) and Borton *et al.* (1968) suggest that the ERV phenomenon is not due to the presence of large numbers of bacteria *per se* but to some bacterial action during growth. Jay (1965) inoculated beef with two different proteinases and showed that this caused a drastic fall in ERV value. He tentatively suggested microbial proteolysis as a cause of increased WHC (decrease in ERV) in spoiling beef, possibly reflecting the unmasking of water-binding groups in the meat proteins, a proposal of Hamm (1960) to explain changes in WHC due to various other factors, e.g. pH. Pearson (1968f), by studying the effect of protein and fat content on the ERV values of fresh beef, concluded that the drop in filtrate volume as spoilage advances is due to changes in protein, which is consistent with the suggestion made above. More recently Shelef & Jay (1969a) stated that the amino sugar content (presumed to be present as polymers) of spoiling beef increased with microbial count and appeared to be responsible for the increase in hydration capacity of spoiled meats. The evidence is far from conclusive, because all bacteria contain a mucopeptide layer in their cell walls, and consequently, amino-sugars like glucosamine, the concentrations of which are therefore likely—with a given spoilage flora—to reflect bacterial numbers and hence ERV (see above).

In summary, although there can be no doubt that the ERV of non-packaged meat does decrease on spoilage, the conclusion of both Jay and Borton, that at the recommended ERV values for discrimination between acceptable and spoiled meat both beef and pork flesh exhibit off odours (and in the latter case sliminess), makes the predictive value of the recommended test seem little better than that of other tests. Also, because bacterial numbers of 400 million/g, which Jay shows correspond to his ERV rejection value in minced beef, exceed slime-producing populations of carcass meat, it is difficult to see what value ERV might have for such meat. Riedel, Burke & Nordin (1967) have shown that the ERV phenomenon in both ground pork and beef is influenced by the type of microbial flora and the changes in pH associated with the storage conditions. For instance, although storage of pork and beef in aluminium foil resulted in a decrease in ERV and growth of a mixed bacterial population, storage under vacuum caused the growth of a predominantly lactic acid bacteria flora without any changes in ERV or pH. There are similar results in some of Jay's work (e.g. Shelef & Jay, 1969a). Furthermore, Riedel *et al.* showed a striking correlation between ERV and pH and the inadequacy of the method for determining the microbial quality of porcine muscle with naturally attained different pH values. Price (1965), however, claimed that the ERV technique appeared to work satisfactorily for estimating the microbial quality of both beef and pork trimmings. There is an obvious need for more experimental data before the usefulness of the method can be fully assessed, though of those described it does appear to be the method of greatest promise.

One would suppose that spoilage of meat would involve breakdown of the fat,

besides the proteinaceous tissue. Indeed, titration of the fatty acids liberated by lipolysis has been used (e.g. in whale oil) as an index of decomposition, and Pearson (1968d) suggested the use of this method in conjunction with other measurements (see above) for assessing the quality of beef. However, the breakdown of fats by microbes is relatively slow so that in raw meat the lean is usually spoiled before fat breakdown becomes noticeable.

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A note on the aerobic microflora of fresh and frozen porcine liver stored at 5°C

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Summary

The microbiological contamination on porcine liver is predominantly on the surface. Storage at 5°C in air resulted in a proteolytic type of spoilage brought about mainly by Gram negative bacteria such as *Alcaligenes* and *Pseudomonas*. Other types such as *Escherichia*, *Microbacterium thermosphactum*, and lactic streptococci were also found.

When stored at 5°C in polythene bags, the level of contamination was much higher in the 'drip', and this appears to be related to the load on the surface of the liver. The souring type of spoilage could be attributed to the predominance in the flora of lactic streptococci and *Leuconostoc*.

Freezing was not found to change the spoilage characteristics.

Introduction

There is currently *c.* 45 ton of liver produced weekly from pigs slaughtered in N. Ireland, most of which is frozen prior to distribution and sale. The 'pluck', which includes the trachea, lungs, heart and liver, is removed from the pig in one piece on the slaughterline. It is then subjected to veterinary examination and may be washed prior to chilling, divided into the four anatomical parts, the livers being packed in polythene bags for freezing in cartons.

There appears to be no published data on the nature of the spoilage microflora, and the work described in this paper is concerned primarily with this aspect. Comparative studies were made between frozen and unfrozen livers stored either in air or in polythene bags at 5°C.

Materials and methods

Livers

All the livers were obtained from one factory and transported to the laboratory in insulated boxes, arriving within 45 min of removal from the pigs. In each of two experi-

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ments livers from the same batch were divided and tested as follows:

(a) Unfrozen.

(b) 'Hot' frozen. The livers in polythene bags were frozen in cartons in a blast freezer operating at an air temperature of −32·2°C and an airspeed of 3·5 m/sec.

(c) 'Chilled' frozen. The livers were chilled overnight at 5°C before freezing as in (b), except that the air temperature was −27·7°C.

In both (b) and (c) the average liver temperature was reduced to −17·7°C in 14–19 hr. After 13 days storage at −17·7°C the livers were defrosted for 2 days at 5°C before an experiment was started.

For each storage experiment, two livers were held in a tray under aerobic conditions and two were stored in a polythene bag, where most but not all of the air was excluded. All livers were stored at 5°C for 7 days.

Bacteriological analyses

With aseptic precautions, 10 cm^2 from the surface of each of the two livers in any one treatment were removed and bulked. The two surface samples were shaken with 20 ml 0·1% (w/v) peptone water for 3 min on a mechanical shaker. From livers in a polythene bag, 1 ml of drip was taken and added to 9 ml of the same diluent. Serial dilutions were plated out by the surface technique of Davis & Bell (1959) on a total count medium (TCM) (Gardner, 1968). Colonies were enumerated on the plates after incubation at 22° for 5–7 days.

The selection and identification of isolates from the total count plates is described elsewhere (Gardner, 1968).

Results and discussion

Preliminary investigation on methodology for the microbiological examination of liver

An experiment was carried out to determine the differences in the numbers and types of micro-organisms on the surface or in the deep tissue of liver. Samples (5 g) of 'deep tissue' were exercised from the liver, using a technique (Gardner & Carson, 1967) which negated any transfer contamination from the surface. All samples were plated out in duplicate for both aerobic and anaerobic incubation. The results of investigations on two fresh livers are given in Table 1. The aerobic counts differed little from the anaerobic counts. The deep tissue samples had a much lower level of contamination than the surface samples. It is difficult to compare counts related to weight with counts related to area. However, the mean weight of 288 surface samples each of 10 cm^2 was 5·2 g. Thus 1 cm^2 of tissue would be approximately equivalent to 0·5 g. Flora analysis indicated that the types of bacteria on the aerobic plates were similar in both sites.

The results of the same livers after storage for 7 days at 5°C are given in Table 2.

Aerobic counts were higher than anaerobic counts on the surface of livers stored aerobically. Aerobic counts on deep tissue samples and surface samples of livers stored in a

TABLE 1. Microflora of fresh porcine liver

Liver	Sample site	Total count at 22°C* Anaerobic	Aerobic	No. of isolates	Flavo-bacteria	Coryne-form bacteria	Flora analysis of aerobic plates Incidence % of *Micro-coccus*	Lactic strepto-cocci	*Leucon-ostoc*	*Acineto-bacter*	*M.thermo-sphactum*	Unclass-ified
A	Surface	65×10^3	87×10^3	14	21	14	29	7	7	14	0	7
	Deep tissue	$2{\cdot}4 \times 10^2$	$1{\cdot}7 \times 10^2$	17	29	23	12	6	23	6	0	0
B	Surface	46×10^3	41×10^3	20	30	0	55	10	5	0	0	0
	Deep tissue	$0{\cdot}7 \times 10^2$	$2{\cdot}2 \times 10^2$	20	25	25	25	10	0	0	5	10

* Surface counts: /cm². Deep tissue counts: /g.

TABLE 2. Microflora of porcine livers stored for 7 days at 5°C

Storage condition	Liver	Sample site	Total viable count at 22°C* Anaerobic	Total viable count at 22°C* Aerobic	No. of isolates examined	Flora analysis of aerobic plates Incidence (%) of *Pseudomonas*	*Escherichia*	Lactic streptococci	*Leuconostoc*	*M. thermosphactum*	Miscellaneous
Aerobic	A	Surface	28×10^6	78×10^6	20	75	10	15	0	0	0
		Deep tissue	26×10^3	44×10^3	20	0	0	85	15	0	0
	B	Surface	24×10^7	46×10^7	17	0	12	65	6	18	0
		Deep tissue	$7{\cdot}6 \times 10^6$	$10{\cdot}6 \times 10^6$	18	0	0	100	0	0	0
Polythene bag	A	Surface	$17{\cdot}5 \times 10^6$	$18{\cdot}9 \times 10^6$	19	0	0	47	53	0	0
		Deep tissue	45×10^3	51×10^3	20	0	0	75	10	0	15
	B	Surface	$27{\cdot}7 \times 10^6$	$24{\cdot}2 \times 10^6$	19	0	0	68	21	5	5
		Deep tissue	$3{\cdot}6 \times 10^6$	$3{\cdot}6 \times 10^6$	20	0	0	100	0	0	0

* Surface counts: /cm². Deep tissue counts: /g.

polythene bag were, if anything, only slightly higher. In one of the livers (A) the level of contamination in the deep tissue was lower than that on the surface, but the difference was less marked on liver B. Analysis of the flora on the aerobic plates showed that surface spoilage in air was due to the growth of *Pseudomonas*, *Escherichia*, *Microbacterium thermosphactum*, and lactic streptococci. The types found in deep tissue samples and on surface samples of bagged liver were mainly lactic streptococci and *Leuconostoc*.

Thus it would appear that surface sampling coupled with aerobic incubation of plates gives the highest counts.

The microflora of refrigerated frozen and unfrozen liver

The numbers and types of bacteria on frozen and unfrozen livers stored at 5°C for 7 days were examined, and the results of the two experiments are combined and given in Table 3. The fresh livers had a similar flora to that found in the preliminary study (Table 1). After 7 days the microbial load on the livers stored in air exceeded $10^9/cm^2$; colonies were visible on the surface of the liver. The spoilage flora was primarily composed of *Alcaligenes*, *M. thermosphactum* and lactic streptococci. There was no appreciable difference in the numbers and types of spoilage organisms between frozen and unfrozen liver.

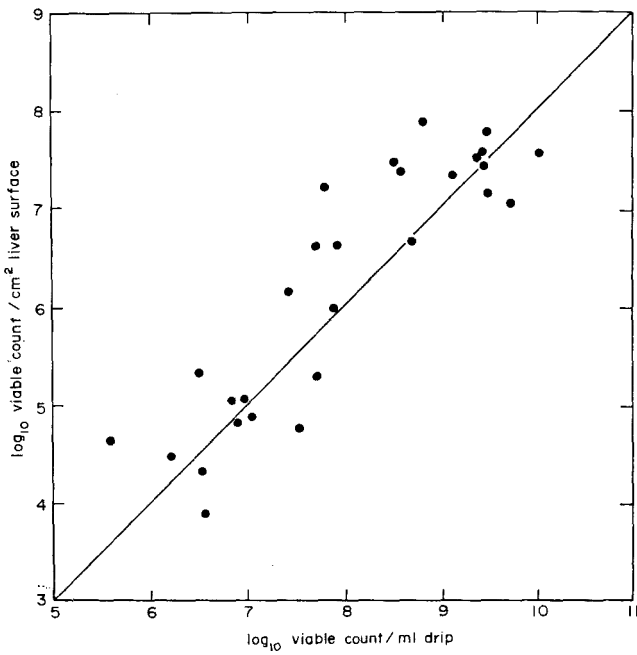

FIG. 1. Relationship between the total microbial load in the drip and on the surface of frozen and unfrozen liver stored at 5°C in polythene bags.

TABLE 3. Numbers and types of bacteria on frozen and unfrozen porcine liver stored at 5°C for 7 days

Storage condition	Treatment	Mean viable count/cm²	Total no. isolates examined	Analysis of the flora Incidence (%) of								
				Alcaligenes	Lactic streptococci	*Leuconostoc*	*M. thermosphactum*	*Acinetobacter*	*Micrococcus*	Coryneform bacteria	Flavobacteria	Unclassified
None	Fresh	$66{\cdot}5 \times 10^3$	81	1	12	5	4	7	9	49	11	1
Aerobic	Unfrozen	$18{\cdot}3 \times 10^8$	41	73	7	0	7	10	2	0	0	0
	'Hot' frozen	$23{\cdot}2 \times 10^8$	41	73	12	0	7	5	0	0	0	2
	'Chilled' frozen	$41{\cdot}6 \times 10^8$	42	43	9	2	38	0	0	0	0	7
Polythene bag	Unfrozen	$24{\cdot}0 \times 10^6$	38	13	60	26	0	0	0	0	0	0
	'Hot' frozen	$17{\cdot}1 \times 10^6$	38	10	58	18	7	3	0	0	0	3
	'Chilled' frozen	$29{\cdot}0 \times 10^6$	36	14	64	14	8	0	0	0	0	0

In contrast to the proteolytic type of spoilage found on the livers stored in air, those stored in polythene bags exhibited a spoilage of a 'souring' nature. The flora was mainly composed of lactic streptococci and *Leuconostoc* with lower proportions of *Alcaligenes* and *M. thermosphactum*. Again, freezing did not affect the spoilage characteristics. Generally speaking most meats, when stored in low oxygen tensions, spoil due to the growth and activities of the facultative anaerobic lactic acid bacteria (Ingram, 1962), and pork liver does not seem to be an exception.

As in each bag of livers there was 4–6% (w/w) liquid, the system is biphasic, i.e. the liver and the drip. The relationship between the microbial counts in the drip and the counts on the surface of corresponding livers is shown in Fig. 1. Although there is considerable variation and also assuming that all the organisms on the surface of liver are in the drip, the slope of the line would indicate that there is *c.* 0·01 ml drip/cm^2 of liver or that there are *c.* 100 times more organisms in 1 ml drip than on 1 cm^2 liver surface. Hence it would be expected that the drip would show signs of gross spoilage before the liver, and this probably contributes to the fact that these livers (Table 3) were spoiled with a relatively low level of surface contamination.

These experiments show that porcine liver stored at 5°C is spoiled in 7 days. When stored in air, a proteolytic type of spoilage is associated with a microflora dominated by *Alcaligenes* and *Pseudomonas*, whereas in a polythene bag the souring type of spoilage is associated with a predominance in the flora of lactic streptococci and *Leuconostoc*.

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The bacteriology of 'scampi' (*Nephrops norvegicus*). III. Effects of processing

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Summary

The effects of handling and processing on the bacteriology of *Nephrops-norvegicus* has been examined in five processing factories. Production of frozen 'scampi' and frozen breaded scampi were examined along with, in one case, the bacteriological aspects of automated polyphosphate treatment.

Apart from some contamination during hand peeling, there was no significant increase or decrease in the numbers of any of the various types of bacteria during processing. Studies of the spoilage flora developing during ice storage show that, in common with other fishery products, bacteria of the *Pseudomonas-Achromobacter* group predominate.

Introduction

Most of the scampi landed in the UK are marketed as frozen tail meats. Another product is coated with butter and breadcrumbs before a final freezing; another, for overseas markets, is left in the shell and then frozen.

Essentially, processing consists of the removal, in one operation, of the head, claws and thorax, followed by removal of the tail meat from the shell, then deveining, i.e. removal of the gut, and freezing of the individual meats. In practice, there are many variations in the details of processes (Early, 1965). Throughout there is considerable manual handling of the product, though machines are gradually being introduced for all stages of processing.

The important aspects of processing from the bacteriological viewpoint are the effects of ice storage, the effects of freezing, thawing and re-freezing and the general hygiene of the process.

The bacteriology of spoilage of fish and fishery products was reviewed by Shewan & Hobbs (1967) and work on the spoilage flora of crustacea has been published by Harris (1932), Tobin & McCleskey (1941), Alford, Tobin & McCleskey (1942), Green (1949), Campbell & Williams (1952), Puncochar & Pottinger (1954), Fellers,

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Gagnon & Khutchikian (1956), Kachikian, Fellers & Litsky (1959), Sreenivasan (1959) and Early (1967).

It is clear from the published work that spoilage of fishery products at ice temperatures generally results from the growth of Gram negative bacteria of the *Pseudomonas* and *Achromobacter* genera, though at higher temperatures the Gram positive coryneforms and *Micrococci*, along with some *Enterobacteriaceae* contribute significantly. This situation is presumably a result of the ability of the *Pseudomonas-Achromobacter* genera to grow actively in ice whereas the other genera grow poorly or not at all at this temperature (Torry Research Station, unpublished data).

The effects of freezing on the spoilage flora of fishery products were reviewed by Shewan & Hobbs (1967). They concluded that 60–90% of the population was killed: the Gram positive flora surviving better than the Gram negative flora. Storage of fish in the frozen state resulted in a slow decline in the number of viable organisms present.

The bacteriology of *Nephrops* when newly landed at the ports has been described in Parts I & II (Walker *et al.*, 1970; Cann *et al.*, 1971). In the present investigation the starting material was *Nephrops* examined on arrival at the processing factory or taken from bulk chill or cold stores and thawed out for processing. Because of the complexity of the marketing and distributing system it was not practicable to follow individual consignments of *Nephrops* from the catching vessels to the processing factories.

Materials and methods

Five processors of *Nephrops*, situated in different areas of Britain, were visited during the period December 1968 to September 1969 and an examination of the various stages of processing was made. Individual processing methods varied; an outline of the two major lines follows.

A. Frozen scampi processing

1. Shell-on *Nephrops* tails are taken into the processing line from either frozen or iced batches. Factories A and C used *Nephrops* frozen in the shell, factories B and E used iced *Nephrops* and factory D used both. Running tap water was used both for thawing of frozen *Nephrops* and for removal of ice from iced batches.

2. Grading for size and weighing in lots for issue to individual peelers.

3a. Peeling by hand or blowing out the meats with compressed air, followed by deveining by hand and washing in water.

3b. Large *Nephrops* with shell on, for overseas markets, are washed and then deveined by machine.

4. Grading by hand and check weighing for purposes of estimation of yield, peelers' bonus, etc.

5. Treatment with polyphosphate solution by either dip or spray.

6. Freezing by either air blast or liquid nitrogen.

7. Glazing with water.
8. Weighing and packing by hand.

B. Breaded scampi processing

1. Frozen scampi are coated with batter and breaded by either hand or machine. Some processors repeat this process after an intermediate freezing.
2. Freezing by air blast.
3. Weighing and packing by hand.

Samples for analysis were taken from each stage of these two lines. Wherever possible, two or three peelers were chosen at random and each was given a batch of *Nephrops*, large enough to provide samples throughout the processing line. An attempt was made to follow each experimental batch by tagging, but, due to conditions prevailing in a busy processing plant, this was not always possible. In addition similar samples were carefully peeled by hand by the authors and returned to the processing line.

The two following stages were examined in more detail.

Automated polyphosphate treatment

Most processors treat *Nephrops* with polyphosphate solution, either by spraying or hand dipping. A machine has recently been designed to automate, standardize and improve this procedure. The construction and usage of this machine is described elsewhere (Wignall, Potter & Windsor, 1970). Essentially, the *Nephrops* are passed through a tank of refrigerated polyphosphate solution on a continuous conveyor belt so that the concentration and time of immersion can be carefully controlled. This machine was installed in one factory and during its operation a detailed bacteriological examination of the treatment was carried out.

On each of 2 days four batches of *Nephrops* were passed through the machine and samples were examined at 2-hr intervals both before and immediately after treatment. In addition samples of the solution in the tank were taken. On the third day the entire production of the factory was treated in the machine, and three batches were examined at intervals. Each batch was divided into two, half passing through the machine and the other half being treated with polyphosphate by the usual factory method. Each batch was examined before treatment, immediately after treatment and again as the finished, packaged product. Samples of the solution in the tank were also examined.

Ice storage of Nephrops

Three batches each of two stones of *Nephrops* tails caught within the previous 12 hr, were iced in boxes and stored for 10 days. Total viable counts were carried out at intervals during the storage time; detailed examination of the bacterial flora was carried out on the fresh and 10 day stored samples.

One hundred gram amounts of peeled *Nephrops* were taken for each bacteriological examination. The methods used have been described by Cann *et al.* (1971).

Results

The results of the examination of factory processing are presented in Tables 1–8. Total viable counts at 20°C and 37°C did not show any significant increases throughout processing except for some initial contamination at the peeling stage in each case.

The coliform, faecal coli and faecal streptococci counts are not correlated, when one is high the other two are not necessarily so. For instance in factory B coliforms and faecal coli were generally relatively high whereas the faecal streptococci were very low; in factory D the reverse was found. Similarly a high coliform count does not necessarily mean a high faecal coli count. However, all the counts are generally lower than the existing specified standards for fishery products (Shewan, 1970).

Staphylococcus aureus was frequently demonstrated in samples taken at all stages of processing and occasionally in numbers exceeding the existing standards for fishery products. This almost certainly reflects human handling and even when samples were carefully peeled and washed by ourselves this organism was frequently found.

Clostridium welchii was, for the most part, only found by enrichment techniques and even then in relatively few samples. In factory D it was not found at all.

Salmonella and *Clostridium botulinum* were not found in any of the samples.

Automated polyphosphate treatment

The polyphosphate solution in the dipping tank rapidly acquired a total bacterial load equivalent to that of the *Nephrops* passing through it. Subsequently the load did, however, remain at the same level throughout use on each of the three days. There was no build up of pathogens in the solution; *S. aureus* being demonstrated only once and *Cl. welchii* twice; *Salmonella* was not found.

As far as bacterial load is concerned, samples of *Nephrops* treated with polyphosphate by either the usual factory method or the automated dipping procedure were not significantly different at any stage of subsequent processing. The greater uptake of polyphosphate by the *Nephrops* in the automated process did not affect the bacterial load of the finished packaged product.

Iced storage of Nephrops

The results of the total viable counts and the flora analyses are presented in Table 9. In two of the experiments there was little difference in the numbers of bacteria growing at 37°C throughout the storage period but in the *Nephrops* from Eyemouth there was an increase of almost three orders of magnitude. Increases of the same order occurred in the counts at 20°C from all three storage experiments.

After storage for 10 days the predominant organisms found were *Pseudomonas*, especially groups III and IV.

Of the food poisoning bacteria, *Salmonella* and *Cl. botulinum* were not found and *Cl. welchii* was demonstrated by enrichment in only two samples. *S. aureus* was demon-

TABLE 1. Total viable counts of *Nephrops* processed at Factory A

Handled by	Peeled 'prawns'		Polyphosphate sprayed 'prawns'		Blast frozen 'prawns'		Glazed 'prawns'		Packed scampi	
	37°C	20°C	37°C	20°C	37°C	20°C	37°C	20°C	37°C	20°C
TRS staff	$4{\cdot}26 \times 10^3$	$6{\cdot}78 \times 10^4$								
Processor 1	$4{\cdot}11 \times 10^5$	$5{\cdot}04 \times 10^5$	$3{\cdot}00 \times 10^5$	$7{\cdot}23 \times 10^5$	$3{\cdot}42 \times 10^5$	$7{\cdot}74 \times 10^5$	$5{\cdot}46 \times 10^5$	$1{\cdot}77 \times 10^6$	$5{\cdot}52 \times 10^5$	$8{\cdot}88 \times 10^5$
Processor 2	$7{\cdot}23 \times 10^4$	$2{\cdot}82 \times 10^5$	$2{\cdot}58 \times 10^5$	$4{\cdot}71 \times 10^5$	$5{\cdot}43 \times 10^5$	$9{\cdot}00 \times 10^5$	$2{\cdot}94 \times 10^5$	$7{\cdot}14 \times 10^5$	$3{\cdot}81 \times 10^5$	$6{\cdot}09 \times 10^5$
Processor 3	$3{\cdot}36 \times 10^5$	$4{\cdot}95 \times 10^5$	$3{\cdot}72 \times 10^5$	$5{\cdot}01 \times 10^5$	$8{\cdot}28 \times 10^5$	$1{\cdot}77 \times 10^6$	$4{\cdot}71 \times 10^5$	$1{\cdot}47 \times 10^6$	$1{\cdot}01 \times 10^6$	$9{\cdot}42 \times 10^5$

TABLE 2. Bacteriological examination of *Nephrops* processed at Factory B

		Count at 37°C/g	Count at 20°C/g	*S. aureus*/g	*Cl. welchii*/g	Coli-forms	Faecal coli	Faecal strepto-cocci
						Most probable numbers/g		
Samples taken Day 1								
Peeled *Nephrops*								
TRS	1	$2{\cdot}2 \times 10^6$	$3{\cdot}36 \times 10^6$	4×10^2	present in 0·5 g	> 72	9	0·44
TRS	2	$7{\cdot}84 \times 10^4$	$2{\cdot}16 \times 10^6$	8×10^2	present in 0·5 g	14	1·4	0·16
Processor	1	$1{\cdot}14 \times 10^6$	$1{\cdot}02 \times 10^7$	absent	absent	> 72	> 72	4·4
Processor	2	$4{\cdot}76 \times 10^5$	$7{\cdot}52 \times 10^6$	absent	absent	> 72	> 72	0·68
Polyphosphate treated *Nephrops*								
Processor	1	$1{\cdot}00 \times 10^6$	$1{\cdot}16 \times 10^7$	absent	absent	> 72	> 72	> 72
Processor	2	$1{\cdot}39 \times 10^6$	$7{\cdot}88 \times 10^7$	absent	absent	> 72	> 72	0·8
Frozen *Nephrops*								
Processor	1	$3{\cdot}52 \times 10^6$	$8{\cdot}4 \times 10^6$	absent	absent	> 72	> 72	64
Processor	2	$8{\cdot}4 \times 10^6$	$1{\cdot}22 \times 10^6$	absent	absent	> 72	> 72	7
Double glazed *Nephrops*								
Processor	1	$2{\cdot}6 \times 10^6$	$8{\cdot}32 \times 10^6$	absent	present in 0·5 g	> 72	> 72	10
Processor	2	$3{\cdot}56 \times 10^6$	$8{\cdot}48 \times 10^6$	absent	absent	> 72	> 72	10

Packaged *Nephrops*								
Processor	1	$4{\cdot}04 \times 10^6$	$1{\cdot}25 \times 10^7$	absent	absent	> 72	> 72	14
Processor	2	$3{\cdot}36 \times 10^6$	$8{\cdot}0 \times 10^6$	4×10^2	absent	> 72	> 72	8
Samples taken Day 2								
Peeled *Nephrops*								
TRS	1	$2{\cdot}6 \times 10^6$	$8{\cdot}48 \times 10^5$	absent	present in 0·5 g	> 72	5·2	0·08
TRS	2	$1{\cdot}92 \times 10^5$	$6{\cdot}4 \times 10^5$	absent	absent	> 72	1·4	1·4
Processor	1	$1{\cdot}28 \times 10^6$	$3{\cdot}36 \times 10^6$	4×10^2	absent	> 72	> 72	5·6
Processor	2	$3{\cdot}44 \times 10^5$	$2{\cdot}64 \times 10^6$	absent	absent	72	22	64
Processor	1a	$7{\cdot}12 \times 10^5$	$7{\cdot}68 \times 10^6$	absent	absent	> 72	> 72	5·2
Processor	2a	$5{\cdot}96 \times 10^5$	$2{\cdot}52 \times 10^6$	absent	absent	> 72	> 72	7
Polyphosphate treated *Nephrops*								
Processor	1	$5{\cdot}8 \times 10^5$	$2{\cdot}64 \times 10^6$	absent	absent	> 72	64	2
Processor	2	$4{\cdot}6 \times 10^5$	$7{\cdot}32 \times 10^5$	absent	absent	> 72	> 72	9
Glazed *Nephrops*								
Processor	1	$2{\cdot}24 \times 10^6$	$4{\cdot}08 \times 10^6$	present in 0·5 g	absent	> 72	64	1·8
Processor	2	$6{\cdot}4 \times 10^5$	$1{\cdot}10 \times 10^6$	absent	absent	> 72	> 72	3·8
Packaged *Nephrops*								
Processor	1	$1{\cdot}49 \times 10^6$	$6{\cdot}32 \times 10^6$	absent	present in 0·5 g	> 72	> 72	1·6
Processor	2	$4{\cdot}84 \times 10^5$	$4{\cdot}24 \times 10^6$	absent	present in 0·5 g	> 72	> 72	> 72
Frozen packed *Nephrops*								
TRS and Processor								
Combined		$8{\cdot}68 \times 10^5$	$5{\cdot}56 \times 10^6$	absent	absent	> 72	> 72	22
in freezer		$8{\cdot}2 \times 10^5$	$8{\cdot}36 \times 10^6$	absent	present in 0·5 g	> 72	> 72	> 72

TABLE 3. Bacteriological examination of breaded scampi processed at Factory B

	Count at 37°C/g	Count at 20°C/g	*Cl. welchii*/g	Coliforms (Most probable numbers/g)	Faecal coli (Most probable numbers/g)	Faecal streptococci (Most probable numbers/g)
Samples taken Day 1						
Frozen *Nephrops*	$4{\cdot}32 \times 10^6$	$9{\cdot}8 \times 10^6$	present in 0·5/g	> 72	> 72	11
Battered *Nephrops*	$7{\cdot}28 \times 10^6$	$8{\cdot}8 \times 10^6$	absent	> 72	17	14
Battered and breaded *Nephrops*	$6{\cdot}08 \times 10^6$	$8{\cdot}72 \times 10^6$	present in 0·5/g	> 72	> 72	8
Finished breaded scampi	$3{\cdot}08 \times 10^6$	$8{\cdot}88 \times 10^6$	absent	> 72	> 72	> 72
Fresh batter	$1{\cdot}12 \times 10^5$ ml	$1{\cdot}8 \times 10^5$/ml	present in 2/ml	18/ml	0·25/ml	1·75/ml
Batter from machine	$2{\cdot}08 \times 10^6$ ml	$2{\cdot}09 \times 10^6$/ml	absent	> 18/ml	> 18/ml	2.75/ml
Samples taken Day 3						
Frozen *Nephrops* from Day 2	$2{\cdot}8 \times 10^7$	$1{\cdot}78 \times 10^7$	absent	> 72	> 72	64
Battered *Nephrops*	$3{\cdot}28 \times 10^6$	$1{\cdot}0 \times 10^7$	present in 0·5/g	> 72	> 72	10
Battered and breaded *Nephrops*	$2{\cdot}72 \times 10^6$	$8{\cdot}6 \times 10^6$	present in 0·5/g	> 72	> 72	0·56
Fresh batter mix (after 1½ hr use of mixer)	$6{\cdot}02 \times 10^6$/ml	$6{\cdot}91 \times 10^6$/ml	present in 2/ml	> 18/ml	0·7/ml	0·25/ml
Fresh batter mix (after 7 hr use of mixer)	$3{\cdot}6 \times 10^3$/ml	$9{\cdot}8 \times 10^3$/ml	present in 2/ml	> 18/ml	0·09/ml	2·5/ml
Batter from machine (after 1½ hr use)	$3{\cdot}22 \times 10^6$/ml	$3{\cdot}51 \times 10^6$/ml	absent	> 18/ml	> 18/ml	> 18/ml
Batter from machine (after 7 hr use)	$1{\cdot}75 \times 10^6$/ml	$3{\cdot}8 \times 10^6$/ml	present in 2/ml	> 18/ml	> 18/ml	> 18/ml
Fresh breadcrumbs from sack	uncountable	uncountable	present in 0·5/g	> 72	nil	nil
Breadcrumbs from machine (1½ hr use)	$7{\cdot}36 \times 10^4$	$1{\cdot}02 \times 10^5$	absent	> 72	2	17
Breadcrumbs from machine (7 hr use)	$1{\cdot}00 \times 10^5$	$5{\cdot}32 \times 10^5$	absent	> 72	2	> 72

TABLE 4. Bacteriological examination of *Nephrops* processed at Factory C

	Count at 37°C/g	Count at 20°C/g	*S. aureus*/g	*Cl. welchii*/g	Coliforms	Faecal coli	Faecal streptococci
					Most probable numbers		
Samples taken Day 1							
Peeled *Nephrops*							
Sample A TRS	$4{\cdot}32 \times 10^6$	$3{\cdot}72 \times 10^6$	4×10^1	absent	> 72	1·4	3·2
Sample A Processor	$1{\cdot}25 \times 10^7$	$1{\cdot}48 \times 10^7$	absent	absent	> 72	6·8	2
Sample B TRS	$3{\cdot}92 \times 10^6$	$1{\cdot}64 \times 10^6$	present in 0·5 g	absent	> 72	7	5·2
Sample B Processor	$7{\cdot}76 \times 10^6$	$6{\cdot}32 \times 10^6$	present in 0·5 g	absent	> 72	14	4·4
Graded *Nephrops*							
Sample A Processor	$3{\cdot}48 \times 10^6$	$4{\cdot}8 \times 10^6$	present in 0·5 g	absent	> 72	1·4	4·4
Sample B Processor	$5{\cdot}04 \times 10^6$	$7{\cdot}08 \times 10^6$	present in 0·5 g	present in 0·5 g	14	2	3·2
Frozen *Nephrops*							
Sample A Processor	$2{\cdot}2 \times 10^6$	$2{\cdot}56 \times 10^6$	4×10^2	absent	14	1·4	3·8
Sample B Processor	$6{\cdot}04 \times 10^6$	$5{\cdot}12 \times 10^6$	present in 0·5 g	absent	4·4	0·56	3·8
Glazed *Nephrops*							
Sample A Processor	$3{\cdot}96 \times 10^6$	$4{\cdot}6 \times 10^6$	absent	present in 0·5 g	36	0·8	4·4
Sample B Processor	$8{\cdot}04 \times 10^6$	$8{\cdot}6 \times 10^6$	present in 0·5 g	present in 0·5 g	14	0·32	5·6
Packed *Nephrops*							
Sample A Processor	$4{\cdot}6 \times 10^6$	$1{\cdot}00 \times 10^7$	absent	present in 0·5 g	28	2	3·2
Sample B Processor	$8{\cdot}88 \times 10^6$	$1{\cdot}53 \times 10^7$	absent	absent	32	1·6	2·2

continued overleaf

TABLE 4. Bacteriological examination of *Nephrops* processed at Factory C—*continued*

	Count at 37°C/g	Count at 20°C/g	*S. aureus*/g	*Cl. welchii*/g	Coliforms	Faecal coli	Faecal streptococci
					Most probable numbers		
Samples taken Day 2							
Peeled *Nephrops*							
Sample C TRS	$1{\cdot}96 \times 10^5$	$5{\cdot}44 \times 10^5$	absent	present in 0·5 g	0·8	0·8	0·52
Sample C Processor	$9{\cdot}00 \times 10^5$	$3{\cdot}44 \times 10^6$	absent	absent	5·2	nil	0·28
Sample D TRS	$4{\cdot}92 \times 10^5$	$8{\cdot}76 \times 10^5$	absent	absent	0·8	nil	2
Sample D Processor	$1{\cdot}16 \times 10^6$	$2{\cdot}16 \times 10^6$	absent	absent	6·8	nil	0·56
Graded *Nephrops*							
Sample C Processor	$2{\cdot}24 \times 10^6$	$5{\cdot}88 \times 10^6$	absent	absent	220	1·6	0·2
Sample D Processor	$2{\cdot}73 \times 10^6$	$8{\cdot}61 \times 10^6$	present in 0·43 g	present in 0·43 g	80	0·94	1·18
Frozen *Nephrops*							
Sample C Processor	$2{\cdot}00 \times 10^6$	$3{\cdot}64 \times 10^6$	absent	present in 0·5 g	18	0·8	0·08
Sample D Processor	$3{\cdot}84 \times 10^6$	$5{\cdot}92 \times 10^6$	absent	present in 0·5 g	14	0·8	+ 14
Glazed *Nephrops*							
Sample C Processor	$4{\cdot}72 \times 10^5$	$2{\cdot}2 \times 10^6$	4×10^2	4×10^1	6·8	1·4	9
Sample D Processor	$7{\cdot}6 \times 10^5$	$2{\cdot}6 \times 10^6$	absent	present in 0·5 g	22	0·16	36
Packed *Nephrops*							
Sample C Processor	$1{\cdot}84 \times 10^6$	$3{\cdot}04 \times 10^6$	absent	absent	22	1·8	22
Sample D Processor	$1{\cdot}48 \times 10^6$	$3{\cdot}64 \times 10^6$	absent	absent	36	0·32	36

TABLE 5. Bacteriological examination of breaded scampi processed at Factory C

	Count at 37°C/g	Count at 20°C/g	*S. aureus*/g	*Cl. welchii*/g	Coliforms	Faecal coli	Faecal streptococci
					Most probable numbers/g		
Frozen scampi							
Sample 1	$1{\cdot}16 \times 10^7$	$2{\cdot}58 \times 10^7$	absent	absent	> 72	> 72	> 72
Sample 2 – 3 hr later	$9{\cdot}56 \times 10^6$	$1{\cdot}95 \times 10^7$	4×10^2	absent	> 72	3·2	64
Floured scampi							
Sample 1	$8{\cdot}56 \times 10^6$	$1{\cdot}44 \times 10^7$	absent	absent	> 72	> 72	> 72
Sample 2 – 3 hr later	$9{\cdot}2 \times 10^6$	$1{\cdot}67 \times 10^7$	absent	present in 0·5 g	> 72	14	36
Batter coated scampi							
Sample 1	$6{\cdot}2 \times 10^6$	$6{\cdot}48 \times 10^6$	4×10^2	present in 0·5 g	> 72	> 72	64
Sample 2 – 3 hr later	$8{\cdot}32 \times 10^6$	$1{\cdot}66 \times 10^7$	absent	present in 0·5 g	> 72	36	> 72
Breaded scampi							
Sample 1	$5{\cdot}84 \times 10^6$	$1{\cdot}41 \times 10^7$	absent	absent	> 72	> 72	36
Sample 2 – 3 hr later	$7{\cdot}04 \times 10^6$	$9{\cdot}08 \times 10^6$	absent	present in 0·5 g	> 72	9	> 72
Packaged scampi							
Sample 1	$1{\cdot}02 \times 10^7$	$2{\cdot}5 \times 10^7$	present in 0·5 g	present in 0·5 g	> 72	> 72	> 72
Sample 2 – 3 hr later	$7{\cdot}56 \times 10^6$	$8{\cdot}76 \times 10^6$	present in 0·5 g	present in 0·5 g	> 72	17	10
Breadcrumbs							
after 3 hr use	$5{\cdot}32 \times 10^5$	$9{\cdot}04 \times 10^5$	present in 0·5 g	present in 0·5 g	> 72	0·2	14
Batter after 3 hr use	$1{\cdot}22 \times 10^6$	$2{\cdot}73 \times 10^6$	absent	absent	> 18/ml	16/ml	> 18/ml

TABLE 6. Bacteriological examination of *Nephrops* processed at Factory D

		Count at 37°C/g	Count at 20°C/g	*S. aureus*/g	*Cl. welchii*/g	Coliforms	Faecal coli	Faecal streptococci
						Most probable numbers/g		
Samples taken Day 1								
Peeled *Nephrops*								
TRS peeled and deveined	1	$4{\cdot}00 \times 10^5$	$5{\cdot}84 \times 10^6$	4×10^2	absent	2	Nil	1·8
TRS peeled and deveined	2	$9{\cdot}04 \times 10^5$	$4{\cdot}4 \times 10^6$	absent	absent	6·8	0·08	3·2
Processor peeled and deveined	1	$2{\cdot}8 \times 10^6$	$6{\cdot}88 \times 10^6$	absent	absent	1·6	0·08	2·8
Processor peeled and deveined	2	$1{\cdot}39 \times 10^6$	$5{\cdot}2 \times 10^6$	present in 0·5 g	absent	2·2	0·2	6·8
Graded *Nephrops*								
Processor	1	$5{\cdot}8 \times 10^6$	$1{\cdot}26 \times 10^7$	absent	absent	36	Nil	> 72
Processor	2	$4{\cdot}72 \times 10^6$	$2{\cdot}44 \times 10^6$	absent	absent	> 72	0·44	> 72
Frozen *Nephrops*								
Processor	1	$1{\cdot}55 \times 10^7$	$1{\cdot}72 \times 10^7$	absent	absent	36	0·28	> 72
Processor	2	$1{\cdot}12 \times 10^7$	$1{\cdot}41 \times 10^7$	absent	absent	14	0·08	> 72
Glazed *Nephrops*								
Processor	1	$8{\cdot}24 \times 10^6$	$8{\cdot}6 \times 10^6$	4×10^1	absent	36	Nil	> 72
Processor	2	$7{\cdot}08 \times 10^6$	$1{\cdot}1 \times 10^7$	absent	absent	14	0·2	> 72
Finished product								
TRS	1	$4{\cdot}28 \times 10^6$	$6{\cdot}96 \times 10^6$	4×10^2	absent	> 72	Nil	5·2
TRS	2	$1{\cdot}38 \times 10^7$	$1{\cdot}27 \times 10^7$	present in 0·5 g	present in 0·5 g	> 72	Nil	36
Processor	1	$7{\cdot}68 \times 10^6$	$9{\cdot}08 \times 10^6$	present in 0·5 g	absent	64	0·08	> 72
Processor	2	$1{\cdot}87 \times 10^7$	$1{\cdot}40 \times 10^7$	present in 0·5 g	absent	36	0·08	> 72

Samples taken Day 2							
Peeled *Nephrops*							
Cold-stored stock							
TRS peeled and deveined	$6{\cdot}16 \times 10^4$	$2{\cdot}56 \times 10^5$	absent	absent	1·8	Nil	0·2
Processor peeled and deveined	$5{\cdot}52 \times 10^5$	$7{\cdot}00 \times 10^5$	8×10^2	absent	36	Nil	5·2
New stock							
TRS peeled and deveined	$8{\cdot}08 \times 10^4$	$5{\cdot}28 \times 10^5$	4×10^1	absent	1·2	0·08	36
Processor peeled and deveined	$2{\cdot}52 \times 10^6$	$3{\cdot}4 \times 10^6$	absent	absent	> 72	Nil	36
Graded *Nephrops*							
Cold-stored stock } Processor	$8{\cdot}16 \times 10^6$	$1{\cdot}15 \times 10^7$	present in 0·5 g	2×10^2	> 72	Nil	36
New stock } Processor	$3{\cdot}28 \times 10^6$	$5{\cdot}6 \times 10^6$	4×10^2	absent	64	Nil	> 72
Frozen *Nephrops*							
TRS	$1{\cdot}3 \times 10^7$	$1{\cdot}44 \times 10^7$	absent	absent	2·2	Nil	36
Cold-stored stock } *P*rocessor	$7{\cdot}84 \times 10^6$	$1{\cdot}22 \times 10^7$	4×10^2	absent	36	Nil	0·56
New stock } *P*rocessor	$4{\cdot}00 \times 10^6$	$4{\cdot}52 \times 10^6$	present in 0·5 g	absent	10	0·32	1·4
Glazed *Nephrops*							
Cold-stored stock } Processor	$7{\cdot}2 \times 10^6$	$9{\cdot}92 \times 10^6$	4×10^2	absent	> 72	Nil	1·4
New stock } Processor	$3{\cdot}88 \times 10^7$	$2{\cdot}44 \times 10^7$	present in 0·5 g	absent	10	Nil	64
Finished product							
TRS	$1{\cdot}17 \times 10^7$	$1{\cdot}50 \times 10^7$	absent	absent	2·8	Nil	1·4
Cold-stored stock } Processor	$6{\cdot}6 \times 10^6$	$1{\cdot}1 \times 10^7$	present in 0·5 g	absent	17	Nil	3·8
New stock } Processor	$2{\cdot}34 \times 10^7$	$2{\cdot}44 \times 10^7$	absent	absent	22	Nil	1

TABLE 7. Bacteriological examination of breaded scampi processed at Factory D

	Count at 37°C/g	Count at 20°C/g	*S. aureus*/g	Coliforms	Faecal coli	Faecal streptococci
				Most probable numbers/g		
Samples taken Day 1						
Frozen scampi						
Sample 1	$1{\cdot}54 \times 10^{7}$	$3{\cdot}00 \times 10^{6}$	present in 0·5 g	64	Nil	64
Sample 2	$1{\cdot}78 \times 10^{9}$	$1{\cdot}52 \times 10^{7}$	present in 0·5 g	64	Nil	36
Batter coated *Nephrops*						
Sample 1	$7{\cdot}04 \times 10^{6}$	$1{\cdot}15 \times 10^{7}$	absent	14	0·2	36
Sample 2	$6{\cdot}8 \times 10^{6}$	$1{\cdot}2 \times 10^{7}$	absent	22	Nil	6·8
Powder coated *Nephrops*						
Sample 1	$9{\cdot}92 \times 10^{6}$	$1{\cdot}76 \times 10^{7}$	absent	> 72	0·2	2·2
Sample 2	$1{\cdot}09 \times 10^{7}$	$1{\cdot}59 \times 10^{7}$	absent	22	Nil	22
Finished breaded product						
Sample 1	$5{\cdot}2 \times 10^{6}$	$8{\cdot}8 \times 10^{6}$	present in 0·5 g	64	Nil	22
Sample 2	$8{\cdot}00 \times 10^{6}$	$1{\cdot}08 \times 10^{7}$	absent	64	Nil	> 72
Samples taken Day 2						
Frozen *Nephrops*						
Sample 1	$1{\cdot}45 \times 10^{7}$	$1{\cdot}83 \times 10^{7}$	present in 0·5 g	22	Nil	36
Sample 2	$1{\cdot}34 \times 10^{7}$	$2{\cdot}79 \times 10^{7}$	present in 0·5 g	> 72	Nil	3·2
Finished breaded product						
Sample 1	$9{\cdot}64 \times 10^{6}$	$1{\cdot}37 \times 10^{7}$	absent	64	0·08	> 2·2
Packaged breaded product						
Sample 1	$2{\cdot}73 \times 10^{7}$	$2{\cdot}74 \times 10^{7}$	4×10^{1}	36	0·08	> 72

TABLE 8. Bacteriological examination of *Nephrops* processed at Factory E

		Count at 37°C/g	Count at 20°C/g	*S. aureus*/g	*Cl. welchii*/g	Coliforms	Faecal coli	Faecal streptococci
						Most probable numbers/g		
Peeled *Nephrops*								
TRS	1	$4{\cdot}3 \times 10^4$	$1{\cdot}25 \times 10^6$	nil	nil	5·5	nil	1
	2	$7{\cdot}1 \times 10^4$	$8{\cdot}00 \times 10^6$	present in 0·4 g	nil	2·5	nil	0·4
	3	$5{\cdot}5 \times 10^5$	$1{\cdot}49 \times 10^7$	nil	present in 0·67 g	1·75	nil	0·25
Processor	1	$3{\cdot}00 \times 10^5$	$5{\cdot}1 \times 10^6$	nil	nil	> 90	nil	1·5
	2	$1{\cdot}11 \times 10^6$	$1{\cdot}35 \times 10^7$	present in 0·4 g	nil	80	nil	2·5
	3	$4{\cdot}00 \times 10^5$	$2{\cdot}1 \times 10^6$	5×10^2	nil	> 90	nil	0·65
Sorted *Nephrops*		$1{\cdot}33 \times 10^6$	$2{\cdot}42 \times 10^6$	$1{\cdot}5 \times 10^3$	present in 0·4 g	8·75	0·1	0·65
Frozen *Nephrops*		$3{\cdot}1 \times 10^6$	$6{\cdot}05 \times 10^6$	1×10^3	nil	> 90	nil	1·75
Single glazed *Nephrops*		$3{\cdot}9 \times 10^6$	$8{\cdot}3 \times 10^6$	$1{\cdot}5 \times 10^3$	nil	80	nil	0·65
Triple glazed and packaged *Nephrops*		$8{\cdot}8 \times 10^5$	$3{\cdot}95 \times 10^6$	present in 0·4 g	nil	21·25	nil	0·85

TABLE 9. Bacteriological examination of *Nephrops* stored in ice

Port of landing	Days storage in ice	Total viable count/g		*Pseudomonas*				Percentage numbers of bacteria									
								Aeromonas	*Achromobacter*	*Acinetobacter*	*Enterobacter*	*Micrococcus*	*Staphylococcus*	Coryneform	*Bacillus*	*Cytophaga-Flavobacterium*	No growth
		37°	20°	I	II	III	IV										
Buckie	0	$7{\cdot}56 \times 10^4$	$3{\cdot}08 \times 10^5$	8	3	12	2	1	24	2		2	2	26		17	1
	1	$1{\cdot}48 \times 10^5$	$6{\cdot}12 \times 10^5$														
	3	$3{\cdot}72 \times 10^3$	$1{\cdot}06 \times 10^6$														
	6	$4{\cdot}28 \times 10^3$	$9{\cdot}36 \times 10^5$														
	8	$4{\cdot}24 \times 10^4$	$6{\cdot}16 \times 10^7$														
	10	$4{\cdot}24 \times 10^4$	$2{\cdot}2 \times 10^8$	2	5	59	16		13	1		2				2	
Eyemouth	0	$7{\cdot}68 \times 10^3$	$4{\cdot}12 \times 10^4$		9	7	2	1	12	8	3	10	2	30	3	13	
	1	$4{\cdot}20 \times 10^3$	$5{\cdot}08 \times 10^4$														
	3	$1{\cdot}22 \times 10^4$	$5{\cdot}68 \times 10^4$														
	6	$1{\cdot}47 \times 10^5$	$1{\cdot}91 \times 10^6$														
	8	$1{\cdot}26 \times 10^6$	$8{\cdot}8 \times 10^6$														
	10	$3{\cdot}32 \times 10^6$	$3{\cdot}48 \times 10^7$		18	52	10	7	8				1			3	1
North Shields	0	$2{\cdot}96 \times 10^4$	$1{\cdot}28 \times 10^5$						26	10	2	13	19	23		7	
	1	$4{\cdot}36 \times 10^3$	$3{\cdot}24 \times 10^4$														
	4	$3{\cdot}48 \times 10^3$	$6{\cdot}00 \times 10^4$														
	6	$3{\cdot}96 \times 10^3$	$4{\cdot}88 \times 10^6$														
	8	$3{\cdot}64 \times 10^3$	$4{\cdot}56 \times 10^7$														
	11	$5{\cdot}72 \times 10^3$	$8{\cdot}84 \times 10^7$			66	15		9	1	1			3			5

strated in the 10 day iced sample from Eyemouth at a level of 40/g and in every sample taken during ice storage of the Buckie *Nephrops*, in two by enrichment and in three in countable numbers, the highest count being 400/g. Numbers of coliforms, faecal coli and faecal streptococci were low throughout storage; the highest figures were coliform 36/g, faecal coli < 1/g and faecal streptococci < 2/g.

Discussion

Comparison of data from five factories showed little variation in the degree of contamination during processing. In general, the total viable counts remained unchanged during processing in individual factories and where increases did occur, they were usually less than one order of magnitude. *Nephrops* peeled carefully by ourselves generally had lower bacterial counts than those peeled in factories, indicating that there is an initial contamination which could be reduced by more hygienic handling.

The initial bacteriological quality of the *Nephrops* varied considerably and those with higher counts probably reflected the distances travelled from the port of landing and the extra handling involved. Some processors receive local and distant supplies of *Nephrops* which can be processed successively on the same day, giving widely differing results and making firm conclusions difficult. Nevertheless, the distance of processors B and C from the main ports of landing is reflected in the generally higher figures for total viable counts and coliform organisms in their supplies.

It has been shown that the majority of newly caught *Nephrops* carry total viable counts at 20°C and 37°C of 10^4/g and 10^3/g respectively, and are relatively free from organisms of public health significance. Many of the samples tested in the present work carried initial loads of 10^6–10^7/g at 37°C. Ice storage experiments at Torry Research Station have repeatedly shown that bacterial loads of this order are rarely reached after 10 days ice storage, which is beyond the period of edibility of *Nephrops* (Walker *et al.*, 1970). It appears, therefore, that the high bacterial loads found on these *Nephrops* on arrival at the factories must represent heavy contamination during the procedures of landing, sale at markets and transport rather than spoilage.

Freezing of shell-on *Nephrops* prior to processing is advantageous both from the point of view of subsequent removal of the shell and elimination of the inevitable bacterial growth during the 'conditioning' period in ice. This advantage is lost if the *Nephrops* have been some time in transit or if heavy contamination occurs. On no occasion, however, was any significant bactericidal effect noted after freezing of the *Nephrops* at any stage during processing.

Use of polyphosphate did not affect the bacterial load when applied by either hand or machine. In the study of automated polyphosphate treatment, it was found that the solution in the tank became a pinkish, muddy brown colour and its appearance was aesthetically undesirable. Therefore, although the process was shown to be bacteriologically satisfactory, it is desirable that the machine should be stopped, cleaned and

fresh polyphosphate used at intervals during the day. The precise details of the process would have to take into account all aspects of quality control, economics and the advantages gained in increased weight or yield compared with the hand polyphosphating procedures commonly in use.

The predominance of *Pseudomonas* strains after 10 days ice storage suggests that the flora changes in scampi are similar to those for most marine white fish. It is apparent however from the preliminary work on scampi (Walker *et al.*, 1970), that in some circumstances *Achromobacter* strains can predominate.

Although, in the UK, legislation regarding food processing is not detailed and does not impose precise standards, there is a growing concern among processors about quality and quality control procedures. In international trade detailed legislation and standards are becoming more common, and it is evident that bacteriological standards will be increasingly applied to products such as 'scampi'.

It has been pointed out by Shewan (1970) that existing standards for fishery products are largely based on analogy with other protein foods, and this is a wholly unsatisfactory situation. Details of some existing standards for fishery products are given by Shewan (1970). Total viable counts at 35–37°C are usually $\nearrow 10^4$–10^6/g, a level of $\nearrow 10^5$/g being the commonest; the level of coliforms is $\nearrow$ 10–100/g; *Escherichia coli* $\nearrow$ 10/g; *Enterococci* $\nearrow 10^3$/g; *Staphylococcus aureus* absent to $\nearrow 10^4$/g and *Salmonella* should be absent in a given sample size. These standards apply both to cooked and uncooked products.

From the data presented here it is clear that in the case of scampi processing one or other of these figures is frequently exceeded. For example, as far as total viable counts at 37°C are concerned, only those *Nephrops* processed within 12 hr of catching would meet a standard of $\nearrow 10^5$/g. It is equally clear that, apart from some slight contamination at the peeling stage, the processing itself does not contribute a significant level of contamination. Since 'scampi' is not a cooked product the only aspect of processing which might have been expected to reduce the bacterial load is freezing and thawing The results presented here show that in practice no significant reduction does in fact occur.

If the data on freshly landed *Nephrops* (Cann *et al.*, 1971) are compared with the figures obtained at the beginning of each processing line, it is probable that between landing and arrival at the processors a significant increase occurs both in total bacterial counts and in numbers of bacteria of public health significance. The experiments concerned with spoilage of *Nephrops* in ice show that the count at 20°C rises throughout, but that at 37°C does not necessarily rise at all. In addition, the organisms of public health significance do not grow at ice temperatures. The increase in bacterial load could, therefore, be the result of either storage at too high a temperature or contamination. In a few cases organoleptic assessment of the *Nephrops* indicated that some spoilage had occurred but it was usually known that they had been in ice for some days.

It is suggested, therefore, that the increased bacterial load is predominantly the result of contamination during handling and transportation rather than of spoilage. It should be stressed that a total count of 10^5/g at 37°C does not necessarily indicate a spoiled product or represent a health hazard. Indeed, this figure appears to represent a count which is typical of even good quality scampi produced under existing commercial conditions. It has been stated (Hobbs, 1970) that in the absence of a sound basis for specific bacteriological standards, the only rational basis must be the levels which can be achieved by good hygienic handling, assuming there is no apparent health risk.

The data presented here indicate that the kinds of bacteriological standard mentioned are not realistic for this type of product, especially as 'scampi' is not marketed as a cooked product. On the other hand, the data do suggest that the bacteriological quality of this product could be improved by more hygienic handling. Bacteriological tests are primarily a test of the efficiency of quality control procedures and the practical achievement of optimum processing conditions will only result from continued bacteriological testing and collaboration of quality controllers with everyone concerned from the fishermen to the processors.

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Practical applications of an accelerated stability test to rancidity problems in food processing

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Summary

An oxygen absorption test carried out at 100°C has been used to investigate rancidity problems occurring during processing and storage of foods. A relationship between induction period and flavour deterioration was established for fried foods. In the case of potato crisps the stability test was used to develop plant operating conditions which ensured sufficient shelf-life. Other applications of the test include the effect of artificial light and of emulsifier addition on fats and the comparison of antioxidants.

Introduction

The reaction of oxygen with the unsaturated fatty acid components of triglyceride oils is a two stage one in which tasteless hydroperoxides are first formed, and then decomposed in a second step to give low molecular weight oxygenated products, often with rancid flavours. It is well established that the reaction rates of the three main unsaturated acids present, oleic, linoleic and linolenic acids, with oxygen are very different. The decomposition of the hydroperoxides is known to be catalysed by small traces of metals, which are not completely eliminated in the refining process on the one hand or are quite likely to be introduced during food manufacture on the other hand. On decomposition of hydroperoxides free radicals are formed, which promote further reaction with oxygen.

If natural or synthetic phenolic antioxidants are present, they will remove the free radicals and stop the chain reaction.

The food processor needs to be able to predict when the overall effect of these chemical reactions will produce rancid flavours in his products. A number of tests have been listed (Hoffman, 1970) in which oxygen uptake is accelerated, normally by the use of high temperature, sometimes also by elevated pressure or by exposure to light. These tests usually measure an induction period before rapid oxidation takes place.

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Experimental

This paper describes a number of practical applications of one such test, developed in these laboratories by Sylvester, Lampitt & Ainsworth (1942) and used routinely since then, in which the rate of absorption of pure oxygen at 100°C is measured manometrically. Publications describing essentially similar equipment have appeared at intervals since then, for example, Lancaster, Bitner & Beal (1956), Parsons (1968). The original Sylvester apparatus was modified by Martin (1961). The present results were obtained with this modification, but with a rubber and polythene connecting tube between the flask and the manometer in place of Martin's glass connections, and using a water-cooled condenser.

Results

1. *Relation of induction period to storage*

Samples were stored under the conditions described in Table 1 and tested at intervals of about 4 weeks. The tests were continued for 4–6 months. Table 1 gives the mean loss of induction period per week of storage.

TABLE 1. Loss of induction period with storage

Product	Loss (hr/week)	Storage conditions
Packaged		Room temperature
Shortening A	0·17	Daylight
B	0·24	,,
C	0·31	,,
Fried potato crisps	0·23	Room temperature Darkness
Beef fat flakes		
Opaque package	0·25	Room temperature
Mean	0·24	

The loss of induction period is approximately 0·25 hr per week, independent of product. A faster rate was obtained for shortening C where the package permitted some daylight to penetrate. In the shortenings some flavour deterioration occurred on the surface before the induction period was reduced to nil.

2. *Applications to fried products*

Under conditions of static heating an induction period of 10 hr at 100°C would be equivalent to a few minutes at 180°C. At frying temperature (180–200°C) normal rancid flavours either do not develop or are rapidly distilled out of the system. They develop, however, when the product is stored subsequently.

This point is illustrated by a study made of a potato crisp product while it was being manufactured on the pilot plant scale using a batch fryer. The crisps contained about 25% oil and 3% moisture. It was possible therefore, to obtain oxygen absorption curves directly on the product. Table 2 shows the results of tests made on five separate samples of frying oil, directly on potato crisps sampled at the same time, and on the crisps after storage as indicated.

TABLE 2. Storage of potato crisps induction period (hr)

Age	0 weeks		4 weeks	15 weeks	Decrease per week
Sample	Oil	Crisps	Crisps	Crisps	
	0·25	1	NIL*	NIL*	
	4·5	5	—	2·5	0·17
	7	7	—	3·5	0·23
	4	9	—	6	0·20
	7	10	—	5	0·33
				Mean	0·23

* Rancid flavour

Crisps fried in oil having an induction period of only ¼ hr, developed rancid flavours after 4 weeks storage. No other samples developed rancid flavours during the above test period. It was concluded from these tests that, to obtain a product with a reliable shelf life of 12 weeks, a minimum induction period of 3 hr was required in the fresh product and in the oil used for frying. In order to provide a safety margin and to allow for the observed variability a quality standard of 4 hr was set. This standard was only achieved after modifications of the frying process, leading to adequate control. The effect of the several steps taken is summarized in Table 3.

A study of the thermograph charts (see Fig. 1) indicated the reason for variable results. The induction period of samples of oil is written on the charts against the time at which the samples were taken. It will be seen that with intermittent operation, stoppages resulted in a loss of induction period, since the oil was static at a relatively high temperature and was therefore reacting with oxygen fairly rapidly. During actual frying there was no loss of induction period. This was due to two effects, firstly the

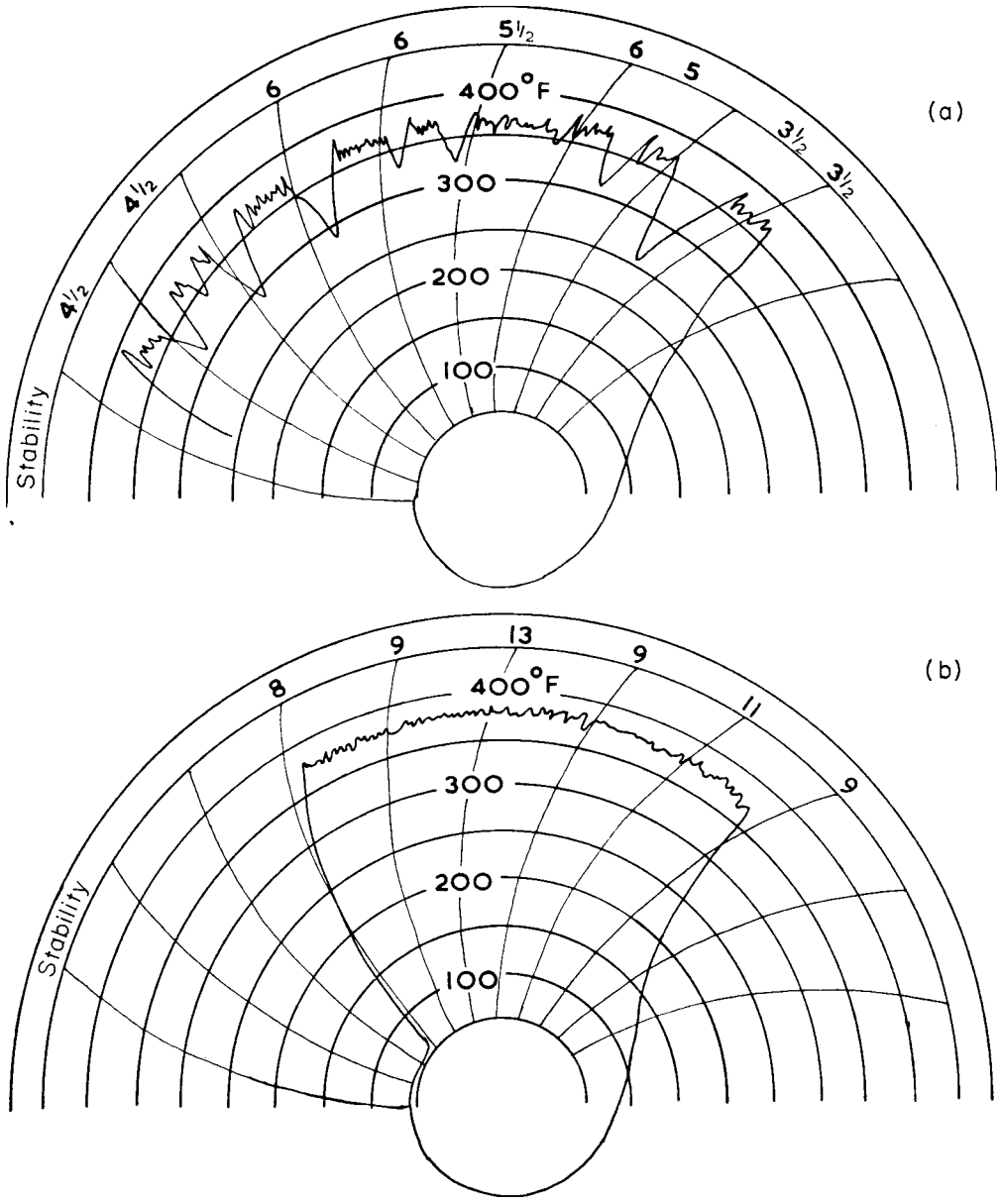

FIG. 1. Thermograph charts from batch fryer (a) discontinuous operation, (b) continuous operation.

TABLE 3. Effect of operating conditions in fryer

Condition of frying	Induction period of oil (hr)	% Rejects	Frying time % of total
Before Investigation	0–8	50	—
Oil Level Controlled	4–7½	0	—
Oil Level Controlled Antioxidant added	3–12	4	—
As above Thermograph fitted	½–12	7	50–70
As above continuous frying	8–13		96–100

blanketing action of the steam generated, and secondly the regular addition, by way of a constant level device, of unused oil containing antioxidant. Satisfactory results were maintained only when truly continuous operation was achieved by providing relief operators during meal and rest breaks.

A correlation between flavour and induction period was also established with fried fish fingers. The product consisted of slices of cod, coated in bread crumbs, fried in ground nut oil and deep frozen. In this instance it was not possible to determine the induction period on the product itself. Instead the test was performed on oil extracted from the product with petroleum ether 40–60°C. The solvent was removed under a stream of oxygen-free nitrogen. Table 4 shows the results obtained on oil extracted

TABLE 4. Storage of fish fingers

Storage period (months)	Induction period of oil (hr) Initial	Final	Flavour
3	0·75	NIL	Rancid, bitter
7	0·5	< 0·5	Poor
3	0·75	< 0·5	Bitter
0·75	—	0·5	Poor
6	1·5	1·5	Fair
6	1·5	1·0	Fair
7	—	1·0	Fair
0·75	—	1·3	Satisfactory

from the product at the time of manufacture and after varying storage periods, and of taste panel tests made on the stored product.

It will be noted that there is little loss of induction period during deep frozen storage. Off-flavour development however does occur and is related to the induction period of the oil as used for frying.

Frying was being carried out in a continuous fryer of about 300 lb capacity. Samples taken during one day's operation (Table 5) showed that a fairly stable equilibrium condition had been reached during continuous operation. On other days some loss of induction period occurred as a result of shorter production runs. In consequence a minimum quality standard of 1 hr for the induction period and a maximum of 1·5% (as oleic acid) for the free fatty acid content was introduced and oil was discarded outside these limits.

TABLE 5. Short term tests of frying oil

Frying time (hr)	Induction period of oil (hr)	Acidity (as oleic acid)
0	0·8	0·85
½	1·1	0·71
1	1·2	1·14
2	1·2	1·14
4	2·0	0·93
5	1	1·14
7½	1	1·14
9½	1·5	1·0

3. *Processing problems*

(a) *Fat in cake icings.* A small proportion of fat is often incorporated in cake icings to improve their texture. The occurrence of rancid flavour in the icings led to an investigation of the method of incorporation of the fat. It was found that fat was being kept molten in a kettle for excessive periods and the kettle was being replenished before it was empty. Table 6 gives the results of the stability test on fat samples taken during the process and from the finished icings.

In the original process each step led to a substantial loss in induction period. Improvement of the process led to a more satisfactory situation without rancid flavours.

In more complicated processes it is useful to test fat from samples taken at each step

TABLE 6. Fat in cake icings

	Induction period (hr)	
	Batch 1 Original process	Batch 2 After process improved
In drum	24	26
Melted in kettle	10	28
From finished icings	NIL	12

and to investigate in detail parts of the process where big losses in induction period occur.

(b) *Effect of artificial light on fats.* In connection with the planning of a processing area for fats, a comparison was made of the effect of tungsten filament bulbs and 'daylight' fluorescent tubes on four fats (see Table 7). In each case a 5 mm layer of sample in a shallow dish was exposed to an intensity of illumination of 20-ft candles and a similar control sample was stored in the dark.

TABLE 7. Effect of illumination on fats

Sample	Hr illuminated	Induction Period (hr) Control	Fluorescent	Filament	Loss of induction period (hr)
Lard	18	2·7	2·4	—	0·3
		2·7	—	2·5	0·2
Hardened whale oil		30	14	—	16
(40–42°C)	70	30	—	23	7
Teaseed oil	114	9·4	8·1	—	1·3
		9·4	—	9·1	0·3
Groundnut oil	70	12·5	11·6	—	0·9
		11·4	—	10·7	0·7

In order to obtain measurable effects with this illumination long exposure times were needed, and it was concluded that for practical purposes neither type of lighting would cause significant deterioration during processing. From the point of view of longer term storage, e.g. retail shelf life, the reduction in induction period observed after 70–100 hr is very significant, and illustrates the need to protect fats from light during storage.

The largest effect obtained was with hardened whale oil and in this instance the fluorescent light had a significantly greater effect.

(c) *Solutions of emulsifiers in fats.* In manufacturing processes where both fats and emulsifier are used it is often desirable to dissolve the emulsifier in the fat and to store the solution as a liquid for a limited period. In this context the possible effects of the emulsifier on fats was studied. In the first place the induction periods of numerous samples of palm kernel oil with and without 3% of glyceryl monostearate (GMS) were determined. The results are summarized in Table 8 where the range of induction periods of the samples is given, together with the % loss in induction period in the presence of the emulsifier. Samples from five different suppliers are coded A to E.

TABLE 8. Effect of GMS on palm kernel oil

Emulsifier sample	Number of oil samples	Range of initial Induction Period (hr)	Range of % loss
A (1)	5	42 to 48	26 to 57
A (2)	4	19 to 51	39 to 74
B	2	39 to 40	NIL
C	1	38	21
D	3	11 to 16	90
E	1	16	6

Only samples B and E could be regarded as presenting no risk in use; sample D had a very marked pro-oxidant effect and its use in manufacture would have presented a serious risk of rancid flavours in the final product.

In a further test series three commercial samples of fats in normal condition were used as substrates to examine the effect of 5% emulsifiers of different types on induction period (Table 9). The letters (F) to (J) are used to indicate different manufacturers, the numbers (1) and (2) indicate different samples from the same manufacturer. The substrates, with their initial induction periods, were palm oil (26 hr), rapeseed oil (6·6 hr) and beef fat (4·3 hr).

TABLE 9. Effect of emulsifiers on induction period

Emulsifier		% Change in induction period Palm oil	Rapeseed oil	Beef fat
Soap containing GMS		—	−39	−52
Soap free GMS	(F)	Nil	−15	−35
,, ,, ,,	(G)	−17	−7	Nil
,, ,, ,,	(H)	−46	−35	−66
Polyglycerol esters	(I)	0	−62	+150
,, ,,	(J)	—	−12	+33
P.G. Esters of oxidized acids		−28	−12	+116
Glyceryl lactopalmitate		Nil	−57	−64
Sorbitan esters	(1)	+10	−50	—
	(2)	+12	−12	+230
Polyoxyethylene sorbitan esters	(1)	−6	−15	−49
	(2)	−60	−39	−30

Changes in the initial induction period greater than 20% are underlined. It will be noted that beef fat is more sensitive in this respect than the vegetable oils, but effects on these are also quite marked.

The causes of these effects are the subject of current investigation. At present it can only be postulated that the losses of induction period observed are due to pro-oxidants formed during manufacture of the emulsifiers, and that the antioxidant effects obtained are due to traces of citric or phosphoric acids or other residual reagents which act as sequestrants. Chemical antioxidants are absent.

4. *Response to antioxidants*

The stability test is of value as a method for the selection of the most appropriate

antioxidant. This is illustrated by the results in Table 10, which cover the use of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and octyl gallate (OG).

TABLE 10. Antioxidants in fats

Sample	Antioxidant	Level % 0	0·001	0·01	0·02	Protection factor at 0·01%	0·02%
Beef	BHA	5	—	96	170	19·2	34
	BHT	3	12	28	—	9·3	
	OG	6·5	15	64	—	10	
Pork	BHT	2		19		9·5	
Chicken	BHA	0·25			14		56
Cottonseed oil	BHA	7·5			7·5		1·0
	BHT				10·5		1·4
	OG				10·5		1·4
Groundnut oil	BHA	12			17		1·4
	BHT	12			23		1·9
	OG	12		24		2·0	
Vegetable shortening	BHA	26			43		1·7
	BHT	22			36		1·6
	OG	25		52		2·1	

In this table a protection factor has been calculated by dividing the induction period of the protected fat by the initial induction period. The protection obtained when antioxidants are added to vegetable fats is an order of magnitude smaller than that obtained with animal fats.

Discussion and conclusions

Accelerated stability tests for fats are of the greatest value in those applications where the correlation with room temperature storage and with off-flavour development has been established. Two such applications of the 'Sylvester' test are described in this paper.

The test is also of value in applications where such correlations have not been fully demonstrated. Thus the test is useful in the investigation of the effects of factory processes on fats because it enables conditions to be specified which maximize induction

period and therefore minimize the risks of the development of rancid flavours. The use of the test for the routine examination of incoming supplies of oils has made it possible in many cases to arrive at a range for the normal induction period of a particular type of oil. Large departures from this range can justifiably be regarded as the result of abnormal treatment before delivery, and therefore as requiring more detailed investigation. Other forms of accelerated test can also be used for these purposes, the 'Sylvester' test is however in the author's experience the simplest to perform, the total operating time required to test four samples being about 1 hr.

Acknowledgment

The author wishes to acknowledge the valuable guidance obtained from Mr N. D. Sylvester in the course of some of the investigations reported and the technical assistance of Mr P. Judge.

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Studies on the quality characteristics of canned grapefruit segments. III. Addition of surfactant agents during the lye-peeling process to improve their quality*

A. LEVI, ESTHER HERSHKOWITZ AND A. LUDIN

Summary

Lye peeling of grapefruit segments greatly facilitates the removal of the carpellary membranes, but causes a decrease in the yield and quality of the whole peeled segments. The HCl treatment used in some industrial plants not only does not improve the quality, but usually causes a decrease in the yield or in the quality. The use of 'wetting' agents, such as Faspeel and Tergitol, helps to reduce the temperature or concentration of the lye solution, thereby bringing about an increase in yield and an improvement in the canned segments' quality.

Introduction

Citrus carpellary membranes in general, and grapefruit membranes in particular, are composed mainly of pectic materials (Webber & Batchelor, 1948; Braverman, 1949). The segments' (carpellary) membranes must be removed during industrial preparation of 'canned grapefruit segments' in order to prevent bitterness (Braverman, 1949) as well as to make the product more easily edible. After removal of the peel and hand segmentation, the segments' membranes are removed manually or more usually by dipping the segments in hot lye solution of about 2–3% NaOH for 10–20 sec, as described by Ludin *et al.* (1969) for grapefruit and by Suryaprakase *et al.* (1969a,b) for orange segments.

The lye peeling process is the most common one, but it may damage the segments severely—as a result of deep penetration of the lye and the high temperatures used in the process. Some producers in Japan use milder conditions, mainly for orange, which incorporates the use of HCl for membrane removal (Suryaprakase, 1969c). These treatments require much more time for removal of the segment membranes and the process hence becomes too expensive, since it is too slow for the modern citrus industry.

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In previous works we studied the factors affecting the quality and means of improving the quality of the canned grapefruit segments (Ludin *et al* 1969; Levi *et al.* 1969). The treatments and additives used improved mainly the drained weight and the textural properties of the segments. The lye-peeling affects mainly the yield, by increasing the peeling losses, but it also affects the quality of the product. Lye-peeling is likely to cause softening of the segments and to increase the quantity of broken segments in the can, particularly at the end of the commercial season, when the segments are already soft and easily broken.

The use of 'wetting agents', such as those employed in peeling tomatoes (Juven, Samish & Ludin, 1969), might reduce the ill-effects of the lye-peeling process, thus raising the yield and quality of canned grapefruit segments.

Materials and methods

Canned grapefruit segments were prepared from fresh grapefruits (variety Marsh Seedless) in A2 cans as described in the first paper of this series (Ludin *et al.* 1969), except for the conditions for removal of the carpellary membranes, which varied according to the requirements of each experiment. When the HCl treatment was investigated the segments were dipped in HCl solution at room temperature and then dipped in lye for different times (see Results and discussion). The times and concentrations chosen approximated to those in use by the industry.

In trials to improve the yield and quality of the segments following lye-peeling, we investigated the influence of the 'wetting agents', Faspeel and Tergitol, which facilitate NaOH penetration and its action on the membranes. The agent was added to the lye peeling bath at different concentrations, as described in Results and discussion. The action of the agents was investigated at different concentrations (0·1, 0·2, 0·25 and 0·3%) and the lye peeling conditions (temperature, NaOH concentration) were varied accordingly.

For each given condition at least 10 kg of fruit was peeled; when quality characteristics of the canned segments were investigated, at least ten A2 cans were prepared and processed, as described in the first paper of this series (Ludin *et al.* 1969).

Yield is expressed as percent of the quantity of whole fresh segments used to prepare the peeled segments. Firmness, drained weight, tendency to break, and quantity of broken segments were measured as described by Ludin *et al.* (1969); firmness is expressed in shear press units (S.P.U.) For drained weight determinations, the filled cans were first stored for 6 weeks at 21°C.

Results and discussion

HCl treatment

In some factories in Israel as well as elsewhere, this technique is used to facilitate the removal of the carpellary membranes and to improve the quality of the peeled seg-

TABLE 1. Effect of HCl treatment on the quality of canned grapefruit segments

HCl		NaOH 2% (99°C)	Yield*	Quality of Canned Segments**			
	Dipping time			Drained	Broken		Tendency
%	(min)	(sec)	%	weight g/can	segments %	Firmness S.P.U.	to break %
0	(Control)	12	76·7	302	1·5	678	27
0·3	20	12	60·6	305	2·5	749	35
0·3	20	15	80·0	301	19·0	550	74
0·5	15	15	71·0	288	19·0	582	57
0·5	15	12	69·5	292	5·0	881	71
0·5	20	15	86·8	288	10·0	510	50

* Expressed as $\frac{\text{weight of whole segments after lye-peeling}}{\text{weight of whole segments before lye-peeling}} \times 100$

** Mean values after 6 weeks' storage at 21°C, of ten A2 cans per treatment.

ments. The dipping in HCl is usually done at room temperature, before the lye peeling. The dipping times and concentration of HCl used were similar to those in use by the industry. The results as well as the conditions of the experiment are given in Table 1.

The results of the HCl treatment are clear enough when compared with the control (no HCl treatment) even without statistical evaluation. Dipping in 0·3% HCl for 20 min followed by NaOH for 12 sec reduced the yield from about 77% (control) to about 61%, slightly improved the firmness and had almost no effect on the tendency to break or the quantity of broken segments. Increasing the time of the NaOH treatment to 15 sec facilitated the removal of membranes, slightly increased the yield, decreased the firmness, increased greatly the tendency to break (from 27% to 74%) and the quantity of broken segments. The detrimental effects were probably a result of the damage caused by deeper penetration of the NaOH.

Reducing the time of the HCl treatment to 15 min, while increasing its concentration to 0·5% followed by dipping in NaOH for 15 sec, did not decrease the yield but caused a decrease in the drained weight, probably by weakening the juice sacs, which burst during the heat treatment (pasteurization), with a resultant loss of juice from the segments. As a result, there was a decrease in firmness and an increase in the tendency to break and in the quantity of broken segments. Decreasing the duration of the lye treatment to 12 sec while keeping the other conditions constant, did not alter the yield or the drained weight markedly, but improved the firmness and decreased the quantity of broken segments. This last finding is questionable since the tendency to break for this treatment was much higher (71%) than in the control. Further increasing the time

of the HCl (0·5%, 20 min) and NaOH (15 sec) treatments facilitated the removal of the membranes giving an even higher yield. However, quality decreased (compared with the control), drained weight and firmness were low, and the tendency to break and the quantity of broken segments were high.

To summarize, it seems that the HCl treatment has a negative effect on the quality of the canned grapefruit segments, causing undesirable changes in their textural properties.

Use of wetting agents

Specific detergents (wetting agents) were added to the lye solution in order to assist the spread of NaOH on the membranes, thereby facilitating their removal from the fruit. Adding Faspeel to the lye solution, without changing any other conditions of the treatment, improved the yield and textural properties of the fresh grapefruit segments (Fig. 1). Adding 0·1% or 0·2% Faspeel increased the yield from 51% to about 70%; 0·3% Faspeel decreased the yield – but this was still above that of the control – possibly due to deep undesirable penetration of the NaOH into the segments. The firmness increased and the tendency to break decreased following inclusion of Faspeel in the treatments.

An industrial-scale experiment aimed at reducing the damage caused by heat during

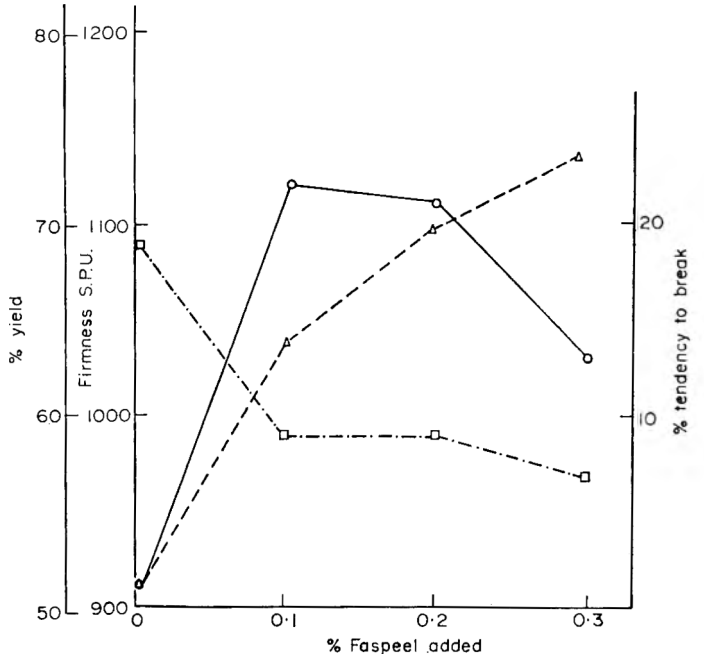

FIG. 1. Faspeel as a peeling agent and its influence on the improvement of the quality of canned grapefruit segments. O———O, Yield; △ - - - - △, firmness; □·-·-·-□, tendency to break.

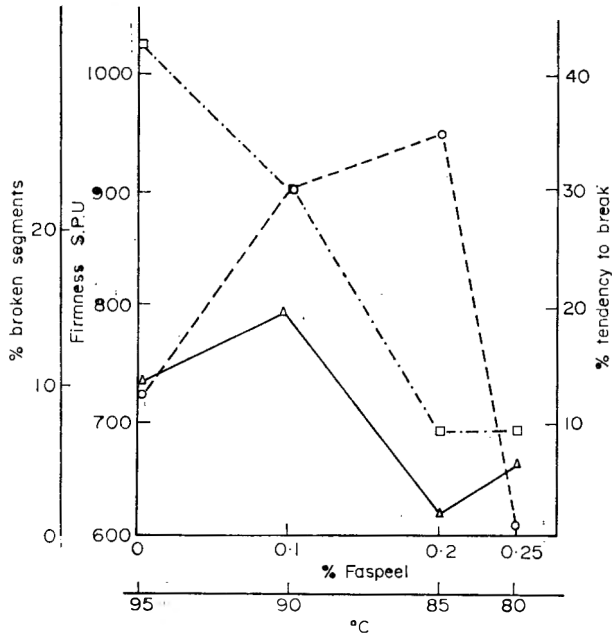

FIG. 2. Improvement of textural properties of grapefruit segments following the use of Faspeel and reduction of the lye-peeling bath (2·5% NaOH) temperature. △———△ % broken segments; O – – – – O, firmness; □·–·–·□ Tendency to break.

the lye treatment while facilitating the lye-peeling process with the addition of Faspeel, gave good results (Fig. 2). Reducing the lye temperature from 95° to 85°C, while adding 0·2% Faspeel, decreased the quantity of broken segments in the can (24 A2 cans per treatment) from 10% to 2%, increased segment firmness and decreased their tendency to break (from 42·5% in the control to 9%). The yield was not determined, as the experiment was performed in a factory during the commercial season. The subjective quality evaluation by the workers was that the manual removal of residual membrane particles (after the lye-peeling process) was much easier after the addition of Faspeel.

The influence of temperature reduction was investigated on a pilot-plant scale, while comparing two detergents—Faspeel and Tergitol (Table 2). Both compounds increased the yield and improved the quality of the product. Good results were obtained when 0·2% Tergitol was added (at 90°C), increasing the yield from 54% in the control to more than 70%, improving the firmness and decreasing the tendency to break; 0·2% Faspeel (at 90°C) gave similar results.

While the results of these experiments do not establish the best conditions for lye peeling with the addition of wetting agents, they do indicate the improvement in yield and quality resulting from the reduction in temperature due to addition of such an agent.

TABLE 2. Effect of addition of Faspeel and Tergitol to the lye-treatment at reduced temperature, on the quality of canned grapefruit segments (2% NaOH, 12 sec)

Treatment		Quality of canned segments*		
Wetting agent added %	Lye temperature °C	Yield %	Firmness S.P.U.	Trendency to break %
0 (Control)	100	54	320	21
0·1 Tergitol	95	78	280	16
0·2 Tergitol	90	72	520	10
0·1 Faspeel	95	61	500	6
0·2 Faspeel	90	68	530	2

* Ten cans prepared from the segments of each treatment.

The yield and quality of the segments may also be improved through milder treatment conditions by reducing the lye concentration while adding a wetting agent (Table 3). This treatment improved the yield and firmness, but did not reduce the tendency of segments to break.

Conclusions

The HCl treatment used in the industry does not increase the yield of lye-peeled segments without bringing about a concomitant decrease in their quality. The addition

TABLE 3. Effect of addition of Tergitol and reduction of lye concentration on the quality of fresh grapefruit segments*

Treatment*		Quality of fresh segments		
%Tergitol	% NaOH	Yield %	Firmness S.P.U.	Tendency to break %
0 (Control)	2·0	42	670	25
0·2	1·75	79	840	21
0·2	1·50	73	830	24

* Peeling at 99°C for 12 sec

of surfactant (wetting) agents to the lye enables milder treatment conditions, which in turn result in improved yield and quality of the segments.

Acknowledgment

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The examination of starch gelling by microscopy

C. D. FREKE

Summary

A microscopic method of determining the gelling temperature of starches has been developed. The apparatus is described and some results showing the effects of varying concentrations of sucrose at two pH values on two starches are presented.

Introduction

The methods generally used to investigate the properties of starches, in particular their gelling temperature and the effects of various additives, are physical and tell one little of what is actually happening to the grains. One disadvantage of this is that since work is being done on the gel it may behave differently from a gel formed undisturbed. For some purposes it is preferable actually to observe and record the changes taking place. The method described here depends on direct observation of the swelling of the starch grains. An objective microscopic method has been described by Berry & White (1966) using loss of birefringence measured by a photometer as the criterion of gelatinization. This method is more rapidly performed than the method described here and has some of the advantages mentioned. However, birefringence is lost before any swelling of the grains occurs and the temperature would tend to be somewhat lower than that given by a physical method. With the method described below, additional information can be gained, if required, on the duration of swelling time, final size or grain, etc.

Materials and methods

Microscope

A Cooke, Troughton & Sims M2525 microscope was used fitted with a vertical, monocular body and using an external light source for transmitted light.

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Camera

The Exacta Varex IIb with its microscope attachment and a 'Magnear' viewfinder containing a clear screen with cross wires.

Stage heater

A slide heater was manufactured from glass and aluminium. The heater contained decolourized liquid paraffin as the heating medium. A length of 40 S.W.G. Nickel/Chromium resistance wire giving a total resistance of 80 ohms was used as a heating element. The whole was lagged with asbestos tape (Fig. 1). Current was provided by a constant voltage supply.

Recorder

Ether, 2 mV, single channel.

Sample cell

The sample cell was constructed by fixing a brass washer of about $\frac{1}{2}$ in. internal diameter to a slide with 'Araldite'. A slot was cut in the washer and a Copper/Constantan thermocouple fixed so that the tip just projected into the cell. The 'cold junction' of the thermocouple was held at a constant temperature in an oil bath.

The apparatus was assembled as set out in the block diagram (Fig. 2).

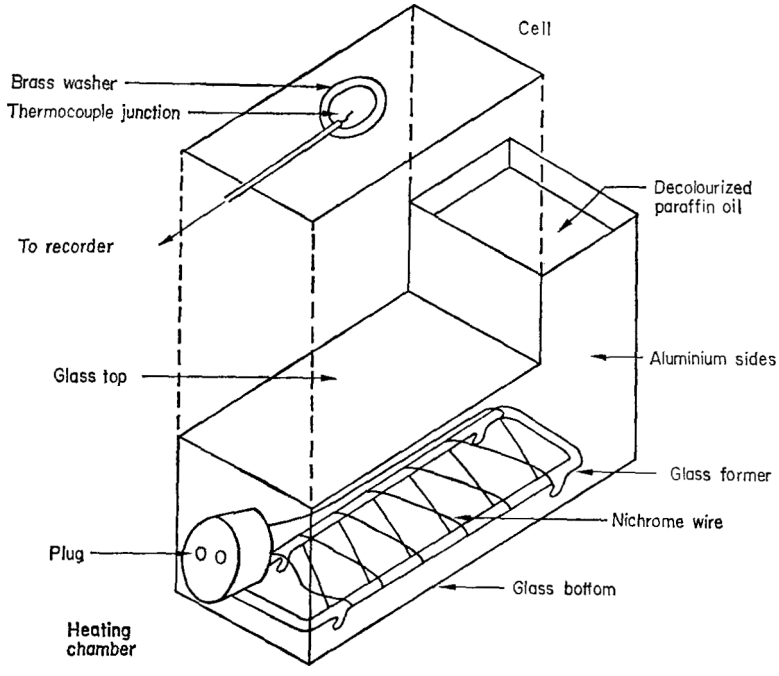

FIG. 1. Construction of heating chamber and sample cell.

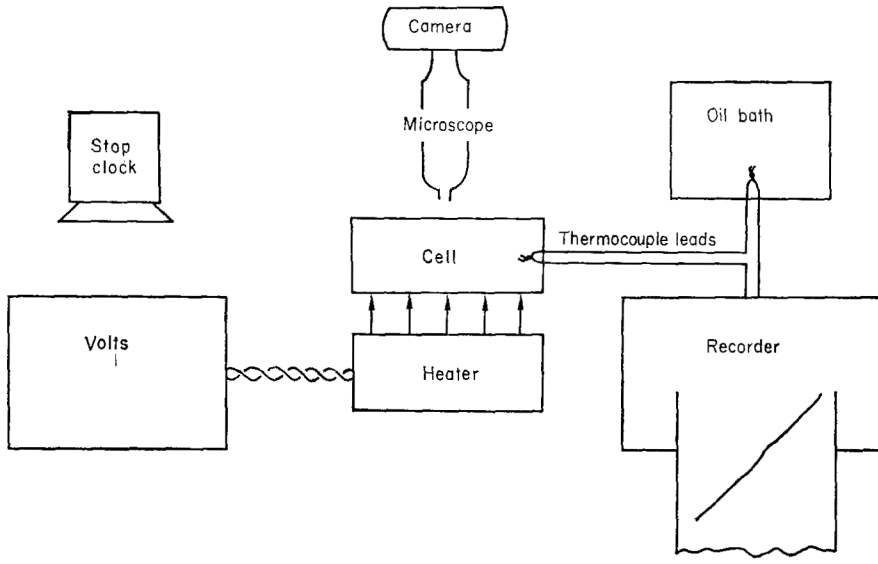

FIG. 2. Block diagram of assembled apparatus.

A sparse suspension of starch in the menstruum under investigation was placed in the sample cell and a coverslip sealed on with silicon grease. The sample cell was clipped onto the heater. The other junction of the thermocouple was held at 53°C (± 0·01°C) in an oil bath and the temperature recorded on the recorder. As starch does not normally swell below 60°C a record of the rate of heating below 55°C is not of much interest. Recording from 55°C upwards allowed an expansion of the scale. With the particular conditions used, full scale represented about 30 Centigrade degrees and $\frac{1}{8}$ in. was equivalent to 1 Centigrade degree. The recorder was run at 30 in./hr so that $\frac{1}{6}$ in. was equivalent to 20 sec. Thirty-five volts at 0·44 amp. from the constant voltage supply gave a temperature rise of about 4 Centigrade deg/min between 55°C and 75°C and 37·5 volts gave the same rate above 75°C. The thermocouple, recorder combination was calibrated at intervals or if any alterations were made to the conditions.

A field containing 50–100 grains was selected and was observed as the temperature increased until a grain started to swell. At this point a photomicrograph was taken, a mark made on the recorder chart and the timer started. Further photomicrographs were then taken of the same field at 20-sec intervals until all or most of the grains had swollen (usually about eight shots).

In early experiments (unpublished) it was shown that individual starch grains started swelling at intervals over about 5 min and that the distribution was normal. Thus the point at which half the grains had started to swell was coincident with the mean. The following method of assessing the result was used.

After development, the series of photomicrographs were examined by projecting them on to a white surface. Starting at the first frame the total number of unswollen grains and the total of swollen grains was counted using a hand tally. In each successive frame only the unswollen grains were counted, since they were easier to recognize. By subtracting the number of unswollen grains from the total number of grains, the cumulative total of swollen grains in each frame can be found.

Expressed algebraically:

$$(U + S) - Ux = Sx$$

U = grains not swollen in frame 1;
Ux = grains not swollen in frame x;
S = grains swollen in frame 1;
Sx = total number of swollen grains in frame x.

These totals were plotted against temperature and the temperature at which 50% of the grains had swollen was found by interpolation. The temperature at each time interval was found from the trace made by the recorder by measuring from the mark made when the run was started. An example is shown in Fig. 3.

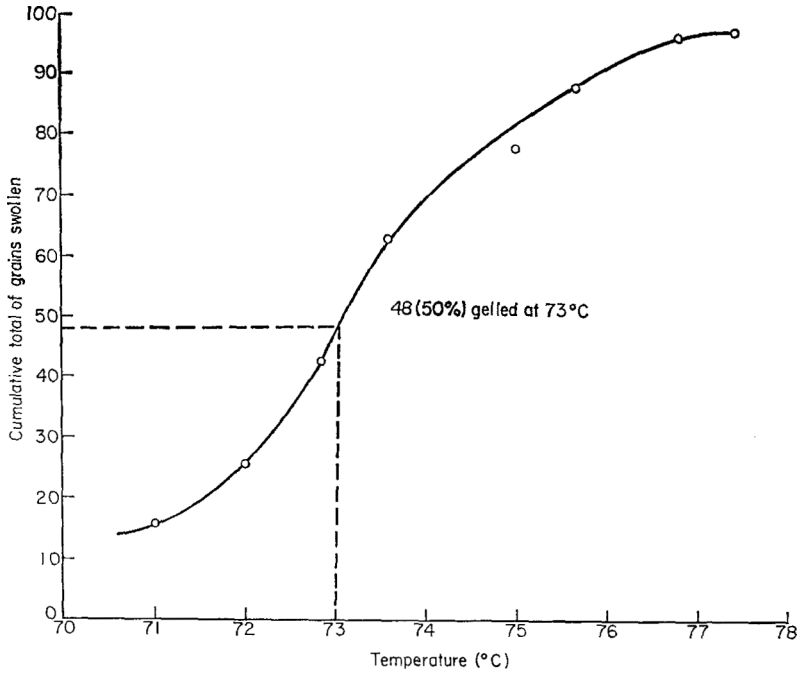

FIG. 3. Run 106. Swollen grains plotted against temperature to find 50% value.

Using the method described, two starches were examined to find the effect of sucrose concentration and pH value on their gelling properties. One was an unmodified waxy maize starch (Amioca) and the other a modified waxy maize starch (Col-flo 67). Their gelling temperatures were determined at pH 4·0 and pH 2·8 in sucrose solutions of 0, 5, 10, 15, 20, 30, 40 and 50% w/v. Duplicate runs were made with each combination of variables. If the difference between runs was greater than 0·9°C a third determination was made. When the third value was close to one of the previously determined values a mean of these two was taken and the odd value ignored otherwise a mean of all three was taken.

Results

The results are shown in Figs. 4 and 5.

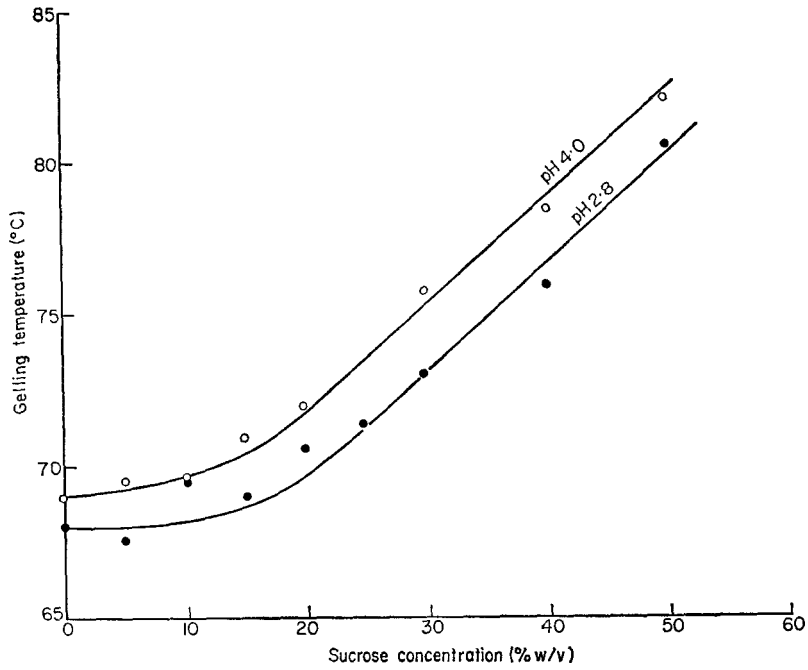

FIG. 4. Effect of sucrose and pH concentration on the gelling temperature of 'Amioca' starch.

Discussion

The differences between duplicate runs were in no case greater than 2·0°C. None of the values in the third runs differed from the duplicate values by more than 1·0°C. This is a very reasonable degree of accuracy considering the innate variability of

biological material and the range of temperature over which the individul grains start to gel.

The method has the advantage that a permanent record in the form of the 35-mm negatives, may be kept from which further information, such as rate of swelling of individual grains, final size, etc., may be gained if desired.

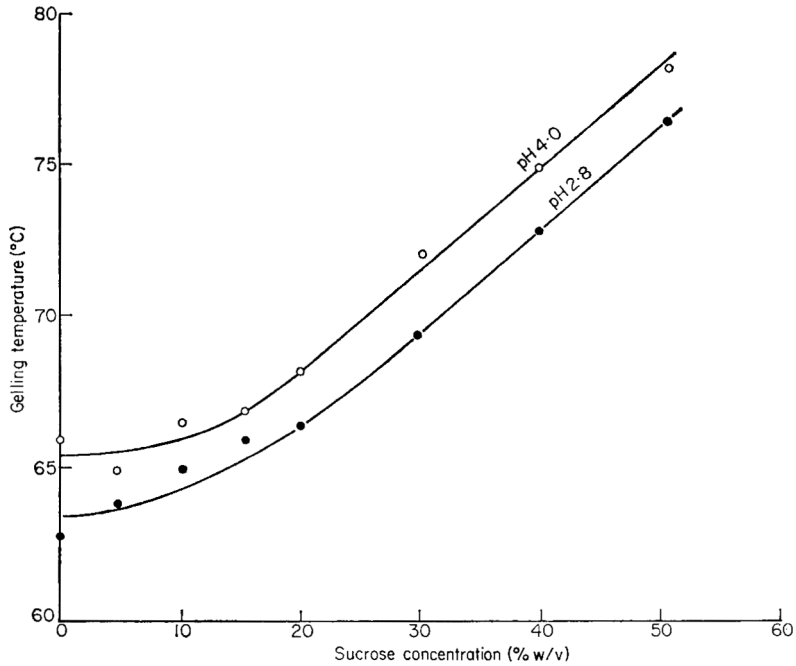

FIG. 5. Effect of sucrose and pH concentration on the gelling temperature of 'Colflo' starch.

The result of the series of runs on the two starches shows a relationship between sucrose concentration and gelling temperature which seems to be independent of the state of the starch and pH value. The gelling temperature increased by 3·5°C for each 10% increase in sucrose concentration above 20% w/v. Acidity had an effect in that the more acid slope was 2·4°C lower and 2·1°C lower for Amioca and Col-flo 67 respectively. Previous work (unpublished) had showed that the gelling temperature of Amioca was stable down to about pH 3·5 but dropped sharply by 2°C at lower pH values. Col-flo 67 was stable down to about pH 3·0 and declined slowly below that value. The difference in the two responses to pH value is not reflected in the response to changing sucrose concentration. Since the gelling temperature increases with increasing sucrose concentration, the implication is that more energy is required before the starch can start to take up water. The possible mechanism of the effect of sucrose on starch gelation will be discussed in another paper.

Acknowledgments

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Some observations on the mechanism of starch gelation

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Summary

The temperature at which starch gelled in systems with varying dielectric constants was examined. Starch was also examined by differential thermal analysis (DTA) and thermogravimetric analysis (TG). Findings showed that the temperature at which starch gels depended on the polarity of the environment. Implications of this and of the DTA and TG findings are discussed.

Introduction

In a previous paper Freke (1971) showed that increasing sucrose concentrations in the suspending liquid caused a corresponding increase in the temperature at which starch starts to gel. This observation raised some interesting speculations as to the mechanism of starch gelatinization. The competition for water cannot be purely osmotic as can be deduced from the following observations. Osmosis requires the existence of a membrane but if one is present it does not play a large part in the properties of the starch. Leach & Schoch (1961) have shown that fragmented starch grains continue to behave as whole grains, i.e. they retain their shape when heated in water until the gelling temperature of the starch is reached when they swell, take up water and lose their shape. Intact grains can be stained by iodine, primulin and other dyes (Sterling, 1964, 1965) showing that the pores in the starch are sufficiently large to admit fairly large molecules, certainly most solvents.

As the effect of sugar cannot be explained as an osmotic phenomenon, the polarity of solvents was investigated since sucrose dissolved in water would lower the dielectric constant of the solution and dielectric constant may be taken as a measure of polarity.

In addition, the number of sites available for bonding in the starch polymer are far too few to account for the amount of water taken up when starch gels. It was therefore of interest to see how much of the water was bound to the gelled starch.

The experimental work therefore falls into two parts—the effect of dielectric constant on gelling temperature, and the determination of bound water in gelled starch.

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Materials and methods

The effect of dielectric constant on the gelling temperature of starch

The starch used in the study was Colflo 67, a modified waxy maize starch. The solvents shown and the following solvent mixtures were used in the study. Water/glycerol, water/sucrose, water/propylene glycol, water/ethylene glycol, water/ethyl methyl carbamate, formamide/benzyl alcohol, formamide/ethylene glycol, formamide/ethyl methyl carbamate, formamide/propylene glycol. All binary systems were tested at intervals of 10% v/v concentration except sucrose which was tested at 5% w/v intervals. The starch was suspended in the various solvents or solvent mixtures and the gelling temperatures determined by the method described by Freke (1971).

Study of bound water in gelled starch

Attempts were made to gel starch with minimal amounts of water. These were unsuccessful as the starch gelled completely locally and not at all elsewhere. Instead a completely gelled starch suspension was vacuum-dried at 80°C and the resultant clear film ground to a powder. This powder showed no regular grains and no birefringence. If placed in cold water it swelled immediately. This pregelled powder and a sample of ungelled starch were submitted to thermogravimetric (T.G.) and differential thermal analysis (D.T.A.).

D.T.A. Slurries of 20% starch in water and in glycerol were examined using the suspending fluid as a referent in each case.

T.G. Col-flo 67, pregelled Col-flo 67, and pregelled Col-flo 67 which had been held in a humid atmosphere for 24 hr were examined by T.G. in a gas stream.

Results

The results are given in Table 1 and Fig. 1.

TABLE 1. Gelling temperatures in solvents of various dielectric constant

Solvent	Dieletric Constant (25°C)	Gelling Temp. °C
Formamide	109·0	60–70
Water	78·5	62
Glycerol	42·5	120
n-Propanol	20·1	does not gel

The following solvents did not gel the starch under the conditions of test. Their dieletric constants range between 17·1 and 2·0. n-butanol, iso-butanol, ethyl-n-methyl carbamate, n-amyl alcohol, benzyl alcohol, methyl salicylate, morpholine, n-butyric acid, dioxan and petroleum ether.

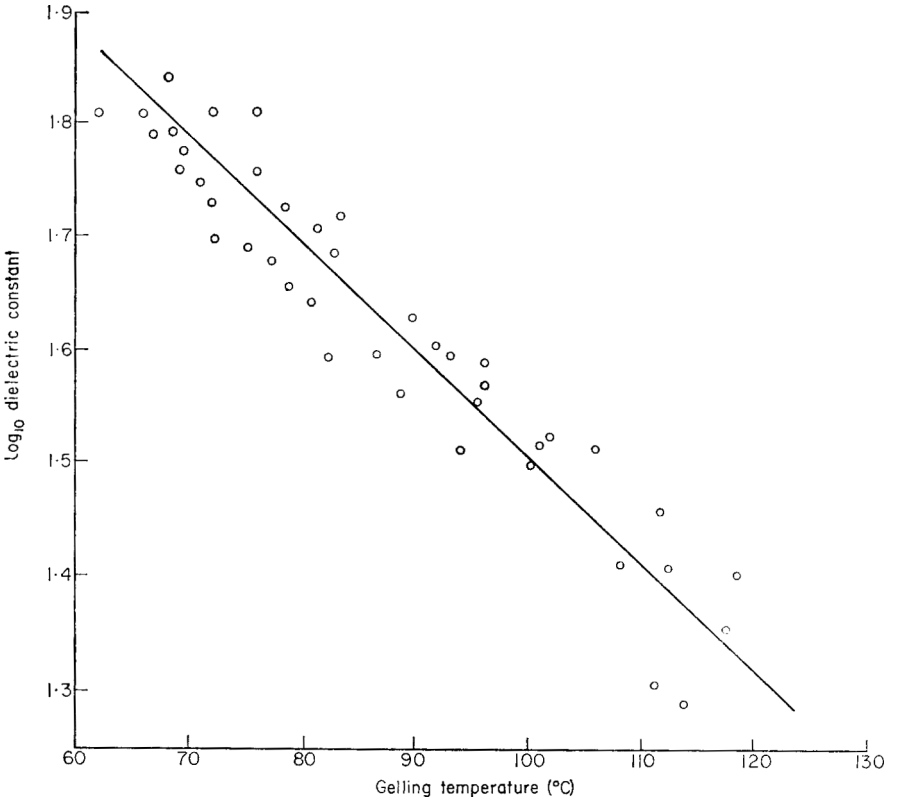

FIG. 1. The effect of dielectric constant of the suspending menstruum on the gelling temperature of 'Colflo' starch.

D.T.A. Water: A small peak with a maximum at 72°C was observed. Glycerol: a small peak with a maximum at 120°C was observed.

T.G. Col-flo 67: 13% by weight of water was lost between room temperature and 175°C. Pregelled col-flo 67: 10% by weight of water was lost between 60°C and 220°C when the starch decomposed. Pregelled and humidified Col-flo 67: 18·6% by weight of water was lost between room temperature and decomposition at about 230°C.

Discussion

It was not possible to measure the dielectric constants of the various solvents and solvent mixtures. In some cases the values for binary mixtures at various temperatures were available (Timmermans, 1959) and in others they were calculated from values given in the literature (Weast, 1969). The latter procedure is not exact but the values would not be very different from the true value.

It is clear from Fig. 1 that there is a semi-log relationship between dielectric constant

and gelling temperature. Halving the dielectric constant leads to an increase of about 32°C in the gelling temperature. Deviations from linearity may be due to experimental error or due to the relative sizes of the molecules involved. It would be expected that the larger molecules may take a little longer to enter the pores in the starch than smaller ones. Other factors such as pH value and water activity may also affect the gelling temperature and modify the effect of any given binary system.

Peaks observed by D.T.A. at 72°C and 120°C for water and glycerol respectively are close to the gelling temperatures in those solvents and show that small amounts of energy are used at those temperatures. The following hypothesis is put forward to explain these findings. The energy, as heat, needed to gel starch is required to break the internal starch–starch bonds.

If the disruption of the starch when it gels is due to replacement of starch–starch bonds by starch–solvent bonds of a polar kind, then any decrease in polarity of the solvent would necessitate an increase in the energy needed to form the bonds. Conversely, the greater the polarity of the solvent the less energy is required, since the starch solvent bonds will be stronger and more easily formed.

The thermogravimetric observations show that ungelled starch loses water continuously from room temperature. This water is the normal unbound moisture of starch. The pregelled starch lost water at a higher temperature indicating that this water was more firmly bound. Assuming that one water molecule is bound to each glucose unit, the water lost by the pregelled starch (10%) agrees well with the theoretical value (9·4%). The pregelled humidified starch lost water from room temperature as did the ungelled starch but continued to lose water up to 50°C higher indicating the loss of both bound and unbound water.

This evidence is consonant with the following hypothesis. Only the initial 10% of water taken up by starch is bound to the molecules. This binding of water disorganizes and weakens the structure which increases in volume to accommodate the new bonds and passively admits more water.

Acknowledgments

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An electron microscopical investigation of fat destabilization in ice cream

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Summary

A limited amount of fat destabilization occurs when ice cream mix is aerated and frozen, and this emulsion breakdown imparts some desirable properties to ice cream. A method of measuring the amount of de-emulsified fat is described, and the results of an investigation of the process by means of electron microscopy are given. The effects of fat composition and processing variables on fat destabilization are summarized, and an overall picture is presented of the part played by fat in the structure of ice cream.

Introduction

Ice cream is made by aeration and freezing of a mix, and it has been known for many years that this operation induces a certain amount of destabilization in the fat emulsion (Dahle, 1936; Sommer, 1944).

Valaer & Arbuckle (1961), in describing the state of dispersion of butterfat in ice cream, distinguished between small homogenized globules, improperly homogenized globules, small clumps, agglomerated fat, coalesced fat and butter particles. To avoid confusion in the present contribution, the general or partial break down of the fat emulsion will be referred to as *destabilization* or *de-emulsification*. We distinguish two cases:

(a) when the globule membranes are largely ruptured and the globules lose their identity, joining together to form large masses of continuous fat; this will be called fat *coalescence* or *churning*, and

(b) when the fat globules retain their form, but join together like bunches of grapes; this will be called fat *clumping* or *clustering*.

A certain amount of fat de-emulsification is desirable in frozen ice cream in order to obtain a dry product (Keeney, 1958; Keeney & Josephson, 1958; Kloser & Keeney, 1959a, b), good shape retention and slow melt down (Keeney, 1958; John & Sherman, 1962), and firmer texture (John & Sherman, 1962). Excessive fat coalescence or churning, however, is undesirable and is associated with poor whipping properties,

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fat and ice separation in the freezer, unsatisfactory extrusion, a buttery texture, and a 'does not melt' defect accompanied by separation of a thin watery serum. The situation is analogous to that of whipped cream where a controlled amount of fat clumping promotes stability (Graf & Muller, 1965).

The extent of fat destabilization in frozen ice cream is affected mainly by the type and amount of fat, emulsifier and stabilizer, by the milk protein stability and mineral salt balance in the mix, and by such processing variables as ageing time, freezing time (batch freezers) and extrusion temperature (continuous freezers). The influence of some of these factors will be discussed later.

The present investigation is concerned mainly with the effects of different extrusion temperatures (continuous freezers) and of different types of fat on the fat destabilization.

Materials and methods

Ice cream

Ice cream mixes were prepared by full scale procedures to the following formula:

Fat	9·0%
Milk solids not fat	10·5%
Sugar	16·5%
Emulsifier	0·4%
Stabilizer	0·14%

After homogenization, pasteurization and ageing, freezing was carried out in Vogt continuous freezers. The ice cream was hardened at − 23°C to − 30°C before examination.

Measurement of fat destabilization

Various methods for the measurement of the extent of fat destabilization in ice cream have been described by Keeney & Josephson (1958), Schulz, Voss & Gutter (1959), and Alsafar & Wood (1965).

The method developed in our laboratories involves centrifugation of the sample of ice cream mix or melted ice cream, under conditions which have been calculated to separate all fat globules greater than 2 μm in size. Sixty grammes of the sample, at room temperature, is centrifuged at 3500 rpm for 15 min, and the bottom 20 g in the centrifuge tube is slowly run off for a fat determination (Mojonnier method). The resistance of the emulsion to break down is calculated as a stability coefficient (s.c.), given by

$$\text{s.c.} = \frac{F_B}{F_M} \times 100 \qquad (1)$$

where F_B is the fat content of the bottom third of the centrifuge tube, and F_M is the original fat content of the mix. It is clear that the percentage of destabilized fat could be taken as (100—s.c.), although we have preferred to work with stability coefficients.

Consistency measurement

Consistency was measured at − 5°C, with the F.I.R.A./N.I.R.D. extrusion apparatus, on samples of ice cream taken at the outlet from the freezer.

Specimen preparation for electron microscopy

Specimen preparation was by the freeze-etching technique developed by Moor, Waldner & Frey-Wissling (1961), Moor & Muhlethaler (1963) and Moor (1964), and was carried out in the Balzers apparatus (Bohler, 1968). Freeze-etching of the specimens involved (a) very rapid freezing of the specimen (~ 100°C/sec), (b) partial cutting, but mainly fracture of the specimen under vacuum at − 100°C, (c) etching away of the surface layers of ice by low temperature sublimation (− 100°C for 1·5–2·0 min), (d) metal shadowing at an angle of 25° with Pt–C, and (e) replica making by sputtering on carbon at right angles. The replica clean-up techniques, employed before examination in the electron microscope, involved removal of (i) water solubles, (ii) milk proteins, and (iii) fat, in this order. The replica was passed sequentially through the following thirteen liquids: water, 30% HCl, 70% HCl, conc. HCl, 70% HCl, 30% HCl, water, 20% ethanol, 50% ethanol, 80% ethanol, ethanol, ethanol/fat solvent, fat solvent. The fat solvent used was methanol : chloroform = 1 : 2. The replica was then picked up on an EM grid and the solvent gently evaporated.

In two cases, specimen preparation was by freeze fracture only, the etching stage being omitted.

Results

Microscopical examination of the homogenized ice cream mixes before freezing invariably showed very few fat globules above 2 μm in size, and the stability coefficient of the mixes used in these experiments was 90–95%. The stability coefficient after freezing and thawing was much lower and is a measure of the proportion of fat globules still below 2 μm in size.

Fig. 1 shows the effect of refrigeration on the stability coefficient. A relatively small increase in the applied refrigeration causes a marked decrease in stability coefficient when palm oil is used, whereas a palm kernel oil mix is not as sensitive. Fig. 2 illustrates the relationship between the applied refrigeration and the consistency of the ice cream leaving the freezer.

Plate 1 shows the replica of a vanilla ice cream *mix* before aeration and freezing, and the fine dispersion of the emulsion is clearly visible. However, the freeze-etching technique does not necessarily present the maximum diameter of individual fat particles but rather a random selection of all possible diameters up to the maximum.

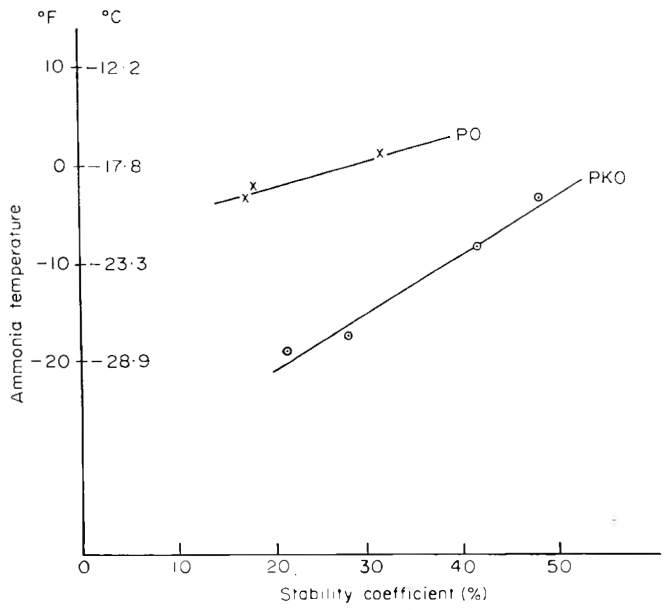

FIG. 1. Effect of refrigeration on stability coefficient.

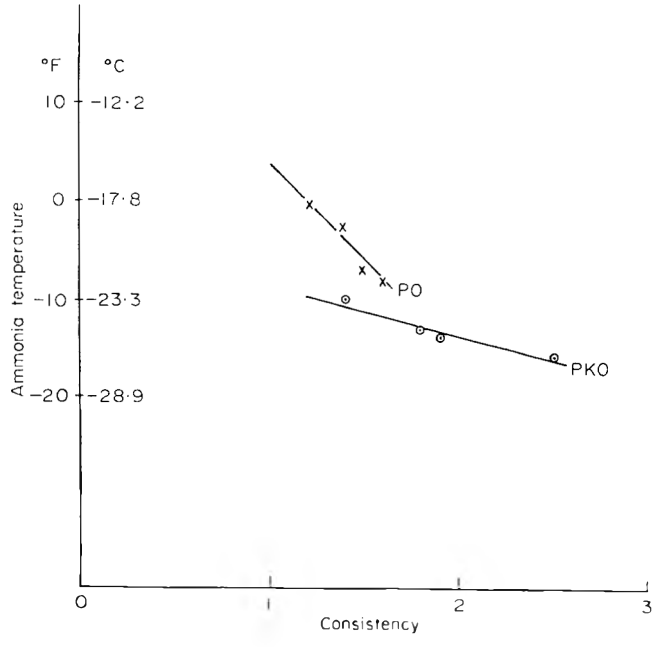

FIG. 2. Effect of refrigeration on the consistency of ice cream.

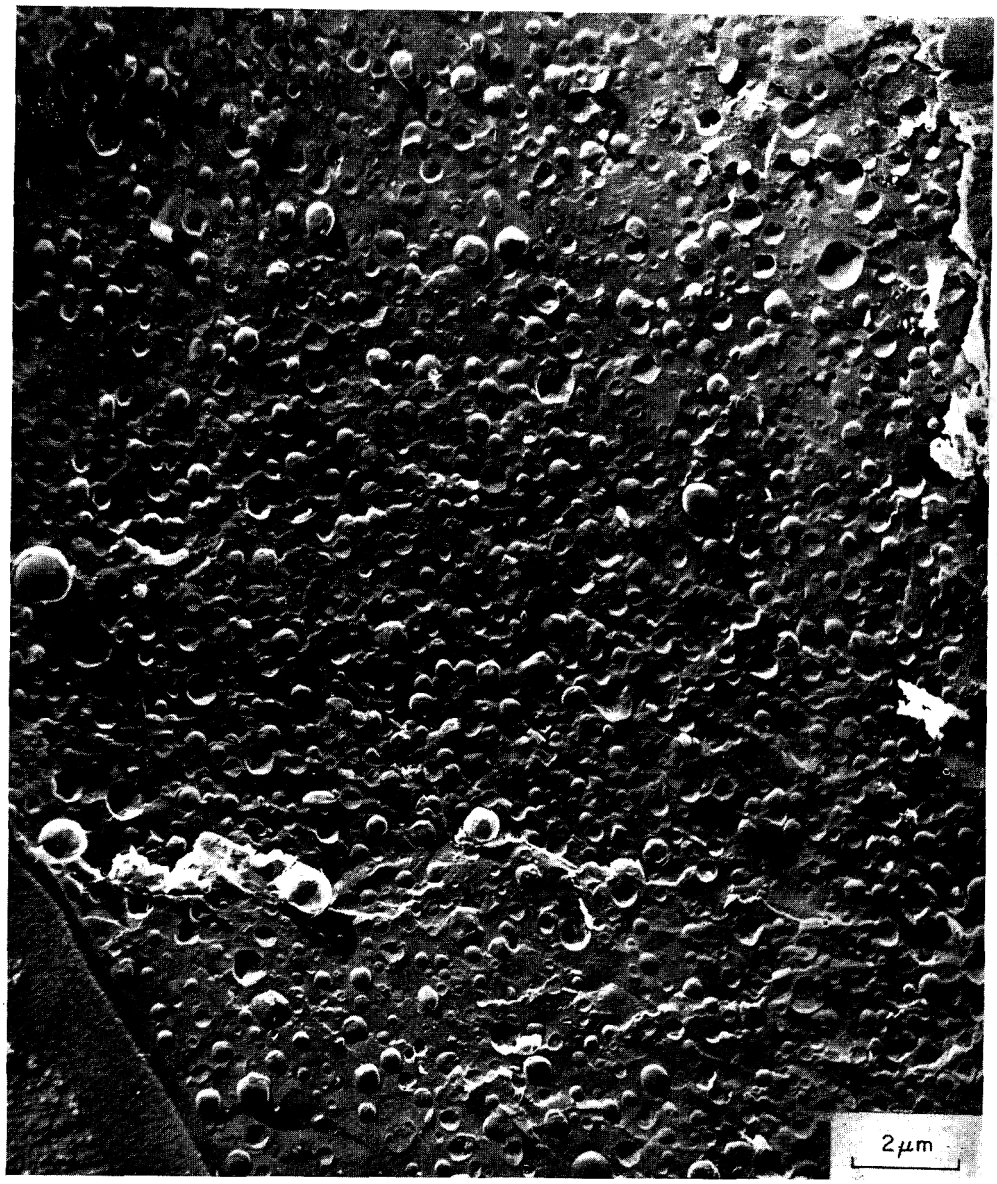

PLATE 1. Replica of freeze-etched ice cream *mix*.

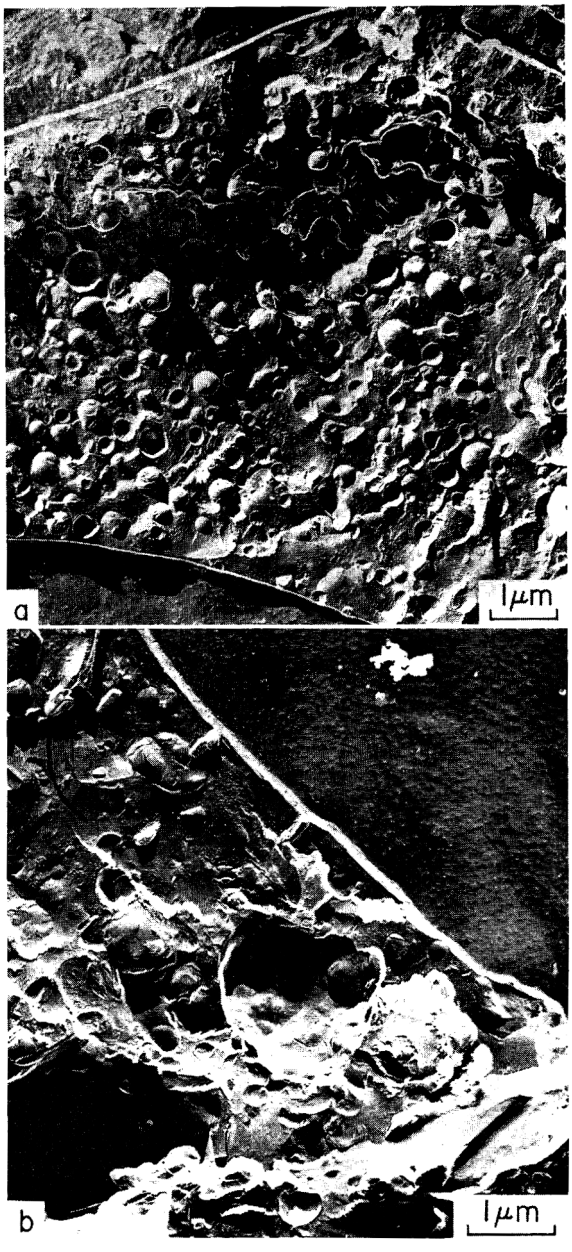

PLATE 2a. Replica of freeze-etched, −5·0°C extruded, vanilla ice cream.

b. Replica of freeze-etched, −8·2°C extruded, vanilla ice cream.

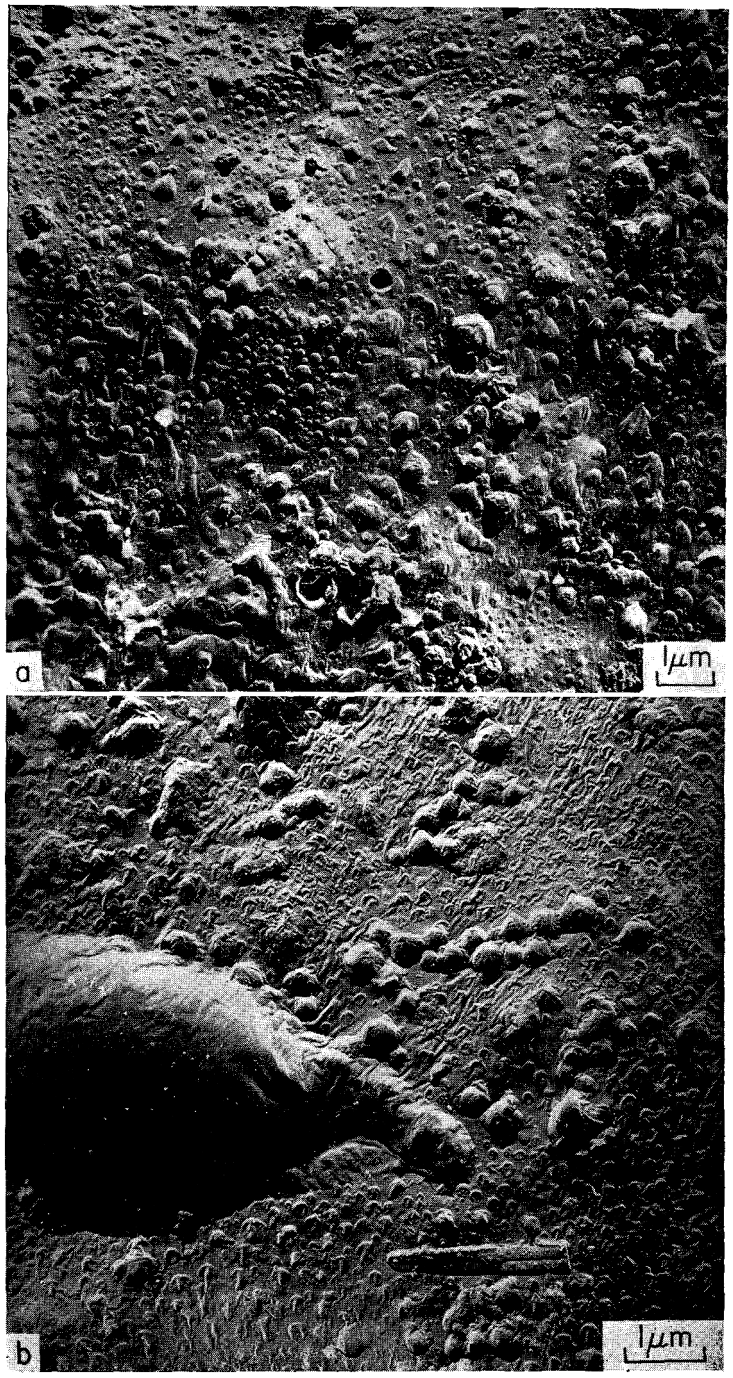

PLATE 3a. Replica of freeze-fractured ice cream, containing palm kernel oil.
b. Replica of freeze-fractured ice cream, containing palm oil.

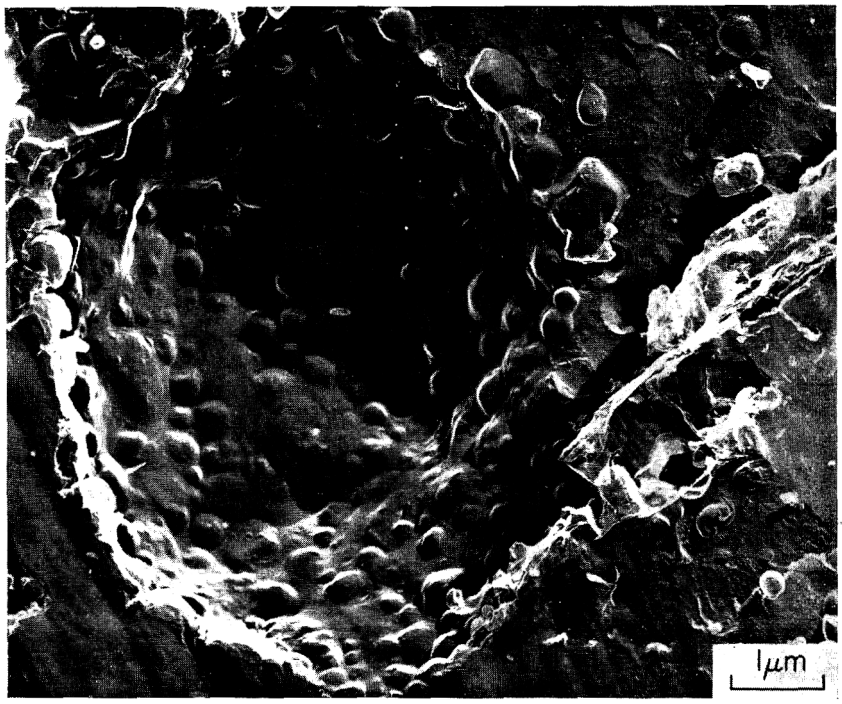

PLATE 4. Replica showing interior of typical air cell in ice cream.

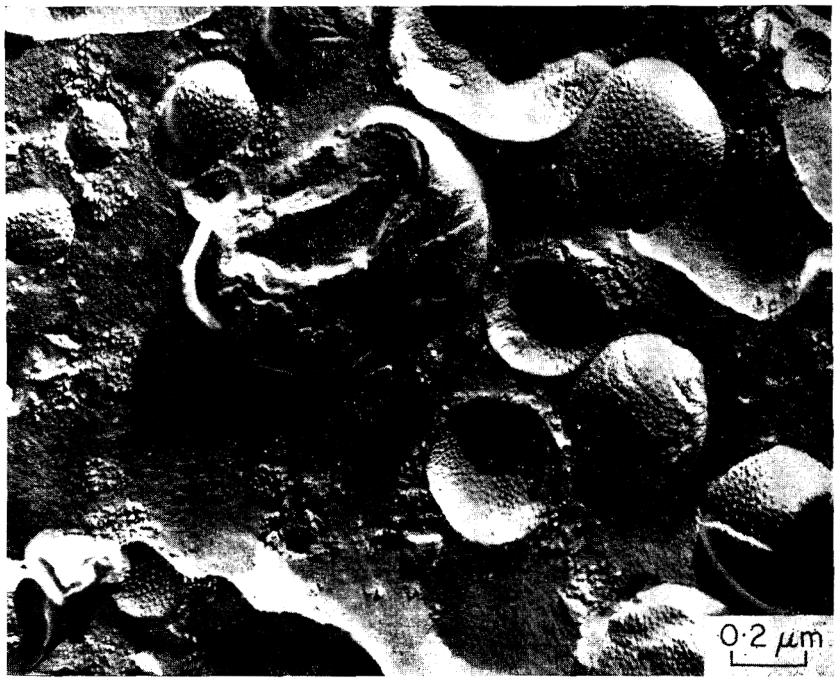

PLATE 5. Replica showing fat globules with cascin sub-units in the membrane.

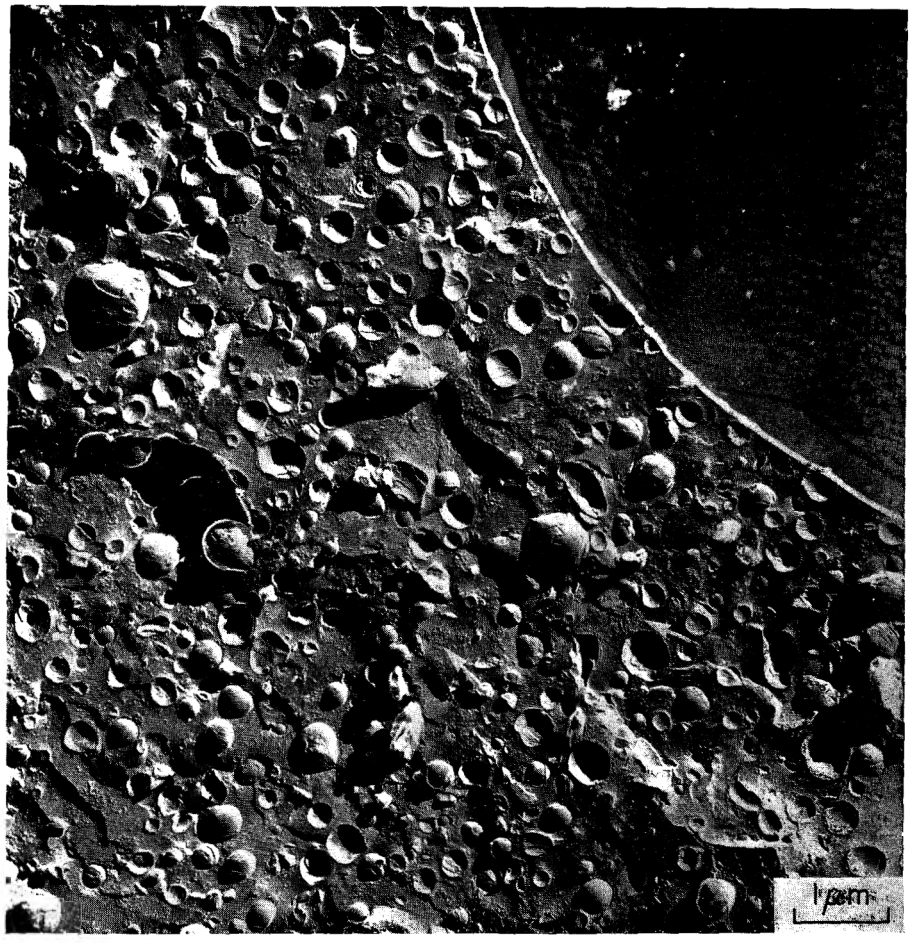

PLATE 6. Replica of ice cream showing fat globules and casein micelles.

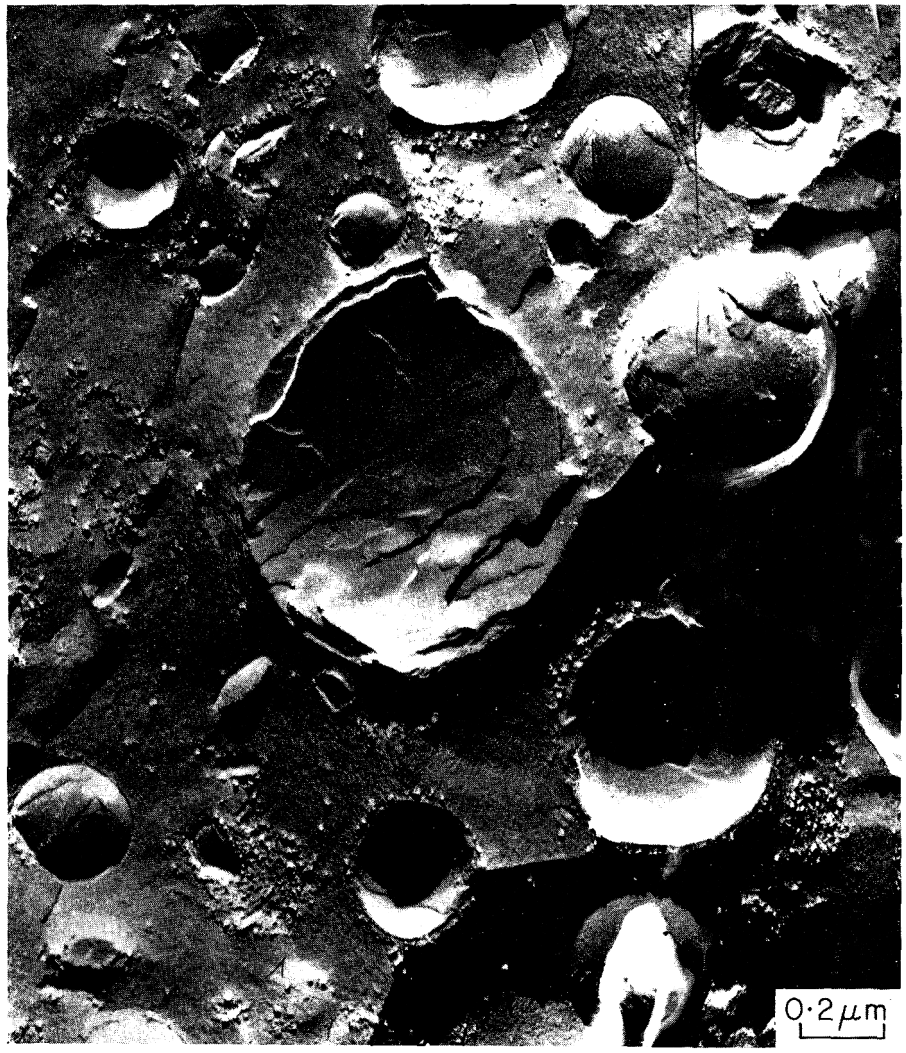

PLATE 7. Replica of ice cream showing casein sub-units lining fat globules.

The particle sizes obtained by direct measurement, therefore, have been corrected by a factor of $4/\pi$, obtained by mathematical integration. The size range obtained in this way was 0·04 to 3·0 μm, with a mean value of 0·45 μm. This distribution is in good agreement with results obtained by other methods.

Plate 2(a) and (b) show replicas of two freeze-etched ice creams that had been extruded at − 5·0°C and − 8·2°C respectively. The higher extrusion temperature leaves the fat emulsion still fairly well dispersed, but the lower extrusion temperature has caused coalescence and distortion of the fat globules, which are reduced in number.

The effects of using fats of different liquid oil contents are illustrated in Plate 3(a) and (b), which show replicas of freeze-fractured ice creams that had previously been extruded at − 5·4°C. It can be seen that palm kernel oil remains reasonably well dispersed in the frozen ice cream but that palm oil shows not only chains and clumps of fat globules, but also large masses of coalesced fat and crystals, some of which have been found to be several micrometers in length.

Measurements of the liquid content of these two oils have been made using NMR. It was found that in the temperature range + 5 to − 5°C, the liquid content of palm oil was higher by 5–9%. It is thought that these measurements, made under equilibrium conditions, do not fully represent the dynamic conditions obtaining in a scraped surface heat exchanger. Under these conditions, it is probable that the difference in liquid oil content will be greater because of the differing rates of crystallization of the two oils. Further experiments were done to compare ice creams made with (a) palm oil, and (b) cottonseed oil. It was found that fat globules were much less numerous in the ice cream made with the more liquid, cottonseed oil, and that many areas of coalesced fat, and some fat crystals were visible.

We have found that as the amount of fat destabilization in frozen ice cream is increased, the emulsion breakdown in the case of continuous freezers does not usually result in the formation of clusters or clumps of fat globules, but rather in the formation of coalesced or churned fat. This is accompanied by a large decrease in the number of fat globules per unit area on the electron micrographs.

Fig. 3 shows the results of fat globule counts made on electron micrographs from ten separate experiments. The best straight line was calculated by the method of least squares. A correlation coefficient of 0·884 was obtained (significant at the 0·1% level).

Plate 4 shows one of the air cells in ice cream, and it appears that the surface is lined with smooth material, characteristic of coalesced or liquid fat, and also with many individual fat globules which protrude into the air cell.

The structure and strength of the artificial membrane around the fat globules is an important factor controlling fat destabilization in ice cream. Some information on this membrane can be obtained from electron micrographs at very high magnification. Plate 5 shows a rather granular coating on the outside of the fat globules. These are the sub-units of the casein micelles and the sub-units form part of the fat globule membrane. Normally these are lifted off in fractured and freeze-etched specimens to

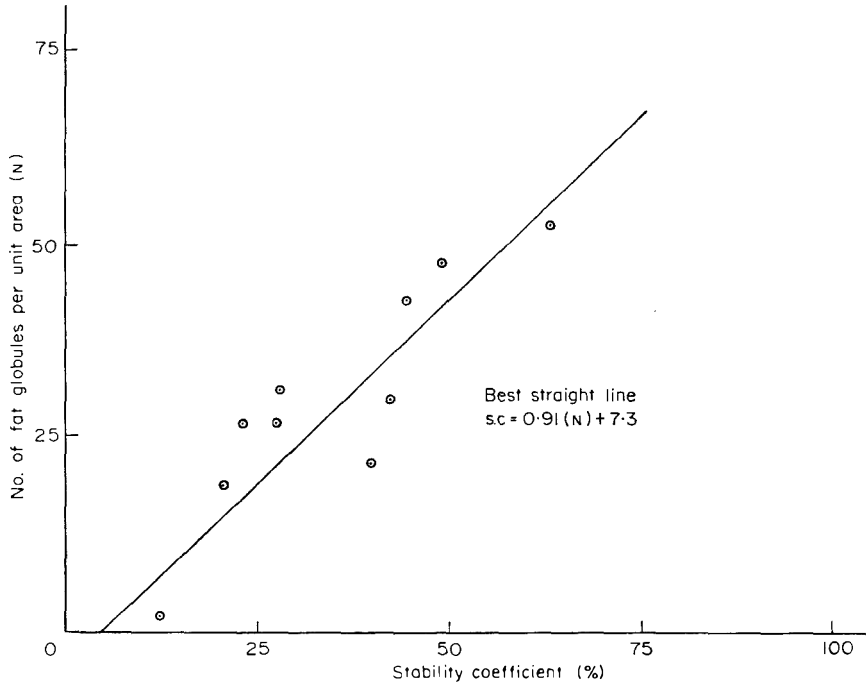

FIG. 3. Fat globule concentration versus stability coefficient.

reveal the steps, of molecular thickness, in the lipid layers at the surface of the globules (Plates 2, 6 and 7). Plate 6 shows a replica of ice cream extruded at − 5°C, in which the casein micelles may be seen between the fat globules. The micelles show a granular internal structure of sub-units, and the latter are also found freely distributed in the continuous phase. Plate 7 shows a replica of the fat globules and casein micelles under high magnification (105,000 ×), and the sub-units from the micelles can be seen lining the fat globules.

Discussion

The results represented in Fig. 1 demonstrate the quantitative relationship between applied refrigeration in the continuous freezer and the destabilization of the emulsion, and also the different effects obtained with palm oil and palm kernel oil.

Fig. 2 shows that this destabilization is reflected in the texture of the frozen product. A linear increase in consistency is obtained with increased refrigeration. A stiff consistency, indicating good 'stand-up' properties can be attained when palm kernel oil is used. With palm oil, however, the destabilization of the emulsion is more rapid, and a stiff consistency cannot be attained without excessive destabilization.

Before interpreting the phenomena of fat destabilization in ice cream, it is helpful to summarize the literature on the effects of processing and fat composition on emulsion breakdown in frozen ice cream.

It has been noted that in batch freezers the percentage of destabilized fat increases with aeration-freezing time (Keeney, 1958; Alsafar & Wood, 1965, 1966, 1968). Similarly, in continuous freezers, lowering the extrusion temperature increases the fat destabilization (John & Sherman, 1962). An observation made by Holland & Herrington (1953), on the effect of temperature on the churning time of milk fat, was that the fat must be partly liquid and partly solid for churning to occur. When the fat was totally liquid or solid the normal churning phenomenon did not occur.

The effect of fat content has been discussed by Kloser & Keeney (1959b) and Knightly (1959), and it was concluded that the degree of fat destabilization during freezing increased as the fat content of the mix increased. The effect of type of fat has been discussed by Frazeur (1959), Frazeur & Noller (1960), and Alsafar & Wood (1966, 1968), and it is apparent that churning is encouraged when the liquid fraction in the fat is greater. Keeney & Josephson (1958, 1962) suggested that the overrun obtained in ice cream depends on the activity of the emulsifier at the air cell-serum interface (promoting aeration) relative to its destabilization action at the fat-serum interface (inhibiting aeration). The effect of type and level of emulsifier on ice cream emulsion stability has also been discussed by Knightly (1959) who recommends the more liquid-type emulsifier, such as glyceryl mono-oleate, to provide controlled fat destabilization. John & Sherman (1962) investigated the effects of the emulsifier in ice cream, and found that monoglyceride increased the percentage of churned fat, decreased the subsidence at body temperature under constant load, decreased the rate of softening and increased the shape retention.

Govin (1969) has investigated emulsifier action in ice cream utilising the HLB concept. He found that the level of milk solids non-fat normally used in ice cream has sufficient capacity to emulsify the fat present. It was further found that the added emulsifier, irrespective of its HLB value or concentration, contributed little to the emulsification of the mix; instead, it promotes destabilization in the freezer, the extent of de-emulsification being proportional to the HLB value of the emulsifier—the higher the HLB number, the greater is the destabilization.

The evidence given above can now be interpreted in the light of the present state of knowledge. When ice cream is frozen, the combined effects of aeration, rapid freezing and mechanical agitation cause rupture of some of the protein membranes that protect the fat globules. The free oil is then available for migration to the air-mix interface. The strength of the protein membrane is clearly a significant factor in the emulsion destabilization that occurs during freezing.

Since the monograph of King (1955), many studies have been made of the *natural* membrane at the surface of the milk fat globule (*Comm. Bur. Dairy Sci. Technol.*, 1969). Comparatively little work, however, has been done on the *artificial* membrane at the

surface of the fat globules in homogenized milk and cream, or in ice cream mixes. The rheological work of Sherman (1961) suggested that the fat globules in ice cream were surrounded by a protein shell of thickness 0·3 μm, and that 80–90% of the total protein was involved. Sherman suggested that the emulsifier film around the oil globules was penetrated by the non-polar side groups of the first layer of adsorbed milk protein, and that the many films of protein within the protein layer have undergone different degrees of denaturation and coagulation, depending on their proximity to the O/W interface.

Our results, obtained by electron microscopy, suggest that this picture may need some modification. Plate 5 shows a group of fat globules in ice cream, each coated with a granular coating consisting of casein sub-units, 10 nm in diameter. These sub-units normally form the granular structure seen in casein micelles; such micelles and also free sub-units may be seen in Plates 2, 5, 6 and 7. No sign of a thick protein shell can be detected in Plate 7 or in any other electron micrographs in our work. Plate 7 does show however, a thin shell of casein sub-units, one unit (10 nm) thick, round some of the fat globules and we have observed this in other cases also. Another feature of our electron micrographs is that the protein covering surrounding fat globules is normally lifted off, in the fracture stage of specimen preparation, to reveal the steps, of molecular thickness, in the crystalline lipid layers at the surface of the fat globules. This suggests that the protein layer is only weakly bound to the fat surface in the frozen ice cream.

A number of other workers have used the electron microscope in recent years to study dairy products, and their observations confirm and supplement our findings. Muller & Eggmann (1969) also found that the globules from homogenized milk were surrounded by a layer of casein sub-units and this has been confirmed by Schmidt & Henstra (1970) and Henstra & Schmidt (1970a, b). In slowly cooled products such as sterilized cream (Graf & Muller, 1965), milk (Buchheim, 1969, 1970a) and in triglyceride emulsions (Buchheim, 1970b), electron microscopy has shown that the outer layers of the fat globules consist mainly of the high melting fraction. It has not yet been demonstrated that this is also the case in *rapidly* cooled O/W emulsions.

To sum up, the structure of the artificial membrane at the fat globule surface in homogenized products is probably, working from the globule outwards:

Crystalline fat — emulsifier — protein layer.

If this membrane is ruptured in the freezer, and the fat is still partially liquid, coalescence of the liquid fraction then occurs leading to churned fat as in Plate 3(b), and to the appearance of free oil at the A/W interface.

Our results in Fig. 3 show that when marked churning occurs, the number of fat globules per unit area in the electron micrographs decreases very markedly. This is not accompanied by the appearance of an appreciable number of particles of clumped fat, as might be expected from the observations in the literature, and we conclude therefore

than much of the free oil migrates to the air/water interface. Our interpretation of this difference is that the published observations have been made on ice cream after thawing. During thawing the fat globules lining the air cells are cemented, by the free oil which forms the envelope of the air cell, into 3-dimensional clumps, but these are largely absent in the structure of the frozen ice cream.

The air cell of Plate 4 shows a typical smooth surface which is different from the granular appearance of the continuous phase in the freeze-etched preparations; we have come to associate such smooth surfaces with fat, and it can also be seen that the cell is lined with fat globules. It is well known that oil will spread at an A/ W interface, and there is confirmatory evidence of this explanation from the literature. Arbuckle & Cremers (1954) found that the fat globules in ice cream were dispersed individually in the unfrozen phase, and individually and in chain-like arrangement round the air cells. Mohr & Mohr (1955) found that butter oil and butter fat crystals formed a layer round each air bubble in whipped cream. Keeney (1962), in work on foam fractionation of ice cream melts, found that the fat content of the foam was always higher than that of the serum, but the emulsifier content was almost always smaller. Alsafar & Wood (1968), using the electron microscope, confirmed the chain-like arrangement of fat globules around the air cells in ice cream frozen in a counter freezer. They also found that the fat globule membrane was thinner when emulsifier was used, when Tween 80 rather than Span 60 was used, and in samples subjected to prolonged agitation in the freezer.

In conclusion, our work on the phenomena of fat destabilization in ice cream, using electron microscopy, has thrown light on the role played by fat coalescence and the migration of liquid oil to the air/water interface. Much still remains to be investigated.

Acknowledgments

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A study of some of the factors affecting the spray drying of concentrated orange juice, on a laboratory scale

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Summary.

Concentrated orange juice (a) without additives and containing (b) sodium carboxymethyl cellulose, (c) Gum Acacia, and (d) liquid glucose as additives was spray dried in a laboratory drier. Liquid glucose was found to be the most satisfactory additive, producing a powder with good flavour, free-flowing characteristics and a minimum of wall deposition. Variations in air inlet temperature, feed temperature and rate and atomizer speed, within a limited range, resulted in no significant changes in the bulk density and particle size of the product. The higher temperatures did result in some change in colour and an increase in insoluble solids.

Cooled plate experiments indicated that the problem of wall deposition is related to wall temperature and is minimized when the wall temperature is below the sticky point temperature of the product.

Introduction

Dehydration has proved to be one of the most difficult of all preservation techniques to apply to fruit juices. Such materials are heat sensitive and contain a high proportion of hygroscopic substances such as sugars, which lead to problems in controlling the drying time, removal of the product from the drying zone and subsequent handling of the product.

Spray drying techniques have been applied to fruit juices. However, relatively high temperatures are encountered in spray drying, even taking into account the evaporative cooling which occurs. On the other hand, the drying times are much shorter than those required by other methods (freeze drying, vacuum drying) and so heat damage can be controlled provided the residence time of the dried particles in the hot zone of the drier is controlled. This latter condition is difficult to fulfil with fruit juice powders because of the properties referred to above. The extreme hygroscopicity, combined with the thermoplastic nature of such products gives rise to difficult wall deposition problems and hinders subsequent handling of the products.

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The two most fruitful lines of approach towards solving these problems appear to be the use of additives as drying aids and the use of specially-designed equipment to facilitate removal of the product from the drying chamber.

Additives which have been used to produce physical changes in the product and thus reduce wall deposition and product handling problems include air and compressed gases to produce foams, alginates, soya protein, glyceryl monostearate, natural gums, sucrose, corn syrups and dextrins. Bohm & Bornegg (1931) suggested the use of comparatively small amounts of water-soluble gums such as gum arabic, gum tragacanth and agar-agar. They found that 2% of gum arabic, on the basis of the total solids content of the feed, gave a product of low hygroscopicity. Perech (1946) used salts of carboxymethyl cellulose and suggested that 2% of the sodium salt was the most suitable for producing citrus fruit juice powders. Eddy (1950) used 1% of methyl cellulose as a drying aid for spray dried orange juice. Glycerol monostearate in the proportion of 1% of the total solids content of orange juice was used by Strashun (1951). However, low yields, flavour changes and poor reconstitution were encountered.

During the Second World War spray dried lemon juice powder was produced containing 20% lemon juice solids and 80% of corn syrup solids (Strashun & Talburt, 1954; Tressler, 1956). The reconstituted product possessed a modified taste and was limited in application. Holzcker (1943) produced an orange juice powder of good taste and colour containing 25% orange juice solids and 75% corn syrup solids.

Skimmed milk was used as a drying aid for a wide range of fruit juices by Coulter & Breene (1966). The product had good physical characteristics but there were obvious limitations as to its use.

Lazar *et al.* (1956) used a special design of spray drier in which cool air was admitted into the lower part of the drying chamber and an aspirator provided to transfer the powder from the first cyclone separator to warm dehumidified air in the second cyclone. A good quality tomato powder was produced when sodium bisulphite and sodium chloride were used as additives. Yields, however, were low, of the order of 60 to 65%. A 'cool wall' spray drier was used by Robe, Malvick & Heid (1968) to produce tomato powder. Air was drawn through a hollow jacket surrounding the drying chamber, maintaining a wall temperature of between 100 and 122°F (38 and 50°C). The air on leaving the jacket was further heated and directed into the drying chamber. The product was of good physical and organoleptic quality.

Much of the published work in this field is of a somewhat general nature. Published data on the effects of additives on the physical and organoleptic properties of the product, the effects of variations in operating conditions on product quality and drier performance and the factors influencing wall deposition, are surprisingly limited.

A study was undertaken to obtain further data of this type and experimental evidence on some of the more general observations made in the literature. Part of this work,

that which is concerned with the physical properties of the product and the drier performance, is presented in this paper.

Materials, methods and equipment

Materials

Orange juice. The composition of the orange juice concentrate used in this work is given in Table 1.

TABLE 1. Composition of concentrated orange juice

Total solids	60%
Ascorbic acid	227·5 mg per 100 g solids
Total sugar (as sucrose)	80·6% of solids
Sulphur dioxide	1990 ppm
pH	3·4

Additives: First grade, hand-selected Gum Acacia, from Senegal, was used.

After some initial trials, Edifas B, grade 10 (*Sodium carboxymethyl cellulose*) was selected for use.

B.P.C. liquid glucose. Acid-converted with a dextrose equivalent of 39 to 43 and a Beaumé reading of 43 $\pm$ 2° was the main sugar additive employed.

Methods

Insoluble solids were determined according to British Standard Specification No. 4288 Part 2 and A.O.A.C. 1955. Results are expressed as a percentage.

Colour was evaluated using a Lovibond Tintometer as described by MacKinney & Little (1962). All samples were reconstituted to 12% total solids for this purpose.

Particle size and size distribution were determined by a microscopic technique as described by Orr & Dallavalla (1959). The sample was dispersed in a 20% solution of celloidin in butyl acetate. Five hundred particles were sized in each case. Average particle diameters were calculated and expressed in microns. Size frequency diagrams were constructed when appropriate.

Bulk density was determined by placing a weighed sample of powder into a graduated cylinder. The cylinder and contents were vibrated on a sieve vibrator at a fixed frequency for a fixed period of time, after which time the volume occupied by the powder was noted and the bulk density expressed in g per ml. (Breene & Coulter, 1967).

Sorption isotherms for the powders were constructed from results obtained by the flask method used by Stitt (1958), Taylor (1961), and Karel & Nickerson (1964).

Sticky point temperature was measured by an empirical method as suggested by Lazar *et al.,* 1956.

A test tube containing a standard size sample of the powder was immersed in a water bath and the temperature of the bath raised slowly. The powder was stirred by an impeller. The sticky point was the bath temperature at which the current to the stirrer motor increased sharply. (Notter, Taylor & Downes, 1959).

Equipment

A Niro Minor Laboratory Spray Drier was used in all the drying trials. This is a vertical, cocurrent drier with a vaned centrifugal atomizer driven by an air turbine at speeds up to 40,000 rpm (See Fig. 1). The feed was metered into the drier by means of a peristaltic pump (H.R. Flow-Inducer, type MHRE/30, Watson-Marlow Limited, England) via a heat exchanger.

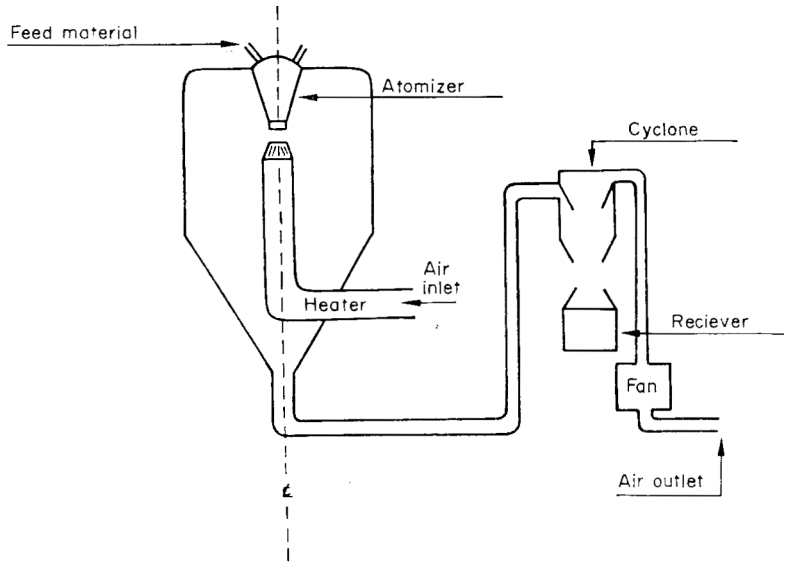

FIG. 1. Niro Minor Laboratory Spray Drier.

Experimental, results and discussion

Concentrated orange juice, adjusted to 40% total solids content, was spray dried under the range of operating conditions shown in Table 2. In all trials a heavy wall deposit was formed. Yields of product collected in the receiver were very small, of the order of 1 or 2% of that calculated from the feed solids. The product was difficult to remove from the drier walls even for cleaning purposes. The small quantity collected in the receiver was tacky and hygroscopic and very difficult to handle.

TABLE 2. Conditions under which standardized orange juice concentrate was spray dried without additives

Inlet air temperature °C	Outlet air temperature °C	Feed rate g/min	Feed temperature °C
120	75	22·5	20
120	72	28·5	20
120	70	40·5	20
125	81	22·5	20
125	70	28·5	20
125	67	40·5	20
150	97	22·5	20
150	94	28·5	20
150	89	40·5	20
175	114	22·5	20
175	109	28·5	20
175	104	40·5	20
200	118	22·5	20
200	112	28·5	20
200	106	40·5	20

Speed of the atomizer: 31,000 rpm
Air flow rate 1700 ft³ per hr

Use of sodium carboxymethyl cellulose

After some preliminary trials a feed material containing 41·5% total solids (40% orange juice solids, 1·5% Edifas B-10) was chosen as being the most suitable in terms of viscosity and suitability for handling in the feeding system. This feed material was dried under the range of operating conditions shown in Table 2. No improvement was noted as compared with the standardized juice concentrate without additives.

Use of Gum Acacia

Preliminary trials led to the selection of a feed material containing 37·5% total solids (35·25% orange juice solids and 2·25% Gum Acacia). The feed material was dried under the operating conditions shown in Table 2 but the runs at 120 and 200°C air inlet temperature were omitted. In these experiments yields of the order of 30–35% of a relatively free-flowing powder were obtained. The extent of the wall deposition, 70–65% of the powder produced, did not vary significantly with changes in operating

conditions. The products were analysed in terms of moisture content, bulk density and particle size. Sampling for analysis was done after the powder adhering to the wall had been brushed down and mixed with the product in the receiver. Moisture content results showed an expected pattern. The overall variation in bulk density was not great (0·45 to 0·60 g/cc). There was a trend towards lower bulk densities with increasing air inlet temperature. Particle sizes were generally within the range expected of the equipment used (22·5–30·5 μ). There was a general tendency for bulk density to decrease with decrease in particle size.

Use of liquid glucose

Preliminary trials, using liquid glucose of low D.E. value, malt dextrins and dextrins as additives, gave products with good free-flowing characteristics and low wall deposition, but an unacceptable flavour, as judged by a taste panel. The most satisfactory glucose additive proved to be B.P.C. liquid glucose, 34–43 D.E. and the best feed one containing 52·5% total solids (29·85% orange juice solids and 23·65% glucose). The conditions under which this feed material was dried and the results obtained in these trials are given in Table 3. The feed rates used in these trials were the maximum attainable under each set of temperature conditions and were selected by preliminary trials.

The products from these trials were of good quality, exhibiting free flowing properties and an acceptable flavour. The highest feed rate was possible at an air inlet temperature of 140°C (284°F). At the lower temperature 130°C (266°F) if the feed rate was increased much above 15 g/min, wetting of the chamber wall occurred. At an air inlet temperature of 150°C (302°F), feed rates greater than 20 g/min greatly increased the wall deposition and even at a feed rate of 20 g/min relatively low yields were recorded (40–58%). As in the previous experiments the product adhering to the wall was brushed down and added to that in the receiver prior to sampling. At a fixed air inlet temperature, moisture content of the product decreased with increasing feed temperature. The insoluble solids content of the product increased markedly at an air inlet temperature of 150°C (302°F) as compared with 130 or 140°C (266 or 284°F). The results at 150°C are not directly comparable with those at the lower temperatures because of the low yields, resulting in extended hold-up time and generally poor drier performance. There is no apparent correlation between particle size and bulk density nor between these two properties and the air inlet temperature or feed temperature. Colour measurements on the reconstituted products revealed a reduction in yellow units and saturation values with increase in air inlet temperature, indicating some loss of carotenoids.

Further experiments using a wider range of air inlet temperatures, 120–155°C (248–311°F), a constant feed rate of 15 g/min and feed temperature of 20°C (68°F) gave results which confirmed the observations made above in relation to bulk density, particle size and insoluble solids.

TABLE 3. Effects of operating conditions on bulk density, particle size, moisture content, insoluble solids and yield of spray dried orange juice powder using liquid glucose as additive

Operating conditions								
Inlet air temperature °C	Outlet air temperature °C	Feed rate g/min	Feed temperature °C	Bulk density g/cc	Particle size microns (μ)	Moisture content g of H_2O per 100 g. of dry solids	Insoluble solids %	Yields %
130	83	15	20	0·71	24·72	2·42	0·095	77·00
130	85	15	50	0·70	29·15	2·10	0·106	85·00
130	85	15	80	0·70	18·20	2·00	0·182	90·00
140	85	34	20	0·75	24·46	2·65	0·118	71·60
140	90	34	50	0·72	26·50	2·00	0·107	62·26
140	94	34	80	0·67	30·35	2·00	0·105	61·10
150	80	20	20	0·69	17·45	2·25	0·337	40·00
150	84	20	50	0·64	21·72	2·10	0·430	43·00
150	85	20	80	0·72	20·19	2·00	0·450	58·40

Speed of the atomizer	31,000 rpm
Air flow rate	1700 ft^3/hr
% insoluble solids in the feed material	0·07

Sorption isotherms were constructed for selected samples of the product. The sorption isotherm constructed for the powder immediately after production (air inlet temperature 140°C (284°F), air outlet temperature 80°C (176°F), feed rate 34 g/min) is shown in Fig. 2. The shape of the isotherm is typical of fruit and vegetable powders (Karel & Nickerson, 1964; Labuza, 1968). The results were plotted in accordance with the B.E.T. theorem and the water held in the monolayer estimated as being 0·707 g per 100 g of solids. Karel & Nickerson (1964) found a value of 1·09 g per 100 g solids for orange juice crystals.

Another experiment was performed in which atomizer speed was varied from 25,000 rpm to 37,500 rpm, other conditions being kept constant. Within this range atomizer

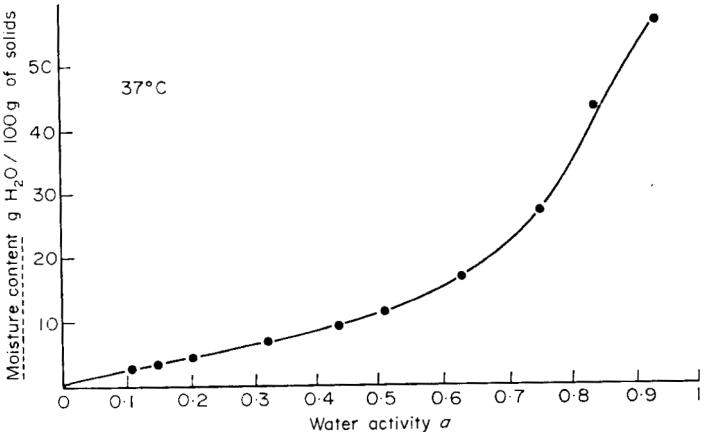

FIG. 2. Water sorption isotherm, at 37°C, for spray dried orange juice powder containing liquid glucose as additive. Measurements made on freshly-produced powder.

speed had no effect on the moisture content, particle size and insoluble solids content of the product. Bulk density increased slightly with increase in atomizer speed.

Wall temperature effects

In spite of the improvements in yield resulting from the use of liquid glucose as additive, wall deposition amounting to 10–60% was still encountered. Modifications to the drier, including the use of an aspirator and secondary cyclone in the product collecting system, as suggested by Lazar *et al.* (1956), failed to improve the yields significantly. These heavy deposits could be accounted for either by non-uniform drying (perhaps resulting from non-uniform atomization) or too high a wall temperature. Size analysis of the products (including that brushed from the walls) showed that particle sizes fell within quite a narrow range. A typical size-frequency diagram is shown in Fig. 3. Attention was therefore turned to the problem of wall temperature.

Copper-constantan thermocouples were attached to the inside walls of the drying chamber as shown in Fig. 4(a). The temperature registered during subsequent drying trials were recorded on a multi-point potentiometric recorder. Typical results are given in Table 4.

The sticky point temperatures of product samples, conditioned to different moisture contents, were measured. The results are summarized in Fig. 5. It can be seen that sticky point temperature is a moisture–dependent property and that for an orange powder with a moisture content of 2 g per 100 g dry solids the sticky point temperature is of the order of 44°C (111°F). These findings agree generally with those of Lazar *et al.* (1956) for tomato powder except that they found that the sticky point temperature of their product with a 2% moisture content was 60°C (140°F). It is clear from Table 4

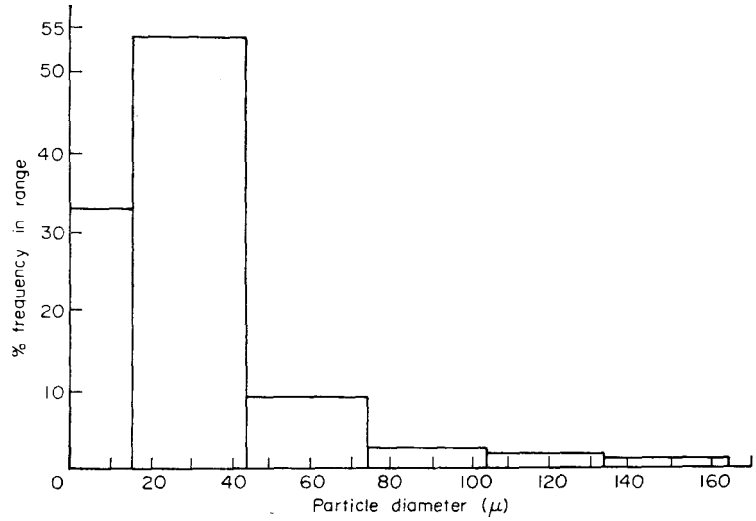

FIG. 3. Typical size frequency curve for orange juice powder with liquid glucose as additive.

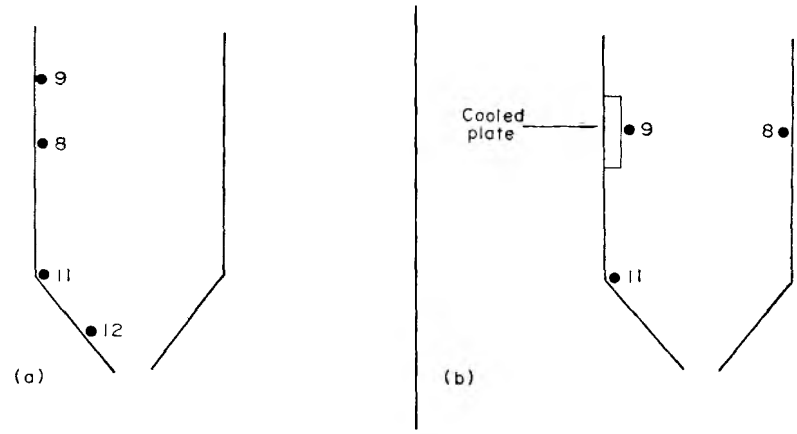

FIG. 4. Location of thermocouples on drier walls and cooled plate.

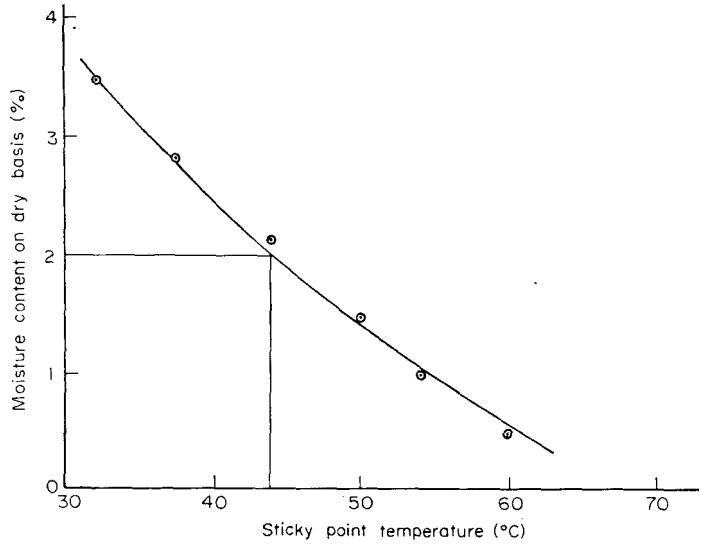

FIG. 5. Relationship between sticky point temperature and moisture content of orange juice powder with liquid glucose as additive.

TABLE 4. Wall temperatures recorded under different operating conditions (Ref. Fig. 4(a))

Operating conditions				Temperature recorded in each point °C				
Inlet air temperature °C	Outlet air temperature °C	Feeding rate g/min	Feeding temperature °C	Point 9	Point 8	Point 11	Point 12	Moisture content g of H_2O per 100 g of dry solids
150	83	34	20	84	84	81	81	2·00
140	83	34	20	75	75	75	75	2·00
130	79	34	20	74	77·5	69	69	2·42
150	88	34	50	85	85	78·4	82·4	1·95
140	82	34	50	80	80	77	73·0	2·00
130	78	34	50	80·5	80·5	74·7	70	2·10
150	93	15	50	135	140	113	120	1·91
140	89	15	50	127	122·5	103	115	2·10
130	84	15	50	126·5	126·6	105	110	2·15

that the wall temperatures encountered in the drying trials were well in excess of the sticky point temperatures of the product which had moisture contents generally of the order of 2%.

A water-cooled plate was fitted into the drying chamber of the laboratory spray drier and a thermocouple attached to its surface. Further drying trials were carried out with this plate in position. The temperatures were recorded at the points indicated in Fig. 4(b) and the extent of wall deposition both on the plate surface and on the drier walls noted. These results are given in Table 5. Although no means of accurately

TABLE 5. Drier wall and cooled plate temperatures recorded under different operating conditions (Ref. Fig. 4(b))

Operating conditions		Temperature °C		
		Point 9	Point 8	Point 11
Inlet air temperature	140°C			
Feed rate	15 g/min	40·5	55·5	56·5
Feed temperature	20°C	No deposition	Deposition on the wall	
Outlet air temperature	61°C			
Inlet air temperature	140°C			
Feed rate	22·5 g/min	35·0	52·0	53·0
Feed temperature	20°C	No deposition	Deposition on the wall	
Outlet air temperature	62°C			
Inlet air temperature	140°C			
Feed rate	34 g/min	35·0	49·0	53·0
Feed temperature	20°C	No deposition	Deposition on the wall	
Outlet air temperature	58°C			
Inlet air temperature	130°C			
Feed rate	15 g/min	29·5	49·0	53·0
Feed temperature	20°C	No deposition	Deposition on the wall	
Outlet air temperature	60°C			
Inlet air temperature	130°C			
Feed rate	22·5 g/min	26·0	49·0	53·6
Feed temperature	20°C	No deposition	Deposition on the wall	
Outlet air temperature	58°C			
Inlet air temperature	130°C			
Feed rate	34 g/min	23·5	49·0	53·0
Feed temperature	20°C	No deposition	Deposition on the wall	
Outlet air temperature	56°C			

controlling the plate temperature were readily available it is clear from these results and from visual observations that when the plate temperature was below 40·5°C (105°F) little or no deposition occurred on it, whereas drier wall temperatures as low as 49°C (120°F) still resulted in heavy deposits forming. These results indicate that if the drier wall temperature is in excess of the sticky point temperature of the product, wall deposition will occur and low yields will result.

Conclusions

Concentrated orange juice without additives cannot be satisfactorily spray dried because of the hygroscopic and thermoplastic nature of the product. Under the conditions of these trials, sodium carboxymethyl cellulose proved ineffective as a drying aid. Gum Acacia was partially effective but heavy wall deposition still occurred. B.P.C. Liquid Glucose (39–43 D.E.) proved to be the most effective additive, reducing the wall deposition markedly and producing a product of acceptable flavour and with good free-flowing properties. Variations in air inlet temperature in the range 120–155°C (248–311°F), in feed temperature in the range 20–80°C (68–176°F), in feed rate in the range 15–34 g per min and atomizer speed in the range 25,000–37,000 rpm produced no significant change in the bulk density or particle size of the product.

There was an increase in insoluble solids and also some change in colour at the higher temperatures. The size analysis of the product together with the observations in the cooled-plate experiments suggest that wall deposition is related to wall temperature. Cooling the wall to a temperature below the sticky point of the product eliminates wall deposition but no account has as yet been taken of the effects of such cooling on the performance of the drier.

Naturally these conclusions are strictly related to this size and type of spray drier and are not necessarily applicable to larger plant or plant of different design.

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Part of this work (by J.H.) constitutes work for a higher degree at the National College of Food Technology (University of Reading).

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Protein, lactose and lactic acid separation from cheese whey using reverse osmosis dynamically formed membrane

A. M. FREEDMAN AND H. I. SHABAN

Summary

Hydrous zirconium oxide dynamically formed membrane was used in reverse osmosis apparatus to separate protein, lactose and lactic acid from cheese whey directly. An unexpected feature was 40% rejection of pure protein within 1½ hr from commencement of experiment. However, protein rejection figure changed to 44% with undesirable 17·4% lactose rejection and 29% lactic acid rejection after 4 hr approximately. The permeation rate of solvent across the membrane was constant and the colour rejection of cheese whey was almost complete (approx. 100%).

Introduction

Reverse osmosis is a membrane permeation technique for separating water and other small molecules from solutions containing large molecules. At present reverse osmosis is used for desalination of some brackish waters and sea water but on a small scale compared with flash distillation which produces millions of gallons of fresh water daily.

More recently reverse osmosis processes have been used to concentrate fruit juices, egg white and whey, to refine sugar and in biological applications as reported by Harrison (1969).

The membranes currently used in research are made from cellulose acetate a few microns thick. The use of cellulose acetate membranes is based upon the work of Reid and Breton, as described by Merten (1966), later modified by Loeb and Sourirajan (Sourirajan, 1970a) to produce better salt rejection and higher water flux.

It has been reported by Thomas & Watson (1968) that two of the most promising types of membranes considered for reverse osmosis are cellulose acetate and dynamically formed membranes, the latter formed from a variety of additives. The most attractive features of dynamically deposited membranes are their high production rates and their self healing characteristics by retaining in the feed solution a small amount of the membrane forming additive, as shown by Kraus, Shor & Johnson (1967).

In June 1965, at the Oak Ridge National laboratory, Marcinkowsky observed that

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by circulating a pressurized salt solution containing a few hundred ppm of an additive ($Th\ Cl_4$) past a porous silver frit, a high flux sodium chloride rejection layer was formed dynamically on the filter surface, as reported by Sourirajan (1970b).

Kraus *et al.* (1967), and Sourirajan (1970a) have mentioned suitable additives for membrane formation. Examples are: organic polyelectrolytes, hydrous oxides and salts of hydrolysable metals (Zr (iv), Fe (iii), and U (vi)), ground, low cross-linked ion exchange beads and certain natural products, such as clays and humic acid.

Whey is a by-product of cheese manufacture and mainly used for lactose production, animal feed and food additives. Fractionation of whey yields mainly lactose, lactic acid and protein.

The purpose of this experiment was to explore the technical feasibility of applying reverse osmosis to separate protein from lactose and lactic acid directly in cottage cheese whey using a hydrous zirconium oxide dynamically formed membrane.

No work has been reported on direct separation of protein, lactose and lactic acid by dynamically formed membranes. The use of cellulose acetate membranes for cheese whey concentration to a level suitable for spray drying has been reported by Harrison (1969, 1970), McDonough (1968) and Horton (1970). Similar work has been reported by Marshall, Dunkley & Lowe (1968) but applying the crystallization method for extracting protein, lactose and salts from concentrate.

Rejection and permeation across the membrane depends on the concentration, viscosity and feed circulation velocity.

A summary of relevant equations of filtration of solute across the membrane has been given by Spiegler (1966) and can be written in terms of observed rejections (Ro) as

$$Ro = 1 - \frac{Cp}{Cf} \qquad \ldots (1)$$

where Cp represents concentration of product and Cf represents concentration of feed in g/l in the turbulent annulus. In fact, Cf is taken as equal to the solute concentration at the membrane feed interface.

The rate of flow of solvent across the membrane can be represented by equation (2) due to Merten (1966), i.e.

$$\text{Water flow} = \frac{K\ A\ (\Delta P_i - \Delta P_2)}{t} \qquad \ldots (2)$$

where

K = permeability coefficient of membrane for water
A = membrane area
ΔP_1 = pressure differential across membrane
t = thickness of the membrane
ΔP_2 = osmotic pressure differential across membrane

Membrane porosity and thickness play an important role in obtaining good permeability and solute rejection. It is difficult to apply this equation due to difficulties in obtaining value for (K) and (t) for hydrous zirconium oxide membrane. Consequently, flow rate was measured directly.

Materials and methods

Membrane

The basic material for the membrane was hydrous zirconium oxide. Kuppers *et al.* (1967) have investigated filtration of organic solutes using inorganic hydrous zirconium (iv) oxide and have shown that glucose, sucrose and raffinose rejection increased with increasing molecular weight. Kraus *et al.* (1967) have reported similar work, using alcohols. Hydrous zirconium oxide membranes have also been used in the pulp and paper industry to reject most of the colour present in the liquors generated in the sulphite process as reported by Perona, *et al.* (1967). Hydrous zirconium oxide membranes have been found to reject salts (Mg Cl_2, Na Cl and Na_2SO_4) as shown in the *Saline Water Conversion Report* (1967, 1969), Sourirajan (1970b) and Kraus *et al.* (1967).

Membrane formation

The membrane was prepared by boiling 2 g zirconium chloride in 1 litre distilled water under reflux for 20 hr. The solution acquired a milky appearance indicating the formation of hydrous zirconium (iv) oxide. A similar method has been described by Marcinkowsky *et al.* (1966).

The solution so obtained was made up to 8 litres with distilled water and then circulated to the outside of the porous ceramic tubing in the reverse osmosis apparatus, shown in Fig. 1. The porous ceramic tube used as the membrane support was 12 in long, 1·0 in outside diameter and $\frac{1}{2}$ in inside diameter with 32% porosity and pore diameter less than 1·5 microns.

A starting pressure of 100 psi was used to deposit the membrane on the porous surface. The pressure increased automatically to 200 psi within the first 20 min of the experiment as a result of membrane formation. The permeation rate was approx. 48 gal/ft^2/day. After 4 hr of continuous running the permeation rate decreased to 30 gal/ft^2/day and the pressure gauge recorded a constant pressure of 400 psi. The membrane formation at this stage was complete and the rest of the solution in the reverse osmosis cell was drained and replaced with whey solution.

Operating condition after membrane formation

The operating pressure was kept at 400 psi for the main part of the experiment. Operating was attempted at 600 psi to investigate the change in production; it was found, however, that under this condition the membrane deteriorated. The whey solution feed was at 20°C with a pH of 6. A feed circulation rate of 10 gal/hr was used.

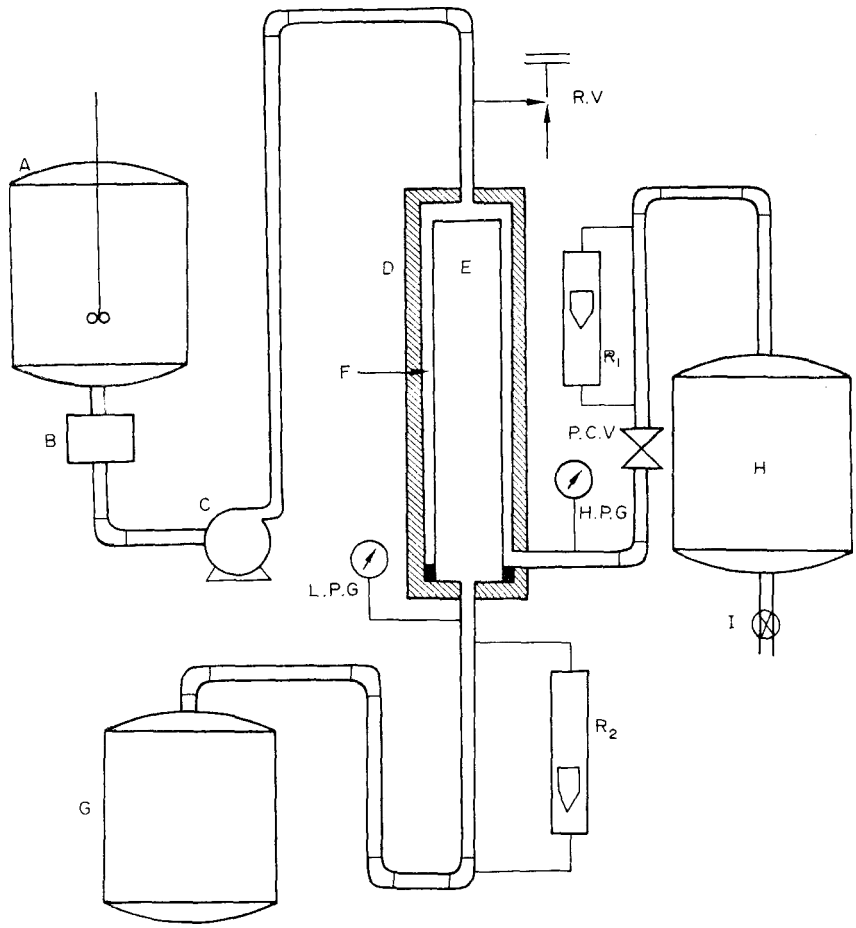

FIG. 1. Diagram of reverse osmosis apparatus. A, Feed storage tank (whey); B, filter; C, high pressure pump; D, reverse osmosis cell; E, porous ceramic tubing; F, thin layer of membrane; G, product storage tank; H, waste storage tank; I, shut off; R_1, rotameter at waste; R_2, rotameter at product; H.P.G., high pressure gauge; L.P.G., low pressure gauge; P.C.V., pressure control valve; R.V., relief valve.

Results and discussion

Cheese whey was diluted five times with distilled water. Dilution was necessary due to the small scale of the system.

It has been found whilst testing the dynamically formed zirconium hydroxide membrane, that reverse osmosis occurs with dilute and concentrated solutions of brine, the rejection being greater with the more dilute feed. More concentrated solutes of whey, such as handled industrially, could not conveniently be tested at this stage because of system limitations. It is believed, on the basis of brine and other tests, that con-

centrated whey feed could be expected to behave similarly.

The permeate and feed samples were collected at fixed intervals and each analysed for protein, lactose, lactic acid, fat and ash. The fat and ash content of the feed was negligible and no traces were found in the permeate (product), hence no calculations were made for fat and ash. Lactic acid gave a not inconsiderable rejection being close to lactose rejection. Thus Fig. 2 represents the overall results obtained for protein, lactose and lactic acid rejection in $18\frac{1}{2}$ hr.

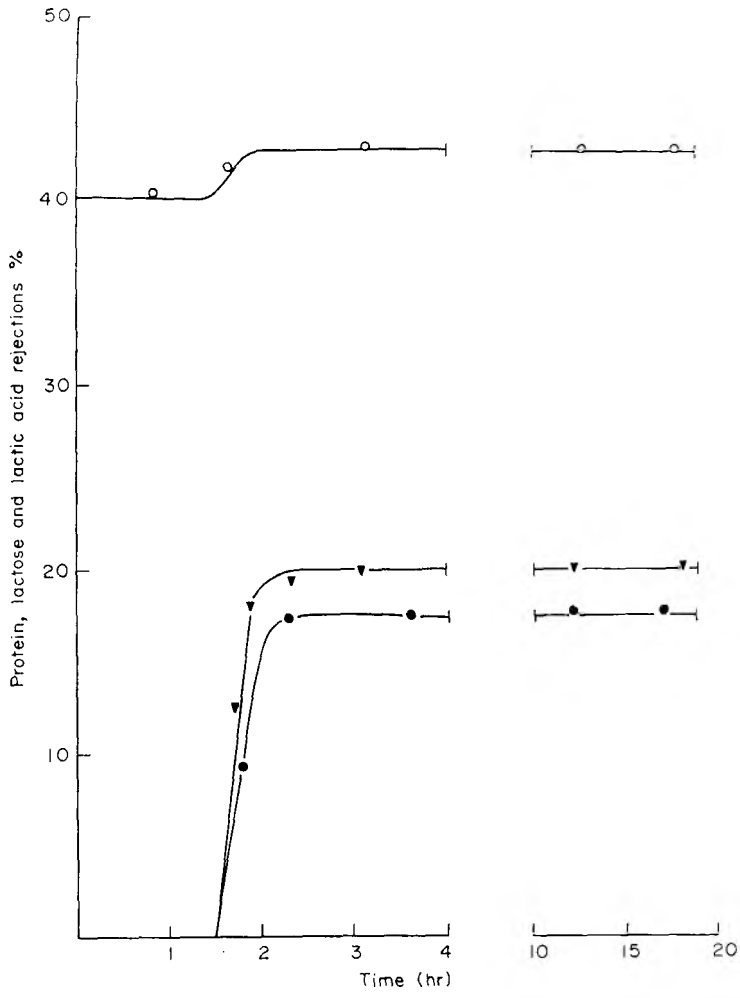

FIG. 2. Solute rejections % versus time (hr). Hyperfiltration by hydrous zirconium oxide dynamically formed membrane on porous support (porcelain tubing of 32% porosity at 400 psi). O, Protein; ●, lactic acid; ▼, lactose.

Equation (1) was used to calculate the solute rejection in the whey solution.

The most interesting observation (see Fig. 2) was the rejection of pure protein up to a level of 40% in the first 1½ hr of the experiment. After 3½ hr protein rejection increased to 44% but with some lactose (Ro = 16·3%) and lactic acid (Ro = 20%). Solute rejection became constant after approx. 4 hr of continuous running, i.e. protein, lactose and lactic acid rejection were 44%, 17·4% and 20% respectively. The lactose and lactic acid rejection rose sharply after 1½ hr giving undesirable rejection rates. This was probably due to a new membrane formation on top of the previous hydrous zirconium oxide membrane. The new membrane could be caused by proteins or other solutes present in the whey solution.

At 400 psi the permeation rate of solvent across the membrane was steady at about 30 gal/ft²/day.

The colour rejection of cheese whey which had a milky appearance was also recorded and equation (1) was used again to calculate colour rejection. For the first 3 hr of the experiment the colour rejection was 99·99% (clear permeate), after which the colour rejection reduced to 99·6% for the remaining time (15½ hr).

When the pressure was altered from 400 to 600 psi the permeation rate increased to 36 gal/ft²/day and the permeate became milky as a result of membrane deterioration. The colour rejection was based on optical absorption made at 684 nm.

The fat, ash, lactic acid, lactose and protein were tested by Rose-Gottlieb, gravimetric, titration with N/9 NaOH, Benedicts quantitative solution and Kjeldahl methods respectively.

Conclusion

It has been shown that dynamically formed membranes using hydrous zirconium oxide in the reverse osmosis apparatus are very promising in separating protein, lactose and lactic acid in cheese whey solutions.

Since this kind of membrane has a high porosity, it shows great promise in the colour rejection applications in the field of food technology where large molecules are involved. High production rates may be obtained using this method.

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Differential thermal analysis of frozen food systems. I. The determination of unfreezable water

R. B. DUCKWORTH

Summary

A method is described for the determination of the amounts of unfreezable water in food materials by the use of differential thermal analysis (D.T.A.). Results for a range of different food materials and food constituents are summarized and compared with a smaller number of values obtained by other workers using a variety of other techniques. The method described is simple and direct and is free from the uncertainties which attend the procedures involved in most of the methods previously employed.

Introduction

The immobilizing influence of the solid constituents of a biological material, particularly of the skeletal macromolecular solids, on a substantial part of the water present in such a system is a phenomenon well known to biological scientists. The amount and condition of such partially-immobilized water in a processed foodstuff can have an important effect on both the initial quality and the storage behaviour of the material concerned.

Various criteria have been used from time to time to distinguish the extent of this water-binding in biological systems and in some cases quantitative determinations have been made of fractions of water having properties which contrast sharply with those of the bulk liquid. One criterion which has been used more than any other is non-freezability at low temperatures (Kuprianoff, 1958; Duckworth & Smith, 1963). A number of different methods have been employed to determine the size of the non-freezing fraction, either directly or, more commonly, by measuring the amount of ice formed and therefore the quantity of water remaining unfrozen by difference.

Moran, in some early studies (Moran, 1926, 1931) first used a dilatometric method to determine the amounts of ice forming in gelatine gels. He later discarded this, in work on egg albumin and on muscle tissue (Moran, 1935), in favour of a method in which ice was caused to form only at the surfaces of a test specimen and in which the

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residual non-freezing water within the bulk of the specimen could, therefore, be determined directly. Most workers in the field have, however, employed calorimetry (Plank, 1925; Mennie, 1932; Daughters & Glenn, 1946; Riedel, 1956, 1957, 1960, 1961, 1969; Nemitz, 1961; Fleming, 1969) while, in recent years, nuclear magnetic resonance (Sussman & Chin, 1966; Toledo, Steinberg & Nelson, 1968) differential scanning calorimetry (Davies & Webb, 1969; Parducci, 1970; Youngs, 1970) and differential thermal analysis have each been added to the list of techniques used for this kind of determination. The present paper is concerned with the last of these methods.

Differential thermal analysis (D.T.A.) is a technique for recording the difference in temperature, Δt, between a test substance and an inert reference material as samples of the two are warmed or cooled, preferably at a constant rate, over a suitable range of temperature. If the test substance is thermally active, then the resulting curve, plotting Δt against sample temperature, shows one or more irregularities or peaks. These indicate the occurrence and measure the extent of energy-involving reactions, transitions or phase changes within the test sample over the range of temperature employed.

The history of use of the method since its initial development at the end of the last century (Roberts-Austen, 1899) has recently been traced by McKenzie, R. C. (1970). For half a century, application of D.T.A. was limited almost exclusively to the examination of inorganic materials, but during the last twenty years its use has spread rapidly, first to the study of relatively simple organic substances and then later to that of more complex systems of biological origin. Reviews of some of these more recent applications have been published by Mitchell & Knight (1965), Murphy (1966), Ladbrooke & Chapman (1969) and MacKenzie, R. C. (1970).

Low temperature D.T.A. of aqueous solutions, utilizing the latent heat requirements to study freezing patterns appears to have been first used by Rey (e.g. 1960, 1961) and later Greaves & Davies (1965) in work on the freeze-drying of biological systems and on the possible use of cryoprotective substances to prevent damage to living organisms caused by ice-formation. Further detailed studies of a similar kind have been carried out more recently by Rasmussen and his co-workers (e.g. Rasmussen 1969, Rasmussen & Luyet, 1969), while Mazur (1963) and MacKenzie A. P. (1970) have used low temperature D.T.A. on aqueous systems actually containing living micro-organisms.

Differential thermal analysis of frozen food materials and the use of the method for the direct determination of unfreezable water in solid systems of biological origin were both pioneered by the author and various research students (Ralph, 1964; Hood, 1965; Gauldie, 1966; Burnett, 1967; Kelly, 1970; Youngs, 1970; Parducci, 1970). The purpose of the present communication is to describe the method used for the determination of unfreezable water in food materials and to summarize some of the results obtained.

Materials and methods

The materials examined during the course of the work are listed in Table 1. Treatment prior to thermal analysis was essentially similar in each case. Since all the materials to be examined were solid over the range of moisture content within which they were to be analysed, it was necessary to prepare them in a physical form in which they would most readily fill, and make good thermal contact with the surfaces of, the chamber of the D.T.A. assembly, viz, in a powdered form. Each material was therefore first dried in warm air sufficiently to permit powdering and then put through a laboratory hammer mill. Samples of the resulting powder were subsequently moistened (or dried) to suitable water contents for examination by D.T.A. The moisture content was increased either by subjecting the sample to an atmosphere of high relative humidity within a desiccator and monitoring the weight increase until the desired end point was reached. Alternatively, if this procedure proved too protracted, direct addition of the necessary amount of water was made from a micrometer syringe or microburette, followed by thorough mixing of the sample. In either case, a minimum period of 6 days was allowed for complete re-equilibration within the sample after the moisture level was changed and before thermal analysis was performed.

An initial screening run was necessary with each material in which a number of samples showing relatively large incremental differences in moisture content covering a wide range were analysed to determine over what narrower range a more detailed examination would be necessary. Subsequently, a further set of samples falling within this latter range of moisture content and showing relatively small differences was prepared and subjected to thermal analysis.

Moisture contents were determined on the samples actually used for D.T.A. by a standard oven method. Duplicate samples of between 2 and 2·5 g were dried in a vacuum oven (< 10 mm Hg pressure) at 70°C for 24 hr, the moisture content being calculated from the loss in weight. Results are expressed in all cases as g water per 100 g dry solids.

The differential thermal analysis itself was carried out for the most part on simple apparatus designed and constructed in the author's laboratory—see Fig. 1 (a few of the data quoted in Table 1 were obtained by a similar procedure but using the Du Pont 900 Differential Thermal Analyser which became available at a late stage). The test sample and corresponding reference sample were accommodated, in the simpler apparatus, in opposed chambers drilled out from a solid cylindrical block of aluminium 7·5 cm in diameter, each chamber being large enough to hold between 3 and 5 g of a typical powdered test material.

Any dry finely-divided material which remains inert over the range of temperature employed could be used as reference. During the course of the work a number of possible alternative reference materials, including glass beads, sand, kaolin, sawdust and the test material itself (water free) were tried in an attempt to match the general

TABLE 1. Unfreezable water contents of some food materials and food constituents

Material	Unfreezable water, determined by differential thermal analysis (actual values lie between the limits shown)		Other values for non-freezing water from the literature		
		Lowest freezing temperature employed		Method	Lowest freezing temperature employed
Cellulose (cotton)	13·1–14·7	−78°C			
,, (potato)	18·0–18·45[1]	−180°C			
Starch (potato)	32·0–32·7[1]	,,	38–40[2]	Calorimetry	−40°C
,, (soluble)	22·1–30·2[3]	−78°C			
Pectin (apple)	28·52–32·43[1]	−180°C			
(orange)	36·0–36·78[1]	,,			
Agar	41·16–43·21	,,			
Albedo (orange)	30·0–32·23[1]	,,			
Bean (green)	22·5–22·9	−78°C	11·0, 68·5[4]	Calorimetry	−20°C
Brussels Sprout	23·3–25·1	,,			
Cabbage	18·2–22·2	,,			
Carrot	22·7–24·5	,,			
Celery	19·72–20·14[5]	,,			
	19·75–23·75[5]	−180°C			
Pea	19·1–19·7	−78°C	4·1–32·1[4]	Calorimetry	−20°C
Potato	23·1–23·7	,,			
Gelatine	36·23–46·11	−180°C	53–56[6]	Dilatometry	−19°C
	48–50[7]	−30°C	49–58[8]	Calorimetry	−20°C
Egg white	26·76–29·32[5]	−180°C	28·6[9]	Calorimetry	−70°C
			25·5[10]	Calorimetry	−30°C
			41[11]	Dilatometry	−20°C
,, ,, (heat denatured)	22·36–25·57[5]	,,	22·0[10] (desalted)	Calorimetry	−30°C
Egg albumin			26, 35–37[11]	Direct	−20°C
,, ,, (denatured)			26, 30[11]	Direct	−20°C
Beef muscle (raw)			+25[13]	Dilatometry	−20°C
			43[11]	Direct	−40°C
,, ,, (cooked)	24·3–24·7	−78°C	22·5[12]	Calorimetry	−180°C
Cod muscle	25·7–27·0	,,	39·0[14]	Calorimetry	−70°C
	24·05–24·45[5]	,,	36·8[15]	Nuclear magnetic resonance	−20°C
	24·02–24·80[5]	−180°C			

continued

TABLE 1. Unfreezable water contents of some food materials and food constituents

Material	Unfreezable water, determined by differential thermal analysis (actual valves lie between the limits shown)	Other values for non-freezing water from the literature	
		Method	Lowest freezing temperature employed
Milk (whole)	8·7–12·8[16]	– 180°C	
,, (skim)	16·7–18·1[16]	,,	
Casein	24·1–29·5[16]	,,	
Whey	19·9–24·8[16]	,,	

(1) Youngs, 1970
(2) Riedel, 1960
(3) Ralph, 1964
(4) Daughters & Glenn, 1946
(5) Parducci, 1970
(6) Moran, 1926
(7) Hood, 1965
(8) Mennie, 1932
(9) Riedel, 1957
(10) Nemitz, 1961
(11) Moran, 1935
(12) Riedel, 1961
(13) Moran, 1931
(14) Riedel, 1956
(15) Sussman & Chin, 1966
(16) Kelly, 1970.

thermal characteristics of the hydrated test material as closely as possible and thus reduce the problem of baseline slope. Mixtures of sand and sawdust were generally found to be at least as satisfactory as any other materials for the purpose and their use was latterly adopted as standard procedure.

Two diametrically opposed pairs of chambers were drilled into each block so that two test samples with corresponding reference samples could be accommodated at one time. The temperature difference between reference and test samples was monitored in earlier work using a simple iron-constantan thermocouple pair. One couple was positioned centrally within each sample, the constantan wire forming the bridge between the two and the two iron wires leading directly to the input terminals of the linear amplifier of a modified electronic recorder. The signal was amplified fifty times, so that one complete traverse of the 11-inch chart of the recorder corresponded to a temperature difference of 0·4°C. In later work, this sensitivity was increased several fold by using differential thermopiles consisting of up to five pairs of thermocouples in series, the couples in this case being constructed from 24 gauge chromel and alumel wires supported and insulated near the junctions by fine ceramic sleeves—Fig. 1. Sensitivity was increased in approximate proportion to the number of pairs of thermocouples used and was such as to permit detection of the thawing of appreciably

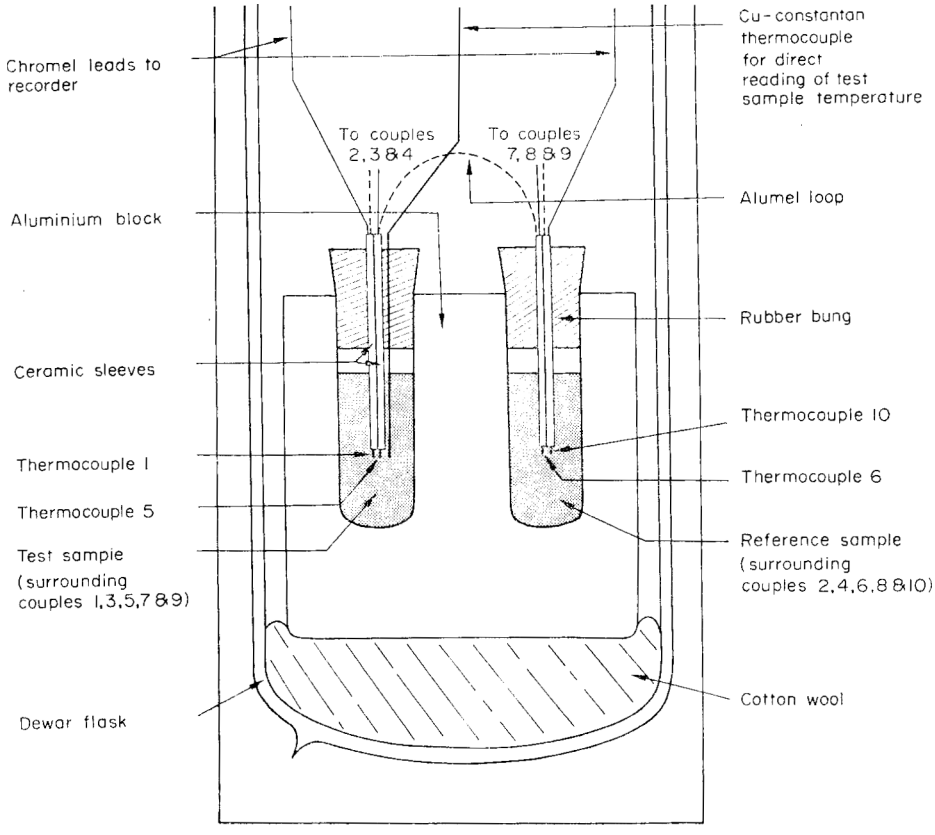

FIG. 1. Diagram of the block assembly used for differential thermal analysis with test and reference samples and thermocouples in operating positions.

less than 1 mg of a eutectic sodium chloride solution. A copper-constantan thermocouple was also inserted in each test sample to provide simultaneous monitoring of sample temperature. Full details of construction of the D.T.A. block assembly are shown in Fig. 1.

In most of the work summarized in this paper the range of temperature used for the D.T.A. was between − 78°C (or − 70°C) and ambient. The block containing the samples was first cooled with powdered CO_2 and subsequently, after equilibration and rapid removal of excess coolant, allowed to warm up inside a dewar flask held in a well insulated box. Alternatively, the block assembly was cooled down, already insulated, in a low temperature cabinet, the refrigeration of which was switched off when the assembly had reached equilibrium at the cabinet temperature of − 70°C.

The rate of warming was controlled by varying the amount of insulation and, although not constant throughout a run because of the changing temperature differ-

ence between the block and the laboratory atmosphere which served as the heat source, the change in warming rate over the temperature range of interest was in fact small. Warming rates of between 5° and 8°C per hour were most commonly used. This provided a suitable period of time in the early part of a run for the system to recover after the initial disturbance caused by removal of the coolant and also permitted the accurate pin-pointing of significant transition temperatures. With continuous automatic recording of Δt and of the test sample temperature, analyses could be conveniently run overnight.

Results

Representative sets of D.T.A. curves for samples of potato (scalded), of green bean (scalded) and of cod muscle showing small differences in moisture content around the respective unfreezable water levels are illustrated in Fig. 2. Prominent features of the curves from the moister samples in each series are the broad asymmetrical peaks produced as a result of the thawing of ice formed during the initial cooling process. It is not easy to distinguish precisely in these curves the points at which melting was initiated during warming, but the temperature range over which the thawing process reached completion is indicated clearly by the sharp reduction and virtual disappearance of Δt at the upper limit of each peak. The reduction in size of the peak for samples containing lesser amounts of freezable water is also clearly shown and the area under each peak, though difficult to measure with accuracy, is directly related to the quantity of freezable water present in the sample. Over a particular range of moisture content, encompassing the highest level at which no freezable water remains, evidence of thawing during warming completely disappears as shown by the completely smooth form of the lowest curve in each series. Were any of the substantial amount of water contained within the respective samples capable of undergoing a normal freezing process during cooling to − 78°C, subsequent thawing of the ice formed would inevitably manifest itself on the D.T.A. curve. The absence of any such indication is therefore taken as establishing the non-freezability of the water present in these samples over the range of temperature employed. In the case of some materials, results for which are included in Table 1, the temperature range was extended downwards by using a Dupont Differential Thermal Analyser with liquid nitrogen as a coolant. No evidence was obtained that cooling to temperatures below − 78°C can induce the formation of additional ice. Indeed, in those cases, e.g. celery, where the content of unfreezable water has been determined by both procedures, no difference was found in the pattern of results (see Table 1).

A further feature of interest illustrated by the curves in Fig. 2 is the variation between different materials in the temperature relations of the thawing process. Usually the temperature at which melting begins during warming can be distinguished with greater confidence when the curves for samples containing relatively small amounts of

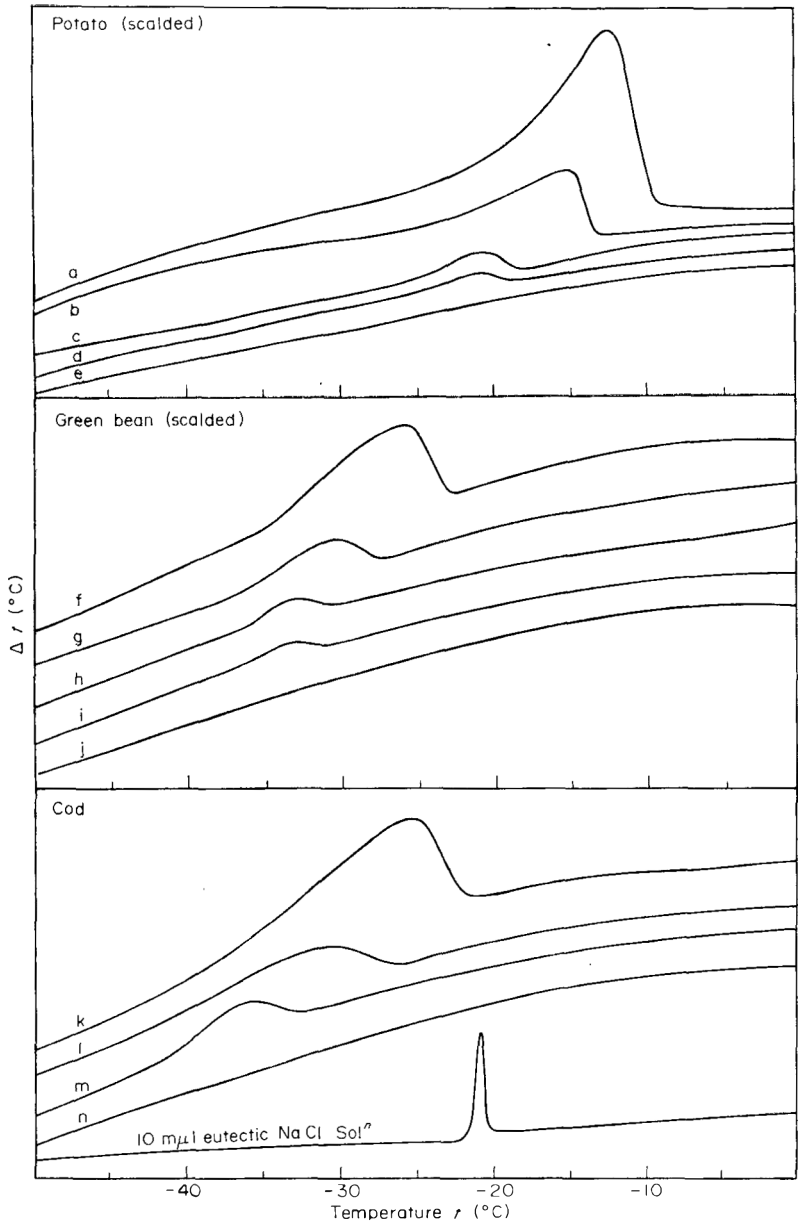

FIG. 2. Series of $\triangle t$ curves for samples of potato, green bean and cold muscle containing different amounts of water ranging upwards from the unfreezable water level in each case. The $\triangle t$ scale, though equivalent in each case, has been shifted for each successive curve so as to show the curves separately. Potato moisture contents (g/100g dry), a, 35·30; b, 30·18; c, 24·72; d, 24·14; e, 22·83. Green bean moisture contents (g/100g dry), f, 29·80; g, 24·97; h, 23·27; i, 22·92; j, 22·49. Cod moisture contents (g/100g dry), k, 33·84; l, 28·56; m, 27·0; n, 25·72.

freezable water are examined. Thus it would appear that the temperature at which thawing is initiated during warming is characteristic for each particular material examined.

A summary of results obtained by this method for the content of unfreezable water in a wide range of food materials and food constituents is given in Table 1. Also included in this table for purposes of comparison are a number of values for unfreezable water in comparable materials obtained by other methods and reported in the literature.

Discussion

Most previous estimates of the amounts of water remaining unfrozen in food materials at low temperatures have been derived indirectly from calorimetric or dilatometric data as the difference between the calculated quantities of ice formed within fully hydrated samples at given temperatures and the determined total water contents of the respective materials. In the absence of exact data for the specific heats or co-efficients of thermal expansion of these components of the system other than ice (i.e. of the solids and of the unfrozen water itself which, because of its very nature, would be expected to exhibit a unique behaviour), calculations of this kind must inevitably lack precision. As Moran (1935) has pointed out, further uncertainty arises in the case of dilatometry because of the possibility of the release of dissolved gases during the freezing process. The accuracy and reliability of values for unfreezable water obtained by these methods must, therefore, remain open to question however carefully the techniques themselves were applied.

A few further data regarding the amounts of 'liquid' water persisting in frozen foods have been obtained relatively recently using wide-line nuclear magnetic resonance (Sussman & Chin, 1966; Toledo, Steinberg & Nelson, 1968). The latter authors monitored the proton resonance signal from flour samples containing different amounts of water as the temperature was reduced through the freezing zone. They found that where the sample contained more than a certain proportion of water (29 g/100 g dry flour) a sharp drop in signal, attributable to freezing, occurred between 30 and 20°F, but that below this range of temperature, the signals from different samples, irrespective of total water content, became similar and fell together slowly during further cooling. This persisting signal they attributed to the continuing presence of unfrozen 'bound' water. However, they were unable to distinguish a temperature at which the freezing process became complete and, in order to calculate the quantity of non-freezing water, they arbitrarily selected the resonance signal at 0°F as a basis for their calculation. Similar difficulties attend the calculation of a precise value for the content of unfreezable water in cod muscle from the data of Sussman & Chin (1966).

The method described in the present paper is relatively simple and direct, depending, as it does, on the mere detection, with high sensitivity, of a small phase transition

within the sample. The results unquestionably confirm that, for each of the materials examined, there exists a definite and fixed amount of water of hydration which is incapable of undergoing a normal freezing process. Additional water above this level freezes in a normal manner and begins to thaw out during rewarming at a temperature determined by the spectrum of soluble constituents present and, therefore, characteristic of the particular material concerned. This recognition of a characteristic 'eutectic' temperature which can be determined, in spite of the lack of sharpness of the transition, within quite narrow limits, and which is unaffected by the freezing treatment applied, suggests the existence of a lower limit to the range of temperature over which freezable water can normally be converted to ice. In respect of the materials for which results are illustrated in Fig. 2, the 'eutectic' temperatures lie between $-25°$ and $-40°$C. It would therefore appear to be quite possible, indeed likely, that at the temperatures used by some earlier workers for the determination of values for unfreezable water included in Table 1, the freezing process was not complete and that the values themselves must therefore be erroneously high.

The precision with which the quantity of unfreezable water is determined by D.T.A. depends on the incremental difference in water content between the most moist sample showing no evidence of the phase transition and that of the driest sample for which such evidence is present—a difference which can be reduced at will by the careful preparation and analysis of additional samples. For a reasonable degree of precision, an appreciable number of samples of a given material need to be examined. Fundamental to the whole operation, of course, is a reliable and reproducible method of moisture determination.

The need to prepare the material in a finely divided form, which in turn necessitates at least a partial prior dehydration, may be considered a disadvantage since it can be argued that, as a result of this initial treatment, the material may have undergone irreversible changes affecting its behaviour. However, the significance of the results described here relates primarily to the condition and properties of the constituent water of foods which have been preserved by freezing or by dehydration, and in either of these cases comparable degrees of water-removal producing changes of a similar kind will have taken place.

The results of further studies by differential thermal analysis bearing on the behaviour and properties of the unfreezable fraction of water in food materials will be reported in later papers.

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Book Reviews

The Fat-Soluble Vitamins. Ed. H. F. De Luca and J. W. Suttie.
University of Wisconsin Press, 1970. Pp. xviii + 531. £7.10.

This volume contains thirty-three chapters on various aspects of the biochemistry of fat-soluble vitamins. It is the published version of a symposium held in honour of Harry Steenbock, one of the great pioneers in the study of these fascinating substances. It is an impressive tribute and illustrates how much his early studies have stimulated a new generation of able investigators to deploy the powerful tools of modern biochemistry to delve deeply into the mysteries of the biological function of these compounds.

In spite of many profound advances recorded in this book the problem of the basic functions of the fat-soluble vitamins is still essentially unsolved. This contrasts sharply with the situation with water-soluble vitamins whose universal roles in cellular activities are well characterized. The difference in our knowledge of these two groups of vitamins simply reflects the variation in our knowledge of basic biochemistry. The water-soluble vitamins are concerned with basic metabolic processes which are well understood, while the fat-soluble vitamins are concerned with specific activities of differentiated tissues, for example bone, and the biochemistry of differentiation is in its infancy. However, developments are likely to be considerable in the next few years and with this increasing knowledge will come much deeper insight into the functional role of the fat-soluble vitamins.

Meanwhile this volume gives an excellent account of the most up to date attempts to come to terms with these difficult problems and as such it is essential reading for vitaminologists. It is well edited, well produced and carries a good index; all in all it is a very fitting memorial to Harry Steenbock.

T. W. Goodwin

Modern Food Microbiology. By James M. Jay.
London: Van Nostrand Co., 1970. Pp. vii + 328. £4.65.

This book which is designed primarily as a second course in microbiology might also be entitled 'A Comprehensive Introduction to Food Microbiology'. It covers this complex field at a depth which whilst instructive is not too involved for students approaching the subject for the first time. In a logical approach to food microbiology the author describes the factors which control the growth of micro-organisms and spoilage. The core of the book deals with the central topics of preservation and food poison-

ing. There is a useful chapter which introduces the complex subject of microbiological standards and another which deals with biological hazards other than food poisoning bacteria. Whilst serving as a thorough introduction to a new field of study this also provides much useful data in tabulated form which the student may find useful in further work.

J. M. Wood

Books Received

Glucose Syrups and Related Carbohydrates. By G. G. Birch, L. F. Green and C. B. Coulson.
London: Elsevier, 1970. Pp. x + 118. £3.25.

Eggs, Cheese and Yogurt Processing. By George Wilcox.
USA: Noyes Data Corporation, 1971. Pp. viii + 280. $35.

Food and Beverage Processing Industries.
USA: Noyes Data Corporation, 1971. Pp. 169. $20.

Milk, Cream and Butter Technology. By George Wilcox.
USA: Noyes Data Corporation, 1971. Pp. viii + 311. $35.

Introductory Foods. A Laboratory Manual of Food Preparation and Evaluation. By Mary L. Moor and Theodore F. Irmiter.
London: Collier-MacMillan Ltd, 1971. Pp. 294. £2.50.

Man and Food. By Magnus Pyke.
London: World University Library, 1970. Pp. 256. £1.75.

International Sugar Confectionery Manufacturers Association Methods of Analysis Volume 2.
Paris: 1971. 100 Francs.

The Master Chefs. A History of Haute Cuisine. By E. B. Page and P. W. Kingsford.
London: Arnold, 1971. Pp. viii + 248. £3.50.

Managing to achieve quality and reliability. By Frank Nixon.
Maidenhead: McGraw-Hill, 1971. Pp. xvi + 290. £4.25.

Beverage Literature. Compiled by A. W. Noling.
USA: Scarecrow Press Inc, 1971. Pp. xvii + 865. £10.00.

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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

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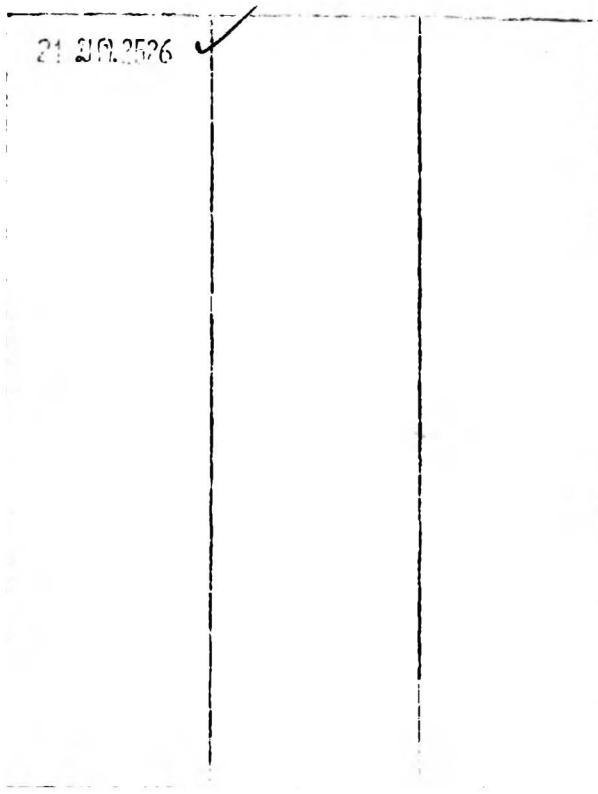

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