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Salt as a preservative for foods

M. INGRAM AND A. G. KITCHELL

Introduction

Salt is probably the most widely used of food preservatives. Though its use dates from antiquity, it is only fairly recently that the reasons for its effectiveness have begun to be understood. It will be appreciated, upon reflection, that salt is not applied to preserve foods which do not putrefy, like fruits or cereals. Fundamentally, its success depends on the facts that while we tolerate salt, it inhibits objectionable putrefaction and dangerous micro-organisms, and those which it does not inhibit are more or less unobjectionable. The intention of this paper is to give a broadly ecological review of these relations.

General effects of salt on microbial growth

As a rule, relatively low concentrations of salt stimulate micro-organisms while higher concentrations inhibit them. This stimulation is the reason for the common inclusion of small quantities of salt in laboratory culture media. Here, however, we are concerned with the inhibitory effect of higher concentrations.

The concentration ranges over which these effects are exerted may be very different for different organisms (cf. Ingram, 1957). At one extreme is the organism which is stimulated (if at all) by rather low salt concentrations and inhibited by concentrations which are still quite low. At the other is the organism stimulated by high concentrations, resisting even saturated solutions, and perhaps even requiring a high concentration for growth. These two types of behaviour may be broadly distinguished as salt-intolerant and salt-tolerant or halophilic, and various intermediate types as salt-facultative. Table 1 gives examples of organisms which display the diverse kinds of reaction to salt concentration; there are similar relations to sugar. There is a confusion of names for these diverse behaviour patterns (Ingram, 1957), but this is not the place to discuss them.

The selective action of salt depends, of course, on these differences in behaviour between individual organisms: the intolerant organisms are excluded from a salted food. This is simply illustrated by contrasting the behaviour of a *Pseudomonas* isolated as a dominant species on putrefying chilled pork with that of a *Micrococcus* typical of those found on cured meats. A 'brine concentration' exceeding about 5% will exclude the *Pseudomonas* but permit the growth of the *Micrococcus*.

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TABLE 1. Examples of organisms with different degrees of tolerance to high concentrations of sugar or salt

Type	Sugar	Salt
I A Intolerant, strongly	*Pichia* spp.[1]	Coliform bacteria[1]; Pseudomonads[4]
B Intolerant, moderately	*Sacch. cerevisiae*[2]	Staphylococci[6]
C Intolerant, slightly	?	Urobacillus XII[5]
II A Facultative, low optimum	'Normal' organisms	Urobacterium VI[5]; *Bacillus* spp.[4]
B Facultative, moderate optimum	*Sacc. stellatus*[3]	Urobacterium XXIV[5]
C Facultative, high optimum	*Zygos. polymorphus*[3]	Micrococci[4]
III A Tolerant, weakly	?	*Vibrio costicolus*[7]
B Tolerant, moderately	*Zygos. nussbaumeri*[2]	Colourless halophiles[4]; *Bacterioides halosmophilus*[4]
C Tolerant, strongly	*Zygos. richteri*[2]	Red halophiles

References: [1] Scarr (1954).
[2] Lochhead & Heron (1929); Scarr (1954).
[3] Kroemer & Krumbholz (1931).
[4] Shewan (1942).
[5] Hof (1935).
[6] Scott (1953).
[7] Christian (1956).

The effect of salt is often similar to that of drying. When this is the case, sodium chloride can be replaced approximately by molecular equivalent concentrations of other salts or even non-electrolytes like sugars, and their respective effects can be equated in terms of the 'activity' of the water in the system. Scott (1957) has reviewed many examples of this kind of behaviour, which may be called 'substitutive'. It implies that the microbiological effect of salt in a food probably depends on osmotic withdrawal of water, and that it will reflect the water activity in the food, which will depend on the molecular ratio between the amounts of dissolved solutes and of water in the food. In a salted food, where salt is the main solute, this ratio is often expressed as the so-called 'brine-concentration' (Hankins *et al.*, 1950). It is only an approximation, however, for there is an additional contribution by soluble constituents of the food itself; though this is usually small (because proteins, etc., have high molecular weight) it has been shown to be significant microbiologically (Scott, 1957). The 'brine concentrations' in salted foods are often surprisingly high. In butter, for example, they may reach 10%, and even higher in some cheeses and bacon. The foods often do not taste correspondingly salty, apparently because non-aqueous constituents act as diluents to the salty flavour (Ingram, 1949).

There are, however, occasions when the situation cannot be explained thus, in terms of water activity (Rockwell & Ebertz, 1924). This is evidently the case with organisms tolerating, or requiring, high concentrations of sodium chloride which cannot be substituted by other salts – in an extreme case, not even by closely related salts like

potassium bromide. This 'non-substitutive' behaviour, more or less extreme, is very common among the coloured obligately halophilic bacteria (cf. Ingram, 1957). The reason for the specific requirement for sodium chloride is now clear: it is partly accounted for by the salt requirements of enzymes extracted from the cells (review Ingram, 1957), and Norwegian work (Mohr & Larsen, 1963) suggests that it is needed to maintain the stability of the cell wall.

The difference between the substitutive and non-substitutive type of halophile may be of considerable practical importance, for the latter type of organism remains sensitive to quite ordinary concentrations of other salts which might accordingly be used to inhibit it. Thus, for example, it was once suggested privately that growth of salt-tolerant bacteria on salt beef could be prevented by small concentrations of calcium salts; but no attempts to explore such possibilities in practical experiments are known. This might be one of the reasons for the use of potassium rather than sodium nitrate in traditional curing mixtures.

Influence of other conditions on the action of salt on growth

There may be large differences in the action of salt under different conditions and these differences are noteworthy, not only in practice, but also because the conditions in practice are often very different from those prevailing in laboratory experiments. Knowledge of the influence of these various conditions is therefore essential in comparing the results of laboratory experiments with practical experience.

The influence of temperature

For instance, experimental investigations of the action of salt on micro-organisms are usually carried out, for reasons of convenience, at temperatures considerably higher than often prevail in practical operations. It is therefore important to realize that temperature has a great influence on the action of salt.

The lethal action of salt, like that of other disinfectants, is less at low temperatures. There is a striking illustration of this in the unexpectedly long survival of pathogenic organisms in curing brines kept industrially at temperatures near 5°C. Salmonellas, for example, are rapidly destroyed in such brines at normal temperatures, but survive for weeks at the low temperature which exists in actuality (Shipp, 1958; Buttiaux & Moriamez, 1958); *E. coli* behaves similarly (Ingram, 1958a, p. 281). The same effect of temperature on the action of salt on the survival of salmonellas has been demonstrated in culture media (Blanche Koelensmid & van Rhee, 1964) and in vacuum packed bacon (Bardsley & Taylor, 1960).

The influence of temperature on the inhibitory (as distinct from lethal) action of salt is less clear. It has been found that the maximum salt concentration in which growth occurs is greater at lower temperatures—high sugar concentrations likewise have a smaller effect at lower temperatures (Kroemer & Krumbholz, 1931)—which appears

to parallel the influence of low temperature in diminishing the lethal action. A remarkable instance was the reported growth of *E. coli* and typhoid bacteria in 25% salt at 5–8°C, though not in 10% salt at 37°C (Dumesh, 1935); but our, and other (Ingram, 1958a, p. 281; Patton, 1958; Shipp, 1958) experience has not confirmed this. Labrie & Gibbons (1937) reported that the preservative action of salt on fish increased with reduction of temperature from 21 to 10°C, which seems to infer a reduced salt tolerance at lower temperature, but it is possible, though unlikely, that different organisms were involved at the upper and lower ends of this temperature range. With moulds, however, several workers agree that the greatest tolerance exists at the optimum temperature for growth (e.g. Tomkins, 1929; Heintzeler, 1939) with a much lower tolerance near the temperature limits (Stille, 1948). The same is true for sporing bacteria, e.g. *Cl. botulinum* type E. The maximum salt concentration permitting growth of this bacterium is 5·8% at 30 and 25°C, 5·1% at 20°C, and 4·3% at 15°C (Ohye, Christian & Scott, 1967). Salt concentrations ineffective at high temperatures may become inhibitory or at least result in markedly delayed outgrowth as the temperature falls to 10°C or below (Segner, Schmidt & Boltz, 1966).

The temperature optimum and the maximum temperature for growth are raised when an organism grows in the presence of salt, and the rise is large when the organism tolerates high salt concentrations (Christian; see Ingram, 1957). It is not clear whether the same applies to the minimum growth temperature, and this is sometimes a question of practical significance. For instance, it is known that the growth of staphylococci is prevented below 10°C in media of low salt content—would a higher temperature suffice in the presence of salt?

In view of its practical importance, and the uncertainties which still exist, the influence of temperature merits further investigation.

The influence of pH

The influence of pH is supplementary to that of salt. It was shown long ago, with a pure culture of *E. coli*, that smaller concentrations of a salt are needed to inhibit at pH values remote from the optimum (Sherman & Holm, 1922) and more recently with *Cl. botulinum* (Ohye & Christian, 1967) and salmonellas (Blanche Koelensmid & van Rhee, 1964). It is clearly a general rule that, as the acidity rises, less salt is needed to prevent growth of individual bacteria and yeasts.

There is, moreover, another way in which pH and salt may act together. It has been found, among the bacteria from cured meats, that those which resist salt tend to be unusually sensitive to acidity, and vice versa (Ingram, 1958b).

A combination of acidity and salt is thus very generally inhibitory, a suggestive fact. It seems to imply, for example, that less salt might be needed to preserve the comparatively acid meat of well-rested animals; that more salt may generally be needed to preserve fish than meat, because of the generally higher pH of fish; and that, in bacon for prepacking, less salt might be tolerated because of acid production by lactobacilli

which dominate the microflora under vacuum. Unfortunately, exploitation of this principle is hindered by the fact that acidity in flesh appears to accentuate the flavour of the salt in it (Ingram, 1949).

Synergistic effects

As for pH, the action of sodium chloride is synergistic with those of various other inhibitory agents.

A well-known example is its synergistic action with preservatives like benzoic acid, which can be used in lower concentrations when salt is present (von Schelhorn, 1951). This phenomenon has obvious importance in attempts to preserve salted products with the aid of such preservatives, as in investigations with semi-preserved fish (e.g. Erichsen & Molin, 1964; Joergensen & Bak Henriksen, 1964). The reason is not elucidated, to the writers' knowledge. The addition of salt, even if it is not itself acid in reaction, by raising the ionic strength in solution frequently has the effect of reducing the pH, which latter is known to increase the effectiveness of weakly acidic preservatives like benzoic acid (cf. Ingram, Ottaway & Coppock, 1956); but the concentrations of salt needed to change the pH appreciably are comparatively large, and some additional explanation may be necessary. Reports that salt 'sensitizes' bacteria to carbon dioxide (Rockwell & Ebertz, 1924) may arise from similar causes.

This synergistic action of salt should always be remembered by the practising bacteriologist working with salted foods. He habitually seeks particular groups of bacteria by transferring portions of the food to selective media, which contain substances more or less inhibitory to bacteria, even to those bacteria which the medium is designed to select. The transfer of salt with the bacteria is likely to increase the inhibitory action of the selective agent so that even the resistant bacteria may be inhibited. There is an example in attempts made by us in the past to count lactobacilli from salted meat on acetate agar of rather low pH; if dilutions for counting are made in non-saline solutions, a proportion of the bacteria are killed by the osmotic shock, but if salt solutions are used, we run into the above difficulty and a proportion of the organisms fail to grow. For similar reasons, it is not safe to modify the amounts of sodium chloride in published formulae for selective media.

Action of salt on properties other than growth

Consider now the action of salt, not upon the ability to grow, but upon other properties of micro-organisms.

Broadly, the action is similar to that on growth: low concentrations of sodium chloride, or other salts, stimulate while higher concentrations depress. This has been shown, for example, for retention of viability (Fabian & Winslow, 1929), for respiration (Ingram, 1940), fermentation, motility, and for sporogenesis (Fabian & Bryan, 1933). Further, such action is not merely broadly similar, but is usually roughly in parallel

over the various properties. An organism able to grow at high salt concentrations is able to respire and carry out other metabolic activities at high salt concentrations—if it were not so, growth would be impossible.

Nevertheless, there may be important differences in detail. For example, by increase of salt concentration, motility may be stopped before growth, so that on lightly salted meat the bacteria are still able to grow but their colonies remain isolated instead of spreading to form a continuous slime (salting diminishes the amount of free liquid at the meat surface, which may also contribute to this effect). It may be suspected that, in a like manner, salt has more influence on the proteolytic activities of bacteria than on their growth, as was roughly indicated by Rockwell & Ebertz (1924), for low concentrations of salt diminish the production of putrid odours and flavours from meat more than seems to be explained by the change in the bacterial flora. Nitrate reduction ceases at salt concentrations still permitting growth (Patterson, 1963). Also, temperature as well as salt influences nitrate and nitrite reduction in curing brines, the rates at 10°C in 25% salt being comparable with those in a weaker brine (20% salt) at 5°C (Eddy & Kitchell, 1961).

In spite of indications to the contrary (Williams & Purnell, 1953) it has been claimed (Halvorson, 1958) that lower concentrations of salt prevent outgrowth of bacterial spores than are needed to stop vegetative growth, especially if the spores have been heated: this property is important for the stability of partially sterilized canned meats (see below). Germination of spores, however, continues up to much higher salt concentrations (Gould, 1964).

The importance of inter-relations of this kind is illustrated by work on *Cl. botulinum*. In meat of low salt content, putrefaction accompanied growth and toxin formation, so it is perhaps unlikely that dangerous meat would be eaten (Greenberg *et al.*, 1958). But with salted meats having 'brine concentrations' in the range 6·25–7·12%, toxin formation occurred though putrefaction was prevented, so that there was here no spoilage to indicate that the meat was dangerous (Greenberg, Silliker & Fatta, 1959). Similar results with ham have been obtained at 'brine concentrations' from 3·6 to 4·5% (Pivnick & Barnett, 1965).

Considering their practical significance, we know very little about such effects of salt in changing the properties of micro-organisms. They might conceivably be very striking. Dumesh (1935), working with salt-sensitive and salt-tolerant bacteria, reported profound differences in biochemical and serological properties with change of salt concentration, differences sufficient in some cases to alter the whole character of the organism. Similarly, the biochemical activities of halophilic bacteria change as the salt concentration changes (Gibbons, 1957). Unfortunately, no general pattern emerges from these investigations, and more study in detail is evidently required.

It is, of course, well known that the size and shape of bacterial cells may differ greatly at different salt concentrations (reviewed Ingram, 1957). This, coupled with the just-mentioned variation in biochemical properties, makes it sometimes very

difficult to establish the identity of organisms found in saline environments; especially as useful techniques like serology are unsuitable for this kind of situation. Probably because of such difficulties, it has not been possible to account for most of the bacteria seen under the microscope in bacon curing brines (Ingram, Kitchell & Ingram, 1958).

Reactions of individual organisms to salt

As the preservative action of salt depends on the different reactions of different organisms to salt, it is necessary to consider individually the reactions of organisms of particular interest. It is convenient to deal with these organisms in two broad groups which have rather different significance.

First are those which inhabit the bodies of warm-blooded animals including man, and which are accordingly adapted to the conditions there. Thus, they grow best at temperatures near that of the animal body, and are called mesophilic. Most of the organisms dangerous to man belong to this group, for obvious reasons. Thus the food poisoning bacteria, and the related organisms which may be used as indicators of their possible presence, come mainly from the bodies of warm-blooded animals and grow best near 37°C, which is why refrigeration protects against them. For similar reasons, these bacteria are not adapted to resist salt, which must be one of the fundamental reasons why salting became accepted as a satisfactory method of preserving foods. Troubles naturally arise, in this connection, if enough salt is not used, and many present-day difficulties follow from the continued trend of taste towards less strongly salted foods.

The second group of microbes come from outside the animal body (e.g. from soil, water or air), and may collectively be designated as saprophytes. Corresponding to the variety of possible habitats, they exhibit great variety of behaviour. In temperate climates, at least, most of them are capable of growing under cool conditions, many even at freezing point; and in soil there are types capable of resisting even saturated sodium chloride solutions. Salt-tolerant and salt-requiring types are especially common in saline soils or in the sea (Hof, 1935; Stuart, 1938). The organisms causing spoilage of salted foods, in temperate climates, belong to this second group.

Mesophilic bacteria significant in public health

As typical of the first group, one may take the salmonellas, pathogenic inhabitants of the gut. Their growth is prevented by concentrations of sodium chloride exceeding about 6%. Much the same applies to *Escherichia coli* (Hof, 1935) which is widely used as an 'indicator' of the possibility that salmonellas may be present through faecal contamination. Such an indication is, however, valid only if the two organisms react in a generally similar way to the environment, and this is not well established for salted foods. It has been shown that the tolerance of *E. coli* to sodium chloride cannot be increased above 6·5% by adaptive training (Hof, 1935); we know of no similar tests

with salmonellas. There are indications that salmonellas survive better than *E. coli* in ham jelly containing different quantities of salt (Blanche Koelensmid & van Rhee, 1964). At 5°C, in 12% salt, no *E. coli* survived 15 days whereas 10–40% salmonellas remained viable under the same conditions. More systematic comparison seems necessary to establish the usefulness of the coliform index in salted products.

The faecal streptococci, unlike *E. coli*, are not inhibited in growth (at 37°C) by 6·5% of NaCl. This is, indeed, one of the 'Sherman criteria' for enterococci (Sherman, 1937). Some strains tolerate up to 10·5% (Orla-Jenson, 1919). These organisms are also relatively resistant to acidity and to heat, and can grow down to 0°C. Consequently, because they are widespread, they frequently develop in salted foods—e.g. in canned hams (Ingram & Barnes, 1955) and in vacuum packed bacon (Cavett, 1962, 1963)—and they survive well in curing brines (Riemann, 1958).

A second food poisoning organism of faecal origin is *Cl. perfringens*: so far, the ability to cause poisoning is associated only with a minority of strains, but all are likely to cause spoilage in meat if it is kept warm. *Cl. perfringens* is, however, also found in soil ('telluric' origin), and it is reported that strains from soil are more resistant to salt than those of faecal origin (Beerens & Delcourte, 1958). Correspondingly, the vegetative cells of soil strains are said to survive in brines for longer than those of faecal strains (Beerens, 1958). It should be noted, however, that these experiments were made with a raw brine, which was presumably saturated with oxygen which itself rapidly kills the vegetative cells of clostridia; in a mature brine of E_h 0 to +100 mV and containing soluble protein, such cells might survive longer.

Among the clostridia, most interest attaches to *Cl. botulinum* because of the peculiarly fatal toxin produced when it multiplies in a food; and, in order to ensure its absence from cured products, the effect of salt on its development has been investigated many times. The classical investigations of Tanner & Evans (1933) suggested that surprisingly high concentrations, up to 12%, were needed to prevent growth and toxin formation: probably, however, the inocula used contained only lightly heated spores, the significance of this point not being understood at that time. Smaller concentrations are needed to prevent germination if the spores have been heated, as first became evident from unpublished work of Yesair, Bohrer & Cameron of the National Canners' Association. Still smaller concentrations are active if spores have been strongly heated, as in canning. According to Gross, Vinton & Martin, between 3·5 and 1·5% is significant in this case. Silliker (1959) and Abrahamsson, Gullmar & Molin (1966) report similar findings.

The spores of other bacteria are likewise inhibited by comparatively low concentrations of salt if they have been heated (Roberts & Ingram, 1966), and it is believed that this is an important reason for the stability under commercial conditions of many canned products known to contain viable spores. Similarly, irradiated spores, like heated spores, are prevented from germinating by low salt concentrations (Hansen & Warnøe, 1960; Roberts, Ditchett & Ingram, 1965). The fact that such spores are

unusually sensitized towards salt, in rough proportion to the amount of heating (or irradiation) they have undergone, must be remembered when it is proposed to diminish the heating or salt through the use of some auxiliary preservative like an antibiotic.

A food-poisoning mesophilic organism not of faecal origin is *Staphylococcus aureus*, and this is noteworthy in resisting unusually high concentrations of salt, up to 15% or occasionally even 20%, while remaining capable of growth at very low concentrations (Fagreus, 1949). Some, but not all, staphylococcal phages tolerate 10% of salt (West & Kelly, 1962). Without becoming unpalatable, few foods can contain enough salt to prevent the growth of these staphylococci; nevertheless, they are not common on raw cured products, even after storage under warm conditions. It seems that this happens because there are far more numerous organisms which are similar save that they do not form enterotoxin, and these latter crowd out the poisonous strains (Ingram, 1960; Eddy & Ingram, 1962). It is when the harmless organisms are removed, apparently, that development of food poisoning staphylococci becomes likely, e.g. in cooked ham. One would wish to know whether enterotoxin can be formed over the whole range of salt concentrations within which growth is possible, but so far as is known, 5% is the highest salt concentration at which toxin formation is recorded. If this is indeed the limit, most raw cured products would fall above it, and many corresponding cooked products below, so that toxin formation would be possible in the cooked but not in the raw product. This situation needs clarification.

The salt tolerances quoted for these food-poisoning organisms are, as a rule, those measured at 37°C because this is the optimum temperature commonly used in studying mesophilic organisms. Unfortunately this temperature is too high to be realistic, and the limiting salt concentration is almost certainly different at the practical temperatures, as indicated earlier. A detailed study by Ohye *et al.* (1967) using spores of *Cl. botulinum* type E indicated that the water requirement for growth and toxin production was increased appreciably by a 15°C reduction in temperature.

Moreover, the limiting salt concentration for growth depends on the nature of the medium, as is well illustrated by the observations of Tanner & Evans (1933) with several strains of *Cl. botulinum*: where concentrations about 8% of sodium chloride prevented growth and toxin formation in nutrient broth, 10·5% was necessary in a pork infusion medium containing pieces of pork. Similar findings with salmonellas (Blanche Koelensmid & van Rhee, 1964) and *Staph. aureus* (Parfentjev & Catelli, 1964) are reported. More detailed information is needed about such modifying influences; in the meantime, quoted figures for salt tolerance serve as no more than a comparative guide, especially with mesophilic bacteria.

It is remarkable that a concentration about 6·5% salt is critical for several of these bacteria from the gut, and it would be interesting to know what the fundamental significance of this particular concentration may be. The coincidence is heightened by the fact that the telluric strains of *Cl. perfringens* resist higher concentrations, while

Staph. aureus resists much higher concentrations. With these organisms one can imagine that a greater range of tolerance might be acquired because of the large fluctuations in water content of habitats like soil and skin.

Saprophytic micro-organisms

Because of the great variety among these organisms, attention must be confined to a few examples of particular importance. Most of these examples are, of course, drawn from experience in temperate conditions, and somewhat different relations probably prevail in hot climates.

Pre-eminent under cool conditions are the bacteria of the so-called *Pseudomonas–Achromobacter* association, which are the usual agents causing spoilage of perishable foods like eggs, fish and meat (Mossel & Ingram, 1955). As already said, the value of salt as a preservative depends primarily on its power to inhibit these putrefactive organisms. Comparing the bacteria of this type found on meat and on fish respectively, it seems at present that there may be many more which require salt for growth on fish than there are on meat. Whether those on fish are, in general, correspondingly more salt resistant is not clear (Watanabe, 1960).

The nature of the flora which, under cool conditions, replaces the *Pseudomonas–Achromobacter* association at higher salt concentrations depends on the circumstances, broadly whether conditions are anaerobic or aerobic.

Under aerobic conditions, salt tolerant micrococci, yeasts or moulds may occur up to the highest salt concentrations. It is, of course, probable, though it is not proved, that the organisms which occur at different salt concentrations are not the same, within each of these groups, or even among the same species. Among the micrococci, for example, Pohja (1960) has attempted to distinguish strains on the basis of their salt tolerance; there is, however, a possibility that this character may be insufficiently stable, as otherwise similar strains sometimes show a variety of responses towards salt (Ingram, 1957). Among the yeasts, there are certainly differences in tolerance between different isolates of the typically salt-tolerant species *Debaryomyces guilliermondii* and *D. kloeckerie* (Etchells, Bell & Jones, 1953). There are similar differences between strains of the salt-tolerant mould *Sporendonema epizoum* (Frank & Hess, 1941).

A flora of this type is characteristic of the surface of most types of salted meat and fish, and some of the saltier kinds of cheese, if they are exposed to air. In cases where yeasts and moulds are apt to cause undesirable flavours, they can be excluded by covering the surface. This is the microbiological advantage of putting a floating wooden lid on the sauerkraut barrel, or of covering a dried sausage with a more or less impermeable skin; similarly, vacuum-packed bacon does not go mouldy in the package, unless the latter is perforated. The activities of the micrococci seem less objectionable, though it is not clear why. Some of them are strongly proteolytic when tested at low salt concentrations, but this activity must be limited at higher salt concentrations, otherwise salted foods would not be so durable. Many of the micrococci are also lipolytic, and

perhaps contribute in this way to the characteristic flavours of salted products, though little is yet known about this (Cantoni *et al.*, 1964).

It is not altogether clear what circumstances cause micrococci, yeasts or moulds to predominate. Under drying conditions, as on dry salted hides or fish, moulds like *Sporendonema epizoum* may occur (Frank & Hess, 1941; Vaisey, 1954); but bacteria may occur too. It has been suggested that a low pH favours yeasts rather than bacteria on bacon (Gibbons & Rose, 1950); and, similarly, the low pH caused by a lactobacillus fermentation may cause the prevalence of yeasts on the surface of sausage.

This flora may also penetrate the food if it is aerated; and the large number among these organisms which are capable of reducing nitrate (e.g. Kitchell, 1958; Pohja, 1960) and probably nitrite likewise (Eddy, 1958) are capable of growing within otherwise anaerobic systems containing these salts, such as bacon and ham, or barrelled or canned cured fish (Lindberg, 1958). Similarly, micrococci and yeasts may be found in the depths of nitrate-containing meat curing brines, but moulds do not occur there, and yeasts are usually much more common on the surface where they form films.

Under anaerobic conditions, at moderate salt concentrations, lactobacilli usually predominate (Niven, 1952). This is the situation, for example, in vacuum packed cured meats (Hansen, 1960; Alm, Erichsen & Molin, 1961; Cavett, 1962), in the interior of cured sausages, or in the bulk of the liquid in a sauerkraut fermentation (Pederson, 1960), or in a can of fish titbits (Erichsen & Molin, 1964), in all of which the concentration of salt must be not too high, or the product is unacceptable. The metabolism of these lactobacilli lowers the pH, which helps to confer on the product a stability and safety greater than that expectable from the salt alone, for reasons already mentioned, so that it is possible to stabilize a product like sauerkraut by use of a modest salt concentration. There are two hazards in this type of process. The first is that the low salt concentration may not hold off the Gram-negative rods long enough for the lactobacillus flora to become established. The second is that yeasts and moulds growing on the surface are capable eventually of respiring away the acids produced by the lactobacilli, which may again bring the acidity back to a level where the salt alone is insufficient to prevent spoilage (Fabian, 1937; Pederson, 1960).

Apart from this production of acidity, little is known of the effects of lactobacilli in fish or meat products. It is not even certain what their metabolic substrates are, from which the acid is produced. It has, however, been suggested (Lerche, 1960) that in semi-preserved fish, with 10% NaCl and a pH near 4, carbon dioxide is produced by lactobacilli from decarboxylation of arginine, glutamine or tyrosine. Very few of these lactobacilli have been isolated and characterized, the indications being that most, but not all, are homo-fermentative and resemble *Lactobacillus plantarum* (Skovgaard, 1961).

These lactobacilli apparently do not grow well at low temperatures, so that the combination of moderate salting and anaerobic conditions with cool storage is a powerful one. These relations help to account for the short life of commodities like fish titbits or packed bacon, when they are not kept cool.

No clear pattern of events has yet emerged at high salt concentrations under anaerobic conditions. Lactobacilli have been reported as dominant in rapid ham-curing brines containing up to 20% salt (Deibel & Niven, 1958a) but, in our experience, are less numerous in bacon brines with 25% salt. The lactobacilli are believed to be inert towards nitrate and nitrite in the pH range 5·5–6·5 found in such brines. Sulzbacher (1958) reported that nitrate-reducing *Achromobacter* strains predominated in his long-term ham-curing brines. A strongly denitrifying organism resembling *Vibrio costicolus* has been isolated (Henry *et al.*, 1958); and spirilla are reported, as also from 'surströmming'—a Swedish fish preparation peculiar among salted products in being distinctly alkaline.

It is possible, of course, that some of these reports of vibrios and spirilla may be prompted by the pleomorphic forms which are induced by salt in quite different organisms, e.g. lactobacilli (Deibel & Niven, 1958b), and vibrios from brines themselves assume remarkable shapes (Henry *et al.*, 1958). The characters of the strongly salt-tolerant organisms have been little investigated and, especially, little is known of their metabolic activities at salt concentrations such as occur in practice, though it is clear (see above) that such activities might not be adequately represented by investigations under different conditions.

In warm conditions, the spoilage of meat and fish is commonly caused by faecal organisms, and especially objectionable is the internal putrefaction caused by anaerobic *Clostridia*. Such spoilage, as already indicated, can be prevented by salting at concentrations about 10%.

However, in the hot dry climate in which solar salt has traditionally been available, the surface of salted products often dries to the extent that salt crystallizes out there. Under these conditions, strongly halophilic organisms grow on the surface, which they often discolour (Venkataraman & Sreenivasan, 1954; Gutheil, 1960). Such organisms are common in solar salt (Stuart, Frey & James, 1933), and are introduced by it into the food. They are active only at comparatively high temperatures, the optima at high salt concentration usually being near 40°C.

Such organisms are strongly aerobic, and are therefore usually confined to the surface of the food. There must be few anaerobes which tolerate high concentrations of salt, at least in foods, for only one has been thoroughly described (Baumgartner, 1937).

Conclusion

This review should have given some idea of the preservative action of salt on foods, and of the way in which it is related to the effect of salt in inhibiting particular micro-organisms and to the influence of auxiliary factors on that inhibition. It is perhaps surprising that, with a process so old and so widely used, there should still remain so many details which are uncertain or not yet investigated. Certainly, in spite of much recent work with clostridia, there is still a need for more research on this subject.

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Bread as a source of protein

T. MORAN AND J. PACE

Introduction

The protein contributed by various groups of foods to the average British diet is shown in Table 1. These data are derived from the latest Report of the National Food Survey Committee (1966) and refer to the year 1964.

TABLE 1. Protein contributed by groups of foods
(Household Consumption, taken over all households, 1964)

Foodstuff	Protein contributed (g/head/day)
Liquid milk	13·2
Cheese	3·2
Beef, mutton, pork, bacon	11·9
Poultry, sausages and other meat	7·3
Eggs	4·0
Fish	3·7
Vegetables	6·8
Bread	14·4
Bread and flour	16·8

The figures show the quantitative importance of the contribution made by bread and flour to the supply of protein. The total average daily household consumption of protein in 1964 was 75·1 g/head. Of this, 60·1% was of animal origin and 22·4% was from bread and flour (exclusive of other wheaten products such as cakes and biscuits).

The quality of wheat protein

One useful method of assessing the protein values of foods and diets, as customarily eaten, is that devised by Platt, Miller & Payne (1961) in which values are stated in terms of Net Dietary Protein calories per cent (NDp cals %). This term represents the utilizable protein in the foodstuff or diet, and is a function of both quality and quantity.

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Provided the total calorie requirements are met, then on the NDp cals % scale a value of 4·6 represents a satisfactory level of protein allowance for adults, other than pregnant and nursing women. For children up to about 3 years old the desirable figure is about 8 and for pregnant and nursing women about 9·5 (Platt *et al.*, 1961).

Wheat by itself has a value of 6·0 while rice (5·1) and maize (4·7) are lower in the scale (Aykroyd & Doughty, 1964). Miller & Payne (1961) have given values of NDp cals % for examples of diets derived from different countries when the staple foodstuff (i.e. that which is the chief source of calories) is consumed together with additional sources of protein. Their values for dietary examples from Britain, with wheat as the staple foodstuff, are given in Table 2.

TABLE 2. Utilizable protein in diets in which wheat is the staple foodstuff

Origin	Staple*	Additional† effective sources of protein	Observed values	
			Protein calories (% total calories)	NDp cals %
Britain	Wheat	Cheese	9·4	6·9
Britain	Wheat	Milk, meat, eggs	13·1	8·2
Britain	Wheat	Fish, milk	23·0	10·8
Britain	Wheat	Milk	29·0	12·8

* Chief source of calories.

† Foods (excluding the staple) contributing more than 20% of the protein in the diet.

These figures show that diets containing wheat as the staple foodstuff together with appropriate amounts of protein-rich foodstuffs can satisfy the protein requirements of man, as assessed by NDp cals %, at all stages of development from infancy onwards. Indeed, in terms of NDp cals %, wheat, with a value of 6·0, would itself, without supplementation, meet the requirements of adults, other than pregnant and nursing women.

Essential amino acids

The contribution made by wheaten products to the quality of the protein in the average British diet may also be illustrated by calculating the approximate intake of essential amino acids from the various groups of foods consumed in households in Great Britain in 1964. These values are shown in Table 3.

The contribution of essential amino acids made by bread and flour may also be expressed as a percentage of the total intake provided by the sources given in Table 3.

TABLE 3. Estimated contribution of essential amino acids (g/head/day) from groups of foods (Household Food Consumption taken over all households)

Essential amino acid	Dairy products	Meat and poultry	Fish	Eggs	Vegetables*	Total cereals† (including flour and bread)	Bread and flour
Isoleucine	1·05	1·0	0·20	0·25	0·25	0·8	0·6
Leucine	1·7	1·5	0·3	0·35	0·3	1·6	1·25
Lysine	1·35	1·55	0·35	0·3	0·3	0·4	0·3
Phenylalanine	0·9	0·8	0·15	0·2	0·25	1·1	0·85
Tyrosine	0·95	0·65	0·1	0·15	0·15	0·7	0·5
Cystine	0·15	0·25	0·05	0·1	0·1	0·5	0·4
Methionine	0·4	0·45	0·1	0·1	0·1	0·4	0·3
Threonine	0·8	0·85	0·15	0·2	0·2	0·6	0·45
Tryptophan	0·25	0·25	0·05	0·05	0·05	0·25	0·2
Valine	1·2	1·0	0·2	0·3	0·3	0·9	0·7

Sources: Report of the National Food Survey Committee (1966) for the year 1964. Appendix C, Table 1. Data on amino acid composition from B. P. Hughes in McCance & Widdowson (1960) and (for bread and flour) from McDermott & Pace (1957). Calculated on the basis that 100 g protein contain 16 g nitrogen.

* Amino acid composition taken as the mean of those for potatoes and cabbage.

† Amino acid composition taken as that for bread protein.

These figures are given in Table 4 which also includes, as a matter of general nutritional interest, the calculated approximate composition, with respect to essential amino acids, of the total (mixed) protein of the diet.

TABLE 4

	Essential amino acids in total protein in diet* (g AA/100 g protein)	Intake provided by bread and flour (as % of total intake)
Isoleucine	4·9	17·7
Leucine	7·9	21·3
Lysine	5·8	7·6
Phenylalanine	4·6	24·9
Tyrosine	3·8	18·9
Cystine	1·5	34·2
Methionine	2·2	19·9
Threonine	3·9	16·3
Tryptophan	1·3	20·4
Valine	5·4	18·1

* Calculated from the protein sources given in Table 3.

These calculations do not, of course, take into account the factor of availability of the amino acids in different foodstuffs or the possible effects of cooking and processing upon availability, and are consequently subject to the limitations which affect all estimations of protein quality in terms of a chemical score. But they do provide a rough guide to the relative contribution made by the different sources of protein in our average diet. Within this context the figures show that bread and flour supply substantial quantities of all the essential amino acids other than lysine.

The value of wheaten flour as a source of essential amino acids was most strikingly illustrated in the studies of Widdowson & McCance (1954) with undernourished German children. Their subjects obtained most of their protein from bread and vegetables. In one orphanage (Duisberg) wheaten flour provided 75% of the total calories and the average protein intake was 61–73 g/day, of which only about 8 g were obtained from animal sources. Hughes (1955) has calculated the approximate intake of essential amino acids on the diets and compared it with the intake from the diet of pre-war British children which was much richer in animal protein. Some of the data given by Hughes (1955) are reproduced in Table 5.

TABLE 5. Amounts of amino acids (g/day) consumed by children

Amino acid	Diets		
	At Duisburg		
	Containing 100% extraction flour	Containing 70% extraction flour	Pre-war British
Arginine	3·2	2·3	3·3
Histidine	1·5	1·1	1·7
Isoleucine	3·1	2·7	3·2
Leucine	5·0	5·0	5·1
Lysine	2·5	2·0	4·2
Phenylalanine	3·4	3·5	2·8
Methionine	1·3	1·1	1·4
Threonine	2·2	1·8	2·5
Tryptophan	0·9	0·7	0·8
Valine	3·4	3·0	3·7

The Duisberg diets provided quantities of the essential amino acids similar to those of the pre-war British except for lysine, which was lower in both the wholemeal and the white flour diet. The lysine content of the wholemeal diet was higher than that of the white flour diet but, in the circumstances of the German experiments, both appear to have been adequate in this respect. Wholemeal flour has a slightly higher lysine content than white flour and this alone explains the superiority of the protein of wholemeal flour to that of white flour when these are the sole sources of protein in the diet of the weanling rat, cf. Hutchinson, Moran & Pace (1956).

The calculations given above illustrate the comparative value of bread protein as a source of essential amino acids, both in the extreme case of a very high bread diet and also in a mixed diet such as that of the average consumer in this country. But while such calculations based on amino acid analyses are a guide to protein quality they cannot entirely replace feeding trials, which are the ultimate test of dietary value.

Feeding trials

(i) *The rat*

Over 50 years ago Osborne & Mendel (1914) showed, in studies with the young rat, that wheat protein was deficient in lysine. Since then many studies involving bread and flour as sources of protein have been made with the weanling rat and in recent work the data obtained have been related to the content of essential amino acids in the protein. Thus with about 12% bread protein as the sole source of protein the

addition of 0·2–0·3% l-lysine to the diet approximately trebles the rate of growth of the weanling rate (Rosenburg & Rohdenburg, 1952; Hutchinson *et al.*, 1959). The growth rate is still not so good as that obtained, at the same level of protein, with a high quality protein such as casein. But with an addition of about 0·5% l-lysine and 0·1% l-threonine the growth rate and efficiency of protein conversion are comparable to those obtained with casein (Hutchinson *et al.*, 1959). If the lysine deficiency of the bread protein is corrected by the addition of another protein then this concomitantly provides the threonine, and the growth rate on the mixed protein is of the same order as that on casein. Alternatively if the bread protein content is increased this improves the threonine level sufficiently so that the addition of lysine alone then produces a high rate of growth (Hutchinson *et al.*, 1962).

These experimental observations are consistent with the composition of bread protein with respect to the essential amino acids and with the relatively high lysine requirement of the rapidly growing young rat. Although the rate of growth of the weanling rat is slow on unsupplemented bread protein, we have observed, in a recent experiment, that growth continued without any mortality, to sexual maturity on diets consisting solely of bread, white or wholemeal, supplemented only with vitamins A and D and calcium.

In previous work six successive generations were successfully grown and bred on a diet in which the lysine deficiency of wheat protein was corrected by supplementation with another protein. The diet in this exacting test contained 73% by weight of dried bread, 5% wheatfeed and 10% casein as the sources of protein (Hutchinson *et al.*, 1964).

Osborne & Mendel (1919) found that the body weight of adult rats could be maintained by diets containing about 6·5–7% of whole wheat protein. In recent work (unpublished) we have extended this observation by examining the performance of rats which had reached sexual maturity, but were still growing at a slow rate, where white bread was the only source of protein in the diet. In this experiment 16-week-old rats were used and two matched groups of litter-mates were compared. One group was fed a good stock diet of standard cubes with supplements of meat and milk, the other was given a diet in which all the protein was supplied by dried white bread. Over a period of 22 weeks there was no difference between the two groups in respect of rate of gain in weight and general health. It thus appears that bread protein alone is adequate for the final stages of growth and maintenance of the rat.

(ii) *Human subjects*

(a) In Sherman's (1920) classical review of the evidence then available for estimating the protein requirement for *maintenance* in adult man he gives the details of one of his own experiments, with an adult male subject, in which over 95% of the protein consumed was derived from commercial white bread. With this particular subject over a period of 15 days he found that 'practical equilibrium was maintained on an intake

of a little less than 0·5 g of protein per kilo of body weight per day'. It would seem dangerous to generalize from this but Sherman himself comments:

'Thus the protein of bread showed as high an efficiency in the maintenance metabolism of man as would be expected of the protein of mixed diet in general'.

(b) More recently Mickelsen, Bolourchi & Friedemann (1966) have carried out feeding trials at the University of Michigan with adult human subjects. This study consisted of a 21-day control period during which the subjects received a 'typical American' diet, containing animal protein, with the protein restricted to 72 g/head/day. This was followed by 50 days on the experimental diet which provided 67 g protein/head/day, with 90% of this coming from wheat flour and the remainder from fruits and vegetables. The subjects remained in good health and physical condition. The authors state: 'Throughout the study 24-h urine and stool samples were collected. Analyses of these samples and the diets indicated that during the control period the men were in negative nitrogen balance which approached equilibrium at the end of the third week. During the first half of the experimental period the subjects showed an average daily loss of 100 mg of nitrogen; for the second half there was an average retention of 1380 mg. Throughout the study there were fluctuations in body weight of individual subjects. However for the entire group there was no change in mean body weights'.

These feeding trials with adult human subjects are consistent with the expectation to be drawn from the NDp cals % value of 6·0 for wheat which indicates that wheat by itself can meet the protein requirement of the adult male human.

(c) The outstanding and most exacting investigation on human subjects is, of course, that of Widdowson & McCance (1954) on young, undernourished growing children. In these studies 60–80% of the total protein came from the protein of wheat. The authors state: 'Probably the most important finding concerns the high nutritive value of wheat in any of the forms customarily consumed by man. Thus it has been shown that diets in which 75% of the calories were derived from wheat flour and 21% from vegetables and which contained only 8 g of animal protein a day, provided undernourished children aged 5–15 years with all the nutrients required for a high rate of growth and development for a period of 18 months'.

It is also of interest to note that one of the two groups of children taking part in the investigation obtained 12·0% of their total calories from protein whilst the other group obtained only 8·8% from the same source. Yet growth and development were excellent in both cases.

Discussing this investigation Davidson & Passmore (1963) comment: 'McCance & Widdowson's only firm deduction from this experiment was that wheat in any form is an excellent food for growing children. Their results show that wheat proteins are capable of maintaining high rates of growth for a long period. Bread indeed is a good source of protein and has been wrongly condemned as a "starchy food" '. The evidence given in this paper supports this view.

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Isolation of structural elements of food products by preparative ultracentrifugation: ice cream and ice-cream mix*

J. J. WREN AND B. K. BULLIMORE

Summary. The potential value of this technique is illustrated. With minimal risks of rearrangement or degradation it resolves a variety of food products into layers containing structural elements of different density. When ice cream was melted at 2°C and centrifuged for 90 min at 176,000 *g* (maximum) it yielded eight layers (other than the air layer) that were different in appearance, texture and chemical composition. The largest layer was clear serum; above this were fat-rich layers containing some protein and below were protein-rich layers containing some fat. Under the same conditions, ice-cream mix gave a somewhat different pattern of layers, which could be related to the effects of 'fat clumping' and air incorporation during the freezing of ice cream. Analyses indicated that the layers contained a system of lipoprotein particles of different sizes, all coated with monoglyceride, diglyceride and protein. The triglycerides of all layers of both ice cream and mix had very similar fatty-acid compositions.

Introduction

Chemical and physical studies of the microscopic structures of food products, and how these structures are affected by processing and ingredients, often depend upon the isolation of structural elements. Ideally, such isolation should give sizeable specimens, of reasonable purity, and not rearranged or degraded in any way. However, isolation procedures that are commonly used, for example selective extraction, enzymic digestion, and sedimentation or flotation in water or aqueous solutions, do tend to rearrange or degrade the structural elements.

Conventional centrifuges are used widely for 'creaming tests' on ice cream, to measure emulsion stability and degree of homogenization. It was observed in these Laboratories some time ago (Wren & Wiggall, 1962, unpublished experiments) that a preparative ultracentrifuge gave a much more dramatic result, namely, complete breakdown of the ice-cream structure into at least seven layers. This separation

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of the structural elements and some analytical studies that it made possible are the main subject of this paper.

Mauritzen & Stewart (1963, 1965) have successfully applied ultracentrifugation to wheat-flour doughs. From our further observations it seems that the technique is applicable to a wide variety of liquid and plastic food products.

Methods

Ultracentrifugation of ice cream and ice-cream mix

The centrifuge used was an M.S.E. 'Super-Speed 40', with the small, swing-out rotor, holding three polypropylene tubes each of nominal capacity 5 ml. At maximum speed this gave an average force of 137,000 ***g***, and a force of 176,000 ***g*** at the bottoms of the tubes. The centrifuge bowl was refrigerated at maximum setting throughout an experiment and for 30 min beforehand. The rotor was pre-cooled to − 20°C before being loaded with the tubes. These precautions ensured that the temperature inside the tubes never rose above 2°C during an experiment.

Small samples of ice cream were allowed to melt at 2°C, with occasional gentle stirring, during 40 min.

Mix or melted ice cream was poured into the tubes, which were balanced, capped, and then kept for 15 min at 2°C. They were then mounted in the rotor and spun at maximum speed for 90 min.

After observation, the centrifuged tubes were frozen by immersion in a dry ice–methyl cellosolve bath. If a tube was entirely frozen while upright, the serum layer (particularly from mix) tended to expand through the middle of the upper layers. To avoid this, the stoppered tube was first cooled gently, until the serum became opaque, then inverted to immerse the upper layers in the bath, so making them contract from the walls of the tube; finally, the tube was immersed in the upright position.

After freezing, the tubes were uncapped and placed in a wooden template (Fig. 1). A sharp knife-blade was then placed in the slot and struck with a mallet, so cutting the tube from top to bottom. The tube was then rotated and cut on the opposite side, and folded back (Fig. 1) to discharge the solidified contents intact. (This technique avoided the risk of 'smearing' material from one layer to another.) The layers were then dissected as well as possible on a slab of dry ice and transferred to tared tubes, which were well stoppered and stored at − 80°C until sampled for analysis. Before sampling the contents of each tube were always warmed rapidly to room temperature and thoroughly mixed.

Ultracentrifugation of other products

Essentially the same method was used, but excluding pre-cooling and refrigeration of the centrifuge bowl. The temperature did not exceed 30°C.

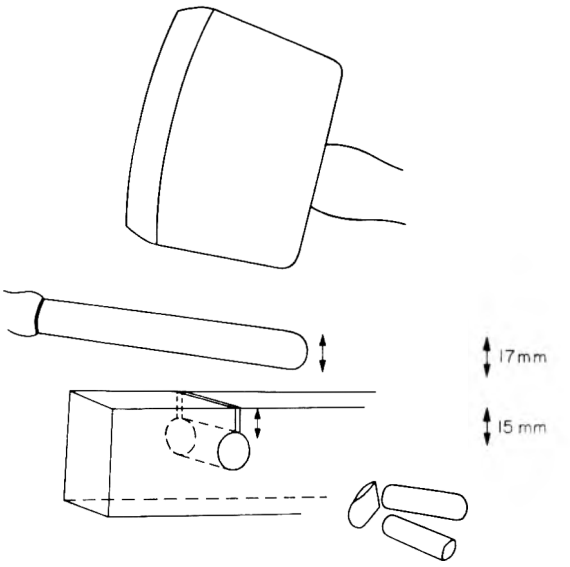

FIG. 1. Tube cutting: template, knife, mallet and an opened tube.

Total solids

The sample was evacuated at 100°C to constant weight.

Protein

The sample was mixed with water and washed three times with diethyl ether, by shaking and centrifuging. The aqueous layer was heated at 40°C for 30 min to remove all the remaining ether. Aqueous 3 N-trichloroacetic acid was added to give 1 N concentration in the mixture, which was then incubated at 37°C for 60 min, cooled to 0°C, and centrifuged at *c.* 100,000 ***g*** for 15 min. The supernatant liquid was well drained from the precipitated protein; this was then determined by the micro copper-binding method of Westley & Lambeth (1960) using bovine serum albumin as standard (dry-weight basis).

In model experiments trichloroacetic acid gave nearly quantitative recovery of milk proteins, but only *c.* 50% recovery of gelatin. The micro copper-binding method was found to be unsuitable for samples containing starch.

Lipid

The sample's contents of fat, emulsifier and skim-milk lipids were measured together as a single quantity, the 'hydrolysate lipids' content. This was determined by complete hydrolysis, as described by Wren & Wojtczak (1964).

Sugar

The trichloracetic acid supernatant (see above) was subjected to the anthrone method described by Scott & Melvin (1953), using sucrose as standard.

Proline and hydroxyproline

Proline (Wren & Wiggall, 1965) and hydroxyproline (Leach, 1960) were determined in protein precipitated with trichloroacetic acid.

Mono-, di- and triglycerides

The sample was twice extracted with chloroform–methanol (Bligh & Dyer, 1959), the combined extracts were evaporated, and the residual lipid was redissolved in chloroform containing 50 ppm of BHT (Wren & Szczepanowska, 1964) and stored at −23°C.

The lipid was resolved into the three glyceride classes by thin-layer chromatography in light petroleum–diethyl ether–methanol–acetic acid (90 : 20 : 3 : 2, by volume) (Brown & Johnson, 1962) on non-activated plates of Silica Gel G, spread at 0·25 mm thickness. Zones were located with iodine vapour, removed (the 1,2- and 1,3-diglyceride zones being combined), and eluted with 20% (v/v) methanol in ether.

The eluates were subjected to glycerol analysis (Moore, 1962), with appropriate blanks, and with highly purified tripalmitin as standard.

Fatty acid composition of triglycerides

Lipid was extracted and fractionated as above, except that the Silica Gel G was spread at 0·5 mm thickness and the developing solvent was 30% (v/v) diethyl ether in light petroleum. The isolated triglyceride was methanolysed and the product subjected to gas–liquid chromatography at 200°C on a poly (ethylene glycol adipate) column.

Results

The product that was most studied in this work was a commercial ice cream, prepared from non-fat milk solids, sucrose, refined palm kernel oil, gelatin stabilizer, monodiglyceride emulsifier and vanilla flavouring. It was made in continuous freezers of the Vogt type and hardened according to normal practice. When centrifuged, this ice cream gave eight visibly different layers made up of liquids and solids, as depicted in Fig. 2. These filled only about half of the original volume, the remainder being occupied by air from the gas cells. An equal weight of the mix from which the ice cream was prepared gave an appearance that was broadly similar, but different in two respects: (a) layer (i), the thin 'scum' of flaky solid, was missing and (b) the other top layers (ii–iv) were nearly 50% deeper at the expense of the bottom layers (vi–viii). The densities of the serum layers obtained from mix and ice cream were indistinguishable, within the limits of experimental error (± 2%).

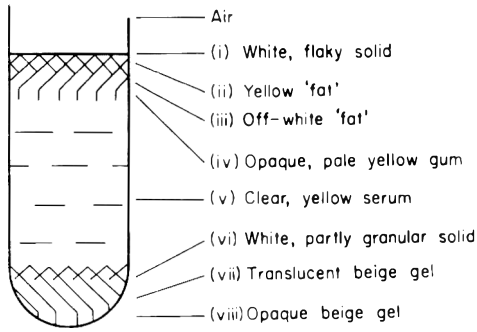

FIG. 2. Layers obtained from ice cream.

Equilibrium seemed to be fully established during the 90-min period of centrifugation, because no further changes in appearance occurred when tubes were re-centrifuged. The separation was impaired by reducing the period to 15 min, or by halving the speed, although marked layering and complete separation of air (from ice cream) still occurred. By alternately pre-cooling the rotor and tubes to $-20°C$ and spinning for short periods it was possible to maintain temperatures ($\not> -9°C$) at which a large proportion of the water in ice cream was in crystalline or vitreous state (White & Cakebread, 1966). However, at these temperatures no visible separation occurred.

The distribution of total solids, protein and lipid in a centrifuged tube of ice cream is represented in Fig. 3. Sucrose and lactose made up nearly all of the solids other than protein and lipid. Of the protein present in whole ice cream, 16% was found in the top layers (i–iv), 21% in the serum layer (v), and 63% in the bottom layers (vi–viii). Of the lipid, the proportions were 92, 1 and 7%, respectively.

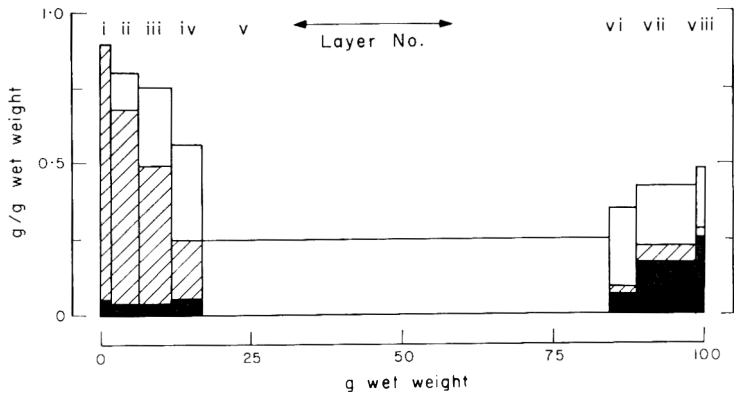

FIG. 3. Distribution of solids in layers obtained from ice cream. Black columns, protein portion; cross-hatched columns, lipid portion. Abscissa represents cumulative wet weight from top to bottom of a tube, scaled up to 100 g. Area designated for a layer therefore represents actual weight of solids in the layer.

During the course of this work another study of lipid distribution was made by determining the lipid contents only of the top and bottom halves from each tube. The proportion of lipid thus found in the bottom half varied from 0·2% to 18·6% for some twenty samples of ice cream, prepared in freezers of several types from mixes containing various types and proportions of milk and fat ingredients (including butterfat). For the mixes, the proportion found in the bottom half varied from 1·5% to 39·9%. The proportion usually fell markedly when a mix was frozen into ice cream, especially if the load on the freezer's motor was high. Occasionally the proportion underwent no significant change on freezing.

The ratio of lipid to protein in each layer appeared to be characteristic (Fig. 4), and varied between 25,300 mg/g, in layer (ii), and 65 mg/g, in layer (viii). The ratio of protein to water also varied characteristically (Fig. 4), approaching 500 mg/g in

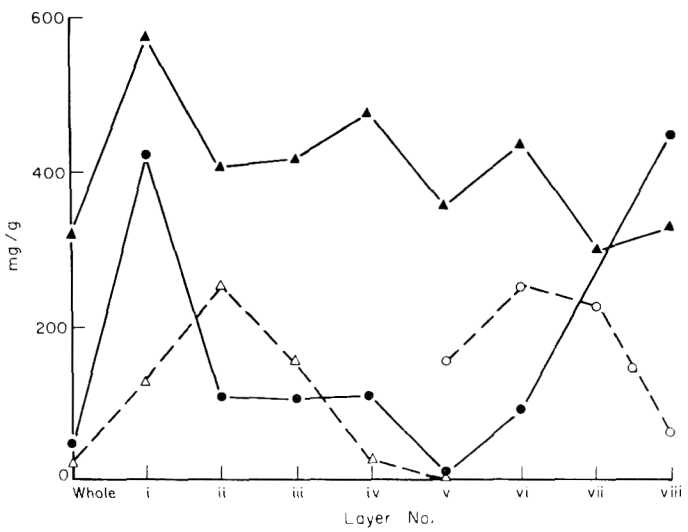

FIG. 4. Ratios of main constituents in layers obtained from ice cream. ●, Protein–water; ▲, sugar–water; ○, lipid–protein; △, lipid/100 × protein.

layers (i) and (viii) and falling to 14 mg/g in layer (v). The ratio of sugar to water varied more randomly, but was markedly low in layers (vii) and (viii) (the protein-rich gels) and high in layer (i).

Fig. 5 gives some indication of the distribution of individual proteins in the layers. It was anticipated that hydroxyproline, specific to the gelatin ingredient, would tend to occur in a constant ratio to water, whereas proline, abundant in both gelatin and caseins, would not. Accordingly, the hydroxyproline–water ratio varied only about two-fold whereas the proline–water ratio varied about forty-fold. The hydroxyproline–protein and proline–protein ratios were highest in the serum layer (v), where

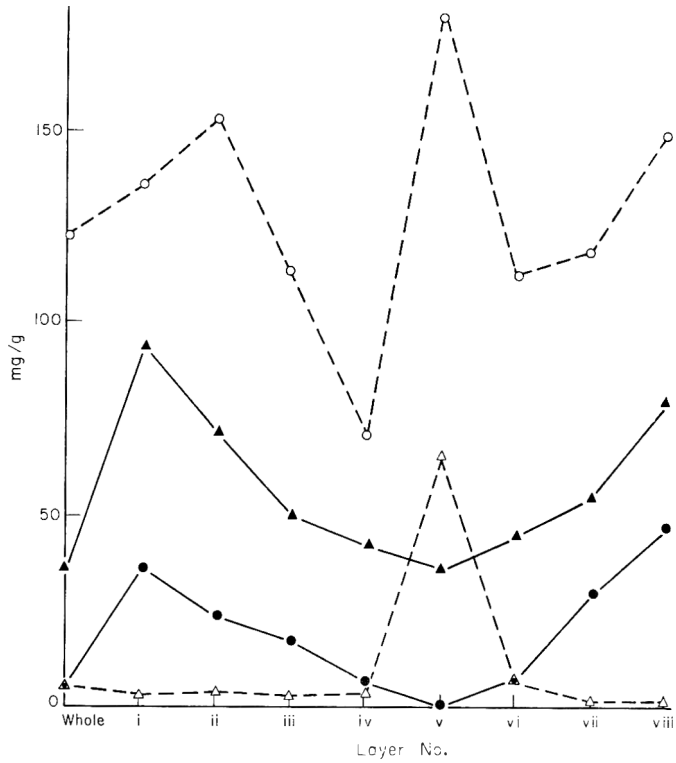

FIG. 5. Proline and hydroxyproline contents in layers obtained from ice cream. ●, Proline–water; ▲, 100 × hydroxyproline–water; ○, proline–protein; △, hydroxyproline–protein.

most of the gelatin presumably occurred. In other layers, where the hydroxyproline–protein ratio was low and fairly constant, the proline–protein ratio varied markedly, showing that the various milk proteins were distributed in a non-uniform manner.

Ice cream and mix of a slightly different formula, containing carob bean gum as stabilizer in place of gelatin, were subjected to a detailed study of lipid composition. Although the mix did not give a 'scum' the topmost portion was taken and designated layer (i), to facilitate comparisons with ice cream. Some of the results thus obtained are illustrated in Fig. 6. The monoglyceride–triglyceride ratio increased markedly from the top to the bottom layers, as also did the diglyceride–triglyceride ratio. About 20% of the total emulsifier was recovered in the bottom layers (vi–viii). The monoglyceride–triglyceride and diglyceride–triglyceride ratios were lower in the bottom layers obtained from mix than in those obtained from ice cream.

Analyses of the triglyceride fatty-acid composition in layers obtained from mix did not seem to vary beyond the limits of experimental error (Fig. 7). However, for ice cream they suggested a tendency for laurate residues to occur preferentially in the

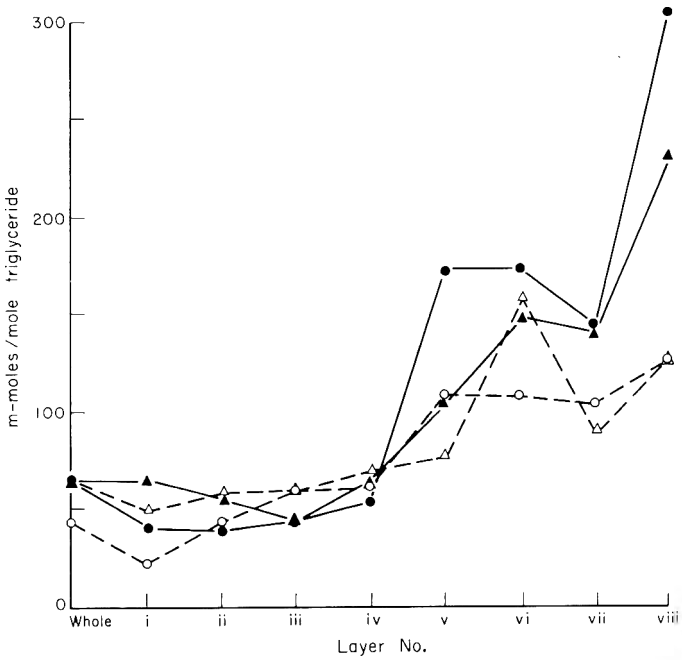

FIG. 6. Molar ratios of mono- and diglycerides to triglycerides in layers obtained from ice cream and mix. ●, Ice cream monoglyceride–triglyceride; ▲, ice cream diglyceride–triglyceride; ○, ice-cream mix monoglyceride–triglyceride; △, ice-cream mix diglyceride–triglyceride.

top layers, and oleate and linoleate residues in the bottom layers. Preliminary results of gas–liquid chromatography of the intact triglycerides (Kuksis, 1965) also suggested that triglyceride composition varied, but relatively slightly. The *gross* fatty acid composition varied more, as was predictable if the content of monoglycerides and other polar lipids varied.

Discussion

By the two simple steps of warming to 2°C and applying gravitational force, ice cream was resolved into nine layers (including air) that differed in appearance and chemical composition. This is convincing evidence for at least nine structural elements. One more structural element, ice, could not be resolved because centrifugation was not successful at temperatures below the melting point. However, because many differences were found between centrifuged, melted ice cream and centrifuged ice-cream mix, it seems reasonable to believe that the elements resolved had not been degraded or rearranged significantly by melting.

Because equilibrium was achieved between the layers it follows that particle density was the controlling factor, and not particle size or shape, which are equally important factors in 'creaming tests' made with lower gravitational force. Thus the

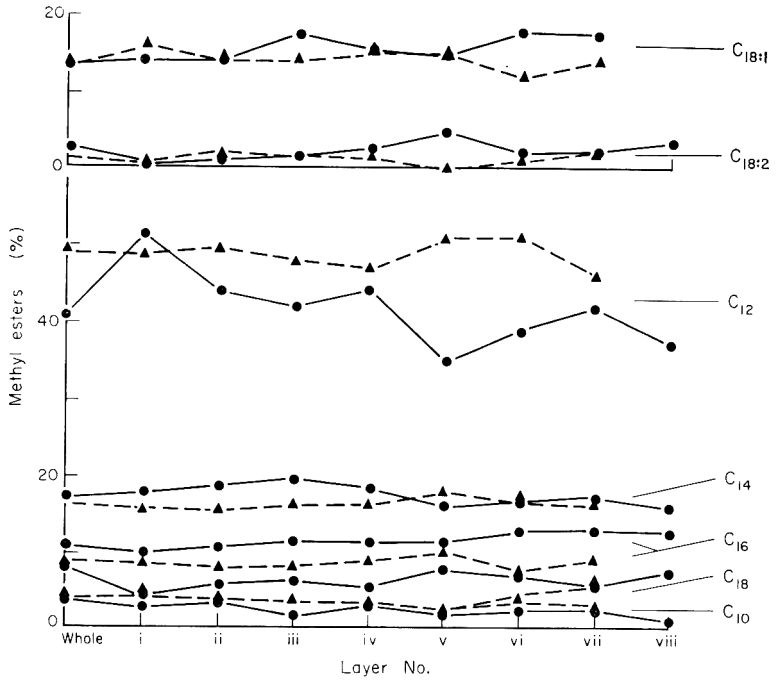

FIG. 7. Fatty-acid compositions of triglycerides in layers obtained from ice cream (———) and mix (- - - -).

resolution achieved, besides being much more complete than that obtained in 'creaming tests', is potentially easier to interpret in terms of a single property.

Good as the resolution was, it was imperfect for at least three reasons: (a) different structural elements did not necessarily have different densities, (b) serum remained in interstices and gels, and (c) boundaries between the layers were not always sharp.

The protein and lipid analyses can be interpreted remarkably well by an analogy with the lipoprotein system of blood (Gurd, 1960). This consists of fat particles ranging in diameter from about 1 μ (chylomicra) to about 0·01 μ (high-density lipoproteins). The surfaces of these particles are coated with polar lipids and proteins, which are more dense than the fat, and the ratio of surface area to volume increases with decreasing size. Hence, particle density is related inversely to size, and directly to polar lipid–fat ratio and protein–fat ratio. The monoglyceride–triglyceride and protein–lipid ratios for ice cream were indeed higher in the bottom than in the top layers (see Figs. 3, 4 and 6). Moreover, the relatively high protein–water ratios in the top layers and the occurrence of triglycerides in the bottom layers can only be explained by the occurrence of stable lipoprotein particles. High-density lipoproteins have previously been found to occur in homogenized milks (Fox *et al.*, 1960; Fox, 1963; Patton, Durdan & McCarthy, 1964). However, it is quite remarkable that in some ice-cream mixes a third or more of the total fat occurred in them.

Diglyceride content was related much more to monoglyceride content than to triglyceride content (Fig. 6). Contrary to a widely accepted view (Durham, 1963) this indicates that diglyceride is surface-active in ice cream.

Recent reports by Stistrup & Andreasen (1962) and Keeney & Maga (1965) suggest that freezing mix into ice cream induces a change in triglyceride composition within the lipoproteins. According to the present results, the extent of such a change, if it occurs at all, is only slight.

Two changes that would be expected to affect the lipoprotein system when ice cream is frozen into mix are: (a) the formation of lipoprotein membranes at the air–cell surfaces, and (b) the 'clumping' of fat (lipoprotein) particles (Doan & Keeney, 1965). Change (a) may well be responsible for the appearance of flaky solid (layer i) on centrifuged ice cream; this solid could be the membrane material. It is assumed that 'fat clumps' are not disrupted by centrifugation. If they are formed randomly from lipoprotein particles of different sizes, then their densities are expected to be much more uniform than those of their constituent particles, and nearest in value to those of the larger particles. If their formation releases some protein coating this will appear in the bottom layers instead of the top. Hence change (b), 'clumping,' may well be responsible for the nett transfer of lipid to the top layers, the contraction of the top layers, and the expansion of the bottom layers.

The lipoprotein system of ice cream differs from that of blood in having most of its triglyceride in the solid state. 'Clumping' appears to be a result of this, perhaps dependent upon loosening of the protein coating.

The method of ultracentrifugation described could clearly be used further to study structural and biochemical aspects of ice cream. As Plate 1 illustrates, it also promises to be an effective tool for the study of numerous other food products.

Acknowledgments

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Thanks are due to Mr E. C. Bishop for fatty acid analyses and Miss K. P. Brown for photography.

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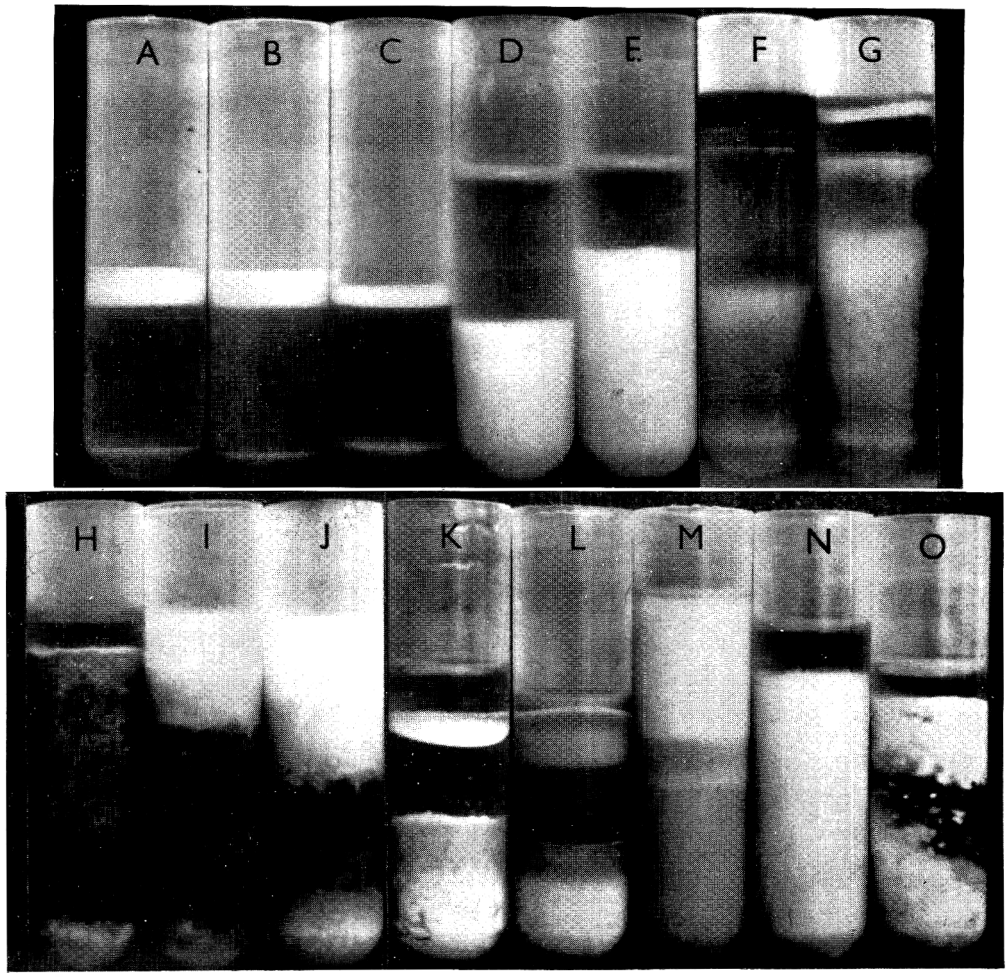

PLATE 1. Tubes after ultracentrifugation. The number of layers discerned before photography is noted in parentheses. A, ice cream (8); B, ice-cream mix (7); C, dairy ice cream (8); D, lard (2); E, compound fat (3); F, condensed cream of tomato soup (9); G, beef and vegetable broth (11); H, frankfurter sausage (5); I, pure pork sausage (6); J, pork sausage containing cereal (8); K, short-pastry dough mixed with equal weight of M-NaCl (7). L, sponge-cake batter (8); M, mayonnaise (8); N, butter (3); O, blue cheese mixed with equal weight of M-NaCl (4).

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Studies on mechanical factors affecting dough development*

P. W. HEAPS, T. WEBB, P. W. RUSSELL EGGITT
AND J. B. M. COPPOCK

Summary. Doughs of wheat flour, salt and water have been mixed at various rates and to various levels of mechanical work input. Rheological tests have confirmed that there exists a definite level of work input which gives maximum dough development for any one flour; the results suggest, moreover, the existence of an optimum rate of work input for producing the most stable dough and the best bread.

Baking tests have been used to relate the rheological parameters of the doughs with their baking performance.

Introduction

The replacement of the normal 3–4 hr bulk fermentation stage of conventional bread-making by a very short period of intense mechanical mixing has been one of the most notable advances in cereal technology during the past decade. Important changes taking place during fast mechanical mixing involve the development of a three-dimensional gluten network by hydration and simultaneous re-orientation and cross-linking of the protein chains originally present in the flour. Several authors have postulated that the mechanical scission and subsequent re-formation of a more orderly system of disulphide bonds, to produce a stable, expandable protein network, is an essential feature of mechanical development (Axford & Elton, 1960).

The originators of the Chorleywood Bread Process (Chamberlain, Collins & Elton, 1962), (now widely used in this country), claimed that optimum development of a dough is achieved by expending a fixed amount of work on the dough in special batch mixers (0·4 H.P. min/lb dough or 11 Watt hr/kg dough). Later they reported that this critical level of work should be carried out in not more than about 5 min (Chamberlain, Collin & Elton, 1965).

Much emphasis has been placed on the optimum *level* of work required to develop a dough but relatively little experimental effort has been directed towards investigating

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the importance of the *rate* of work input. It is well known that too low a rate of work input produces bread with a poor texture and it is reasonable to suppose that a certain minimum mixing time is required to allow for adequate protein hydration and for the other changes involved in dough development. Thus there is likely to be an optimum *rate* of work input, as well as an optimum *level*, with a particular mixing system. Our object was to investigate this possibility.

Materials and methods

A strong bread flour was used which had been milled from 80% Manitoban and 20% Hard Winters Wheats without bleaching, treatment or the addition of solid improving agents. Its protein content was 13·4% on a 14% moisture basis.

Doughs containing 2% salt and 53% water (both based on the weight of flour) were mixed in a stainless steel-clad Farinograph bowl attached to a Brabender Do-corder. The factors governing the amount of water used were: (i) ease and reproducibility of handling, and (ii) a consistency which allowed work level and rate of work input to be varied over a reasonable range using a constant dough weight of 470 g. With a fixed quantity of dough, the rate of work input is directly proportional to the product of the speed of the mixer and the torque produced by the dough in mixing. This product must therefore be kept constant in order to achieve a constant rate of work input.

Doughs were mixed at a series of constant rates over the range 0–0·45 H.P. min/lb/min and the levels of work introduced into the doughs were varied up to 3·6 H.P. min/lb. The doughs were divided into 3 × 150 g pieces after mixing, moulded, allowed to rest for 45 min in the humidified cabinets of the Brabender Extensograph at 30°C and subjected to the 'work technique' (Muller *et al.*, 1963). The work technique is a rheological procedure whereby the work required to stretch a material (in this case, dough), may be separated into recoverable (elastic) and irrecoverable (viscous) components.

Parallel baking tests were carried out using the above flour–salt–water doughs but with the inclusion of 2% yeast on the flour weight.

Results

The viscous and elastic components of the stress work (i.e. the work required to stretch the dough on the Extensograph) considered here are those obtaining at 8 cm non-recoverable extension (W_v, 8) and 5 cm recoverable extension (W_E, 5). Values of W_v, 8, W_E, 5 and maximum extensibility were plotted, for each rate of work input, against the total work level, and for a number of work levels against the rates of work input. Table 1 summarizes the experimental data.

TABLE 1. Experimental data of maximum extensibility (E_{max}) and stress work components for various rates and levels of work input

Rate of work input (H.P. min/lb/min)	Total work input (H.P. min/lb)	E_{max} (cm ± 1 cm)	W_v, 8 (erg/g)	W_E, 5 (erg/g)
0·1	0·1	29·2	51	31
	0·2	31·0	53	31
	0·3	28·2	58	31
	0·7	26·3	62	36
	1·0	23·7	72	33
	1·2	21·5	76	38
	1·5	19·6	85	39
	2·0	17·0	88	43
	2·3	15·9	93	43
	2·7	13·1	96	44
	3·6	11·4	88	44
0·175	0·1	31·5	—	—
	0·4	27·3	72	38
	0·8	25·1	69	33
	1·2	20·0	84	41
	1·6	18·2	92	47
	2·0	16·0	99	52
	2·4	12·9	104	52
0·25	0·1	30·3	—	—
	0·5	25·2	70	35
	0·9	21·8	81	38
	1·3	19·5	88	37
	1·7	16·2	97	46
	2·1	12·9	114	53
	2·5	11·8	114	51
	2·9	10·6	104	52
	3·3	10·9	109	52
0·35	0·1	32·4	—	—
	0·4	28·9	74	37
	0·8	23·0	84	41
	1·2	19·6	89	40
	1·6	14·7	102	45
	2·0	12·8	114	49
	2·4	11·2	122	57
	2·8	11·8	108	52
0·45	0·1	32·3	54	30
	0·45	27·3	61	30
	0·8	24·3	77	38
	1·15	21·3	89	38
	1·5	16·1	97	45
	1·8	15·6	100	51
	2·15	13·6	107	54
	2·5	10·8	112	59
	3·0	10·9	104	53

Maximum extensibility

Maximum extensibility decreases as the total work input increases, at each rate studied. A typical example of this is shown in Fig. 1, where the vertical lines indicate the estimated spreads of maximum extensibility.

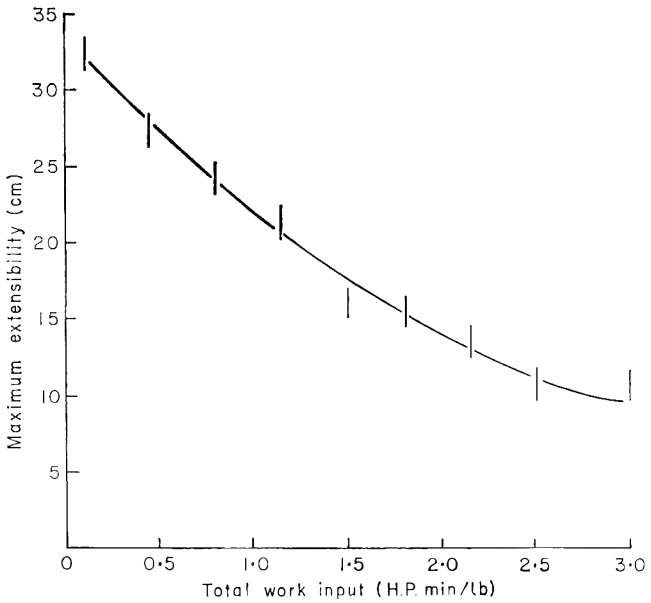

FIG. 1. Graph showing the decrease in maximum extensibility with total work input at constant rate (0·45 H.P. min/lb/min).

When maximum extensibility, at any given level of work input up to about 2·5 H.P. min/lb, is plotted against the rate of work input, as in Fig. 2, a minimum value is observed at 0·35 H.P. min/lb/min.

Stress work components

Fig. 3 shows that W_v, 8 and W_E, 5 pass through maximum values with increasing total work input. This effect was observed at all rates of work input studied and is in general agreement with earlier work from our laboratory (Heaps, Russell Eggitt & Coppock, 1965). W_E, 5 appears to be less sensitive than W_v, 8 to changes in total work input.

Dependence of the stress work components on the rate of work input, at a given level of work input, is shown in Fig. 4. W_v, 8 exhibits a maximum as the rate of work input increases. The position of this maximum, at 0·35 H.P. min/lb/min coincides with that of the minimum in the plot of maximum extensibility against rate of work input (Fig. 2).

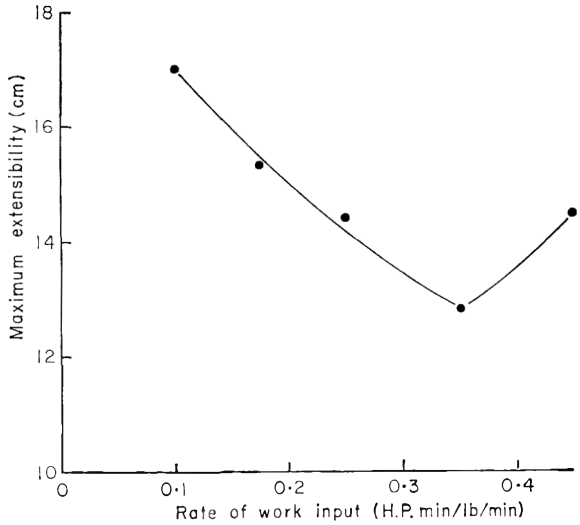

FIG. 2. Variation in maximum extensibility with rate of work input (total work input = 2·0 H.P. min/lb).

Baking tests

Baking tests in which a high level of work was introduced into the doughs at various rates, have given bread the quality of which deteriorated as the rates of work input increased to 0·35 H.P. min/lb/min. No significant change could be detected on

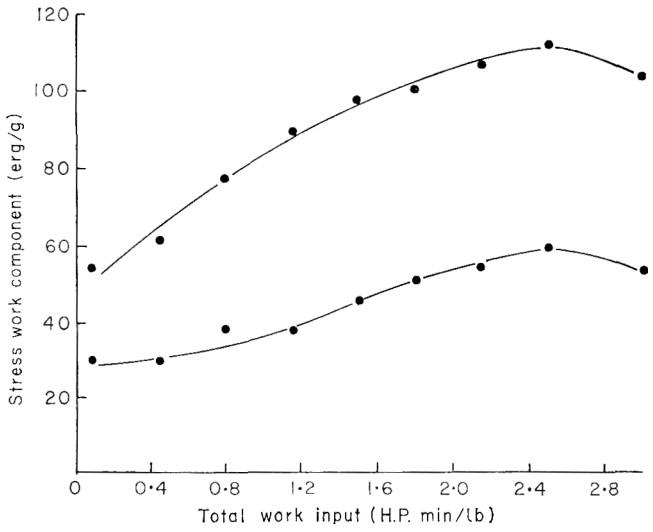

FIG. 3. Development of dough as shown by increasing stress work components with total work input at constant rate (0·45 H.P. min/lb/min). Upper line, W_v, 8; lower line, W_E, 5.

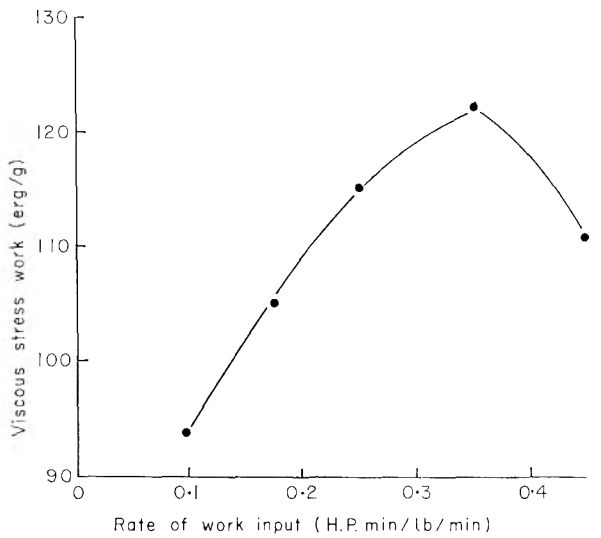

FIG. 4. Dependence of viscous stress work on rate of work input (total work input = 2·4 H.P. min/lb).

increasing the rate still further to 0·45 H.P. min/lb/min. At the high level of work input, the quality of all bread, particularly in regard to specific volume, was inferior to that into which a lower level, e.g. 0·4 H.P. min/lb, was introduced.

Discussion

Development of a dough by the introduction of mechanical work at a given rate, leads to increases in the components of the stress work towards maxima: work beyond the maxima causes over-development and breakdown of the dough structure. Within the scope of the present work, where work input rates are limited by the instrument, there is no evidence that the *level* of work input (about 2·5 H.P. min/lb) at which the maxima are observed is dependent upon the *rate* at which the work is introduced. A further consequence of the extended development of dough appears to be a reduction in its ability to be stretched, probably owing to its highly cross-linked structure. In baking parlance, the dough has become 'short'. This is not a 'shortness' caused by inherent protein weakness, but rather by a lessening, through cross-linking, of protein lengths susceptible to stretching.

We have suggested previously that the greater the components of stress work the greater the development of the protein network (Heaps *et al.*, 1965). If this is so, the development of the dough structure with our instrument appears to be enhanced by increasing the rate of work input to 0·35 H.P. min/lb/min. Thus, in addition to an optimum *level* of work input there appears to be an optimum *rate*, above which molecular degradation, including main-chain rupture, may occur as a result of relatively high

mechanical shearing forces (Angier, Ceresa & Watson, 1958). It is likely that this degradation also takes place at low rates of mixing, to some extent, but it becomes apparent on attaining a rate of 0·35 H.P. min/lb/min. The effects of degradation may, however, outweigh the beneficial effects of higher rates on dough development, thus accounting for a maximum. The increase to a maximum observed for the stress work with increasing rate of work input, at a given level of work input, is accompanied by a decrease of the maximum extensibility to a minimum value. The approach towards this minimum may be accounted for by an increasing degree of cross-linking as the dough structure develops. The observed increase in the maximum extensibility after passing 0·35 H.P. min/lb/min may be attributed to a partial breakdown of the most stable protein network.

In an earlier communication it was suggested that the major requirements for the best loaf of bread are: (a) high stress work, coupled with (b) a maximum extensibility falling between certain specified limits (Heaps *et al.*, 1965).

It is thought that the difference between maximum dough development (occurring at about 2·5 H.P. min/lb) and dough development which is optimum for baking (occurring at about 0·4 H.P. min/lb) may be caused by two independent factors. Firstly, the system used in the present rheological studies consists of flour, salt and water and does not include the additional ingredients which are required to give a good loaf of bread. Secondly, it is possible that the level of work input which is optimal for baking differs from that which gives maximum dough development due to the decrease in maximum extensibility associated with the latter to a value below the range required to permit the production of a satisfactory loaf.

The deterioration observed in the baking tests with increasing rates at the high level of work input is thought to be attributable to the decreased maximum extensibility.

The rheological work we have described, together with the parallel baking tests, serves to emphasize the importance of considering the rate of work input in mechanically developed doughs.

In future work it is hoped to extend the rates of work input beyond the limits imposed by existing machinery.

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J. Fd Technol. (1967) 2, 45–52.

Measurement of the flow properties of powders

G. W. WHITE, A. V. BELL AND G. K. BERRY

Summary. Published literature on the flow of powders through orifices is reviewed. A new powder flow meter is described, and results obtained with some powdered food products are given. The mass flow rate is found to be proportional to the bulk density and to the 2·8th power of the orifice diameter. The flow meter permits comparison of the flow properties of powders and prediction of their behaviour in food machinery and plant.

Introduction

Many powders of widely different properties are used in the food industry. It is usually important that these powders should be free-flowing, so that, for example, hoppers and weighing devices perform satisfactorily. At present there is a need for a simple laboratory apparatus to measure the flow properties of powders.

The purpose of this paper is to describe a powder flow meter that can be used for both research into, and routine control of, the flow properties of powders. A brief review of the literature on powder flow is given first, so that the underlying reasons for the design of the meter will become apparent.

Literature review

Many studies of the flow properties of powders have been reported but, as Pilpel (1965) points out, there is as yet no equation generally applicable to the flow of granular materials and powders of interest to industry.

Where the flow of a powder through a horizontal orifice (in the base of a container) is concerned it might be expected that the mass flow rate would depend on several factors; these might include height of powder, diameter of orifice, particle density, bulk density, flow resistance of the powder, particle shape, particle size and size distribution, moisture content, oil content, static charge, etc.

Unlike liquids, however, powders flow from a container in such a way that the mass flow rate is independent of the height of powder in the vessel (Deming & Mehring, 1929; Takahasi, 1937; Newton, Dunham & Simpson, 1945; Franklin & Johanson, 1955; Brown & Richards, 1959; Fowler & Glastonbury, 1959), provided that the

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height of the powder is at least twice the orifice diameter (Harmens, 1963). This independence of powder height comes about because pressure is not transmitted through a powder beyond a certain distance.

Many investigations have been made of the effect of orifice diameter on the mass flow rate which has been found to be directly proportional to a power of the orifice diameter; values for this power range from 2·50 to 2·96, with a mean value of 2·66.

Many workers have found correlations between the mass flow rate through an orifice and the bulk density of the powder. Generally they found that the mass flow rate was directly proportional to the bulk density. Some controversy centres round the question of whether bulk density or particle density should be used in equations. Franklin & Johanson (1955), Brown & Richards (1959) and Harmens (1963) all used particle density, and this can be justified within certain limits by the observation of Brown & Richards (1959) that for void fractions between 0·37 and 0·54 the flow rate is constant. On the other hand, bulk density depends on the particle density and the void fraction and includes, therefore, the effect of both factors.

By analogy with liquids, it would be expected that there is some property of powders, akin to viscosity, that expresses their resistance to flow. In deriving equations for the mass flow rate of powders, many workers use the coefficient of friction (μ) to express the flow resistance. This coefficient can be defined as the tangent of the angle of repose, determined by allowing the powder to form a cone and measuring the angle with the horizontal. Franklin & Johanson (1955) and Pilpel (1965) have noted that three different angles of repose have been used by various workers – the static angle of repose, the surface kinetic angle of repose and the internal kinetic angle of repose. These three angles are all defined in slightly different ways, and one of the difficulties surrounding their use is that the complex conditions existing above the orifice in the container, when powder is flowing, are different from those used in the measurement of the coefficient of friction.

Brown & Richards (1959) found that the mass flow rate is greater for spherical than for angular particles, and greater for fine than for coarse particles. The effects of particle size distribution have not been greatly studied, but powders with a narrow size distribution tend to flow more readily than those with a wide distribution (Gregory, 1952). Flow is greatly reduced by surface moisture which should, according to Gregory (1952), be less than 1–2%. The effects of additives are complex (Pilpel, 1965; Burak, 1966).

Several workers have developed theoretical and empirical equations to account for the flow rates of different materials through an orifice. By analogy with liquids, it is reasonable to assume that the mass flow rate of a powder from an orifice in the base of a container is given by an expression of the form:

$$\frac{M}{t} = k f(\mu)\ \rho_b^{\alpha} d_o^{\beta} g^{\gamma} \qquad (1)$$

where M/t is the mass flow rate in g/sec, k is a numerical constant, $f(\mu)$ is some function of the coefficient of friction (μ), ρ_b is the bulk density in g/ml, d_o is the orifice diameter in cm and g is the acceleration due to gravity in cm/sec^2.

Many workers have correlated experimental data with equations of the form given in equation (1), and have estimated values for α and β, as shown in Table 1.

TABLE 1. Values reported for α and β in equation (1)

Investigators	α	β
Takahasi (1933, 1934)		2·50
Newton, Dunham & Simpson (1945)		2·96
Kelley (1945)		2·84
Rausch (1948)	1·0*	2·70
Gregory (1952)		2·50
Shirai (1952)	1·0*	2·50
Oyama & Nagano (1953)		2·50
Franklin & Johanson (1955)	1·0†	2·93
Ciborowski & Badzynski (1963)	1·0*	2·50
Mean values	1·0	2·66

* Bulk density.
† Particle density.

The variations in β are probably partly attributable to experimental error, and partly to the fact that equations of this type are inadequate to account for powder flow.

No term involving the height of the powder in the vessel is included in equation (1) because of the experimental evidence, cited above, indicating that the mass flow rate is independent of this parameter. Judging from theoretical equations developed by other workers (Brown, 1961; McDougall & Evans, 1965), it appears likely that the term $f(\mu)$ is dimensionless.

Application of the method of dimensions to equation (1) gives $\alpha = 1$, $\beta = 2{\cdot}5$ and $\gamma = 0{\cdot}5$, so that theory suggests

$$\frac{M}{t} = k f(\mu)\ \rho_b\, d_o^{2{\cdot}5}\, g^{0{\cdot}5} \qquad (2)$$

Apparatus and method

A flow meter with a vibrational attachment has been developed, as shown in Fig. 1. The meter is similar in principle to the flow cups used for measuring the consistency of industrial liquids (British Standard 1733: 1955). It consists of a cylindrical metal

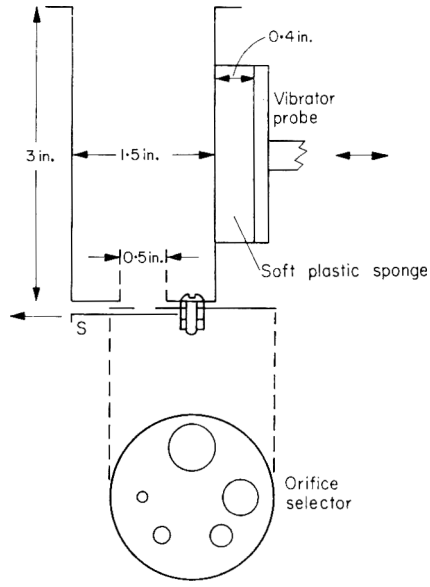

FIG. 1. Powder flow meter.

container with a flat base containing a hole of diameter $\frac{1}{2}$ in. Below the base is a rotatable disc with a series of apertures, of diameters $\frac{1}{8}$, $\frac{3}{16}$, $\frac{1}{4}$, $\frac{3}{8}$ and $\frac{1}{2}$ in., and a sliding shutter S. Mounted at the side of the container is a 50 cycles/sec vibrator (a Pifco massager, set at maximum amplitude, was used). Many powders will not flow from a container unless the vessel is tapped, and the purpose of the vibrator is to standardize the rate and amplitude of agitation. The vibration causes slight fluidization at points of maximum bulk density, and slight compaction at points of minimum bulk density. As a result there is rapid and random variation in the spatial distribution of bulk density which breaks down localized bridges and materially increases the rate of flow, which still, however, depends on inter-particle friction.

The method of using the apparatus is to fill the container, and then to start the vibrator at the moment when the shutter is opened. (Operation of the vibrator with the shutter closed causes marked compaction of the powder and alters the operative bulk density.) The meter is allowed to run until the container is half empty, and the mass flow rate (g/sec) is then determined from the amount that has passed through the orifice.

Results

It was found initially, using the $\frac{1}{8}$, $\frac{3}{16}$ and $\frac{1}{4}$ in. orifices, that the mass flow rate was independent of powder height until the latter fell to $\frac{3}{4}$ in. above the orifice. The mass flow rate through each of the five orifices was then determined for a number of

powdered food products. Other measurements made were of the operative bulk density, i.e. bulk density in the container just before each run, the static angle of repose and particle size, determined by sieving.

The results obtained for each of the powders gave straight lines when log (M/t) was plotted against log d_o as shown for example in Fig. 2. Inspection of equation (1)

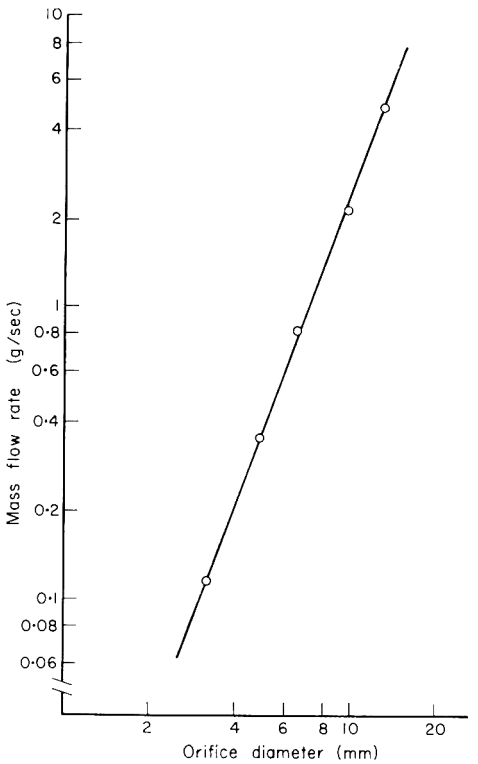

FIG. 2. Dependence of mass flow rate on orifice diameter (instant coffee B).

shows that at a given bulk density, the log–log plot should give a straight line of slope β. The β values were determined for each line, with the results given in Table 2, which also shows the bulk density, mean particle size, and coefficient of friction for each powder.

The mean value of β is 2·84, which is within the range reported in the literature. Mean values of the mass flow rate through the $\frac{3}{8}$ in. diameter orifice are plotted as a function of bulk density in Fig. 3. Evidently the mass flow rate is proportional to bulk density.

The volume flow rates were calculated from $M/\rho_b t$ but no marked correlation could be discovered between these and either the coefficient of friction or mean particle size. The effect of particle size with a single material, however, was demonstrated by

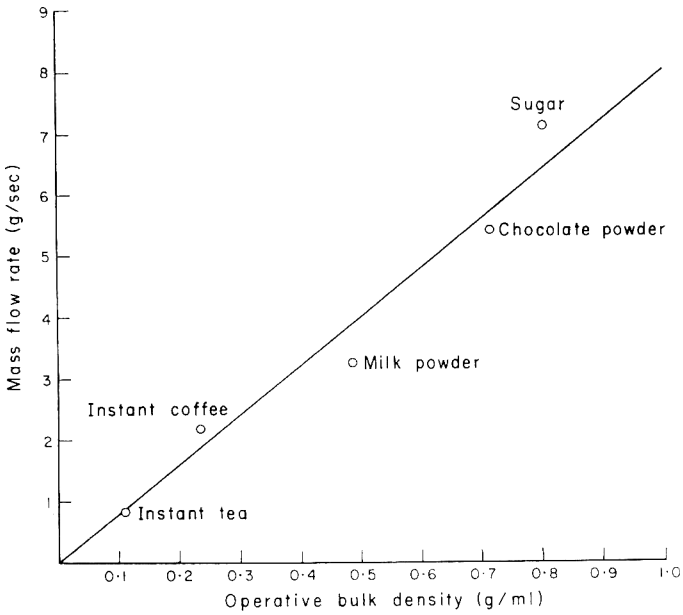

FIG. 3. Dependence of mass flow rate on bulk density.

TABLE 2. Results on powdered food products

Powder	Bulk density (g/ml)	Mean particle size (μ)	Coefficient of friction	Value of β
Granulated sugar	0·838	689	0·596	2·96
Caster sugar	0·764	316	0·680	2·62
Drinking chocolate A	0·728	229	1·072	2·85
Drinking chocolate B	0·721	351	0·982	2·83
Drinking chocolate C	0·692	644	0·654	2·94
Milk powder A	0·549	76	1·150	2·98
Milk powder B	0·537	76	1·158	2·48
Milk powder C	0·379	442	1·200	2·76
Instant coffee A	0·243	247	0·900	2·60
Instant coffee B	0·237	283	0·804	2·70
Instant coffee C	0·224	257	1·032	2·62
Instant tea A	0·147	322	1·060	3·32
Instant tea B	0·100	624	0·856	3·30
Instant tea C	0·087	545	1·032	2·84

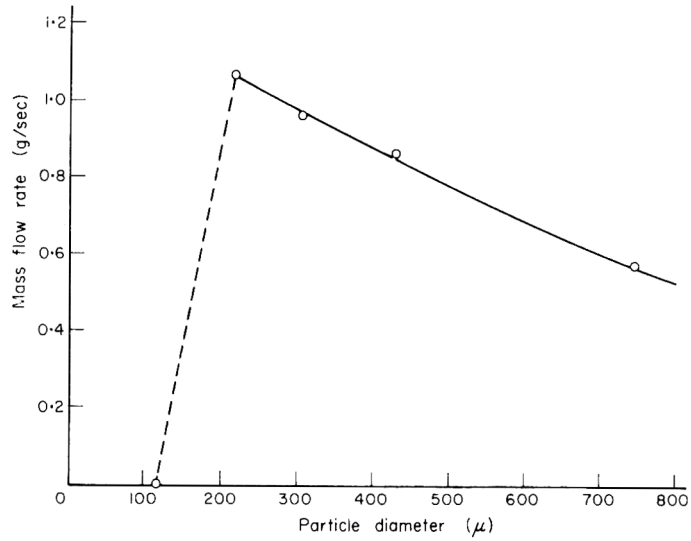

FIG. 4. Dependence of mass flow rate on particle diameter (instant coffee powder, $\frac{1}{4}$ in. diameter orifice).

sieving instant coffee C into five fractions and determining the mass flow rate ($\frac{1}{4}$ in. diameter orifice) and bulk density of each fraction (Fig. 4). The variation in mass flow rate as a function of particle size is largely explainable in terms of the effect of particle size on bulk density; below a certain particle size, the powder will not flow at all. Similar results have been obtained in a larger version of this flow meter, where it was found, with sugars of decreasing particle size, that the mass flow rate increased in the order 'coffee sugar', granulated sugar, caster sugar, while icing sugar would not flow at all.

The effects of moisture content and oil content on powder flow properties are somewhat complex, and still under investigation.

Discussion

It is clear that the flow of a powder from an orifice is not yet properly understood theoretically, or fully explored experimentally. It is generally agreed that the mass flow rate is proportional to bulk density, but the dependence on orifice diameter is nearly always higher than the 2·5th power suggested by theory. No widely applicable equation has yet been derived showing the dependence of flow rate on the coefficient of friction. The effects of particle size, size distribution and shape, of relative humidity and moisture content, of oil content and of temperature need study. The influence of static charges is also important.

The powder flow meter described in the present paper is useful for such studies.

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Shortening as a factor in meat ageing

C. L. DAVEY, HELEN KUTTEL AND K. V. GILBERT

Summary. Shortening during the slow and rapid phases of rigor mortis onset determines largely the extent to which beef ages. For meat stored at 15°C for 3 days, shear-force values are uniformly low at shortenings of 0% to 20% of the freshly excised muscle length. However, there is a four- to five-fold increase in toughness as shortenings proceed from 20% to 40%. This is followed by a decline in toughness as shortenings increase further from 40% to 55%. With increasing shortenings beyond 20%, progressive decreases occur in the extent to which meat ages until at 40% shortening, ageing has declined to zero.

A general study is being undertaken in this laboratory to determine the mechanism of the tenderizing that occurs in meat stored at above freezing temperatures—the so-called ageing of meat. It has been found that the shortening a muscle undergoes during the slow and rapid phases of rigor mortis onset is of particular significance in determining the extent to which meat ages.

In order to obtain the required shortenings relative to the length of the freshly excised muscle (L_0), sternomandibularis muscles removed from a variety of beef carcasses within 30 min of slaughter were held for periods of 0–18 hr at 15°C before being cold shortened at 2°C (Locker & Hagyard, 1963; Marsh & Leet, 1966). In some experiments muscles were clamped in a stretched condition, and held at 15°C for 18–24 hr.

With the onset of rigor mortis both the shortened and stretched muscles became set at the required lengths (L). The degree of shortening (S) is given therefore by the term $1 - (L/L_0)$ which will be negative in value for stretched samples.

For the purpose of this study zero times of ageing for all muscles was considered to be 24 hr post mortem.

Fig. 1, typical of a large number of such experiments, shows the relationship between shear-force value of the cooked meat determined by a tenderometer (Macfarlane & Marer, 1966; Marsh & Leet, 1966) and the time of ageing, for both sternomandibularis muscles of a beef animal at two different degrees of shortening.

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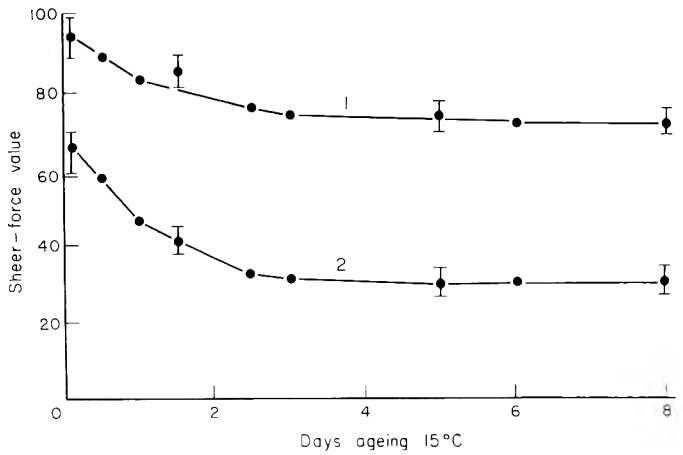

FIG. 1. The effect of the degree of shortening of the sternomandibularis muscles from a 3-year-old steer on the time-course of ageing. At $S = 0{\cdot}25$, curve 1; at $S = 0{\cdot}00$, curve 2. ● , Mean of nine determinations. Vertical lines, standard deviations.

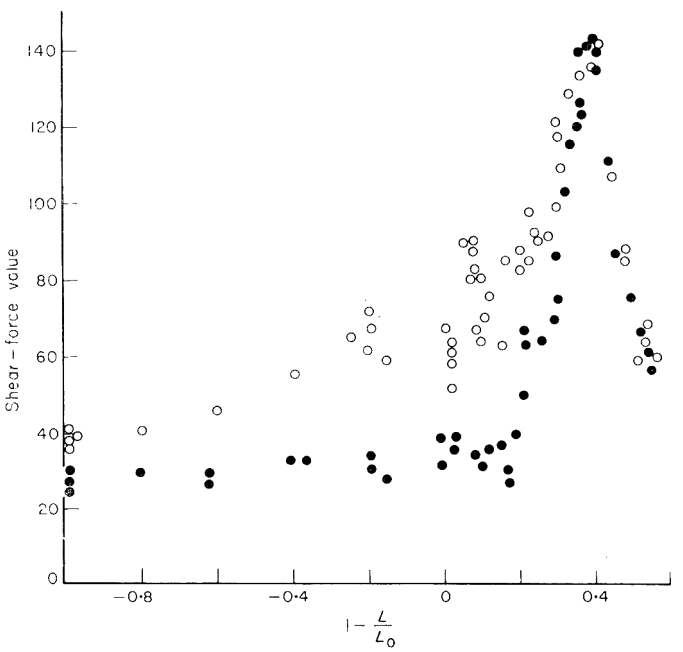

FIG. 2. The effect of degree of shortening on shear-force values of bovine sternomandibularis muscles (ultimate pH values, < 5·90). ○, Mean of five determinations, unaged samples; ●, mean of five determinations, maximum ageing (3 days, 15°C).

The figure shows that meat achieves maximum ageing in 2½–3 days at 15°C and that no further significant changes occur in shear-force values on prolonging this time of storage. However, it is evident that unshortened meat ages to a final shear-force value which is less than that of meat shortened by $S = 0{\cdot}25$.

The effect of ageing maximally (3 days, 15°C) samples of sternomandibularis muscles shortened from $S = -1{\cdot}00$ to $+0{\cdot}55$ is shown in Fig. 2. The relationship between the degree of shortening and shear-force measurements of unaged meat is similar to that obtained by Marsh & Leet (1966), a large peak of toughness occurring at $S = 0{\cdot}40$. Fig. 2 shows that completely aged meat gives a relationship in which a similar peak of toughness at $S = 0{\cdot}40$ appears. At degrees of shortening of $S = -1{\cdot}00$ to $+0{\cdot}20$ approximately, shear-force values are uniformly low (25–40 units); from $S = 0{\cdot}20$ to $0{\cdot}40$ there is a dramatic four- to five-fold increase in shear-force values, whilst from $S = 0{\cdot}40$ to $0{\cdot}55$ an equally dramatic decline in values similar to that obtained for unaged meat occurs. Of particular significance is the finding that with increasing degrees of shortening beyond $S = 0{\cdot}20$, progressive decreases occur in the extent to which meat ages. Indeed, as $S = 0{\cdot}40$ is approached so ageing declines to zero. It appears also that no ageing occurs when values of S exceed $0{\cdot}40$.

Fig. 2 further shows that in the region of shortening from $S = 0{\cdot}00$ to $0{\cdot}20$ approximately, maximum decreases in shear-force units (30–40) are achieved on complete ageing.

Marsh & Leet (1966) have suggested that the peak of toughness ($S = 0{\cdot}40$) may relate in some way to changes within the myofilaments of the muscle cell. Now the sarcomere length of the sternomandibularis muscles from beef animals at L_0 is approximately 2·5 μ (Davey & Gilbert, unpublished data). At peak toughness ($S = 0{\cdot}40$) the sarcomere length should be 1·5 μ, which is the length of the A band of the sarcomere (Locker, 1959). This rather implies that maximum toughness occurs in meat which has so shortened that the I bands of the sarcomere have disappeared allowing the Z lines to abutt the A bands. The dramatic decline in toughness at $S > 0{\cdot}40$ is not so readily explained by changes occurring within the sarcomere. However, the observed relationship between shortening and toughness does call to mind the well-known length–tension relationship for an isolated frog muscle fibre undergoing isometric tetani (Ramsey & Street, 1940; Gordon, Huxley & Julian, 1963). It must be emphasized that progressively less ageing occurs with shortening beyond $S = 0{\cdot}20$ reaching zero ageing at $S = 0{\cdot}40$. The coincidence of the peak of toughness and the onset of zero ageing at this degree of shortening is undoubtedly of much significance to our final understanding of the ageing mechanism.

A full account of this study will be appearing at a later date.

Acknowledgment

The co-operation of the Auckland Farmers' Freezing Co-operative Limited is gratefully acknowledged.

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Structural changes in meat during ageing

C. L. DAVEY AND K. V. GILBERT

Summary. During the ageing of bovine muscle the most notable changes in the pattern of myofibrillar cross-striations are the complete disappearance of the Z lines and the lengthening of the A bands at the expense of the I zones. It is suggested that with the disintegration of the Z lines, the actin filaments of the I zones collapse onto the myosin rods, leading to apparent A-band lengthening. It is concluded that the weakening of the normally refractory Z lines of the myofibrillar structures is an event closely related to meat ageing.

Many histological studies have been made to determine those changes in cell structure that account for the increase in tenderness occurring during the storage of meat at above-freezing temperature – the so-called ageing of meat (Zender *et al.*, 1958; Radouco-Thomas *et al.*, 1959; Sharp, 1963). The most obvious change that has been described is the considerable breakdown in the fibres of the aged meat. This implies no more than that during the preparation of either homogenates or sections for microscopic examination, the more tender, aged, material is prone to disruption. It must be emphasized, therefore, that structural alterations may be observed only in meat that has undergone very appreciable ageing, and then only after special attention has been given to the preparation of samples for microscopic examination. In this respect meat chosen in the present study had not shortened by more than 20% during the onset of rigor mortis (Locker & Hagyard, 1963; Marsh & Leet, 1966). This is an essential requirement, as the extent to which meat ages decreases progressively with increasing shortening beyond 20%. Indeed as 40% shortening is approached, so ageing declines to zero (Davey, Kuttel & Gilbert, 1967). In addition, to minimize the destructive changes wrought by bacterial action, the meat samples were processed under aseptic conditions (Davey & Gilbert, 1966) and at intervals during the storage period sprayed with the antibiotics aureomycin (100 ppm) and chloramphenicol (100 ppm) and dusted with the fungicide nystatin. Bacterial numbers were determined after swab- and rinse-sampling the meat (Ayres, 1960). During ageing aerobic bacterial numbers remained at less than $10^2/cm^2$ of the meat surfaces and were not detected within

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the meat, 2 cm from the surfaces. Anaerobic bacteria were not found, either on the surface or deep within the meat.

Sternomandibularis muscles were removed from a variety of beef carcasses within 30 min of slaughter and held at 15°C. Myofibrils from unaged meat (for the purpose of this study, 24 hr post-mortem) were prepared by homogenizing muscle samples for 2 min in 10 volumes of KCl (0·16 M). With aged material it proved necessary to avoid the excessively disruptive forces of homogenization, so myofibrils were prepared by teasing them from aged meat at least 2 cm from cut surfaces into the solution of KCl.

Plate 1(a) shows a phase contrast photomicrograph of a typical unaged myofibril. Although some ageing will have occurred during the 24-hr period of storage at 15°C needed for the completion of rigor changes with minimal shortening, the classical sarcomere structure of striated voluntary muscle is still clearly seen (Hanson & Huxley, 1957). Dark A bands are shown to alternate with relatively light I zones; the H zones are distinguishable in the central regions of the A bands; and the dense Z lines are clearly shown bisecting the I zones. Considerable departures from this characteristic pattern of cross-striations are observed to have occurred during ageing. Plate 1 (b), (c) and (d), typical of at least 95% of the fields examined, show the phase contrast micrographs of myofibrils prepared from meat (ultimate pH, 5·92) aged for 3 days at 15°C. The two most notable changes are the complete disappearance of the Z lines and the lengthening of the A bands at the expense of the I zones. Although the changes appeared to occur more rapidly in meat of relatively high ultimate pH value, the same changes (Plate 1e) were clearly observed in meat of more typical lower ultimate pH value (pH 5·52). Close examination of the A band regions (Plate 1c) suggests that the fine structure of the unaged myofibrils persists, but that extra protein has accumulated during ageing in the I zones at their junctions with the A bands. This implies that with the disintegration of the Z lines, the actin filaments of the I zones collapse onto the myosin rods of the A bands. It is also shown in Plate 1 (b) and (c) that the structural integrity of the aged myofibrils is still retained, although complete dissolution of the Z lines apparently has occurred. It is possible that either the network of the sarcoplasmic reticulum (Bennett, 1960) or the recently described fibrillen material (Guba, Harsányi & Vajda, 1966) is sufficiently strong to maintain the myofibrillar structure. Certainly almost complete disintegration of the structure into individual A bands occurs on brief homogenization (Plate 1d).

The present results have shown that Z line disintegration occurs in the absence of bacteria which cannot, therefore, be implicated in the reported changes. It is suggested that the changes are due to the meat cathepsins (Landmann, 1963). Certainly the Z-line material is more susceptible to proteolytic attack by trypsin than the other proteins of the myofibril (Ashley *et al.*, 1951; Hama, Maruyama & Noda, 1965). On the other hand, it is probable that active proteolysis of bacterial origin can enhance ageing changes. As such proteolysis would be related to the time-integral of the bacterial population rather than to the numbers of viable organisms present at one time,

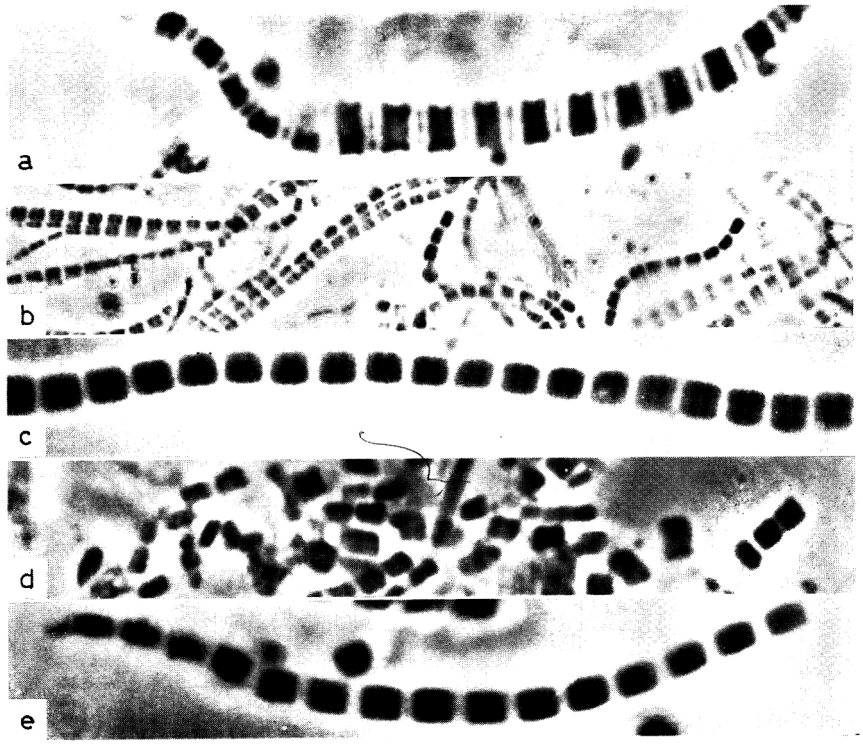

PLATE 1. Phase contrast photomicrographs of myofibrillar preparations from samples of bovine sternomandibularis muscle. (a) Unaged sample (×2100); (b) sample aged 3 days, 15°C (×800); (c) sample aged 3 days, 15°C (×2100); (d) sample aged 3 days, 15°C (×1350); (e) sample aged 3 days, 15°C (×2100).

(*Facing p.* 58)

appreciable enhancement might actually occur in the presence of quite low levels of viable organisms.

An apparent consequence of the changes that have been described is the very considerable loss of tensile strength suffered by meat during ageing. Indeed aged meat (set in rigor mortis in an unshortened condition) can be extended to twice its length when lightly loaded (50–150 g/cm^2). In living muscle, on the other hand, the myofibrils are able to withstand tensions of at least 5 kg/cm^2 (Weber & Portzehl, 1952). For this reason alone it can be assumed that the myofibrils act as a major textural component of meat and thereby offer considerable resistance to transverse shearing forces. It is suggested, therefore, that the weakening of the normally refractory Z lines of the myofibrillar structures is an event closely related to meat ageing.

A full account of this study will be appearing at a later date.

Acknowledgment

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The effect of tylosin lactate on the shelf life of semipreserved herring fillets ('titbits')

INGER ERICHSEN

Summary. The ability of tylosin lactate to increase the keeping quality of canned semi-preserved fish products during storage at 20°C has been investigated, using 'titbits' from two factories as test material. Titbits preserved by the addition of 20 ppm tylosin show no visible gas formation until after 10 and 17 weeks at 20°C for the two factories respectively.

In control samples with no preservatives added, visible gas formation starts after 3 and 4 weeks at 20°C for the two factories respectively.

Addition of 20 ppm tylosin was found to increase storage time at 20°C by 4–10 weeks for the two factories, when compared to samples preserved in the conventional way with a mixture of hexamethylene-tetramine and benzoic acid.

Analysis of the stability of tylosin in the samples during storage indicates that an initial rapid decrease in activity is followed by a stabilization at a level where the tylosin remains active for a considerable period of time.

The results of the storage tests show that visible gas formation may occur when tylosin has decreased to a level of about 1·5–2 ppm in the pickle and 3·5–7 ppm in the fish.

Introduction

The problem of improving the keeping quality of Scandinavian types of semi-preserved fish products by the addition of preservatives has been dealt with by Erichsen, Molin & Teär (1962), Erichsen & Molin (1964), Jörgensen & Bak-Henriksen (1964) and Scheer (1965).

Hexamethylene-tetramine, or a mixture of hexamethylene-tetramine and benzoic acid, are the only preservatives which can prolong shelf-life of Swedish titbits according to Erichsen *et al.* (1962). The highest amount allowed in Sweden for each preservative when added separately to foods is 0·2% benzoic acid or 0·05% hexamethylene-tetramine. When added in combination the sum of the relative amounts of each ingredient must not exceed 100.

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The use of hexamethylene-tetramine has been objected to by public health authorities because of its possible toxic effects. In addition this substance was not recommended by the joint FAO/WHO conference on food additives as a preservative for foods (Anon., 1964). In Germany where hexamethylene-tetramine has been prohibited for use as a preservative in food materials refrigeration is the only method which can be relied upon to increase the keeping quality of semi-preserved fish products.

In the search for a new preservative which could be used as a substitute for hexamethylene-tetramine in preventing gas formation and deterioration of titbits by heterofermentative lactobacilli, tylosin lactate was considered to be of potential value. Tylosin lactate is known to be particularly effective against Gram-positive bacteria, to be stable at high salt concentrations, and to exercise its best effect under acid conditions; accordingly it appeared to fill all the requirements of such a preservative. Tylosin lactate will hereafter be referred to as tylosin.

Material and methods

The samples of titbits used in the experiments were obtained from two different producers. The raw material was in both cases sugar-salted Icelandic herring (*Clupea harengus*). The herring was caught off Iceland during summer and packed on land in barrels with the addition of 14% salt and 6% sugar per 100 kg fish. A brine was thus formed in which the fish was allowed to mature. At the end of the maturing period the salt content of the fish in the brine was 13–14% and the pH of the brine 5·6–6·0.

The fish was then filleted, cut and put into cans at the factories with a pickle containing basically sugar, acetic acid and a little lactic acid. Spices were added according to the recipes of the different factories. After about 1 day an equilibrium was reached between the fish and the added pickle, giving a salt content of 8–9% and a pH of 4·9–5·1 in the cans. The amount of fish relative to the brine was the same in the samples from the two factories. The canning procedure used for commercial manufacture of titbits was described in an earlier report by Erichsen & Molin (1964).

The following experimental series were packed in each factory:

(1) Commercial packs of titbits containing a mixture of benzoic acid and hexamethylene-tetramine in the pickle.

(2) Titbits packed without any preservatives added to serve as controls.

(3) Titbits with 10 ppm tylosin added to the pickle.

(4) Titbits with 20 ppm tylosin added to the pickle.

(5) Titbits with 40 ppm tylosin added to the pickle.

Tylosin was dissolved in the pickle before addition to the cans, the amount to be added being calculated from the net weight of the can.

From each series 230 cans were packed at each of the factories, and the samples were stored at 20°C. An additional ten cans from each series were stored at 6°C during the experimental period.

Samples for tests for residual tylosin at zero time were frozen after holding for 8 hr at room temperature.

Tests for total bacterial count and tylosin residue were performed in all series at zero time, after 1 week, after 1, 2, 3 and 6 months storage at 20°C and after 6 months storage at 6°C.

Samples for bacterial counts were selected from cans with no visible signs of gas formation. Three cans from each series were always examined, and the liquid in each can was sampled. Plate counts for total bacteria were carried out in brain–heart agar (Difco) with the addition of 7% NaCl and 10% sucrose. The sucrose was sterilized by filtration and added to the medium just before plating. The pH of the medium was 6·5. The plates were counted after 5 days incubation at 30°C.

In assaying for tylosin residue, *Sarcina lutea* was used as the assay organism. A standard curve was prepared using the tylosin levels 2, 1·5, 1·0, 0·5 and 0·25 ppm dilutions in the appropriate titbit control.

Plates for the standard curve and for the samples were poured with 6 ml agar seeded with a standardized inoculum of *Sarcina lutea*. After solidification the plates were chilled for some hours before using.

Five stainless steel penicylinders (overall diameter 7·2 mm) were placed on each of eight standard plates and 0·2 ml of each of the five standard curve levels of tylosin was pipetted into a cylinder.

Two plates containing four cylinders each were used for the samples; 0·2 ml sample was added to each of two cylinders and 0·2 ml of the 1·0 ppm/ml standard reference level to each of the additional two cylinders.

All plates were incubated at 30°C for 18 hr, after which time the zones were measured and averaged and the sample values calculated from the standard curve obtained. Tylosin residual tests were performed on the pickle as well as on samples of fish.

Swelling of the cans was measured at intervals by means of micrometer gauge in order to estimate the onset and rate of gas production in the different test series during storage.

Results and discussion

Keeping quality at 20°C

The interval between packing and demonstrable gas production in the cans from the different series is shown in Fig. 1. This may be due to differences in the recipe of the two pickles used, or to differences in types and frequency of the micro-organisms occurring in the factories and in the raw materials used.

The magnitude of the initial infection was of the order of 300–3000 bacteria/ml pickle in samples from both factories.

A comparison of commercially packed samples (hexamethylene-tetramine + benzoic acid) and samples packed with added tylosin clearly showed a considerably better keeping quality of the series packed with tylosin. In commercial packs the first

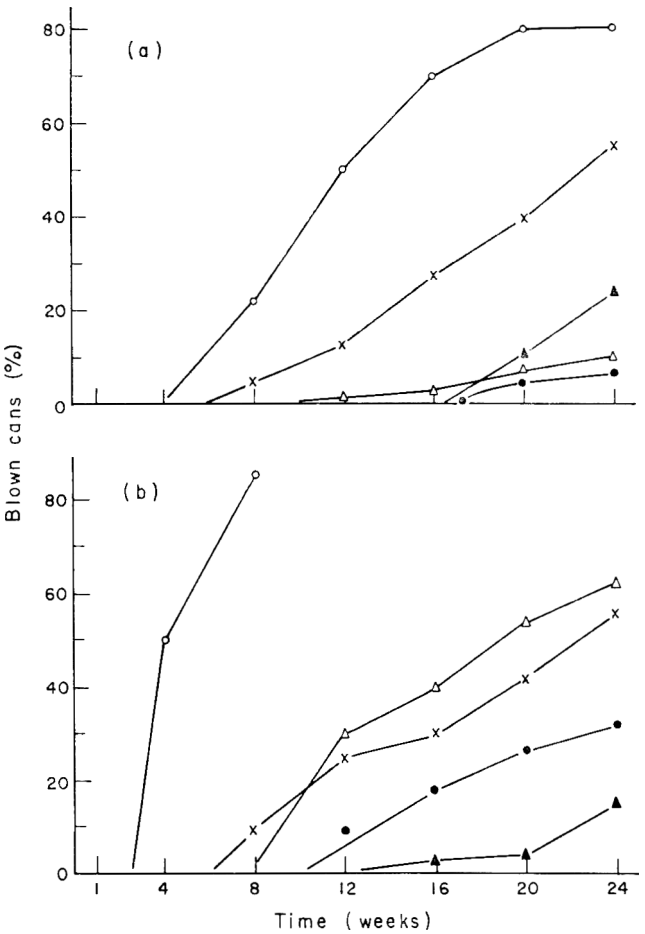

FIG. 1. Gas production in cans of titbits during storage at 20°C. ×, Commercial packs (hexamethylenetetramine + benzoic acid); ○, control packs (without preservatives); △, tylosin packs (10 ppm); ●, tylosin packs (20 ppm); ▲, tylosin packs (40 ppm). (a) Factory A, and (b) Factory B.

sign of gas production was observed after 6 weeks for both factories whereas in packs with 20 ppm tylosin gas formation was detected after 10 and 17 weeks at 20°C for the two factories respectively (Fig. 1).

Residual tylosin after storage at 20°C

The resistance of tylosin to degradation in the pickles with their high salt and sugar content, and with several unknown ingredients, and the minimum concentrations at which it is still capable of exercising an inhibiting activity on the microflora in the

cans under prevailing conditions, are factors of major importance when estimating the value of its application as a preservative for this type of product.

Changes in the biological activity of tylosin in titbits during storage were followed in all series. The results are shown in Table 1 and in Figs. 2 and 3.

TABLE 1. Per cent residual tylosin in samples of titbits stored at 20°C and 6°C

	Tylosin (initial ppm)	Storage time (weeks)						
		20°C						6°C
		0	1	4	8	12	24	24
FACTORY A								
Fish	40	82	48	30	—	18	11	71
	20	75	45	33	—	24	9	95
	10	59	49	41	—	30	10	93
Pickle	40	70	32	15	11	9	4	44
	20	64	32	20	11	11	3	32
	10	77	26	22	12	10	5	38
Factory B								
Fish	40	76	71	32	18	18	9	37
	20	73	72	33	24	17	9	33
	10	59	63	37	—	10	6	47
Pickle	40	53	16	13	11	7	3	28
	20	37	26	16	14	4	3	30
	10	20	22	21	—	10	4	31

A rapid loss of activity during the first weeks of storage was demonstrated, until a level was reached where little further loss took place. The initial loss of activity of tylosin was higher in samples from cans with an initially higher concentration of tylosin. This was, however, compensated for by a higher stable level of biologically active tylosin during the rest of storage.

The activity of the tylosin was invariably higher in the fish than in the pickle. Tylosin was evidently absorbed and concentrated in the fish at an early stage.

Gas formation producing blown cans started when the concentration of active tylosin fell to a level of about 1·5–2 ppm in the pickle. On the other hand, in the fish the tylosin concentration at this point varied between 3·5 and 7 ppm.

Samples stored at 6°C for 24 weeks showed a high percentage residual tylosin, especially those originating from one of the factories, where the fish-meat in some cases contained more than 90% of the amount initially added (Table 1).

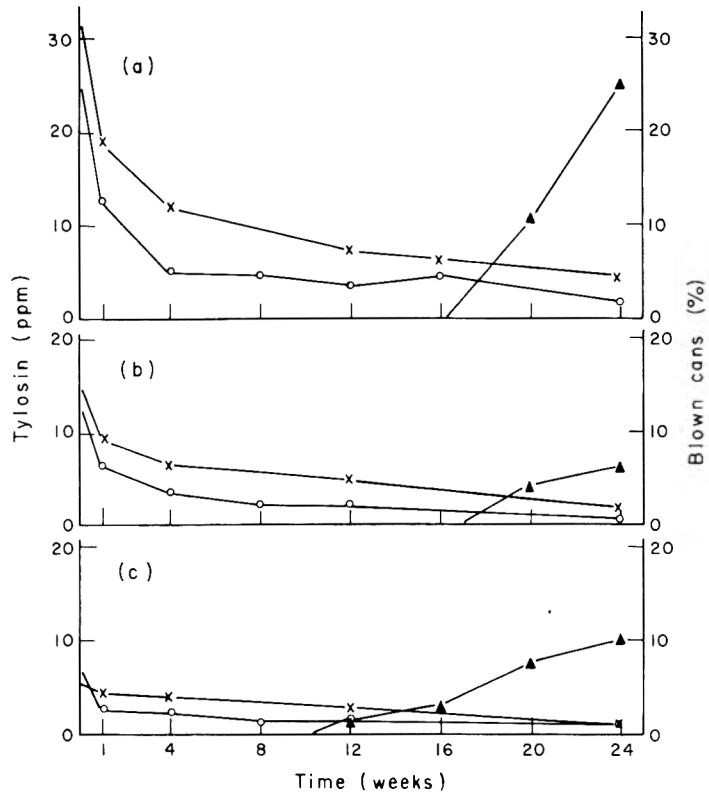

FIG. 2. Residual tylosin (ppm) and swelled cans of titbits (%) during storage at 20°C (Factory A). ×, Tylosin in fish meat; ○, tylosin in pickle; ▲, gas production (%) in cans. Initial concentration of tylosin: (a) 40 ppm, (b) 20 ppm, (c) 10 ppm.

Microflora

The samples for bacterial examination were chosen at random among cans showing no sign of gas formation. Earlier experiments by Erichsen & Molin (1964) have indicated that the number of bacteria has to reach a magnitude of 10^7/ml pickle before visible gas production occurs with the method used. Consequently wider variations in the bacterial count within the individual cans were to be expected at longer storage times.

In all samples examined at zero time the bacteria appearing on the plates were almost entirely of the genus *Bacillus*. During storage, however, this flora was gradually replaced by certain types of Gram-positive, catalase-negative bacteria recognized as *Lactobacillus* spp., which in due time will cause gas formation in the products as described by Meyer (1964) and Erichsen & Molin (1964).

The number of cans, especially in the tylosin series after 6 months storage, which still had a bacterial number of the magnitude found in samples at zero time was

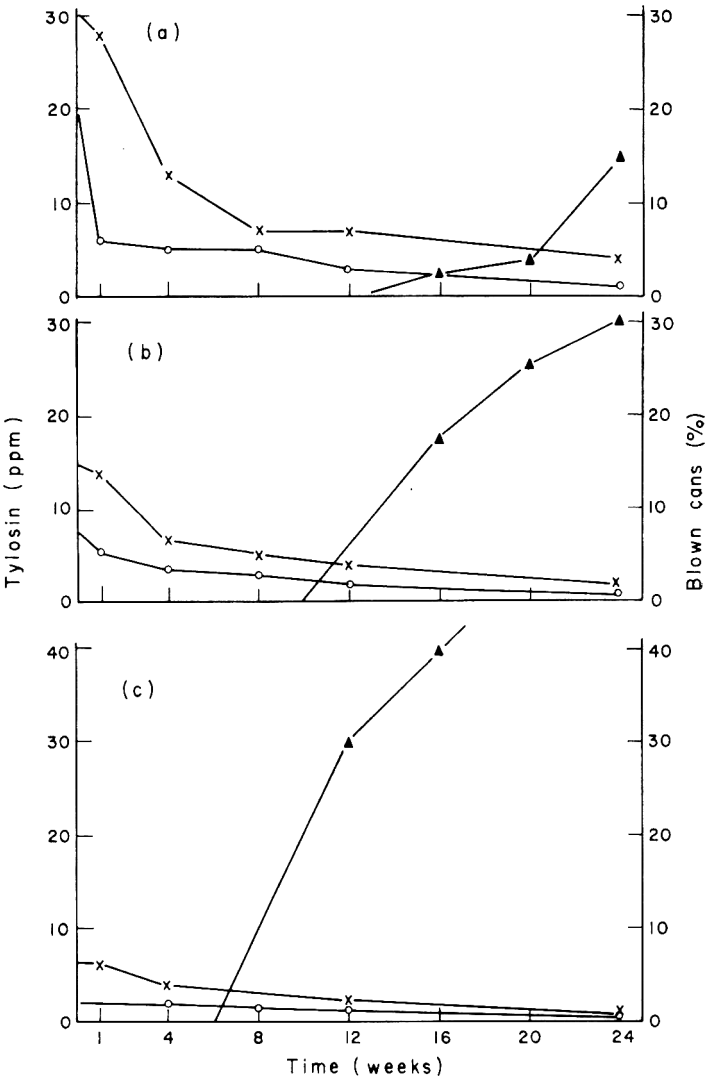

FIG. 3. Residual tylosin (ppm) and swelled cans of titbits (%) during storage at 20°C (Factory B). ×, Tylosin in fish-meat; ○, tylosin in pickle; ▲, gas production (%) in cans. Initial concentration of tylosin: (a) 40 ppm, (b) 20 ppm, (c) 10 ppm.

remarkable. Since the majority of such cans were found in the series with 20 and 40 ppm tylosin added, it may indicate that the tylosin concentrations present effectively inhibited the development of lactobacilli in the cans and that only the pre-existing spores grow out upon plating (Table 2).

TABLE 2. Total bacterial count per ml pickle in titbits

	Storage time (weeks)						
	20°C						6°C
	0	1	4	8	12	24	24
FACTORY A							
Commercial packs	400	800	$1{\cdot}5 \times 10^3$	$4{\cdot}5 \times 10^3$	1×10^6	8×10^5	100
Control packs							
(no preservatives)	250	700	20×10^3	7×10^6	$2{\cdot}5 \times 10^6$	8×10^6	450
10 ppm tylosin	350	150	100	200	10×10^3	2×10^3	300
20 ppm tylosin	450	200	100	100	6×10^3	300	100
40 ppm tylosin	650	150	100	200	800	200	100
FACTORY B							
Commercial packs	3×10^3	150	600	4×10^3	$1{\cdot}5 \times 10^6$	2×10^5	100
Control packs							
(no preservatives)	$1{\cdot}5 \times 10^3$	150	45×10^6*	—	—	—	100
10 ppm tylosin	3×10^3	200	10×10^3	6×10^3	5×10^6	5×10^5	400
20 ppm tylosin	1×10^3	450	2×10^3	1×10^3	800	100	100
40 ppm tylosin	$1{\cdot}5 \times 10^3$	700	200	1×10^3	100	100	100

The total counts are not representative of increase in bacteria number during storage, since samples were taken only from cans showing no sign of swelling.

* All cans in this series were swelled at this stage.

Apparently no bacterial development took place in these cans during storage and where any changes in quality occurred these were most likely of chemical nature. This is confirmed by the fact that the pH in these cans hardly changed during storage, while that in the blown cans decreased to about 4·5 owing to fermentation.

The difference in effectiveness of tylosin in the series from the different factories may also be explained by differences in the types of lactobacilli present in the respective factories. As shown by Malin (1964, personal communication), *Lactobacillus* species vary considerably in their sensitivity to tylosin.

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Preventing denaturation of the proteins in frozen fish muscle and fillets

I. Effects of additives on the quality of frozen minced fish muscle

M. AKIBA, T. MOTOHIRO AND E. TANIKAWA

Summary. In Japan, frozen minced fish muscle has recently tended to be used as the raw material for making Japanese-style fish paste, such as 'Kamaboko', 'Chikuwa', fish sausage and ham. The effect of additives on the quality of frozen minced fish muscle has been studied. Among such, the use of a mixture of sugars (5% by weight) and alkaline polyphosphate (0·5% by weight) was the most effective way to keep high the quality of the frozen raw muscle. In addition to these substances, egg-white (5% by weight) was also effective in preserving the quality. Other high molecular weight substances, such as starches, casein, gluten, carboxymethylcellulose and sodium polyacrylate, showed little effect. Olive oil, shortening oil or lecithin were partially useful as additives accompanying the use of sugars and polyphosphates. Inorganic substances, including potassium bromate, aluminium chloride and sodium chloride, were not effective in improving the quality. A water-leaching procedure prior to the grinding of raw fish muscle always gave a better product than that produced without leaching.

Introduction

Until a few years ago, the raw material for making Japanese-style fish muscle paste, including 'Kamaboko', 'Chikuwa', fish sausage and ham, was the muscle from round fish. Since that time, however, frozen raw fish muscle has tended to be used as the raw material for such products. This is a result of a need to minimize the handling of raw fish and to reduce the number of operations in processing plants.

Frozen raw fish muscle is prepared by the following procedure. Raw fish are beheaded and eviscerated. After washing, the muscle is separated from the bones and skin by means of a meat-separating machine, in which the fish are pressed by means of a plunger. Under pressure the muscle is forced through a perforated iron disc, being separated from the bones and skin which remain behind. The flesh is either soaked in water in a

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tank or rinsed with running water. This has the effect of removing blood, inorganic substances and water-soluble proteins. Excess water is removed, either with a hydraulic press or by means of a centrifugal dehydrator. The partially dehydrated material is crushed with a chopper and ground in a stone grinder, where various additives such as sugars and polyphosphates are added. Finally, the minced muscle is frozen at −25 to −30°C and stored at −20°C.

Denaturation of the protein is an important technical problem in the production of frozen fish muscle, because the protein of fish is easily denaturated by mechanical action or the freezing procedure (Yoshimura *et al.*, 1951; Miyake & Hayashi, 1958; King, 1962). As a means of preventing the denaturation of the proteins in frozen raw fish muscle the leaching of the flesh to remove inorganic substances and water-soluble proteins, and the addition of sodium tripolyphosphate and sodium pyrophosphate (1 : 1) and cane sugar (or glucose or sorbitol) were introduced (Nishiya, 1963). It is now possible to store frozen fish muscle without deterioration for 3 months by using this method.

There are fifty-five frozen fish muscle plants in Hokkaido district, Japan. They produce 25,000 tons of frozen minced fish muscle from 80,000 tons of raw fish per year. The species used are mainly Alaska pollack (*Theragra chalcogramma*), cod (*Gadus macrocephalus*), Atka mackerel (*Pleurogrammus azonus*), squid (*Ommastrephes sloani pacificus*) and flat fishes (*Cleisthenes pinetorum herzensteini*, *Hippoglossoides dubius* and others), which are caught in abundance in the sea near Hokkaido district and in the northern Pacific Ocean.

This paper reports the effect of additives on the quality of frozen fish paste.

Experimental

(1) *Preparation of material*

(a) *Frozen minced product from squid muscle.* The cephalopoditic and fin parts (without mantle cavity), which are by-products from the manufacture of seasoned smoked squid, were soaked in water at 55–65°C for 10 min to remove the skin. They were then leached with solutions of 0·5% hydrogen peroxide and sodium hypochlorite in 10 ppm concentration for 15 min. They were minced and kneaded. Various kinds of additives were mixed with the meat during grinding. The paste was placed in a vessel which contained 7 kg, and covered with polyethylene sheet, frozen at −27°C, and then stored at −18°C. Thirty-seven kinds of samples including the control were prepared by mixing different additives into the paste (Figs. 1 and 2).

(b) *Frozen minced muscle from Alaska pollack.* The Alaska pollack was caught in Funka Bay near Hakodate, Japan, in the winter fishing season. As shown in Figs. 3–5, fifty-three kinds of samples were prepared. The samples which were leached were prepared as follows: After grinding, the raw paste was allowed to stand overnight in 3 parts by volume of water, then centrifuged, after which the water content was 82%. Various

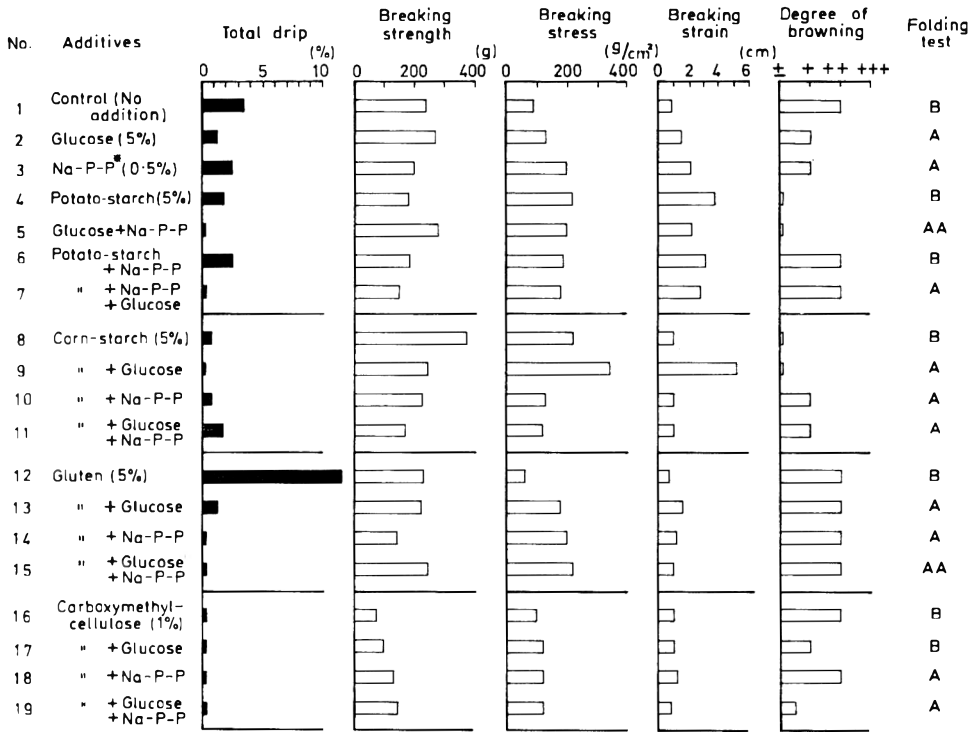

FIG. 1. Results of experiments on frozen squid muscle paste (1) (freezing temperature, −27°C; storage, −18°C; after 90 days).

* A mixture of sodium tripolyphosphate and sodium pyrophosphate (1 : 1).

kinds of additives were mixed with the minced product. Finally, the minced muscle was covered with polyethylene sheet, frozen, and stored at −26°C.

(c) *Frozen muscle from cod.* The samples were divided into two groups. One was leached with water and the other was not. The eleven samples were prepared as shown in Fig. 6. The samples were frozen and stored at −26°C.

(d) *Frozen muscle from Atka mackerel.* Two groups, leached and unleached, were prepared as in section (c). The fifteen samples shown in Fig. 7 were frozen and stored at −26°C.

(2) *Additives used*

Additives used were as follows: (1) high molecular weight substances—potato and corn starches, α-starch, carboxymethylcellulose, raw egg white, gelatin, gluten, sodium-polyacrylate; (2) propylene glycol; (3) fats—plant and shortening oils; and (4) inorganic substances—potassium bromate, aluminium chloride and sodium

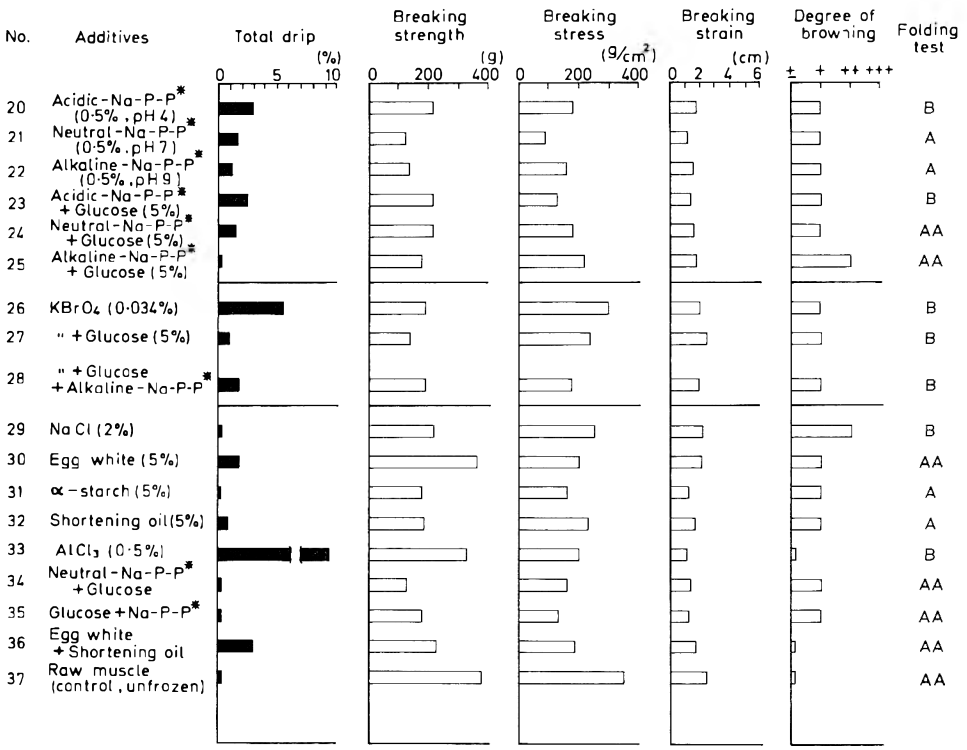

FIG. 2. Results of experiments on frozen squid muscle paste (2) (freezing temperature, −27°C; storage, −18°C; after 90 days).

* A special sodium polyphosphate, named as 'Adecarin'.

† A mixture of sodium tripolyphosphate and sodium pyrophosphate (1 : 1).

chloride. These materials were mixed with raw fish meat paste with or without sugars and polyphosphates. The amount of these materials added to the minced fish muscle are described in Figs. 1–7.

(3) *Assessment of product*

The frozen minced fish muscle after storing was allowed to stand overnight at 10°C for defrosting. The degree of discoloration and the quality of the defrosted paste were estimated organoleptically. The volume of fluid from a portion of the defrosted paste was then measured. In addition to the free fluid which drained out from the paste during defrosting, expressible fluid was obtained by hand pressing (about 0·5 kg/cm²) of the paste. 'Total fluid' was the sum of the free and expressible fluids.

Defrosted pastes which did not already contain them were mixed with sodium chloride (2·7% by weight), and with corn starch (5%). They were then ground for

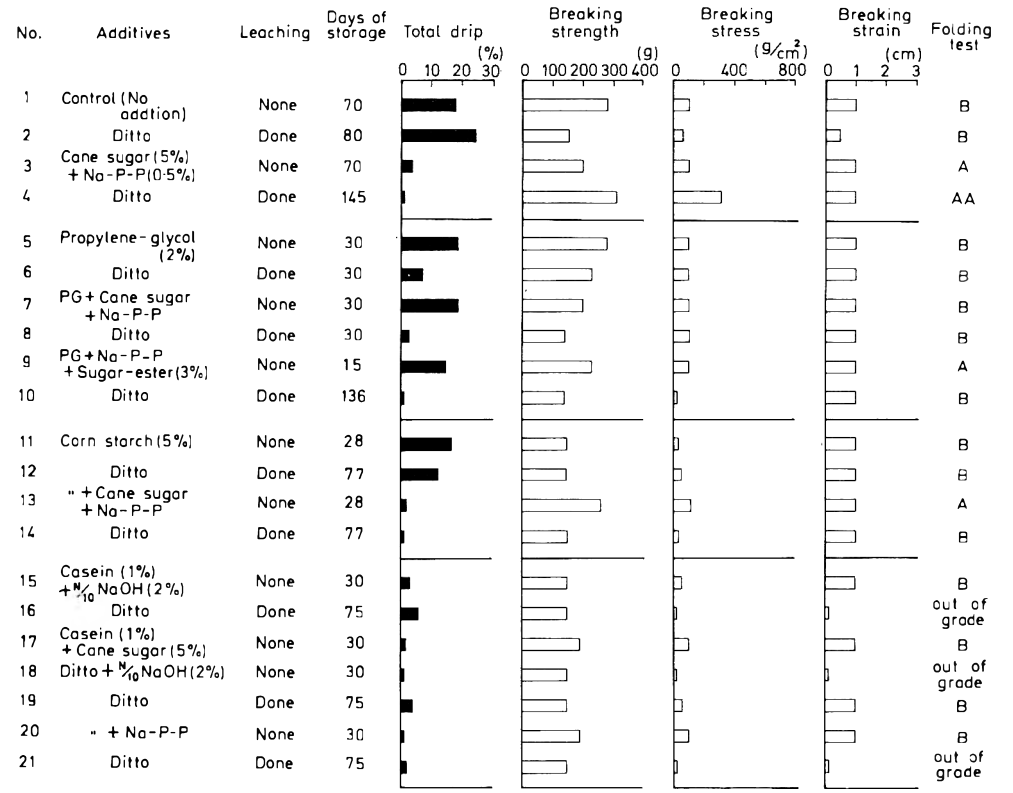

FIG. 3. Results of experiments on frozen Alaska pollack muscle paste (1) (freezing storage at −26°C).

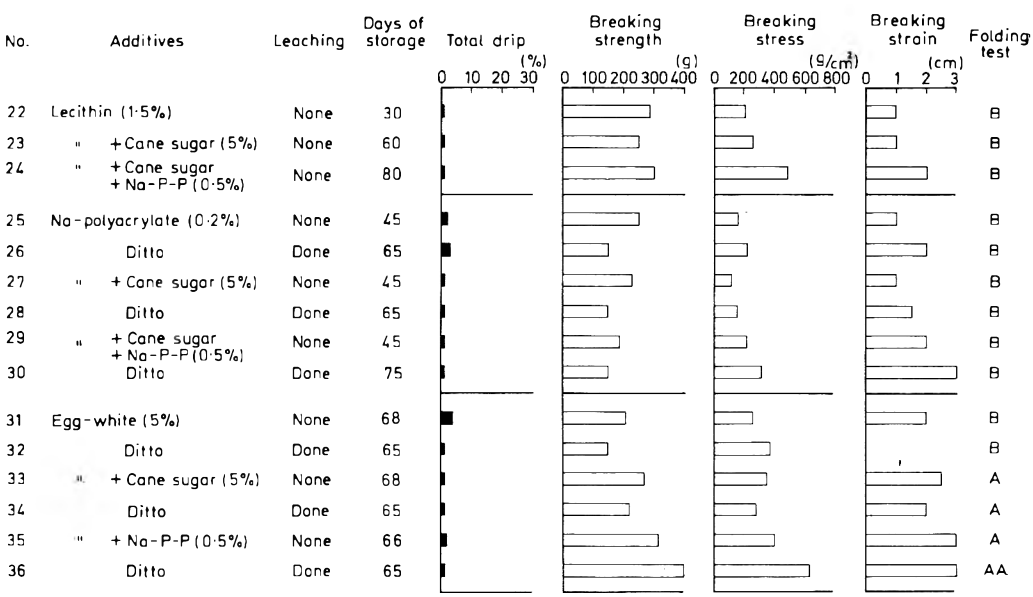

FIG. 4. Results of experiments on frozen Alaska pollack muscle paste (2) (freezing storage at −26°C).

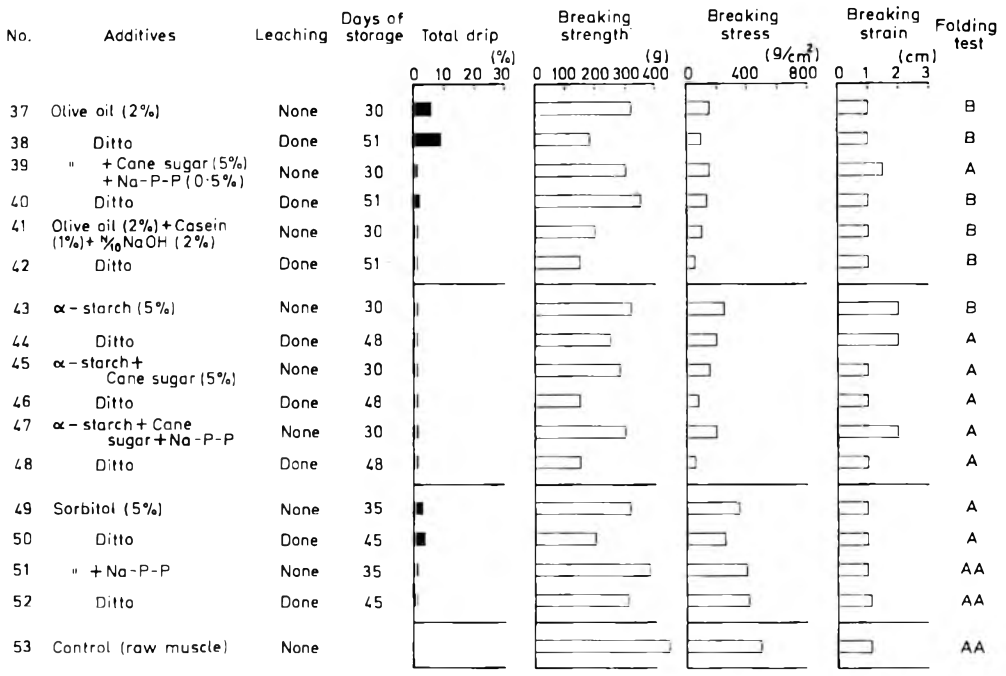

FIG. 5. Results of experiments on frozen Alaska pollack muscle paste (3) (freezing storage at −26°C).

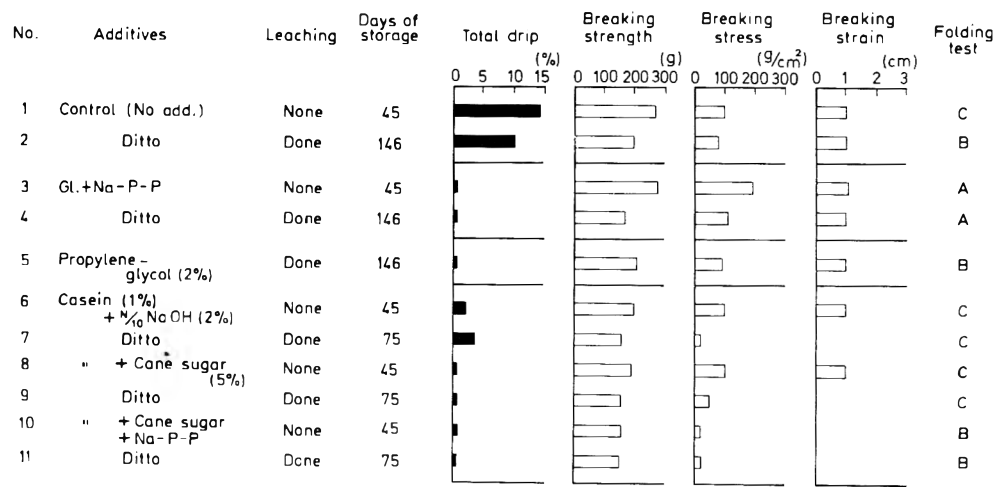

FIG. 6. Results of experiments on frozen cod muscle paste (freezing storage at −26°C).

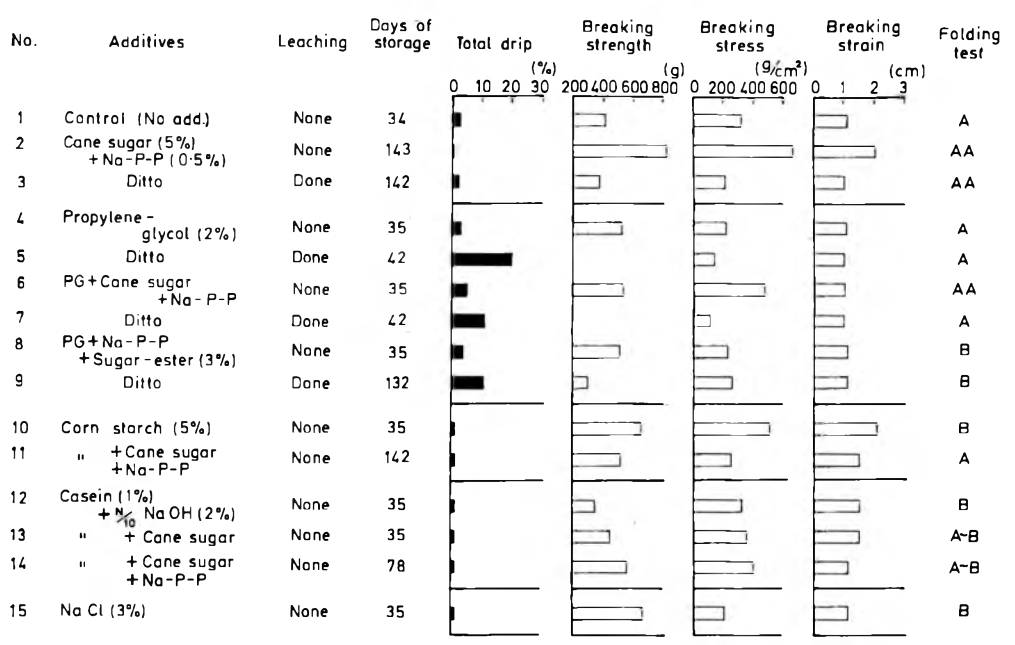

FIG. 7. Results of experiments on frozen Atka mackerel muscle paste (freezing storage at −26°C).

20 min in a stone grinder and their elasticity and viscosity were assessed organoleptically.

After inspection, about 50 g of the paste was placed in a Petri dish, steamed for 30 min at 90°C, and left for one night at room temperature to cool. Breaking strength and penetrometer measurements were then carried out by the methods of Matsumoto & Arai (1952) and Shimizu & Simidu (1953). In addition to the above, the paste was evaluated by the folding method of Nishiya (1963), using the following standards with a 3-mm slice of cooked paste.

AA: Flexible and pliable. Does not show cracks on two foldings.
A: Flexible and pliable. Does not show cracks on folding.
B: Breaks half-way through slice on folding.
C: Breaks into two pieces on folding.
D: Breaks into fragments with finger pressure.

Results

(1) *Frozen squid muscle*

The quality ratings of frozen minced muscle stored for 3 months at −18°C with the various additives are shown in Figs. 1 and 2.

As shown in Fig. 2, even when the raw squid muscle was prepared after skinning in hot water, the quality of the product was excellent. Among samples Nos. 1–36, the

following eight were considered to be acceptable for use as the raw material of fish paste: (1) samples Nos. 5 and 35; (2) sample No. 11; (3) samples Nos. 24, 25 and 34; (4) sample No. 30; and (5) sample No. 36.

There was little change in the qualities of these samples before and after cold storage and the elasticity of the minced fish muscle which was prepared from these samples was acceptable.

Compared with these samples, the control (No. 1) which had been stored for 3 months was of lower quality since it produced a large amount of fluid after defrosting and yielded a lower viscosity on grinding with sodium chloride. On the other hand, the samples which contained additives such as sugars (glucose or cane sugar) and polyphosphates, showed good results. In addition to these additives egg white (5%) or egg white (5%) and shortening oil (2·5%), respectively, were effective in preserving the quality of the frozen raw fish muscle. With respect to the effect of different starches, corn starch (Nos. 8–11) was better than potato starch (Nos. 4, 6 and 7). Apart from starches, other high molecular substances have shown little effect. Concerning polyphosphates, neutral and alkaline polyphosphates (pH 7·0 and 9·0) were more effective than acidic polyphosphate (pH 4·0). Potassium bromate, which as an additive increased the elasticity in the raw material for raw minced fish muscle (Okada & Nakayama, 1961), was not effective in improving the quality of frozen product. Sample No. 33 containing aluminium chloride, which is used for soy-bean paste to prevent discoloration, kept its original colour during frozen storage, though the protein was greatly denatured and gave rise to a large quantity of drip. Thus, the quality of the sample as a raw material for fish paste was inferior to that of the other samples.

(2) *Frozen raw Alaska pollack minced muscle*

The following groups among the samples shown in Figs. 3–5 were usable as raw materials for minced fish muscle.

(a) Cane sugar (5%) and sodium polyphosphate (0·5%) (Samples Nos. 3 and 4).

(b) Egg white (5%), cane sugar (5%) and sodium polyphosphate (0·5%) (Samples Nos. 35 and 36).

(c) Sorbitol (5%) and sodium polyphosphate (0·5%) (Samples Nos. 51 and 52).

Next to these samples described above, the following substances can also be used to improve the quality of this product.

(d) Egg white (5%) and cane sugar (5%) (Sample No. 34).

(e) α-Starch (5%), cane sugar (5%) and sodium polyphosphate (0·5%) (Samples Nos. 47 and 48).

(f) Sorbitol (5%) (Samples Nos. 49 and 50).

Consequently, with minced squid muscle, the quality of minced Alaska pollack muscle was improved by the addition of sugar (cane sugar and/or sorbitol) and sodium polyphosphate. In addition to these materials, α-starch and egg white are also effective in improving the quality of minced Alaska pollack muscle.

Comparing the quality of the product produced with and without the water-leaching procedure, the former was always better than the latter. Thus, the leaching procedure is desirable in the preparation of frozen minced fish muscle.

The addition of such materials a propylene glycol (Nos. 5–10), corn starch (Nos. 11–14), casein (Nos. 15–21), lecithin (Nos. 22–24), sodium polyacrylate (Nos. 25–30) and olive oil (Nos. 37–42) to meat paste, did not improve the quality.

(3) *Frozen raw cod muscle*

The samples (Nos. 3 and 4) which contained glucose and sodium polyphosphate showed a good quality during cold storage among the eleven samples tested (Fig. 6), while the others which contained propylene glycol and casein did not.

(4) *Frozen raw Atka mackerel muscle*

In general the frozen paste of raw Atka mackerel is better in its elasticity than that of squid, Alaska pollack and cod. The samples (Nos. 2 and 3) which contained cane sugar and sodium polyphosphate showed better quality than those of others containing propylene glycol, corn starch and casein.

Discussion

It is probable from the results of Kitabayashi (1954) and Miyake & Hayashi (1956) that the proportion of actomyosin extractable in NaCl is related to the elasticity of the fish paste products. Dyer (1951), Simidu & Simidu (1957) and Yoshimura *et al.* (1951) have found that the fibrous proteins ('actomyosin') are denaturated during cold storage, while the sarcoplasmic proteins are not affected. The present experiment was also carried out on the assumption that frozen fish muscle in which the 'myosins' had become denatured would no longer be usable as the raw material for fish paste products. However, the measurement of protein denaturation during cold storage seems to be somewhat inadequate in this work. Further studies, including measurement of the salt-extractability of the protein, would probably be useful. In the present method for preparing frozen raw fish muscle it is necessary to remove inorganic materials which may cause denaturation of meat proteins by salting out effect during freezing storage. For this purpose, the present method includes a leaching procedure. It was found that the addition of sugars including cane sugar, glucose and sorbitol and polyphosphates improved the keeping quality of frozen fish muscle. This result was in accord with the finding of Nishiya (1963). Besides the above, egg white was also effective for this purpose. It was pointed out by Oshiro (1959) that egg white was effective in improving the elasticity of raw fish paste products, and that it was especially of use with raw fish meat paste of low elasticity. The effect of egg white in preventing the denaturation of 'myosins' in frozen fish muscle during cold storage should be studied. Of all the additives tested, not only egg white but corn-starch, α-starch, and shortening

oil are also beneficial. Since, however, sugars and polyphosphates need to be used at the same time, they might be uneconomical.

Acknowledgments

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Volatile carbonyls in cooked chicken

C. H. LEA AND A. HOBSON-FROHOCK

Summary. Colorimetric determination of volatile carbonylic substances in low temperature vacuum distillates from roast chicken showed marked differences for small variations in treatment.

Introduction

Gas chromatographic examination of distillates or extracts from cooked chicken has proved valuable for the identification of individual compounds, many of them carbonyls (Minor *et al.*, 1965; Nonaka & Pippen, 1966) which might contribute to aroma, but it is difficult to make the techniques of extraction, concentration, application to the column and analysis quantitative at the very low levels required and much less is usually known about the amounts in which the substances identified were originally present in the food. Chemical determination of volatile carbonyls as a class was therefore undertaken, as a prelude to a more detailed examination by gas liquid chromatography, to ascertain whether significant differences arising from small changes in treatment would be likely to be detectable.

The method used to remove the volatile constituents from cooked chicken was based on low temperature (35°C) distillation of all the water from the freeze-powdered meat, with condensation on to a liquid nitrogen-cooled cold finger condenser (Hobson-Frohock & Lea, 1965): procedures involving stronger heating can give much too high results. Since low and higher molecular weight carbonyls might to some extent have different origins, e.g. from browning-type and lipid autoxidation reactions, carbonyl determinations were made not only on the aqueous distillate but also on the contents of the distillate after partitioning between cyclohexane and water.

Variables investigated were: (a) type of meat, i.e. light (breast) or dark (leg); (b) roasting with or without a covering of aluminium foil; and (c) storage of the cooked meat in a domestic refrigerator for 2 days at 5°C.

Methods

Partitioning of the aqueous distillate

Recovery experiments using a mixture of acetaldehyde and 2-hexenal, with

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determination by direct measurement of absorption at 280 and 225 mμ respectively, showed that two extractions of the aqueous distillate with an equal volume of cyclohexane and centrifugal separation gave aqueous and organic phases containing respectively most of the low and higher molecular weight constituents.

Determination of the carbonyls

(a) *In aqueous solution.* The method of Gaddis & Ellis (1963) gave results that could be too high, owing to incomplete removal of excess reagent, or too low owing to variable losses of mainly lower molecular weight carbonyls. A modification of the methods of Lappin & Clark (1951) and Mendelowitz & Riley (1953) was therefore adopted for determination of the total carbonyl content of the whole distillate and of its aqueous fraction after partition.

To 10 ml distillate add 1 ml 0·06% 2,4-dinitrophenylhydrazine hydrochloride in ethanol containing 1 ml concentrated HCl/100 ml. Heat in a water bath at 60°C for 30 min and cool. Add 5 ml 10% KOH in aqueous ethanol (10 g KOH in 20 ml water, diluted to 100 ml with aldehyde-free ethanol), make up to 25 ml with ethanol and read absorption at 430 mμ after the required time of standing, against a blank prepared from 10 ml water and the reagents. Calibration curves with acetaldehyde and 2-hexenal both obeyed Beer's law but their absorption maxima were different (430 and 455 mμ).

A difficulty in determining saturated aldehydes of low molecular weight by the 2,4-DNPH method is the rapidity with which the colour produced fades in alkaline solution (Monty, 1958). Whereas 2-hexenal by this method gave an ε_{430} value of 19,000, virtually unaffected by standing, values for acetaldehyde were 16,000, 15,000 and 11,000 after '0', 2 and 15 min. A reaction time of 2 min was adopted for both aqueous solutions, with an ε_{430} of 15,000 for the aqueous fraction after partition and a higher (approximate) value of 17,000 for the whole distillate, to allow for the presence of longer chain carbonyls.

(b) *In cyclohexane solution.* For determination of carbonyls in the cyclohexane extracts the 2,4-DNPHs were prepared according to the method of Henick, Benca & Mitchell (1954, 1956) and the solution then chromatographed on an alumina column, with elution of the monocarbonyls by benzene for colorimetric determination (Pool & Klose, 1951). At 430 mμ 2-hexenal gave $\varepsilon = 22{,}100$ and n-hexanal $\varepsilon = 20{,}800$: a value of 22,000 was adopted for calculating the mixed carbonyl content of the cyclohexane solution.

Results

The lipid contents of the light and dark meats used were approximately 4% and 9% respectively, and their pH 6·2 and 6·7. The cooked dark meat contained 1–2% less moisture than the light, and the foil-cooked meat 3–4% more moisture than meat cooked without foil.

Effects of variation in roasting and of storage after cooking

The foil-cooked (3 lb) birds were roasted in an oven at 365°F for 2 hr, and then for a further 15 min without foil. The birds cooked without foil throughout were roasted for 2 hr at 375°F.

Cooking without foil considerably increased the amounts of volatile carbonyls (both water-soluble and lipid-soluble) present in the meat (Table 1), and storage in a domestic refrigerator at 5°C for 2 days produced an even greater increase, particularly in the lipid-soluble fraction. The two methods of carbonyl assay gave parallel results, the sum of the two fractions in the partition method averaging 87% of the direct determination on the same distillate: the difference can be attributed in part to retention of dicarbonyls on the alumina column. The concentrations of carbonyls found (μmoles/100 g ≏ ppm) are well above the flavour thresholds for these substances (Lea & Swoboda, 1958). Distillable ammonia also tended to increase in the same way as the carbonyls, but more so in the light than in the dark meat (Table 1).

TABLE 1. Effect of conditions of cooking and of storage on the volatiles* in roast chicken

Meat	Cooking	Storage at 5°C	Carbonyl content				Ammonia
			Total (direct)	Partition method			
				Total	Water-soluble	Lipid-soluble	
Light (breast)	Foil	—	2·3	2·2	1·8	0·4	54
	No foil	—	7·0	6·3	5·1	1·2	84
	Foil	2 days	12·4	9·9	5·7	4·2	84
	No foil	2 days	11·6	10·1	7·0	3·1	133
Dark (leg)	Foil	—	2·2	1·8	1·2	0·6	47
	No foil	—	4·6	4·6	3·4	1·2	61
	Foil	2 days	9·5	7·7	4·2	3·5	48
	No foil	2 days	9·5	8·6	5·5	3·1	65

* All as μmoles/100 g meat.

It would appear that detectable chemical differences that might be correlatable with flavour can result from quite small variations in the treatment of roast chicken.

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New synthetic antioxidants based on caffeic acid

B. H. THEWLIS

Summary. Experimental details are given for the preparation of the synthetic antioxidants 1-caffeyl glycerol, 1-palmityl 2,3-dicaffeyl glycerol, and 1,2,3-tricaffeyl glycerol.

Introduction

Arising out of the work of Martin and Daniels in these laboratories on the isolation from oats of a new family of antioxidants based on caffeic and ferulic acids, King (1962) described briefly the preparation of three new glycerides, 1-caffeyl glycerol, 1-palmityl 2,3-dicaffeyl glycerol and 1,2,3-tricaffeyl glycerol, all of which were effective as antioxidant additives. Attempts by the present author to repeat this work by following the details given in the paper were at first disappointing, the product being in each case a mixture of derivatives which gave a complex pattern of spots on thin layer chromatography. However, the necessary conditions were eventually defined more precisely, and these are given in detail below together with fuller analytical data on the three glycerides.

Materials and methods

3,4-*Dicarbomethoxycaffeic acid.* Caffeic acid (L. Light & Co. Ltd) was recrystallized from water and reacted with methyl chloroformate by the method of Fischer & Oetker (1913)

3,4-*Dicarbomethoxycaffeyl chloride.* 3,4-Dicarbomethoxycaffeic acid (14 g) and dry chloroform (100 ml) were placed in a 500-ml flask fitted with tap funnel and drying tube, and maintained at 50°C. Pure freshly distilled thionyl chloride (20 ml) was added slowly with occasional shaking, when the acid went gradually into solution. When the liquid was quite clear the solvent was taken off under reduced pressure and the residue dissolved in dry carbon tetrachloride (160 ml), boiled with charcoal and filtered. On slow cooling 3,4-dicarbomethoxycaffeyl chloride was obtained as large clumps of glistening needles, melting point 111–112°C, yield 12 g.

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1,2-*Isopropylidene glycerol.* This was made by the method of Newman & Renoll (1948).

Quinoline. This was distilled at ordinary pressure, the clear colourless fraction being collected.

Chloroform. This was washed six times with water to remove alcohol (stabilizer), and dried.

1-*Monopalmitin.* Freshly distilled thionyl chloride (40 ml) was placed in a 250-ml flask and brought to boil under reflux. Pure palmitic acid (28 g) was melted, and run into the flask slowly over ½ hr through a tap funnel. The mixture was boiled under reflux for a further hour, and excess thionyl chloride removed with the aid of a water pump. The dark residue was then distilled, the fraction boiling at 142°C at 0·04 mm being collected and re-distilled. Palmityl chloride was obtained as a colourless oil of refractive index $n_D^{25} = 1{\cdot}4505$.

Palmityl chloride (12·8 g) was added slowly to a mixture of isopropylidene glycerol (8·7 ml), quinoline (10 ml) and chloroform (50 ml); the whole was shaken and maintained at room temperature for 2 days in a stoppered flask. The reaction mixture was then treated with ether (400 ml) and shaken twice with 0·5 N sulphuric acid (100 ml), with dilute sodium bicarbonate solution and finally with water until neutral. The liquid was dried over sodium sulphate and evaporated under reduced pressure until solid began to deposit. Ether (50 ml) and chloroform (5 ml) were added, and the solution cooled to 3°C in the ice bath. Cold concentrated hydrochloric acid (50 ml) was added, and the mixture stood for 30 min. Water (400 ml) was added, and the thick white precipitate was taken up in chloroform (200 ml), washed with water till neutral, dried over sodium sulphate and evaporated until crystals were obtained on cooling. The monopalmitin had a melting point of 76·5°C, yield 7 g. By evaporation of the mother-liquor a further yield of 3·25 g, melting point 75–76°C, was obtained.

1-*Caffeyl-glycerol.* Quinoline (4 ml) and chloroform (20 ml) were mixed with isopropylidene glycerol (3·48 ml, chosen as the excess reagent because of its solubility in water and consequent ease of removal). Dicarbomethoxy caffeyl chloride (5·84 g, 0·668 times theoretical quantity) was added, and the whole shaken in a stoppered flask till solution was complete. It was then allowed to stand for 3 days at room temperature. It was treated with ether (160 ml) and shaken with 0·5 N sulphuric acid (40 ml) to remove the quinoline, then with water till neutral, dried over sodium sulphate and evaporated down to about 20 ml. The liquid was then cooled to 0°C, treated with cold concentrated hydrochloric acid (20 ml), and maintained at 0°C for 30 min. Cold water (400 ml) was then added, and the colourless precipitate filtered off and washed with water till the washings were neutral. The product appeared to be a hydrated derivative of 1-(3,4-dicarbomethoxy caffeyl) glycerol; on intensive drying it reverted to an oil, which became a crystalline solid once more on treatment with water. The melting point of the substance was rather indefinite, ranging from 105 to 120°C, according to the amount of moisture present.

The filtrate from the above process was extracted twice with chloroform, and the extract was shaken with water till neutral, dried, and evaporated, whereby a small further yield of 1-(3,4-dicarbomethoxy caffeyl) glycerol was obtained, and combined with the main yield. This was dissolved in acetone (144 ml) and treated with N sodium hydroxide solution (148 ml) in an atmosphere of nitrogen; the whole was stirred for 3 hr at room temperature. The liquid was then brought to pH 2 with 5 N sulphuric acid, and subjected to continuous ether extraction for 6 hr. The light fawn coloured solid deposited in the boiling flask was removed and recrystallized from the minimum quantity of hot water (about 5 ml) to give 1-caffeyl glycerol, melting point 163–164°C, yield 1 g, 11·5% theoretical based on caffeic acid taken. Found: C, 56·49; H, 5·62. $C_{12}H_{14}O_6$ requires C, 56·70; H, 5·51.

1-*Palmityl* 2,3-*dicaffeyl glycerol.* 1-Monopalmitin (1·4 g), chloroform (24 ml) and quinoline (2·5 ml) were mixed and treated with 3,4-dicarbomethoxycaffeyl chloride (5·5 g, twice theoretical quantity; it was shown that this was the minimum excess necessary to achieve complete substitution). The mixture was held at 40°C for 4 days. Ether (100 ml) and 0·5 N sulphuric acid (25 ml) were added; on shaking, yellow-orange gummy material separated slowly. (This was probably derived from the excess dicarbomethoxy caffeyl chloride; on working up, the only product that could be isolated was caffeic acid.) The gum was re-extracted with ether (100 ml) and the combined ether extract shaken with 0·5 N sulphuric acid to remove quinoline, then with water until neutral, dried with sodium sulphate and evaporated. The residual oil was dissolved in acetone (72 ml) and placed in a 500-ml flask through which nitrogen was then passed. Sodium hydroxide solution (1·5 N, 36 ml) was added (it was shown that this was the minimum concentration necessary for effecting complete removal of the protecting groups), and the liquid was stirred under nitrogen for 3 hr at room temperature. The mixture was acidified with 0·5 N sulphuric acid (120 ml) and extracted with ether (200 ml). The aqueous layer was re-extracted with ether, and the combined extract washed with water till neutral, dried and evaporated (on taking off final traces of ether the residue was liable to spurt suddenly and violently, with consequent loss of material, unless precautions were taken). The residue was taken up in acetone (20 ml) and water added until precipitation of solid was complete and a red oil began to come down. The solid was filtered off and recrystallized from acetone–water to give 1-palmityl, 2,3-dicaffeyl glycerol, melting point 119–120°C, yield 0·3 g, 2·8% theoretical based on caffeic acid taken. Found: C, 68·34; H, 8·91. $C_{37}H_{50}O_{10}$ requires C, 67·89; H, 7·65.

The above red oil was investigated further; however, the only products which could be isolated from it were caffeic acid and palmitin.

1,2,3-*Tricaffeyl glycerol.* Anhydrous glycerol (0·12 g) was dissolved in warm chloroform (60 ml) and quinoline (1 ml) was added. 3,4-Dicarbomethoxy caffeyl chloride (2·5 g, twice theoretical quantity) was added, and the mixture was boiled under reflux for 3 hr, (it was shown that reaction at lower temperatures gave only unchanged

starting material). On cooling, the liquid was shaken with 0·5 N sulphuric acid, then with freshly made sodium bicarbonate solution, then with water until neutral, after which it was dried and evaporated to about 10 ml and treated with ether until an oily deposit was obtained, which solidified on standing for several days. Recrystallization from chloroform afforded 1,2,3-tri (dicarbomethoxy caffeyl) glycerol as a colourless solid, melting point 156–160°C. Found: C, 54·88; H, 4·95. $C_{42}H_{38}O_{24}$ requires C, 54·43; H, 4·10; yield 0·62 g.

1,2,3-Tri (dicarbomethoxy caffeyl) glycerol (0·5 g) was dissolved in acetone (16 ml) and the liquid stirred under an atmosphere of nitrogen at room temperature. Sodium hydroxide solution (1 N, 16 ml) was added, and the whole stirred for 1 hr. The mixture was brought to pH 6 and extracted twice with chloroform, the extract being then shaken with water until neutral, dried and evaporated. The residue was twice recrystallized from chloroform to give 1,2,3-tricaffeyl glycerol, melting point 138–140°C, yield 20 mg, 0·71% theoretical based on caffeic acid taken. Found: C, 61·78; H, 6·27. $C_{30}H_{26}O_{12}$ requires C, 62·27; H, 4·53.

The antioxidant activity of the substances was measured in arbitrary units by the method of Daniels & Martin (1961) using 1 mg of caffeyl glyceride per 100 mg of oat oil substrate. Results were as follows, with methyl caffeate and the established antioxidant propylgallate included for comparison.

Antioxidant	*Activity*
1-Caffeyl glycerol	66·5
1-Palmityl 2,3-dicaffeyl glycerol	22·7
1,2,3-Tricaffeyl glycerol	51·0
Methyl caffeate	55·0
Propyl gallate	50–60

Discussion

The use of the carbomethoxy group as a protecting agent for phenolic acids is well established; however, alternative techniques have been suggested. The latest of these is probably that of Panizzi, Scarpati & Scarpati (1954) in which caffeic acid is reacted with phosgene to introduce a carbonyl protecting group. Carbonyl caffeic acid was accordingly made and converted into its acid chloride; this was reacted with isopropylidene glycerol as above. A complex mixture of products was obtained by this method and the process was not examined further. The Dean–Stark esterification procedure was also tried, but without success. The condensation procedure of Cook & Showler (1965) using trifluoroacetic anhydride was attempted, but did not give the desired product.

3,4-Dicarbomethoxy caffeyl chloride reacted very rapidly with atmospheric moisture, and it was for this reason that it was advantageous to have it in the form of large clumps of crystals, with minimal surface area. All weighing and transferring operations

had to be carried out with the utmost speed; moreover, even when kept in a vacuum desiccator the substance was found to undergo change, so that after about 4 days it would no longer react properly with glycerol derivatives, although its melting point remained unchanged. It was found best to prepare the substance and use it on the same day.

Other workers in the field of synthetic glycerides appear to have found that approximately theoretical proportions of reactants could be used; however, in condensations using 3,4-dicarbomethoxy caffeyl chloride it has now been found necessary to use a considerable excess of one or other of the reactants, otherwise the product was an intractable mixture of partially substituted derivatives, as seen by its failure to solidify and by its complex pattern of spots on a silica thin-layer chromatogram, after development with chloroform or ether. The described reaction conditions were arrived at after numerous others had been tried, such as variation of reaction time and temperature, yielding in most cases a mixture of products.

There is no reason to believe that any of these antioxidants will be in any way toxic, but it is planned to carry out trials to check this.

Acknowledgment

The author is indebted to Dr D. G. H. Daniels for helpful discussions.

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J. Fd Technol. (1967) **2**, 89–94.

Low temperature and the storage of carrots

W. HUGH SMITH

Summary. The long-term storage of carrots grown in the U.K. at 32°F resulted in more fungal rotting than at 34°F. It is suggested that this is due to a form of low temperature injury. At temperatures higher than 34°F rotting developed more rapidly and there was increased growth of shoots and rootlets. For storage up to 2 months temperatures in the range 32–37°F are not critical. For longer periods of storage 34°F would appear to be the optimum temperature.

Introduction

In the U.K. the bulk of the carrot crop is now marketed as it is dug. In exceptionally severe winters like that of 1963–64 this can lead to very heavy losses from freezing. Unlike the potato, the carrot does not lend itself to long-term storage in non-refrigerated buildings. Consequently there is a tendency, in spite of the high cost, to turn to refrigerated storage for holding a buffer stock in good condition for use in late winter and early spring.

The freezing point of the carrot is 29·5°F (ASHRAE, 1964). However, a form of freezing injury has been observed in carrots kept at 31°F. Ice may form on the surface of the root at this temperature with the occurrence of a blister-like lesion. For practical purposes a temperature of 32°F would seem to be the lowest feasible and indeed in the U.S.A. this temperature is recommended for the storage of carrots (Wright, Rose & Whiteman, 1954).

In the course of experiments carried out during the past few years some doubt has been cast upon the validity of this procedure for roots grown in the U.K. In the long term more rotting has been observed at 32°F than at somewhat higher temperature. The evidence for this is presented here.

Materials and methods

Carrots from a number of different sources have been stored in different years over a range of temperatures in constant temperature chambers. In order to maintain a

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high humidity and prevent wilting of the roots the carrots, on either trays or in boxes, were enclosed in a 'tent' constructed of heavy duty polythene film and sufficient forced ventilation was maintained to prevent accumulation of respiratory carbon dioxide above 0·5%.

In a typical experiment samples consisting of at least 100 roots were removed at intervals from storage, examined and subsequently kept under examination at 50°F. Observations were made of the amount of rotting of roots and also the amount of shoot and root growth.

Results

Temperature and fungal rotting

The organisms mainly responsible for rotting were *Botrytis* and *Sclerotinia* spp. Where a form of pitting of the surface occurred *Alternaria* was often found.

Fig. 1 shows the development of rotting in carrots of the Chantenay type (Clucas

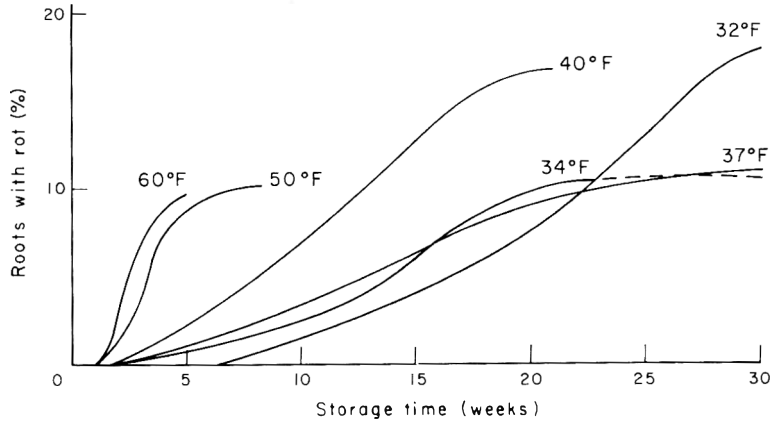

FIG. 1. The development of rotting in Chantenay type carrots during storage at different temperatures.

New Stumprooted) grown in sandy soil in a bed system with high sowing density. The carrots were stored shortly after lifting on 24 November 1964 at 32, 34, 37, 40, 50 and 60°F. The percentage roots with fungal lesions increased more rapidly at higher than at lower temperatures.

Until about the 21st week of storage the amount of rotting was lowest at 32°F, but thereafter it increased more rapidly and by the 25th week was greater at 32°F than that at 34 or 37°F.

Similarly Amsterdam Forcing carrots grown on fine sandy loam under the bed system and dug on 22 November 1965 developed increased rotting at 32°F compared with 35 or 38°F, but after a shorter period (8 weeks) (Table 1).

TABLE 1. Percentage roots with rot lesions after storage at 32, 35 and 38°F (Amsterdam Forcing carrots)

Temperature (°F)	No. weeks stored		
	8	13	18
32	13	10	13
35	8	5	7
38	7	7	7

Additional evidence stems from experimental storage of carrots from two sources, sandy loam soil and fenland in 1956 stored at 34 and 32°F. In Table 2 is shown the percentage of roots with rot lesions after 15 weeks storage.

TABLE 2. Percentage roots with rot lesions after storage for 15 weeks at 32 and 34°F

Temperature (°F)	Sandy loam		Fenland
	On removal	After 2 weeks at 50°F	On removal
32	23	41	17
34	6	15	12

In 1960 carrots of an Intermediate type grown on sandy loam were stored at 32, 34 and 36°F. The percentage roots with lesions at each temperature after successive periods of storage is shown in Table 3.

From the results of these experiments it is clear that less rotting occurred in the long term in carrots stored at 34°F, than at 32°F or higher temperature.

In 1960 the rots occurring in carrots of the Intermediate type were classified according to the position on the root, viz. crown, lateral and tip. In Table 4 is shown the percentage total of roots affected by rots in each of the three positions after 12, 17 and 20 weeks of storage at 32, 34 and 36°F followed by 2 weeks at 50°F.

It will be seen that the increased rotting that occurred at 32°F compared with 34°F after 17 and 20 weeks of storage was confined to the lateral position. This suggests that it is the surface of the root which becomes more susceptible and not the crown or tip.

TABLE 3. Percentage roots with rot lesions after storage at 32, 34 and 36°F (Intermediate type)

Temperature (°F)	No. of weeks stored		
	12	17	20
On removal			
32	10	27	54
34	25	34	37
36	18	47	54
After further 2 weeks at 50°F*			
32	27	35	48
34	32	20	26
36	56	58	49

* At examination on removal roots with visible rots were removed; the rotting after a further 2 weeks at 50°F was therefore additional.

TABLE 4. The percentage roots affected by rotting of crown, lateral surface and tip after storage at 32, 34 and 36°F

Temperature (°F)	Position		
	Crown	Lateral	Tip
Stored 12 weeks			
32	2	24	11
34	6	36	10
36	10	54	10
Stored 17 weeks			
32	8	47	7
34	9	28	8
36	13	78	13
Stored 20 weeks			
32	17	74	11
34	18	34	12
36	30	52	12

Temperature and growth

Growth of shoots and roots on the carrot, both of which are undesirable, is governed mainly by temperature. The effect of temperature on growth of shoots and roots is illustrated by the results of the experiment on Chantenay type carrots 1964–65 (Figs. 2 and 3).

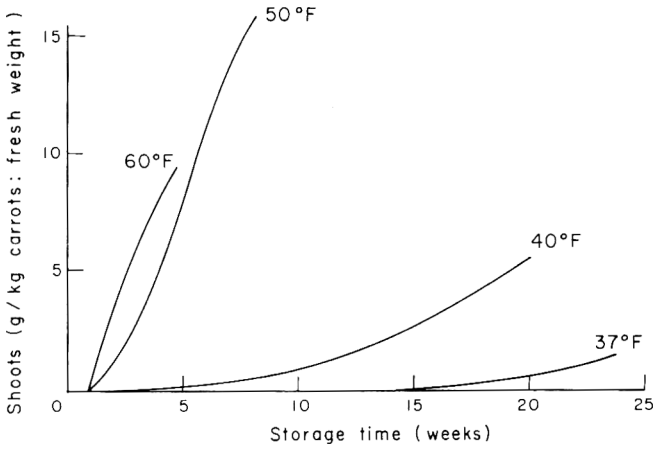

FIG. 2. Effect of temperature on growth of shoots on carrots.

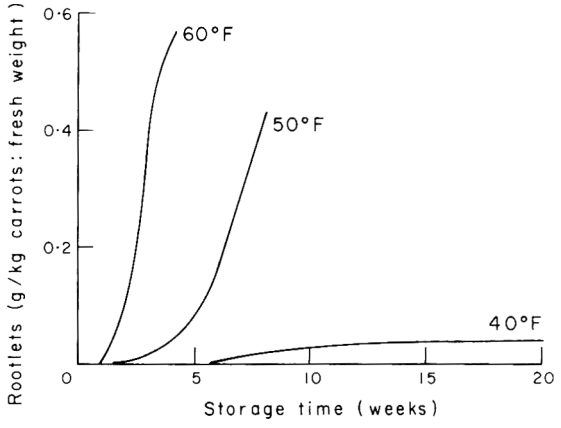

FIG. 3. Effect of temperature on growth of rootlets on carrots.

At temperatures below 37°F mean growth rate of shoots was negligible. Above 37°F the rate of shoot growth increased rapidly with higher level of temperature. Very little growth of rootlets took place at 40°F and none below this temperature.

Discussion

There is clear evidence that after prolonged storage (i.e. in excess of 8–10 weeks) more rotting occurs at 32°F than at 34°F and that during subsequent exposure to 50°F rotting progresses at a higher rate. Since growth of fungi would be expected to be retarded by lowered temperature, it may be inferred that some change of metabolic balance in the surface tissues of the root renders them more susceptible to invasion. No manifest physiological injury appears prior to rotting. However, it would seem justifiable by inference to term the condition 'low temperature injury'.

Growth of shoots and rootlets is well contained by storage at temperatures below 37°F.

Acknowledgment

Acknowledgment is made to Mr J. C. Parker and Miss R. Jamieson who carried out much of the experimental work reported here.

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Note on blue-black spots in bread

L. D. GALLOWAY* AND T. E. RYMER†

An unusual defect was observed in a wrapped loaf of white bread, purchased at a chain store and sliced on the following day. Isolated blue-black spots were then noticed throughout the loaf, later darkening to black.

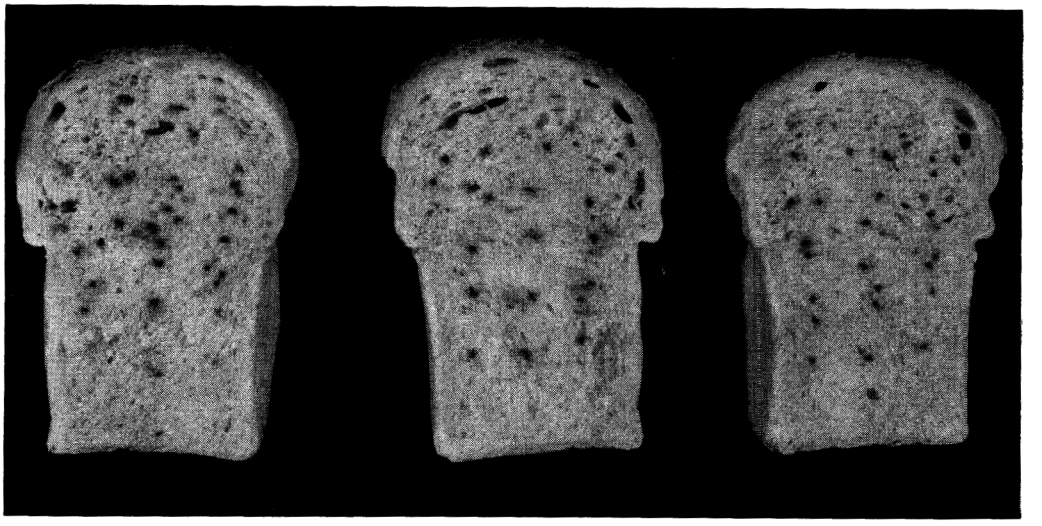

FIG. 1

No dyestuffs or fungal tissues could be detected, but microscopic examination of the spotted areas showed masses of large rod bacteria. In culture the organism proved to be a variant of *Bacillus subtilis*, giving a creamy growth on nutrient agar; on potato agar or on cooked potato slices it gave a blue-black pigment changing to jet black. A number of such pigment-producing strains have been recorded in the literature.

The spots were readily reproduced on bread by slicing a new loaf and giving point inoculations of the cut surfaces with a hypodermic needle dipped into a suspension of the organism. The loaf was then placed in a plastic bag and incubated at 37°C, when spotting developed within 24–48 hr. At 30°C, spots took 48–72 hr to develop.

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No baking experiments have been carried out, but it seems clear that spotting has arisen through the growth of spores that have been present in the dough and have resisted baking temperatures.

This form of spoilage appears rarely, and is no doubt kept in check by the methods customarily adopted for the prevention of 'rope' – which is also caused by strains of *Bacillus subtilis*. Flour and other ingredients should be reasonably free from spore infection, and an acid pH should be maintained in the loaf.

Note added in proof. Since the above contribution was submitted, our attention has been called to a very similar note by R. A. Knight published in 1956 in the *Bulletin* circulated to members of the British Baking Industries Research Association. Here the spots occurred in bread 'produced for a particular purpose' and were described as purple in colour. The organism was considered to agree with *Bac. subtilis* var. *aterrimus*.

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PROCEEDINGS OF THE
INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

Symposium on Transport of Frozen Food held at Grimsby College of Technology, Friday, 28 October 1966

The problem of how to get frozen foods in good condition from the factory cold store to the consumer is one which concerns many diverse groups involved in the transport of frozen foods. This Symposium was organized by the Institute to bring together representatives from these groups so that they might be made aware of, and discuss, various aspects of this problem. It was opened by the Principal of the College, Mr E. S. Green. The morning session was chaired by the President of the Institute, Professor J. D. Mounfield, O.B.E., and the afternoon session by Professor A. G. Ward, O.B.E.

The first paper of the morning, **Technological Requirements of the Frozen Commodity,** by Mr L. P. Hall and Mr S. D. Holdsworth of the Fruit and Vegetable Preservation Research Association, was read by Mr Hall. The authors emphasized the need to choose suitable raw material for the preparation of frozen foods, and to handle it properly before freezing. Relatively rapid freezing rates, that is, cooling from 32°F (0°C) to 23°F (−5°C) in 2 hr or less, are now widely accepted as necessary to obtain good quality products. Numerous and conflicting explanations have been put forward for the well-known and undesirable effects of slow freezing.

The effects of storage temperature on the deterioration of frozen commodities have been widely studied. It is clear that bacterial numbers in frozen foods give no guide to the initial quality, because a proportion of the bacteria die during freezing and cold storage at temperatures below about +20°F (−7°C). Above about 25°F (−4°C) some micro-organisms will continue to grow.

Deteriorative changes are mainly biochemical at storage temperatures below about +20°F (−7°C) and with most foods, with the exception of fish, biochemical deterioration is very slow at a storage temperature of 0°F (−18°C). Fish, however, because of its relatively rapid rate of spoilage at 0°F (−18°C), warrants storage at −20°F (−29°C) if the period of storage is to be prolonged.

At temperatures above 0°F (—18°C) most foods deteriorate relatively rapidly, the rate of deterioration doubling for every 5 degrees F, general appearance and palatability being reduced and in some instances food value impaired.

The need to reduce temperature fluctuations so far as possible is also accepted. The risks of fluctuating temperatures during transport are not always appreciated, especially since transport frequently occupies only a day or two. Nevertheless, the

effects of temperature fluctuations may be considerable, causing not only accelerated deterioration but also breakdown of emulsions, for example in ice cream, and an increase in water loss through drying when the product is returned to low temperature storage. Consequently, it is essential to load containers quickly and prevent or counteract small temperature rises as they occur.

In the discussion that followed it was stressed that although the figure of 0°F (−18°C) was accepted as an upper limit in transport, in fact larger cold stores in the U.K. generally ran at −15°F or below, and general practice was better than codes of practice and recommendations might suggest. Nevertheless, concern was expressed for the possible risks of quality deterioration involved in bulk storage and subsequent repacking. Perhaps for peas and other vegetables there was little danger, but was this equally true for meat and fish where a prolonged period of thawing was sometimes required before packing was possible? Perhaps, also, it was suggested, bacterial enzymes could continue to cause deterioration at temperatures at which the micro-organisms themselves were dormant or even dead. Automatic data logging of temperature during transport is an attractive idea but it is difficult to know where in the load to take the measurements, and certainly a number of positions would be needed. Merely knowing that a part of the load has exceeded a predetermined temperature is less valuable than knowing the integrated time/temperature history, but suitable temperature recorders are generally not sufficiently robust and simple integrating devices are relatively costly and not always reliable. Nevertheless it is useful to know when part of a load has exceeded a certain temperature, given that it may take a considerable time to cool it down again unless special precautions are taken, but where journeys seldom last more than 24 hr, is more information really needed?

The situation with imported frozen food is, however, quite different, since bad handling may cause long delays at docks. It was felt by a number of contributors that great improvements were required here and a request was made that specific, documented evidence of bad handling should be sent to the Importers Committee of the National Association of Wholesale Distributors of Frozen Foods.

Mr K. C. Hales, Director of the Shipowner's Refrigerated Cargo Research Association, in his paper on **Technological Requirements in Transport,** dealt first with the need to load the frozen foods at or below the temperature at which they are to be carried. Insulation will reduce the heat leaking in, and this can be absorbed by a refrigeration system, which must not, however, be expected to cool the load.

The load may lose moisture which will ultimately condense in the air cooler, and unless this loss is prevented by wrapping, it will be proportional to the heat leakage. Adequate circulation of air round the load is also essential if both temperature rise in the load and temperature differences through the load are to be kept small.

The first essential in refrigerating a container is to get as near as possible to the required temperature in every part of it at an acceptable cost. The smaller the heat leak, the easier it will be to keep temperatures uniform and the refrigeration unit

small. Unfortunately reduced heat leak means thicker insulation and reduced payload, and there must therefore be an economic balance between refrigeration and insulation.

Heat entering the container affects the cargo only if heat is transferred to the final cooling surface by way of the cargo and attempts are therefore made to intercept heat by surrounding the load with a jacket through which cold air is circulated. Complete refrigerated jackets are hardly practicable for transport, although some fairly complete ones have been proposed or even built. Less sophisticated is the cold wall container so constructed that free passage of air cannot be prevented by the load.

Mr Hales then dealt with the problems of moisture migration through the insulation of the container. The outside must be as vapour tight as possible and the insulation must have a low coefficient of moisture transference. Although in principle there may be a case for using as inner lining a material having a low resistance to moisture movement, in practice there are good reasons for making the inner lining impermeable.

Air leaks, for example through doors, may account for as much as 60% of the total heat leakage and are at their worst when the vehicle is on the move. Air leaks must clearly be avoided wherever possible.

Another path for heat flow is from the load to any part of the container at a lower temperature, and where heat is transferred, moisture may be transferred also. Damage to the load may occur by transference of moisture within packages or even by loss of moisture to the cooling surface where produce is not wrapped. Clearly, in designing a cooling unit, it is desirable to have a relatively small temperature difference between the air and the cooling surface which must, hence, have a large area, but there are economic considerations affecting size, weight and cost of cooling unit.

The need to make the outer shell vapour tight demands that all joints are vapour sealed internally before the shell is insulated. If sandwich-type insulation panels are used, the edges should be vapour sealed. There now seems a strong preference for polyurethane insulation for land transport of frozen food. Polyurethane is of particular value because of its very low thermal conductivity which, even though it rises slightly over the few months after preparation, still remains lower than polyvinyl chloride or polystyrene. Accelerated ageing tests suggest that this rise in conductivity is almost entirely prevented by sealing the polyurethane between impermeable surfaces. The greater thermal efficiency of polyurethane makes it possible to reduce the thickness of insulation so increasing payload.

A point which is sometimes ignored apparently is that heat leak is increased by intrusion of top hat and other sections into the insulation, so reducing its effective thickness. Heat paths resulting from strength connections between inner and outer linings should be broken by an indifferent conductor of heat.

Refrigeration systems are either mechanical or so-called total loss ones, including solid and liquid carbon dioxide and liquid nitrogen. In the total loss systems the compressor and condenser have been separated from the evaporator, and it is possible to exploit the gain in efficiency by operating a large plant. The maintenance necessary

for a mechanical plant fitted to a container is also avoided. On the other hand, a very low-temperature refrigerant is used in the total loss systems and thermodynamic efficiency is less than in the mechanical system. In addition total loss systems are not really suitable for loads which must be cooled during the journey. The limit of a total loss system has been given as 24 hr, but some operators apparently make journeys of 4 days without difficulty so that the dividing line between the two types of system is not clear-cut. The selection of any particular system will depend very much on the precise conditions of operation which are required.

It should always be remembered that the atmosphere in a container refrigerated with carbon dioxide or liquid nitrogen systems may be dangerous and there is a danger of pressure build-up. The thermostat bulb of mechanical equipment should be placed in the air stream returning to the unit from the container; this is particularly important if merely chilled loads of, for example, fish, fruit and vegetables are likely occasionally to be carried since some freezing of produce is otherwise inevitable.

It was pointed out in discussion that the glass fibre container, where the outer and inner skins were made in one continuous piece, came close to fulfilling the requirements specified by Mr Hales.

Representatives of two organizations mentioned tests which had shown little change in heat transfer properties of glass fibre containers after periods of use; in one instance heat transfer rate increased by 1 or 2% after 4 years' use. Other types of construction appeared less reliable, and examples were given of insulation becoming waterlogged in under 2 years. The question was asked whether the pattern of usage of a container, where there was relatively frequent warming up of the inside, had any effect on the pattern of moisture transmission through the insulation.

The need for a standard of thermal performance of insulated containers was mentioned, and it was stated that the B.S.I. was forming a committee which would draft a suitable standard. Furthermore, the Ministry of Technology is at present considering the possibility of setting up one or more national testing stations for insulated vehicles.

Where cargoes are loaded at a low temperature, for example $-20°F$, the main refrigeration effect will come from the cargo which may be allowed to rise in temperature to the control temperature of the refrigeration plant. Forced circulation plants may do little more than prevent excessive temperature rise in certain parts of the load, the corners for instance.

The design of refrigeration plant was also discussed. The need to use systems with small temperature differentials was stressed. Moisture loss from unwrapped products would thus be kept to a minimum; in wrapped products, considerable moisture migration can still occur within the package, to the detriment of the purchaser.

Difficulties in importing and exporting frozen foods which might be avoided by more effective organization at the ship's side were referred to in discussion. The order of loading was said to be often illogical. Furthermore, the goods frequently wait on the

quayside for long periods at both ends of their journey. Adequate monitoring of temperatures was again stressed.

The afternoon session was opened by Mr C. A. Cardy of Birds Eye Foods who spoke on **Low Temperature Transport Operations.** He concentrated on the problems of bulk haulage from factory to depot. The problem of transport is essentially to prevent frozen food from regaining heat, and this is done first by insulation and secondly by refrigeration. In theory the economic balance between these two can be found, for example, simply by multiplying total heat transfer by capital cost, to give an Index of Performance for comparison of different containers.

Where expendable refrigerants are used it is necessary to calculate refrigerant requirements for specific journeys and Mr Cardy added that his firm found solid carbon dioxide the most economic method. To estimate requirements in practice they use a refrigeration table for each class of vehicle. This gives the weight of solid carbon dioxide for any duration of journey and any prevailing ambient temperature. A 10% excess is allowed in the scale to cover unexpected delays or high ambients.

Rapid turn-round of vehicles is obtained by mechanical handling and pre-assembly of loads on pallets in the cold store. At unloading this procedure is reversed and mixed pallets are removed to cold store where they are broken down. It was doubted by the author whether pre-cooling of the container air space to carrying temperature was worth while, but rapid turn-round should prevent excessive temperature rise of the container itself.

Since some products, if stored for any length of time, require to be held at −20°F, bulk holding stores all operate at this temperature. Delivery to the stockist's cabinet must be at a temperature below 0°F, so that there is a reservoir of refrigeration in the load throughout the cold chain. This is utilized in the following manner:

Bulk storage at −20°F.
Presentation at depot at −10°F.
In delivery vehicle at −5°F.
To stockist at 0°F.

Throughout, the aim is to balance minimum costs against adequate temperature control. To this end, product temperatures and residual amounts of solid carbon dioxide are always recorded. These records are valuable checks on vehicle performance and on costs.

The paper on **Low Temperature Transport Operations** by Mr K. G. Sanders of T. Wall & Sons (Ice Cream) Ltd was read in the author's absence by Mr A. R. Went of Unilever Ltd (Technical Division). This paper was mainly concerned with low temperature transport from depot to stockist, where refrigeration duty is much increased owing to frequent opening of doors, often in high ambient temperatures. The product must be delivered to the stockist's refrigerator at −5 to −3°F and certainly not above 0°F. Since the requirements for insulation and refrigeration are relatively high a chassis of a gross weight of 8–10 tons must be used. The maximum weight is also influenced by

the weight of product that can be handled by one man and by the number and duration of calls he must make during the day. Manoeuvrability must also influence vehicle size, and height is governed by risk of tree damage.

The refrigeration system of a wholesale van must be capable of absorbing the heat leak through the insulation, it must maintain the appropriate internal temperature to be ready for morning loading or holding over unsold produce, and it must be able to deal with the effect of a reasonable number of door openings.

Calculation of requirements are based on the Royal Society of Health recommendation for operation in the climatic conditions of the U.K., namely ambient day temperature 80°F, night temperature 70°F, with allowance for solar radiation and humidity where appropriate. Highly efficient containers are now in use; a heat leak of about 1100 b.t.u. has been recorded on a 500-ft^3 container under test insulated with 5·25 in. polyurethane. At present about half the refrigeration is used to deal with heat entering when the door is opened.

Combating the effects of warm air entering when the door is opened is one of the major problems of the designer. One partial solution is to fix a flexible rubber door or lightweight insulated door behind the insulated container door. The inner door is so hung as always to return to the closed position. Unfortunately these doors make working rather more difficult for salesmen and weighted plastic strip drapes have been used as an alternative. These strips, however, tend to accumulate condensation which may also form ice patches on the threshold step. An alternative is the cold air curtain, perhaps fitted into the sides or stile of the doorway and operating within or outside the container.

Of the various methods of refrigeration available, eutectic holdover plates, with the refrigeration plant recharging the plates overnight on off-peak electricity, offer one of the most satisfactory solutions. Current operation of vans calls for a specified holdover plate temperature recovery from −9°F to better than −24°F in 7 hr. Nevertheless, mention should be made of the Coora system, where sublimation of solid carbon dioxide is used to cool indirectly the air drawn from the container, a secondary refrigerant, Arcton, being used to prevent the build-up of an undesirable atmosphere.

Glass reinforced plastics are also being introduced for wholesale vans, where their superior appearance is an added advantage. The building of body-work below the level of the chassis line is now being avoided and this simplifies maintenance. The author suggested the possibility in future of demountable containers mounted on a single articulated semi-trailer chassis 34–40 ft long, when regulations permit, the containers being on a separate chassis each having single axle and landing gear assemblies. For bulk haulage these are joined and for wholesale operation they go their separate ways. Regional depots with small cold stores are thus eliminated and the advantages of large-scale economics obtained.

The author finally stated his preference for the pre-cooling of vehicles. This was done by backing the open container against the aperture in the cold store wall which is

surrounded by a foam rubber pad. A lightweight inward opening door gives access between store and vehicles and the gap is bridged by an aluminium plate, allowing passage of pallet trucks.

One contributor to the discussion said that the theoretical principles and their practical realization had become so inextricably mixed in the papers and discussions of the day that, from a theoretical point of view, one might wonder what the difficulties were. The concept of heat leak and its measurement was simple, and there were several refrigeration systems to choose from, but a b.t.u. removed by solid carbon dioxide was the same as one removed by a mechanical system. Some systems are thermostatically controlled and some are not and both the authors had come down on the side of systems that were not. How much did this matter? It may be suspected that thermal inertia of the system would alone make it extremely difficult to detect the effect of thermostatic control. Comment was made also that thermostatic control was not of product temperature but of the surrounding air, and the sensing element was placed in a position which might not be representative.

The question was asked whether, in spite of all the care that was taken, more than a small proportion of frozen foods had been delivered to cabinets without reaching temperatures above 0°F at some point in the distribution chain. Although representatives of some firms believed that a very high proportion was distributed under satisfactory conditions, others felt that the major problem was in gaining the cooperation of retailers in the operation of cabinets and in improving delivery vehicle design.

Controlling the conditions of transport demands adequate methods of measuring product temperature and it seemed from the discussion that a cheap and suitable instrument was still needed for this purpose.

The last paper of the day, **Transport in the Next Decade,** was given by Mr E. C. Williams, Chief Scientific Adviser, Ministry of Transport. He spoke, he said, of the whole transport system and not just the frozen food industry, since one could not consider this in isolation.

In intercontinental transport the advent within the next decade of large transport aircraft and of container ships would have enormous effects. Hover-ships over 2000 tons in weight are unlikely within the decade because of structural problems.

Development of the air-bus will greatly influence European travel. The Channel Tunnel will probably be built; geological surveys have been carried out and the probable cost estimated. It will be a rail tunnel and will be served by road and rail links which are already in existence but which will have to be modernized.

So far as the ports are concerned, the container revolution is already taking place. In the future all containers will be standardized in size; an international Commission is already drawing up standards for containers and pallets. Container services will reduce the number of berths required and transform the whole pattern of dock operations. The decision has been taken to maintain extensive rail links within the country and in future speeds will increase, with passenger traffic at speeds of 100 miles/hr.

Internal air services will inevitably be extended and passenger and freight links with the airports improved.

On the motorways there will be improved signalling but—most important—the standards of lighting and braking which will be required will be considerably higher than at present. Within cities there will need to be some restraint on the use of vehicles. Perhaps there will be container transport in cities for delivery to shops.

There will be considerable scope for automation, and experiments are in progress on automatic traffic control, on guided buses, on automatic fare collection and many other projects, all of which will affect transport within the next 10 years. There is, however, still much to be done in the application of information systems to freight and passenger handling. In the immediate future there is scope for the use of computers in speeding documentation procedure.

During discussion the problems of labour relations were raised and it was clear that agreement with Unions on matters of handling will have to be worked out. It was also observed that with faster speeds of transport there were fewer goods actually in the supply pipeline at any one time.

It was felt that some balance had to be found between transport of people and transport of information, and perhaps more attention needed to be given to the transport of information. The present road system is inadequate and extensions as they are built encourage further industrial and urban development, so that transport problems seem to become greater, not less. To reduce the need for people to travel would hence help to relieve road congestion.

The meeting was closed by Professor Ward at 5.15 p.m. In his summing up he commented that there was clearly a need for the dissemination of information, especially to the smaller units in the distribution industry. He also urged that greater attention should be given to the interrelation of economics and technology of transport. There was an obvious need to make sure that all systems of frozen food transport were adaptable to meet changing requirements. He said that he felt the Symposium had been particularly valuable in bringing together engineers and members of the Institute. In this field the contribution of the engineer was as important as that of the food scientist and technologist.

J. Fd Technol. (1967) **2**, 97–98.

Book Review

Nutrition, Vol. III. Ed. by G. H. BEATON and E. W. MCHENRY.
New York: Academic Press, 1966. Pp. xvii +349. 120s.

From time to time statements are published suggesting that there is a significant degree of malnutrition in Great Britain—as, for example, recent statements involving children of poorer families and elderly persons. Some of these statements are based on measurements of food intake and others on clinical assessment. It is not always realized that unless a deficiency is severe enough to result in the obvious symptoms of a disease such as rickets or scurvy it is extremely difficult indeed to diagnose. So many signs supposed to be characteristic of a nutritional deficiency can result from a dozen other causes. Nor can deviations of nutrient intakes from recommended allowances be considered as deficiencies unless they are considerably lower.

This problem is the subject of one third of the book under review, which, indeed, is subtitled 'Nutritional status; assessment and application'.

The nutritionist lays down four approaches to the problem and only if all four point in the same direction is it possible to make a confident diagnosis of malnutrition. Firstly there is the dietary survey to determine whether or not the subjects are consuming less of any nutrient than is generally considered to be the amount recommended for the maintenance of health—this is a rather vague figure. The author of the chapter dealing with this subject describes population sampling, record cards and methodology, in sufficient detail to disabuse anyone who thinks that dietary surveys are easy to carry out. Secondly there are biochemical tests to assess the levels of the nutrients in the tissues. Thirdly the clinician carries out his examination and fourthly there is the final proof—does a therapeutic dose of the missing nutrient alleviate the condition?

The remainder of the book consists of a series of reviews by different authors covering the nutrition of infants, children and adolescents, pregnant and lactating women, and elderly persons, together with one chapter on the principles of therapeutic diets. As is inevitable with books written by several authors the chapters vary considerably in quality. While most of the chapters are more than adequate there are some sad lapses. The chapter on nutrition of the elderly quotes many papers with little critical appraisal and contains several unacceptable statements. For example, the author states that 'circumstantial evidence is overwhelming that limitation in the quantity of the dietary fat and substitution of unsaturated fat for saturated provides an effective measure against atherosclerosis'. No reference is given for this declaration, but it is followed by the statement that 'scientific caution requires modest recommendations' (reference to the American Medical Association).

The same author persists in using the term 'refined' carbohydrates, implying some

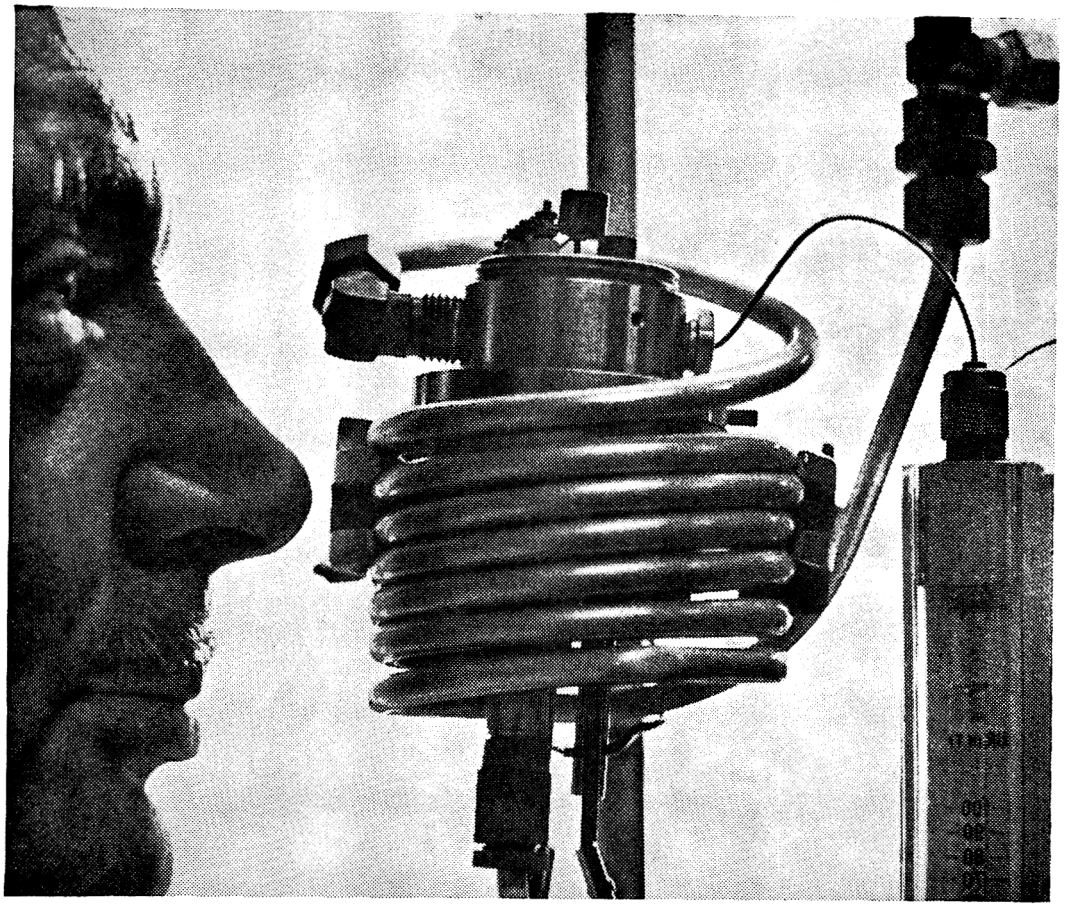
mystical superiority of brown sugar and brown bread. He equates sugar and cornstarch on the one hand (pure carbohydrates) with what he calls 'soft' white bread (which contains 10% protein, plus vitamins and minerals). He states 'with respect to the elderly . . . consuming an adequate diet, there is little evidence that fat-soluble vitamin deficiencies are present'. Surely there can be no deficiency if the diet is adequate!

The same author puts forward a sweeping indictment of all manufactured food and says that conventionally processed and routinely packaged foods present obstacles to the proper nutrition of the aged—because of loss of nutrients, high fat content of certain foods, the use of sodium and sugar, and because the containers are difficult to open and instructions are in small print. He suggests marketing 'entirely new' major lines of food products containing the maximum of nutrients with the minimum of calories and says that all white flour should be fortified by law with vitamins, calcium and iron. Is not this already done in a dozen countries?

Similarly the chapter on infant feeding is open to criticism. The statement that 'infants adequately fed on breast milk are usually fairly plump and may exhibit excessive subcutaneous fat' raises the question of the meaning of the word 'adequately'. Much of the argument on breast-feeding versus cow's milk concludes that breast-fed babies are *no worse* than bottle-fed ones (this is largely in relation to the higher protein retention on cow's milk) but surely the argument is back to front. The same author states that 'the requirement of the infant for energy is more than double that of the adult'.

Apart from these unfortunate lapses the book serves as a useful collection of reviews on topics that are not usually found within one book. It is the third volume of the series but the first two cover the more routine subjects of the macronutrients and the vitamins so this volume can stand in its own right.

A. E. Bender



Nose...meets...automatic nose

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

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

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

Metal Box — brief facts

Leading manufacturers of packaging in metal, paper and plastics.

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

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

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

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

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AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

APPOINTMENT OF CHIEF
DIVISION OF FOOD PRESERVATION

The Executive of CSIRO is seeking to appoint a successor to Dr J. R. Vickery, Chief of the Division of Food Preservation, who will retire in July 1967.

The Executive is seeking a leader who will be responsible for the operation of the Division and its research programme, involving, among other things, the formulation of policy on food research in Australia. He will have the opportunity of pursuing his own research interests.

The Division has a close association with the food industry in Australia, and has an international reputation in research. Its current programme is concerned with physical, chemical and biological properties of foods and food constituents and the changes that occur during preservation, processing and storage. Its work is at present conducted in eight Sections in which a wide range of basic and applied investigations have been developed relating to meat, fish, eggs, fruits and vegetables.

The staff numbers 200, of whom 90 are professional officers, and the annual budget of the Division is approximately $A1,400,000.

The Division's main laboratories are attractively housed in modern buildings at North Ryde, Sydney. The Division also maintains a substantial laboratory for meat research at Brisbane, and smaller units elsewhere in Sydney and at Hobart.

The salary of the appointee will be dependent upon his standing and achievements, but will not be less than $A12,200 p.a.

Senior Scientists interested in this position may obtain further particulars from:

The Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
Africa House,
Kingsway,
London, W.C.2, England (quoting Appointment No. 300/448).

Closing date for applications is 28th April, 1967.

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

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

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

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

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

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

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

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

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

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

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