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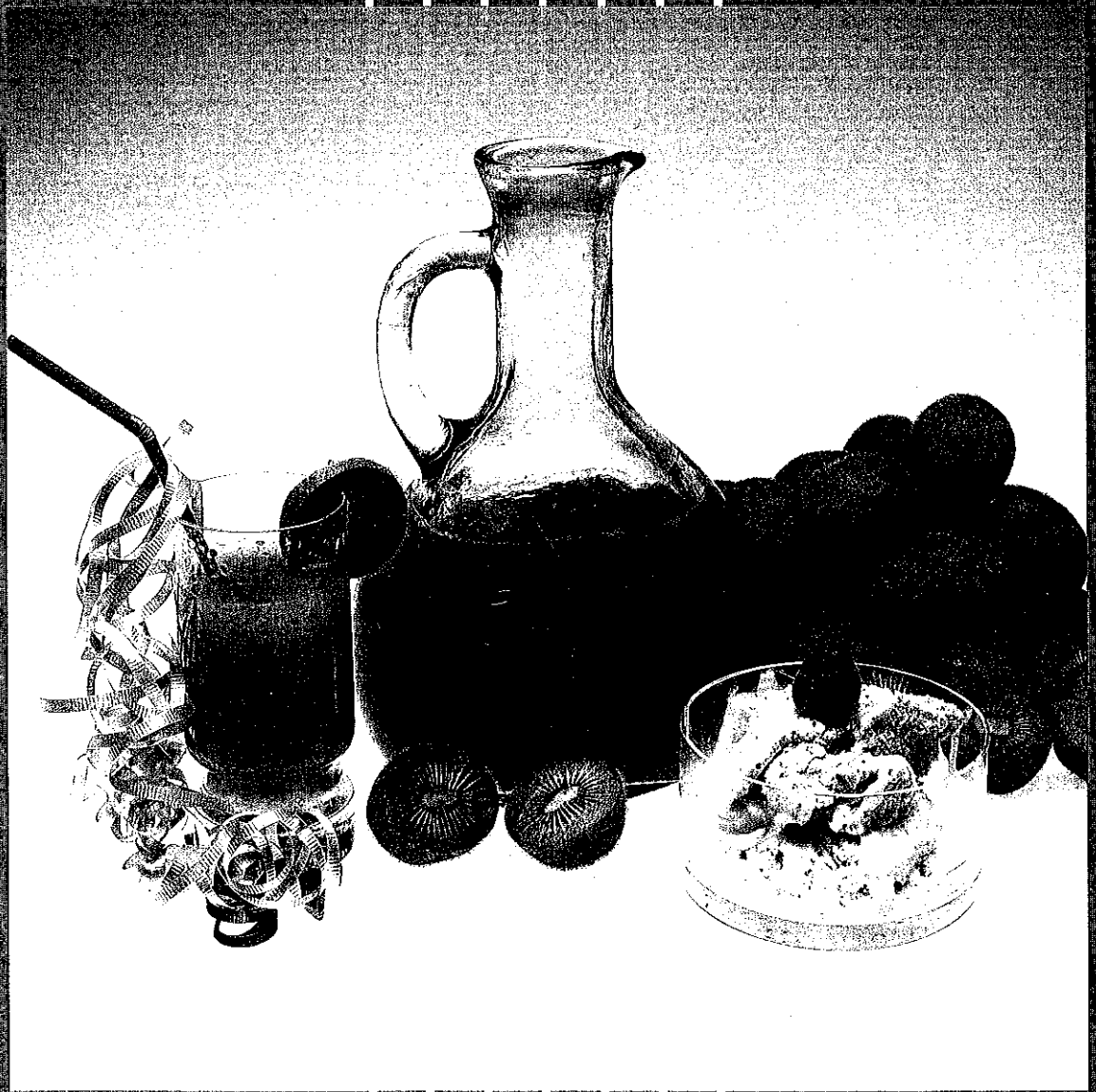

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Contents

Hot Dip Treatments Reduce Chilling Injury During Storage of Citrus Fruit at 1°C

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Introduction

The export of Australian citrus fruit to Japan, New Zealand, U.S.A. and many other countries is often dependent on our exporters being able to satisfy quarantine restrictions that require a guarantee that the exported fruit are free of Queensland and Mediterranean fruit fly.

This, in the past has been obtained by the use of the fumigant ethylene dibromide (EDB), but a recent ban by Japan and the U.S.A. of EDB fumigation has necessitated the development of safe alternative disinfestation treatments.

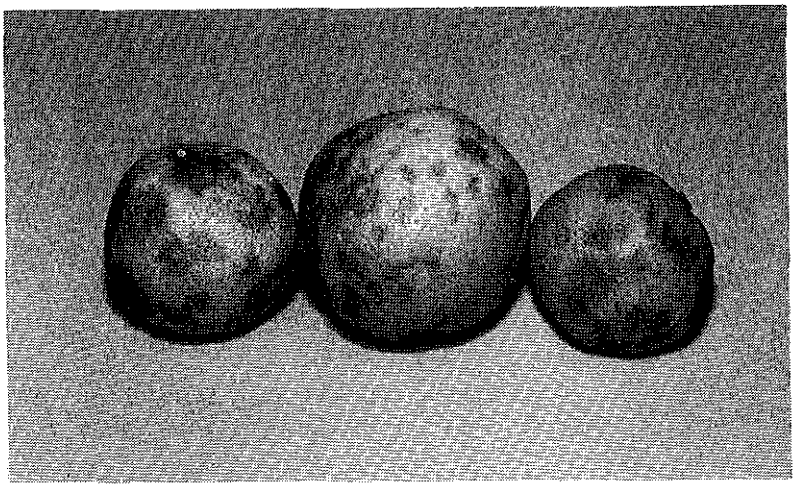
One of the most suitable that has been developed for oranges by the Gosford Horticultural Postharvest Laboratory is cold storage for 16 days at 1°C. This treatment has been established as providing excellent security against Queensland and Mediterranean fruit fly (Hill *et al.* 1988) and has been accepted by Japanese quarantine authorities as a suitable disinfestation process for oranges. It is also being considered for lemons. The only problem with this treatment is that occasionally fruit incur chilling injury when stored at these low temperatures and the rind damage resulting is threatening the commercial adoption of treatment for the export of citrus to Japan.

Additionally, if fruit could be successfully stored at these low temperatures without rind damage, losses due to decay and aging would be reduced (Hall 1938).

Chilling injury

Chilling injury can occur with all citrus fruit species once they are stored for extended periods below their recommended storage temperatures. This temperature varies: for oranges, eg., it is 7°C, grapefruit and lemons 13°C and limes 10°C (Wills *et al.* 1989). Rind injury normally takes the form of dark brown rind pitting which can coalesce as it becomes more severe so that whole sections of the rind can be affected (Fig. 1).

Fig. 1. Chilling injury symptoms on Valencia oranges after 15 weeks storage at 1°C.



Reducing chilling injury susceptibility

Many factors have been investigated for their potential to reduce the risk of chilling injury in citrus fruit. Fruit age (Morris 1982), fruit position in the tree canopy (Purvis 1980), growth regulator treatments (Ismail and Grierson 1977), ethephon treatment (Gilfillan and du T. Pelsar 1985), rind damage (Patterson, personal communication), plastic wrapping (Wardowski *et al.* 1973; McCormack 1976) and prestorage 'curing' (Huelin 1962; Hatton *et al.* 1981; Hatton and Cubbedge 1982) have all been shown to influence the extent of chilling injury in citrus fruit. Additionally, postharvest thiabendazole (TBZ) and benomyl treatment of grapefruit (Schiffmann-Nadel *et al.* 1972; Kokkalos 1974; Wardowski *et al.* 1975; Chalutz *et al.* 1985) has been shown to reduce chilling injury incidence.

To examine further the effects of fungicide treatments on chilling injury and as a possible way of enhancing fungicide response, as in anthracnose control in citrus (Wild 1983), trials were conducted where Valencia and Washington navel oranges and grapefruit were treated with hot dips before storage. In addition, with Valencia oranges, the rind of some fruit were damaged in an attempt to determine if fruit injury induced more chilling injury.

Materials and methods

Valencia oranges

Valencia oranges were obtained in early November, 1987 from two orchards in the Gosford region of New South Wales. On delivery of fruit to the laboratory they were washed, but not waxed, and fruit from each orchard then randomised into 10 treatment units of approximately 130 fruit each.

Five treatment units were subjected to rough handling, where damaged rind was induced by rolling the fruit several times in a barrel containing approximately 2 kg of coarse dry sands, while the other five treatments were carefully handled. One of the following dip treatments was then applied to each unit of fruit from both groups: (a) control, water dip at ambient temperature (approx. 18°C for two minutes). (b) hot water dip, 53°C for two minutes, (c) hot benomyl dip, (500 mg/l) 53°C for two minutes; (d) hot thiabendazole dip, (1000 mg/l) 53°C for two minutes; (e) control (10°C storage, water dip only) same as treatment (a). Treatments (a) to (d) were stored for 15 weeks in a cold room with fan- forced air circulation operating at 1°C ± 0. 5deg. C, whereas treatment (e) was stored under similar conditions but at 10°C. Fruit were held in covered, but vented plastic boxes and stacked in the cold room in the two replicate groups, with treatment positions allocated at random within each stack.

After 15 weeks, fruit in the control treatments had developed extensive chilling injury. All oranges were removed from the cold room, allowed to warm to ambient temperatures and examined for chilling injury. Fruit was classified into one of four chilling injury categories similar to those used by Hatton and Cubbedge (1982): nil, slight, moderate and severe. Each category was assigned a numerical value; 0, 1, 2 and 3, respectively, and a chilling injury index determined for each treatment. The index was determined by summing the products of the number of fruit in each category by the value of each category and then dividing this sum by the total number of fruit assessed. Chilling injury index values were then square root transformed for analysis as a 5 x 2 factorial experiment and means compared by using the Waller-Duncan k ratio LSD rule (Chew 1977) at k = 100. This level is approximately equivalent to the 5% level of significance. The experiment was replicated twice.

Washington navel oranges

This experiment was designed to compare the best hot dip treatment with 'curing' and the effects of waxing on chilling injury development.

Fruit were obtained from the Sunraysia inland area of New South Wales where chilling injury had been reported to be a problem in 1989. On arrival at the laboratory all fruit were washed, then randomly divided into six treatment units, each containing 50 fruits. Each of the following treatments were then applied to two of the treatment units prepared: (a) control, water dip only; (b) thiabendazole 1000 mg/l, two minutes dip at 50°C and (c) fruit 'cured' for one week at 20°C. Half of these treatment units were then dipped in wax (Britseal, 16% solid, Milestone Chemical, Melbourne, Australia) and the remainder unwaxed.

Fruit were then stored for 20 days at 1°C, held for one

week at 20°C and then examined for chilling injury. Fruit were assessed for chilling injury as previously described for grapefruit and a chilling injury index calculated as before.

Data were analysed as a 2 x 3 factorial, replicated three times and chilling injury index means compared using the same technique as previously described.

Grapefruit

Marsh grapefruit were obtained from four locations within New South Wales in July 1989. One source was from the central coast region and the other three from the dryer inland areas. Fruit from each location were used as replicates and postharvest dips applied to treatment units which each contained approximately 40 randomly allocated fruit. The following treatments were either applied at ambient dip temperature (14°C) or 50°C for two minutes; (a) control - water only; (b) thiabendazole, 1000 mg/l, and (c) benomyl, 500 mg/l

Within each replicate, fruit receiving these treatments were divided into two groups, one stored at 1°C the other at 10°C. After four weeks storage, fruit were held for one week at 20°C and then examined for chilling injury.

A chilling injury index value was obtained by using the same method of assessment as for the Valencia oranges with the chilling injury standards shown in Fig. 2. The chilling injury index values obtained were then analysed statistically as a 2 x 2 x 3 factorial, replicated four times. Means were compared using the Waller-Duncan k ratio rule at k=100 (approximately equivalent to the 5/°o level of significance).

Results

Valencia oranges

Statistical analysis showed a significant ($P<0.05$) effect of the method of handling on chilling injury, for all dip treatments stored at 1°C. Chilling injury was highest in the control fruit dipped in ambient water, and then stored at 1°C, with an average chilling injury index of 2.3 and with 98.4% of fruit showing the disorder to some extent.

The dip treatment effects on chilling injury were highly significant ($P<0.001$). Mean separation revealed that the hot water dip treatment and hot benomyl significantly reduced chilling injury ($P<0.05$) to the level of the ambient temperature dip controls stored at 10°C. The hot thiabendazole treatment was significantly better than either the hot water or hot benomyl treatments ($P<0.05$) (Fig. 3). There was no significant interaction between the method of handling and the dip treatments.

Washinqton navel oranqes

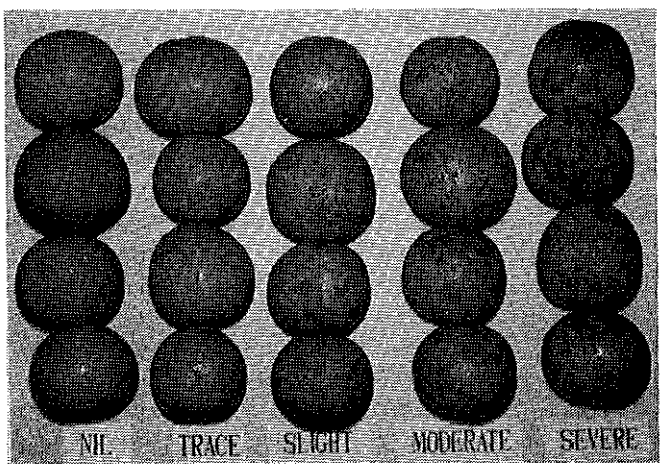
Analysis of variance of the 2 x 3 factorial experiment showed a highly significant effect of wax treatments in reducing chilling injury ($P<0.001$). There was also a significant interaction between wax application and treatments ($P=0.02$) which was reflected in the improvement of the 'curing' step in reducing chilling injury once wax was applied. The most effective prestorage treatment, both with and without wax application was the hot thiabendazole dip (Fig. 4).

Grapefruit

Storage of fruit at 10°C resulted in no chilling injury for any of the treatments tested and consequently no data were available for inclusion in the analysis.

Analysis of variance of data at 1°C, however, showed

Fig. 2. Chilling injury classification standards used for assessing grapefruit chilling injury after eight weeks storage at 1°C.



a highly significant effect of heating all solutions in reducing chilling injury ($P<0.001$). Data also show that thiabendazole at 50°C was significantly better than water at 50°C ($P<0.05$;Fig. 5).

Discussion

Reasons for some of the dramatic reduction in chilling injury with the hot dip treatments could be either the result of physiological changes in the rind, equivalent to that obtained in the 'curing' storage process (Hatton and Cubbedge 1982) or an inactivation of latent fungal infections (Wild 1983), such as *Colletotrichum gloeosporioides* which are often present in the rind of citrus (Brown 1975). Similar infections in mangoes have been controlled by hot dip treatments (Spalding and Reeder 1972; Muirhead 1976).

The effect of waxing on chilling injury with Washington navel oranges has not been shown before, although a reduction in chilling injury with grapefruit was achieved by vegetable oil application (Aljuburi and Huff 1984) and the use of plastic films (Wardowski *et al.* 1973). In this latter work, also, citrus wax application was tested with grapefruit but no positive response was obtained. However, the wax used in that experiment was a synthetic coumarone-indene resin, whereas the wax used in these experiments was a polyethylene based formulation.

The effect of adding thiabendazole to the dip also further reduced chilling injury with Valencia oranges and grapefruit. This confirms the response reported earlier by Wardowski *et al.* (1975) and Chalutz *et al.* (1985), who showed that thiabendazole included in wax coatings on grapefruit reduced chilling injury. The use of the fungicide benomyl however did not improve the hot dipping response or reduce chilling injury by itself, even though it is a benzimidazole fungicide whose mode of action against fungi is identical to that of thiabendazole (Davidse 1977).

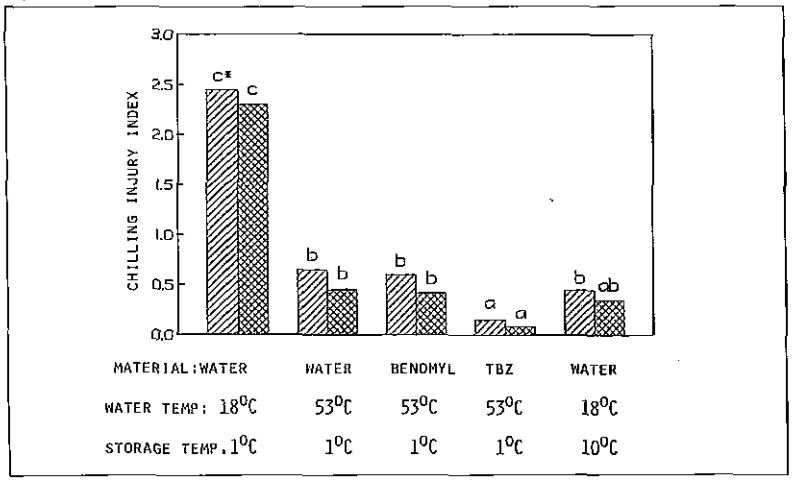
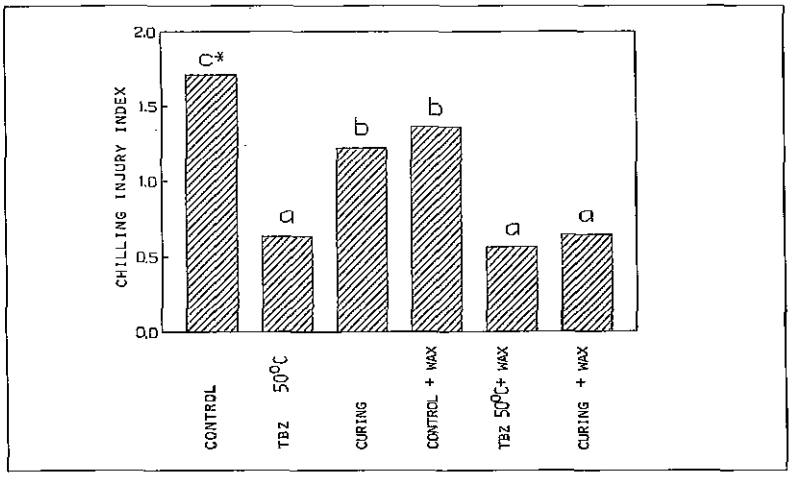


Fig. 3. Effect of method of handling and dip treatment on chilling injury incidence in Valencia oranges stored at 1°C for 15 weeks. Columns covered by the same letter do not differ at the 5% level using k LSD on square root transformed data.

*Fig. 4. The effect of TBZ dips at 50°C for two minutes, fruit 'curing' and waxing on chilling injury in Washington navel oranges stored at 1°C for five weeks. *Different letters denote significant differences between treatments ($P<0.05$).*



Recommendations from this research for the reduction of chilling injury susceptibility with grapefruit and oranges could therefore involve the use of hot thiabendazole dipping of fruit prior to, or in conjunction with, packing line treatments. This treatment would have the advantage of being applied immediately before cold disinfestation and with grapefruit

it may be more convenient than the present 'curing' step of one week's storage at temperatures between 10 and 21°C (Hatton and Cubbedge 1982).

Thiabendazole has the additional advantage that it has a low mammalian toxicity and is accepted by Japanese health authorities as a permitted postharvest treatment on citrus fruit.

Further trials are planned to investigate the hypothesis that, in association with the many other factors that govern chilling injury incidence (Morris 1982), latent infections could be weakening cell walls and predisposing fruit to chilling injury damage when placed under chilling stress.

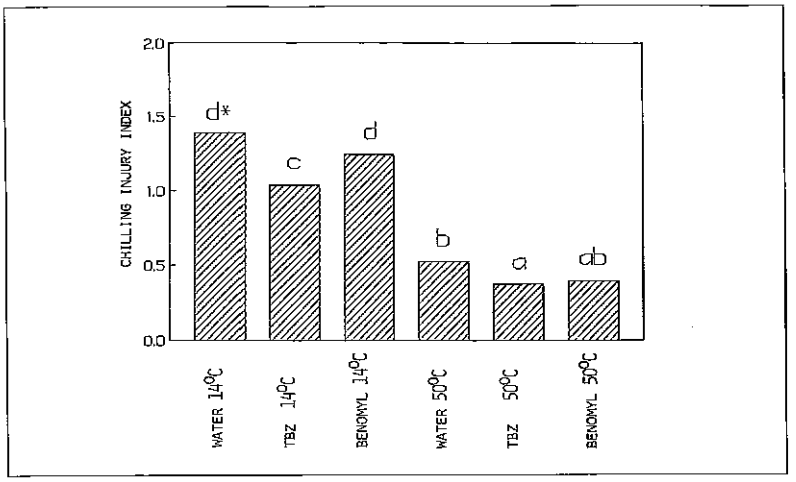


*Fig. 5. Reduction in grapefruit chilling injury as a result of dipping fruit for two minutes in various solutions before eight weeks storage at 1°C. *Different letters denote significant differences between treatments ($P<0.05$).*

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The Hen's Egg as a Model for Food Technology

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Introduction

Although they are not immediately obvious, there are similarities between a laying hen and the food industry. Consider, for example, that, like the laying hen, the food industry is concerned with the packaging of nutrients (i.e., food) in a convenient and compact form such that they resist oxidation and spoilage by microorganisms. It must be admitted that when it comes to short-term food preservation, the hen is more successful than the industry; especially as it has to face some problems not known to the industry, such as a limit to the size and shape of the container. As partial compensation, there are some problems of the industry that the hen does not have to worry about, such as flavours and consumer acceptability.

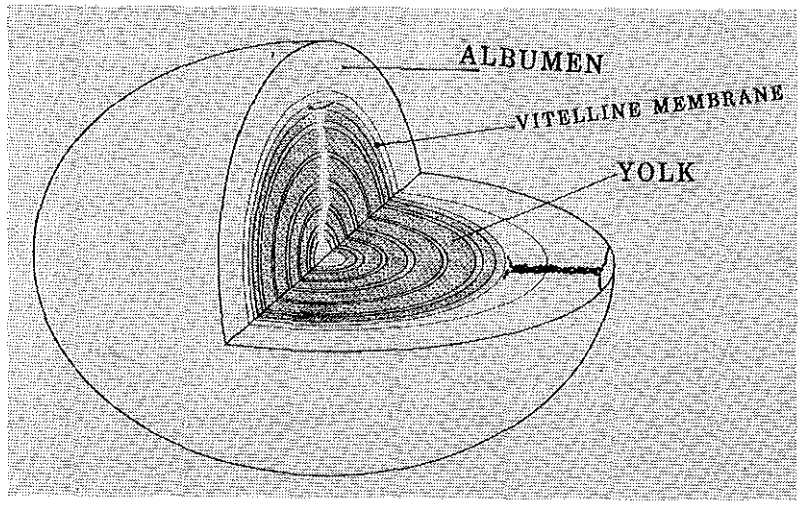
Birds have been thriving for more than fifty million years, so the egg, which at first sight is such a fragile and insignificant thing, must have important properties that enable it to resist microbes and oxidation. This resistance lasts for days, weeks, or even, for some species, for months.

Furthermore, during this time the egg is exposed to temperatures that often exceed 40°C and also to atmospheric oxygen. Of course, in the wild, eggs eventually succumb to microbial attack, but for a considerable period, the constituents of the egg are so little changed chemically that they can be used directly as food for the embryo. This feat is the equivalent of leaving a lamb chop or a baby's bottle full of milk outside for a week or two and then finding it to be still edible. The egg achieves such a result without refrigeration, sterilisation by heat or irradiation, vacuum packing, or any of the other operations of modern food technology. I summarise here what is known about the egg as a successful food package. Some of this information may be of use for food technology in general.

The Egg as an Anti-microbial Package

As far as its anti-microbial properties are concerned, the egg has four parts: shell plus membranes, white (ie, album-

Fig. 1. Drawing of a hen's egg showing main parts.



en), vitelline membrane, and yolk (Fig. 1).

Surprisingly, the hard shell of an egg is not the main barrier to microbes, because it is pierced by innumerable pores that are large enough to permit easy entry to many bacteria. Why more bacteria do not enter eggs through these pores is not quite clear. Possibly it is because on the outside of the shell there is a proteinaceous layer, the cuticle, that may help resist bacteria. Furthermore the pores are often partially plugged with protein. An alternative explanation is that the walls of the pores are hydrophobic and thus repel water, which might carry microbes. The relative importance of these possible explanations is a matter for argument.

On the inside of the shell there are two membranes. These are the first undisputed barriers to bacteria (Parsons, 1982). They each consist of microscopic fibres and they resemble anti-bacterial micropore filters when greatly magnified (Fig. 2); nevertheless, they are not a perfect barrier. Occasionally pores have been observed through them. The best evidence for the fallibility of the shell membranes is that the hen devotes much metabolic energy to the largest part of the egg, the egg white or albumen, which is essentially an anti-microbial gel. It is doubtful if such a large albumen would be necessary if the membranes kept out all microbes.

The *albumen* is an important anti-microbial barrier in the egg, although by itself it would probably not be effective. The anti-microbial properties of the albumen are of biological and medical interest because albumen is an example of passive resistance, by contrast with the immune systems of higher animals that resist microbes by actively synthesising appropriate substances (antibodies, etc.) when needed. Synthesis does not take place in the albumen, as far as is known. Therefore all the anti-microbial substances must be in place by the time the egg is laid. Albumen probably functions in four ways; (i) by immobilising bacteria, (ii) by killing certain bacteria, (iii) by denying nutrients to bacteria, and (iv) by inhibiting enzymes. These are discussed separately.

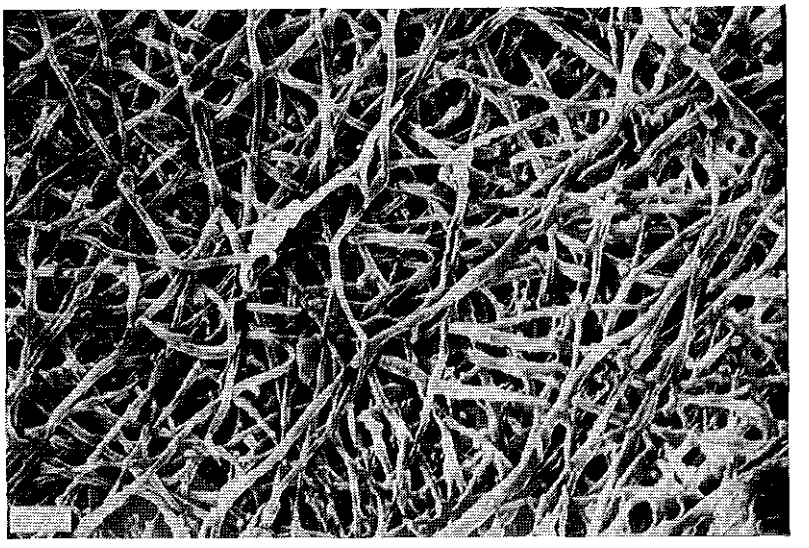
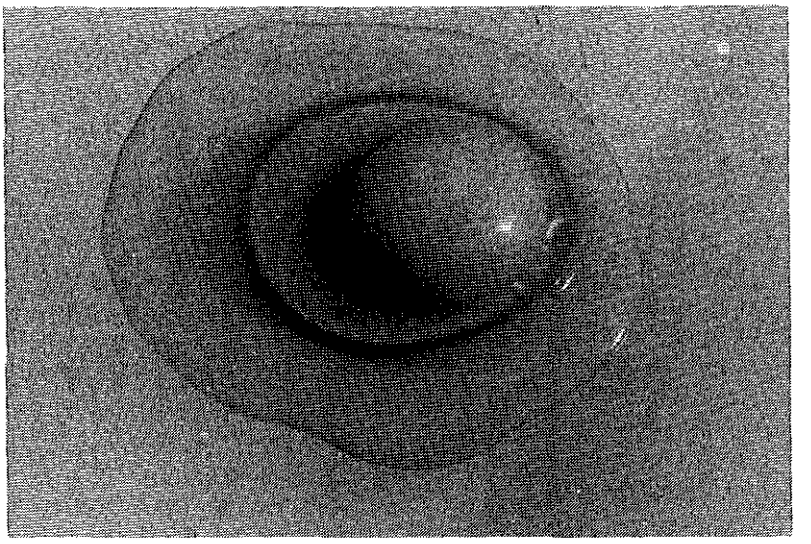


Fig. 2 Scanning electron micrograph of the shell membrane of a hen's egg. Magnification x300. Photograph by M.L. Landrigan.

Fig. 3 Photograph of a broken out egg of good quality showing the albumen gel.



(i) The Albumen Gel

It is well known that the white of a fresh egg of good quality has a jelly-like appearance (Fig. 3). For the consumer, the firmer the white the better the egg. A glycoprotein, ovomucin, is largely responsible for the structure of this gel. How ovomucin functions and how eggs of better quality can be produced, are part of the Division's research on eggs, to be described later in Food Research Quarterly. The natural functions of the gel are obviously not concerned with the food industry or consumers. They are probably to cushion the yolk and also to prevent the movement of micro-organisms. Immobilised micro-organisms are more easily susceptible to other agents in the albumen. In addition, ovomucin itself has antiviral properties (Gottschalk and Lind, 1949).

(ii) Antibacterial Enzymes

In the albumen, bacteria can be killed by antibacterial enzymes, especially if they have been immobilised on the ovomucin gel. Two such enzymes have been identified. The better known is lysozyme, which acts by lysing the walls of Gram-positive bacteria. Most of the lysozyme is in solution in the white, but some is apparently attached to ovomucin. The other enzyme is N-acetylglucosaminidase, which is active in fresh eggs (Winn and Ball, 1975). It inhibits the growth of Gram-negative bacteria. It is present in small amount and it has not been thoroughly investigated.

(iii) Nutrient-binding Proteins

Egg albumen contains several proteins that bind to essential nutrients, particularly to certain metals and vitamins. What these proteins are doing in the egg has not been firmly established, but it would not be surprising if one of their functions is to prevent microorganisms from growing by lowering the concentrations of metabolites to less than that required to maintain growth. Perhaps the best known protein of this group is avidin which binds tenaciously to the B vitamin, biotin. This binding is among the strongest known in biology. The most plentiful binding protein in albumen is conalbumen, now more logically referred to as ovotransferrin from its analogy to other iron-binding proteins, known as transferrins (Komatsu and Feeney, 1967). Ovotransferrin is about 12% of the protein in hens' egg albumen and it has a high affinity for di-and trivalent iron, and for the multivalent ions of several other metals, such as copper.

In the albumen the binding proteins are not normally saturated with the ligand. Their function is therefore difficult to understand unless they act as specific scavengers for essential metabolites. This is certainly the case for the iron and biotin needed by the hens' embryo which are carried in the yolk by other proteins. In the albumen there are many other binding proteins, besides avidin and conablumin. Most of the vitamins have their own protein. For example, there is a riboflavin-binding protein and a thiamine-binding protein. It is less certain that these act as scavengers, because their binding constants do not approach that of avidin-biotin. It should be noted that the binding proteins are not entirely effective by themselves because some bacteria do not need the metabolites they bind, and others are able to take countermeasures. It is therefore likely that their function is to enhance the effectiveness of other antibacterial agents in albumen.

(iv) Antiproteases

The largest class of protein in albumen consists of the enzyme inhibitors. In particular, it includes the antiproteases, inhibitors of proteases, the proteins that break down other proteins. A list, with the enzymes they possibly inhibit is given in the table. Between them, these proteins could effectively inhibit the action of the main classes of protease. Such an ability would be useful to the egg because microorganisms produce proteases. These break down proteins which can then be digested by the microorganism. Bacterial proteases also disrupt the egg structure.

There is controversy about whether or not resisting microorganisms is the main purpose of the egg antiproteases. A possible alternative function is to help control protein synthesis during early embryonic development. Very recently some evidence bearing on this question has been adduced. Laskowski and coworkers (1987) have studied one enzyme inhibitor, ovomucoid, from the eggs of more than 100 avian species. They found large differences in the amino-acid sequences in the region of the active site of the inhibitor; that is, the region that actually binds to the enzyme and inhibits it. This sequence shows hypervariability a variation

Protease Inhibitors in the Albumen of Hens' Eggs

Inhibitor	% in egg	Enzyme inhibited
Ovomucoid	0.8	Trypsin, chymotrypsin
Ovoinhibitor	low	Trypsin, chymotrypsin, elastin, and others
Ovomacroglobulin	low	Most proteases
Cystatin	low	Sulphydryl proteases
Ovalbumin	4.1	Not known

greater than would be expected from the usual rate of mutation. Such variations suggest that there has been selection pressure for variability (Brown, 1987). A speculative explanation of how this could occur is that the amino-acid sequence of the inhibitor has evolved as a result of attacks by specific bacteria which wiped out the entire species apart from a few individuals whose inhibitor was capable of resisting the bacterial protease. These individuals would then survive and continue the species. The amino-acid sequence of their inhibitor proteins would then become dominant and different from that of other species that did not encounter the same micro-organism. Birds that breed in large colonies would be especially susceptible to bacterial infection and thus would be expected to contribute to the proposed hypervariability. Such a course of events, operating over millions of years, would be difficult to prove. It does, however, explain the variability among species.

It is possible that the most plentiful protein in egg ovalbumin, which is about 60% of the albumen proteins is also an enzyme inhibitor. This follows from a comparison of its amino-acid sequence with those of known inhibitors. Ovalbumin is evidently similar to several non-avian antipro-teases in part of its amino-acid sequence (Hunt & Dayhoff, 1980). Unfortunately, the enzyme that ovalbumin inhibits has not been found. It could be from an organism that is now extinct.

The Vitelline Membrane

This is the last barrier to micro-organisms trying to get to the yolk, which is the main store of nutrients. It consists of two fibrous layers with a much thinner continuous layer in between. As with the shell membranes, the vitelline membrane is a physical barrier. The inner and outer fibrous layers could keep bacteria out because they are compact and relatively strong. The role of the continuous layer is not clear. It has not been isolated or analysed because of its thinness. It has been found at the Food Research Laboratory that, in addition to providing a physical barrier, the outer layer of the vitelline membrane contains an insoluble form of the bacteriocide, lysozyme (Back et al, 1982). This protein is not chemically distinguishable from the lysozyme present in egg white. Furthermore this insoluble lysozyme in the membrane is active against bacterial cell walls.

The Yolk

Microorganisms that have penetrated the egg shell and have surmounted the hurdles provided by the albumen and the vitelline membrane find themselves surrounded by excellent nutrients. Whether the egg has any further defenses is not known. It would be surprising if it did not take some precautions although we are not certain what they are. Hen's antibodies (the gamma livetins) are present in egg yolk, but it is doubtful if they are active there: their purpose is evidently to provide protection to the embryonic blood stream. As with the albumen, the yolk has powerful metal binding abilities. The phosphoprotein, phosvitin, binds most of the yolk iron, for example. Whether or not it has an antimicrobial function is not known.

Any discussion of the antimicrobial behaviour of the egg should emphasise that there has been controversy about the relative importance of the various physical barriers and chemical constituents of the albumen (eg, Tranter and Board, 1982). It is clear though that the physical barriers are important, as are the antibacterial proteins, the antiproteases, and the chelating proteins.

The Anti-oxidative Mechanisms of the Egg

As soon as the egg is laid, and possibly a little before, oxygen and other gases can pass through the pores of the shell and through the shell membranes and dissolve in the albumen. The vitelline membrane does not appear to be a barrier to gases, so oxygen can diffuse into the yolk. As with other biological materials, the egg contains chemicals that are susceptible to oxidation. In particular, there are polyunsaturated lipids that readily oxidise to peroxides that are capable of damaging proteins. In addition, several proteins in the yolk and white contain sulphydryl groups that are liable to oxidation and subsequent formation of insoluble protein aggregates. The egg has obviously evolved countermeasures to oxidation, which are different for the albumen and the yolk.

The Albumen

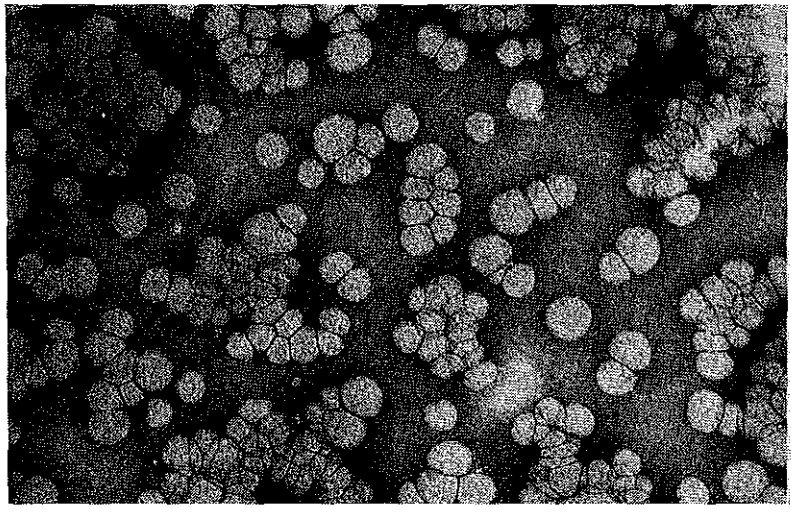
Oxygen has to pass through the albumen to reach the embryo in the initial stages of incubation. The albumen avoids oxidative damage by not having many susceptible chemical groups. The main changes to albumen during storage, such as loss of viscosity, incipient aggregation, and loss of solubility, do not involve oxidation as far as is known. Albumen contains sulphydryl groups, but most of them are well hidden and do not appear to be available to oxygen.

The Yolk

The oxygen-sensitive chemicals of the egg are concentrated in the yolk. Two methods are used in the yolk to prevent oxidation. The first, which is familiar to food technologists, is the presence of antioxidants. The egg contains small amounts of tocopherol, which is probably the main antioxidant. In addition, carotenoids, the yellow yolk pigments, are antioxidants and this may be one of their functions in yolk. It has recently been suggested that the yolk protein, phosvitin, acts as an antioxidant in egg (Lu and Baker, 1986). This property may depend on the ability of phosvitin to bind metal ions, which help catalyse oxidation, so that they do not come in contact with lipid and oxygen. As oxygen is needed for the growth of the embryo, the anti-oxidants must not prevent oxygen from reaching the embryo, which is initially situated on the vitelline membrane, just inside the yolk. It is probable that the embryo gets its oxygen in the earliest stages only from the white. Later the embryo grows an 'artificial lung' on the inside of the shell.

The presence of antioxidants in yolk does not entirely account for its resistance to oxygen. For example, the isolated yolk lipids oxidise more rapidly than the lipids in egg. A possible explanation for the enhanced oxygen resistance of the intact yolk involves the location of the yolk lipid. In egg there is no free lipid. It is all combined non-covalently with special proteins in the yolk in the form of lipoproteins. These

Fig. 4. Electron micrograph of low-density lipoprotein particles of the yolk of a hen's egg.



are submicroscopic particles containing neutral lipid, phospholipid, and protein (Fig. 4). It has been proposed that the compact surface layer of the yolk low-density lipoprotein prevents oxygen from getting to the sensitive lipids, which are on the inside of the lipoprotein particles. A detailed description of this concept has been given previously (Burley et al, 1985).

Conclusion

Evidently an approximate idea of how the egg remains stable before incubation is known. Bacteria are kept out of eggs by means of micropore filters and if they do get in they may be killed or prevented from moving or growing. Eggs also have proteins that prevent bacteria from digesting the albumen. Oxidation is avoided by the presence of anti-oxidants and by excluding oxygen from sensitive regions.

Is any of this information of interest to the food industry? Information about the bactericidal and bacteriostatic proteins in egg white is of immediate interest. In fact, lysozyme has been used as an industrial enzyme on a limited scale for many years. For example, in Australia it is used in cheese making to decrease growth of unwanted bacteria. A great expansion in its use is in prospect. Dilute lysozyme solutions sprayed on vegetables and fruit has been proposed as a method for delaying bacterial growth. Present and proposed commercial uses of lysozyme have recently been well reviewed (Hughey and Johnson, 1987; Proctor and Cunningham, 1988). The other proteins in egg have received less attention. A research project at the Food Research Laboratory involves a study of the large-scale isolation and use of albumen proteins from hens' eggs.

It is possible to predict that other properties used by the egg to enhance preservation will be used by the industry, but at a much later date. Thus in the long term it is possible to imagine the food industry using lipid particles coated with specific proteins that form a surface film impermeable to oxygen. The coating of food with an 'artificial albumen' that excludes bacteria but not gases, is another possibility.

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Further Developments in Adsorption Freeze-Drying[1]

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The development of a novel adsorption freeze-drying process (Mellor and Bell, 1985) arose from a fundamental research project in which it was required to remove water from brain tissues with minimal disruption to their structure and chemistry. Very rapid cooling was used to freeze the water content of small samples into an amorphous ice state, without damage caused by ice crystals (Bell and Mellor, 1984). Freeze-drying offered a means of removing the amorphous ice, with very little change to the material, provided that ice crystals were not allowed to 'recrystallise' during the process. However, since amorphous ice recrystalises at around -70°C, a method of freeze-drying was called for that was both gentle and efficient at ultra-low temperature. Results of experiments with cold desiccants at -20°C resulted in the development of a process which can be applied on a large scale to food processing.

Freeze-drying is a process in which unstable aqueous material is frozen and subsequently dried by sublimation from the frozen state. By this process one can prepare stable, dehydrated products which can be stored at room temperature over extended periods. Thus, the structures and qualities of their biological, nutritional and organoleptic properties can be preserved, provided the dry samples are protected from oxygen, moisture and light.

In conventional methods of freeze-drying, materials are dried in a vacuum chamber at temperatures above -40°C, this being the lowest temperature to which it is practicable to cool refrigeration coils and ensure an adequate vapour pressure driving force. Water as vapour transfers from the subliming ice front in the material, and condenses on the coils. Efficient removal of the water vapour increases the tendency of the water molecules to leave the ice front, and create a vapour pressure driving force which enhances the vapour transfer. The driving force is the difference between the vapour pressures of ice in the material and of ice in the condenser. As ice builds up on the refrigeration coils, they lose their ability to capture water vapour because ice is an effective insulator. Periodic de-icing is necessary to maintain an effective temperature at the surface of the coils, and so sublimation of ice from the product is minimal during defrosting. The product is susceptible to spoilage from thawing during defrosting if the vacuum fails to sustain evaporation of ice from product. This is also a risk which may occur early in the process during pump-down. In addition, if materials do not initially contain sufficient water to allow self-cooling under vacuum, usually down to -15°C or lower, the freeze-drying process must be supplemented by an additional cooling system. This is necessary for many products and further complicates the conventional freeze-dryer.

[1]*Based on a Paper presented at the AIFST Convention, Perth, May 1989.*

The Adsorption Process

The adsorption process depends upon the removal of water vapour by a desiccant, rather than by refrigeration coils. The desiccant creates a high and well sustained vapour pressure driving force, particularly at moderately low temperatures, since the equilibrium water vapour pressure of desiccant decreases as the temperature is lowered. The water vapour does not condense as ice, but is adsorbed into the porous structure of the adsorbent.

Therefore, as water is taken up, the adsorbent's capturing ability diminishes far more gradually than in conventional freeze-drying, since its vapour pressure is not raised by ice build-up. In short, the adsorption freeze-drying process exploits a continuously high vapour pressure driving force produced by cold desiccant. At -20°C the driving force is 0.76 Torr in the adsorption process, compared with 0.68 Torr for ice-free coils at -40°C. By maintaining the desiccant and the product at a prescribed low temperature, many of the engineering complexities (involving balance between vacuum pressure and the heat input to the product which are required to sustain freeze-drying by conventional methods) are avoided. However, heat input to the product will be useful in industrial applications requiring faster drying rates.

The adsorption process consists of a chamber in which the air pressure is reduced and contains a product rack to hold the samples and a perforated container of desiccant, as shown in Fig. 1. The chamber can be fitted with a heater if required.

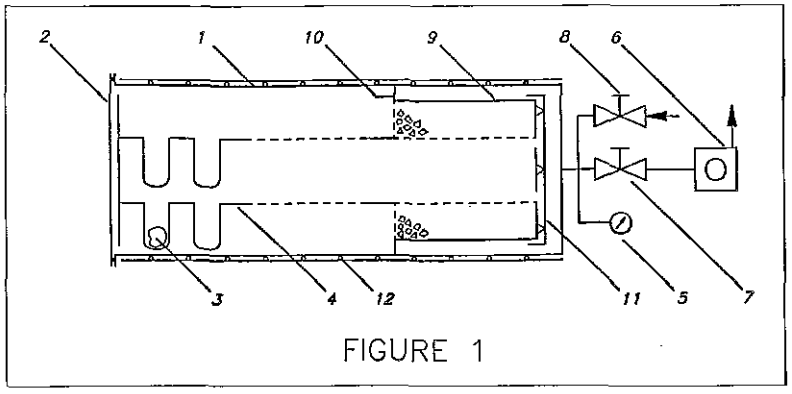
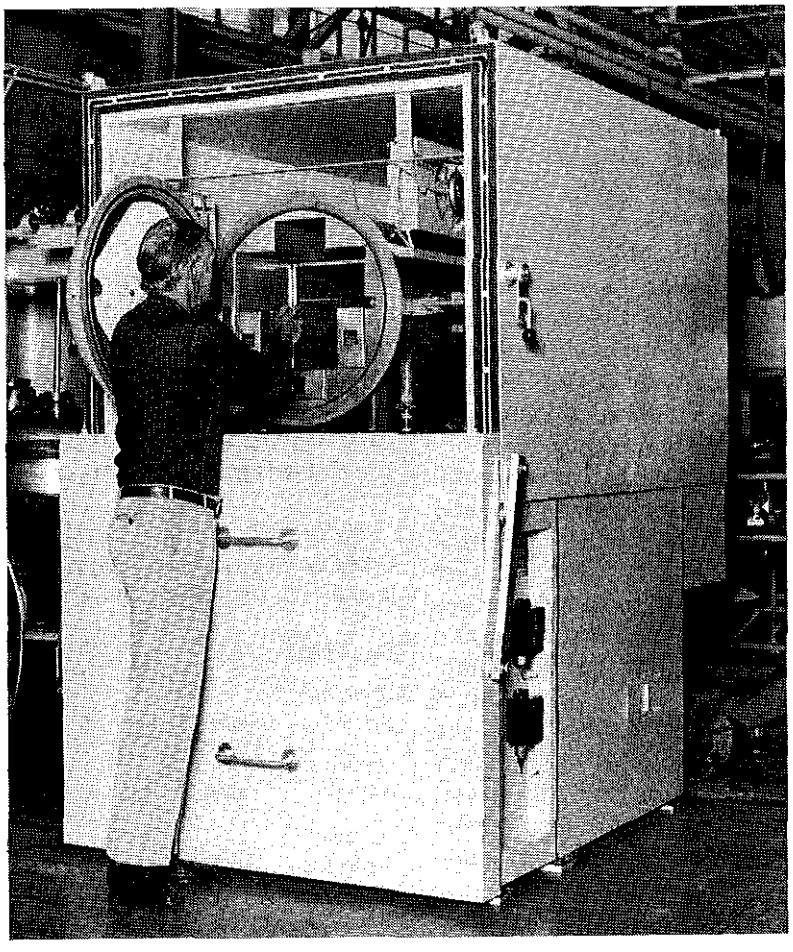


FIGURE 1

Fig. 1: Schematic diagram of original version of the adsorption freezedryer.

Annotations: 1. Vacuum chamber; 2. Cover plate; 3. Product holder; 4. Product rack; 5. Vacuum gauge; 6. Vacuum pump; 7. Shut-off valve; 8. Air admittance valve; 9. Container and desiccant; 10. Perforated end of desiccant container; 11. Baffle plate; 12. Heater and insulation.

Fig. 2: The new 20 kg capacity prototype of the adsorption freeze-dryer, and J.D. Mellor working on the equipment.



The low temperature in the chamber is achieved by cooling the walls of the chamber. A convenient way of doing this is to place the chamber in a freezer store, maintained at a low temperature.

Prototypes

Several small prototypes ranging in capacity from 100 g (Bell and Mellor, 1986) to 3.5 kg have been constructed. A larger prototype with its own refrigeration system operating at a temperature between -10 and -20°C, shown in Figure 2, with a product capacity of 20 kg is located at the Food Research Laboratory, Delhi Road, North Ryde, New South Wales. It is a low energy consuming machine operating on 41 kWh. A convection oven, operating intermittently on 8 kWh, would be required for regenerating the desiccant. A conventional freeze-dryer with the same capacity would require 102 kWh since the refrigeration coil with comparable ability to capture water vapour would require between two and three times the energy input to maintain its temperature at -40°C. There is also a saving in vacuum-pumping requirements.

In a large conventional freeze-dryer the system usually requires two vacuum pumps: a large capacity unit for fast pump-down to avoid surface thawing of frozen samples and a small capacity holding pump. Since the chamber and product are held at -20°C, only the holding pump will be necessary for the adsorption freeze-dryer, and savings will result from reduced costs of purchase and maintenance of the vacuum equipment.

The rate of drying of a typical sliced food product (10 mm) in the 3.5 kg and 20 kg prototypes, operating at a fixed chamber temperature of -20°C and pressure of 27 Pa (0.2 Torr), has been found to be 48 h to 60 h. Improvements in drying time can also be obtained by preparing products to maximise surface area, such as by granulating. Further improvements are possible by agitation of the granulated products.

However, in a heated tray system the drying times were reduced to 24 h which is comparable with conventional freezedrying operations. It is therefore feasible to conduct at least five runs per week. In practice, industrial commercial freezedryers seldom run more than 250 days per annum or five days per week, owing to maintenance and operating requirements (Mellor, 1978, p. 253). The turn-around time for loading and unloading products from the adsorption freeze-dryer is less than 30 min. per load. Defrosting, drying the chamber and vacuum pre-testing is not required for the adsorption freeze-dryer because the inside of the chamber remains dry. This reduces the turn-around time as shown in the following comparison:

Operations times (h)	Conventional freezedrying	Adsorption freezedrying
Defrosting	0.50	0
Drying the chamber	0.25	0
Loading & unloading	0.50	0.50
Vacuum pre-testing	0.25	0
Totals	1.50	0.50

The desiccant used in the adsorption freeze-dryer requires regeneration, which can be achieved in a forced-air oven at 120°C for one hour, outside the chamber, using a second batch of desiccant. An automatic loading and unloading system for replacing the desiccant during the drying run has been designed.

As cryogenic fluids become cheaper it could become practical to use these for adsorption freeze-drying at ultra-low temperatures, e.g. -40° to -140°C.

Practice

A total of nine food products have been tested in the various prototypes. These are shown in Table 1.

Most food products were dried in the more practical temper-ature range of -12° to -20°C, and gave very acceptable products. Final moisture consistency in cheese starter cultures was a problem as the dried product was too hygroscopic and required careful dry handling after processing.

The quality of each freeze-dried product is a function of its physico-chemical nature, its method of preparation and the rate of initial freezing of the product. For example, peas have to be punctured and blanched, strawberries have to be sliced or pulped. Product quality also depends on moisture content as well as oxygen uptake during storage.

After freezedrying, a uniform moisture content throughout a batch can be obtained by storing it in a large sealed drum for 24 hours, prior to packaging. Gas packing with dry nitrogen at atmospheric

TABLE 1

Food Drying Trials In 3 Prototypes of Adsorption Freeze-Dryer

Prototype System (max. kg capac.)		Product	Wet mass (kg)	Moisture loss (%)	Time (h)	Temp (°C)	Product quality
A.	20	Parsley	0.2[4]	-	36	- 12	good
B.	3.5	Abalone	1.0	67 max	18 44	- 19 - 19	good
	3.5	Cheese starter culture	0.5	-	48	- 20	variable
	3.5	Squid (bait)	2.0	max	47	- 18	good
	3.5	Fish: Ling fillets[1]	0.9	max	24	- 15 (tray = 0)	good
	3.5	Strawberry	0.5	39 61 max	24 48 72	- 15	fair
C.	0.3	Liver	0.3	max[2]	72	- 40	excellent
	0.3	Blood (whole)	0.3	max[3]	96	- 40	excellent
	0.3	Brain	0.001 0.003	-	120	- 40 to - 140	excellent

1 deep finned tray, 16 kg/m^2 loading

2 final dry weight moistures = 2.1 to 3.7%

3 final dry weight moistures = 6.3 to 9.7%

4 run with a full load of inedible plant material

TABLE 2
Cost* Comparison: Equipment (20 kg unit)

Cost Component	Conventional Dryer ($)	Adsorption Dryer ($)
Labour	45,000[1]	21,750
Refrigeration system	7,500[2]	750
Vacuum pump	8,250[3]	3,450
Frame	150	150
Cabinet	450	450
Vacuum chamber	7,500[4]	6,000
Heater	1,500[5]	150[6]
Insulation	150	300[7]
Instrumentation	9,000[8]	3,000
25% manufacturer's profit	19,500	9,000
Desiccant and containers	0	1,000
Total cost	99,000	46,000

*Index 1989

1. Greater structural complexity
2. Multiple compression system
3. Two pumps needed
4. Larger, to take refrigeration coil and ice build-up(notwithstanding space needed for desiccant)
5. Heated shelf system
6. Control heater. This item rises with inclusion of a heated tray system
7. Completely insulated chamber
8. Additional instrumentation to operate refrigeration and pumping systems

TABLE 3
Cost* Variations : Processing

	Conventional Freeze-Dryer[1]		Adsorption Freeze-Dryer[2]	
	Pilot[3]	Industrial[4]	Pilot	Industrial
Capacity kg/yr	4000	480000	5000	600000
Total cost, ice removed, $/kg	17.03	1.61	9.34	0.71

*Index 1989

1. Four runs per week
2. Five runs per week
3. Pilot scale = 20 kg unit
4. Industrial scale = 2.4 tonne unit

pressure is preferable to vacuum packing which tends to cause crushing and to enhance migration of water and oxygen into the package.

Costing

Some aspects of costing have been dealt with in the discussion of the prototypes. The major considerations are capital outlay and ongoing processing costs.

Capital cost comparisons between conventional and adsorption freeze-dryers have been calculated for the 20 kg capacity units at current (1989-90) prices. The calculations are based on the analysis of costing (Mellor, 1978). These are shown in Table 2.

Processing costs are summarised in Table 3 for pilot and industrial units. The conventional freeze-dryer would be run for four days a week, because of maintenance and extra loading time required. Much of the cost saving in the adsorption system is due to reduced energy use and labour savings on maintenance and loading, as demonstrated above. Further information will be obtained from future research and development using the 20 kg pilot plant, and this will assist in projecting the costs for large industrial plants, including the cost of the desiccant considered as a running cost.

The initial cost of the desiccant (e.g. silica gel) is unavoidable, but when considered over the life of the material involving a very large number of regenerations, possibly around 1000, the cost per run is negligible. The cost of regeneration of the desiccant could be reduced by using waste heat from an industrial plant.

Conclusions

The adsorption freeze-drying process achieves the following:

- Sublimation of ice in the product to vapour and its removal, both at the same temperature, which can be controlled at any desired level.
- Removal of water vapour by a cooled adsorbent, whose equilibrium vapour pressure is lower than that at the surface of a conventional refrigeration coil.
- An enhanced vapour-pressure driving force.
- Low temperatures which are maintained throughout the drying process and until the product is almost completely dry.
- Equipment costs for the freeze-drying plant will be about half that of a conventional system.
- Adsorption freeze-drying will require less general maintenance and 'down time' than conventional systems.
- The energy required to maintain the cold environment in which the vacuum chamber is contained will be less than that required to drive the refrigeration coil in a conventional freeze-dryer.
- Cost savings will also be obtained in production of the vacuum.
- There will be less danger of the material thawing during the vacuum pumpdown.

Adsorption freeze-drying aims to be a failsafe, dry and constant low temperature process. It presents an opportunity to the Australian food industry to replace its imports of freeze-dried foods and to enter the export market with value-added products with a competitive edge in price and quality.

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Sensory and Other Methods for Assessing Salami Quality

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Introduction

Many sensory and objective methods have been developed and used for the assessment of meat quality (Szczesniak and Torgeson, 1965; Harris *et al.*, 1972; Bouton *et al.*, 1975; Harris and Shorthose, 1988). Manufactured or restructured meat products are much more complex and have many different sensory characteristics or attributes which require multicomponent sensory profiles to describe and quantify them (Berry, 1987). Sensory profiles have been produced for products such as catfood (Jones *et al.*, 1989a), sausages (Jones *et al.*, 1989b), beefburgers (Dransfield *et al.*, 1984) and ham (Nute *et al.*, 1987).

An increasing interest in manufactured meat products has, in turn, increased the need to develop suitable sensory and objective methods to evaluate and define the quality attributes of several products, including restructured (and comminuted) meats, and sausage type products, e.g. frankfurters and salamis.

There has been little of work published on the objective assessment of salamis. In one study (Ziegler *et al.*, 1987) two methods were used. Salami samples 2.6 cm outside diameter by 1 cm high salami samples were subjected to i) two compression-decompression cycles to 25% of original height and ii) five compression-decompression cycles to 80% of original height. However, the type and size of the plunger/compression plate was not specified (Ziegler *et al.*, 1987) making it difficult to repeat these methods. Other workers (Berry *et al.*, 1979) used the Slice Tenderness Evaluator (previously used on cooked pork meat samples by Kulwich *et al.*, 1963) and single blade shear (used on ground beef patties by Gross *et al.*, 1978) to evaluate salami. Skjelkvale *et al.*, (1974) measured the force required to push a 30 mm O.D. plunger 90% of the way into a 20 mm thick salami slice.

In this present paper the development of a sensory profile for salamis is described and compared with some objective methods. The technique used to produce the multi-attribute sensory profile is based on the new technique of Free Choice sensory profiling (Jones *et al.* 1989a).

Materials & Methods

Materials

A survey of the Brisbane area showed that there were a large number of different salamis readily available from large manufacturers and from relatively small specialised ethnic butchers and smallgoods manufacturers. Initially, as many different salamis and salami types as possible were acquired and tested with the aim of obtaining a wide range of textural and other properties. Of these, 12 were selected, based on textural differences and on general availability.

The 12 selected salami types were analysed for percent moisture, fat and protein contents using the standard AOAC (1975) methods.

Sensory panel

Twelve panelists (all with experience in meat texture and

flavour work) who liked salami were asked to attend a tasting of the 12 salami type (presented as 3 mm thick slices and coded). At this meeting, they were asked to produce a list of descriptors that they felt fully described the characteristics of the salamis. They were not restricted to texture alone and could, if they felt it appropriate, include appearance, aroma and flavour factors. As well as deciding on appropriate attributes they were also asked to provide words defining the upper and lower limits of the range of these attributes. This technique is called Free Choice profiling (Jones *et al.*, 1989a).

A second session was held where the panelists were asked to assess six of the salamis (in individual sensory booths under daylight conditions) to finalise their individual descriptive profiles. In this session they were allowed to add extra attributes and omit existing attributes if appropriate.

After these two preliminary sessions, the panelists were asked to use their individual profiles and score all 12 samples (six per session) presented in random order at four taste panel sessions. In this way they gained experience in the use of their individual profiles.

After completion of these six preliminary sessions, where they used their individual profiles, the panelists met as a group. At this meeting, each panelist went through his/her profile explaining what he/she considered each of the attributes or descriptors in their profile meant. After each of the panelists had explained their individual profile there was general discussion within the group regarding the relevance or applicability of the various attributes. From this general discussion a consensus or Fixed Choice profile was established.

This consensus profile was used to quantify the attributes of the 12 salamis at eight sessions (four per week) at which each of six different salamis were assessed. Each panelist was located in an individual booth under daylight conditions. Each salami (presented as two 3 mm thick slices) was given a three-digit code and presented singly according to a balanced incomplete block design (Plan SR22, Bose *et al.*, 1954) so that over the eight sessions each salami was compared with every other salami once.

The attributes were grouped in an agreed logical order of assessment, viz. appearance, aroma, texture and flavour. They were rated by working on an unstructured line or scale with end points defined by consensus. The assessors' ratings for each attribute were coded for analysis by measuring the position of the mark on the line (0-100). Most of these assessments were carried out on a computer system which registered each panelists rating directly as a number.

Objective methods

The four methods evaluated were:-

a) Method I: a 10 cm thick slice of salami, generally with a diameter of 5-6 cm, was subjected to a double 80% compression cycle using a 6.3 mm O.D. plunger. This method was identical to that used at this laboratory for meat (Bouton and Harris, 1972).

b) Method II: 28 mm O.D. cores were cut from 20 mm thick salami slices and compressed 80% under a 65 mm O.D. flat plunger.

c) Methods III and IV: These were both punch tests requiring 20 mm thick slices of salami. Method III used a 24 mm O.D. plunger with a 28 mm I.D. plate, while Method IV used a 6.3 mm O.D. plunger and a 6.5 mm plate. In essence these methods measured the force required to force a plug of material through the hole slightly larger than the plunger.

Three parameters were measured from the force-deformation curves obtained using Method I viz. Hardness or the height of the first peak in kg, Cohesiveness which was the ratio of the work done on the second penetration to the work done on the first penetration and Chewiness which was the product of hardness and cohesiveness. For Method II only the bioyield point (or initial yield) and peak force were measured. Peak forces were measured for both punch tests i.e. Methods III and IV. All measurements were carried out at room temperature (23 ± 2°C).

Measurements of pH and colour

The pH of each salami was measured at room temperature (23 ± 2°C) using a Phillips C64/1 combined electrode with a Townson expanded scale metre. The colour of each sal-

ami was measured using a Minolta Chromometer CR-200. Colour measurements were carried out on a fresh cut surface allowed to bloom, i.e. exposed to air/oxygen for one hour without dehydration. Nine measurements were carried out on each salami - three per slice.

Statistical methods

Analyses of variance were carried out, using the GENSTAT statistical programming system (GENSTAT 5, Copyright 1987, Lawes Agricultural Trust, Rothamsted Experimental Station), to establish standard errors and, hence, least significant difference (LSD) values at the $P<0.05$ level. Principal component analyses were used to reduce the dimensionality of the sensory attribute space.

Results & Discussion

Composition of Salamis

Table 1 shows the percent moisture, fat and protein contents of the 12 salamis. Moisture contents range from 34.7 to 47.5, fat from 23.9 to 36.6 and protein from 15.0 to 25.2. The pH values (Table 1) range from 4.80 to 5.88. There were three different Veneto (nos. 5, 8 and 12) and two different Hungarian (nos. 3 and 7) salamis in the list. Of the Venetos only nos. 5 and 8 were similar in composition while the Hungarian salamis were quite different from each other in composition, i.e. fat content for no. 3 was 32.4% compared with 23.9% for the other.

The percent moisture of any salami will depend on how long it has been aged or conditioned. All these salamis were acquired fresh, i.e. ready for sale and had not, therefore, dried out by prolonged hanging.

Sensory profile

The attributes agreed upon by the panelists have been shown in Table 2 listed in the agreed order of assessment. The profile started with the appearance and aroma attributes, which were assessed before mastication. The attributes for texture and flavour were assessed during mastication. Each attribute was scored (0-100) by either making a mark on a 10 cm long line or directly entering the score onto a computer. The ends or anchor points of each line were labelled as shown in Table 2. Colour was primarily decided by how light or dark and red each sample was. Particle size reflected the apparent coarseness of the structure while fat content covered the obvious fat particles or areas on the surface of each salami. An overall assessment of the acceptability of the appearance was included.

The panelists considered that there were three basic odour attributes, viz. acid/sour, off and smoky. Texture was considered to have three attributes - the first being an assessment of the initial bite or adhesion properties, the second measured how easily the sample could be reduced or masticated to a state ready for swallowing. Greasiness/oiliness was the third textural attribute and was regarded as a measure of the influence of fat or texture, as it was released during mastication. Flavour was possibly the most difficult to both define and assess, but the panel decided that there were four predominant attributes, viz. acidic, spicy, 'off' and smoky. Acceptability ratings for appearance, aroma, texture and flavour were included as separate entities and an overall rating for acceptability was also included.

Results obtained using the Sensory Profile

The mean values obtained for each of the sensory attributes and for each of the 12 salamis after analysis of variance have been listed in Table 3, together with the appropriate LSD values. There were significant differences ($P<0.05$) between most salamis for all attributes so that the panel was able to discriminate between the individual salamis. Salami nos. 1 and 7 had less obvious visual fat content than most of the others (N.B. only 7 and 11 had comparable 'fat content' values in Table 3), but salami no. 1 also appeared to have finer fat particles than any of the others. However, the chemical analysis of no. 1 showed that it had the highest fat content (Table 1). The fat was, therefore, evenly distributed throughout the finer particulate structure of this salami, leading to salami no. 1 being considered the palest of the salamis. The acceptability in appearance of salami no.1 was rated as significantly lower than the other salamis (Table 3). The correlations between the various attributes and the acceptability ratings of the salamis have been listed in Table 4. Apparent fat content and particle size were highly related ($r=0.83$, $P<0.001$) and acceptability of appearance was significantly related to odour ($r=0.66$, $P<0.05$).

The aroma attributes covered a small range for both acid and 'off' aromas (i.e. 38.0 to 56.0 for the former and 14.4 to 33.2 for the latter). According to the correlation results in Table 4 'acid and off aroma' were correlated ($r=0.63$, $P<0.05$) but 'off aroma' was highly correlated with acceptability ratings for aroma ($r=-0.89$, $P<0.001$), flavour ($r=-0.82$, $P<0.001$) and overall acceptability ($r=-0.78$, $P<0.01$). This meant that an unusual aroma, which could be considered 'off', drastically reduced acceptability.

The main textural attributes, while nominally quite different (i.e. initial bite or adhesion rated the ease with which a slice of salami breaks up, but chewiness measured/assessed the toughness of the material when broken up during mastication), were highly correlated ($r=0.96$, $P<0.001$). Neither was significantly related to 'greasiness'. Greasiness was, however, highly correlated with 'off aroma' ($r=0.84$, $P<0.05$) and with all the acceptability ratings except for appearance.

The flavour attributes had no significant relationships with appearance, but off flavour was related to off aroma ($r=0.97$, $P<0.001$) and greasiness ($r=0.76$, $P<0.01$). Smokiness was inversely related to 'off aroma' ($r=-0.87$, $P<0.001$), greasiness ($r=-0.80$, $P<0.001$) and 'off flavour' ($r=-0.88$, $P<0.001$). Aroma and flavour acceptability was inversely related to off aroma and off flavour and directly related to smokiness and 'spiciness'. Textural acceptability was inversely related to off flavour, off aroma and greasiness. Overall acceptability was clearly more strongly influenced by off flavour, off aroma and spiciness than by any other attributes.

The relationships between the acceptability ratings (Table 5) indicated that appearance was a minor factor when compared with flavour. However, since appearance was an attribute likely to influence purchase, it should not be ignored.

Principal Components Analysis

Principal components analyses were carried out on the sensory attributes and on the hedonic (acceptability) assessments. The results have been shown in Fig. 1 (sensory attributes) and Fig. 2 (acceptability ratings). The vector loadings for the first three principal components have been shown in Table 6 for the attributes. The first component accounted

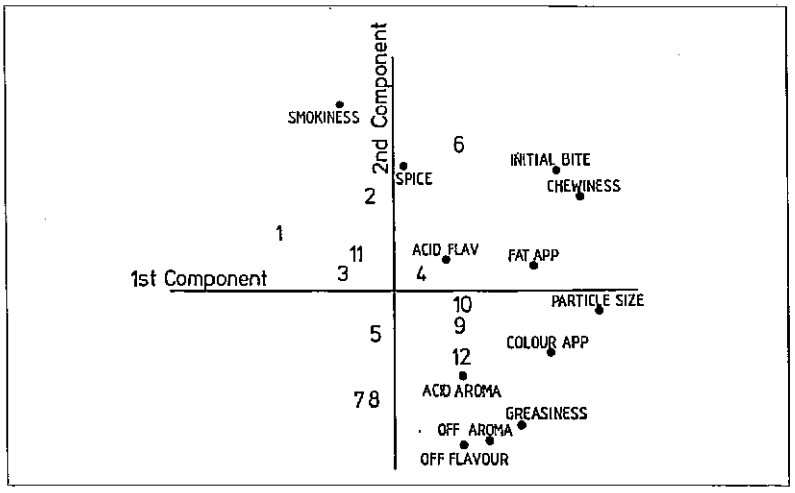
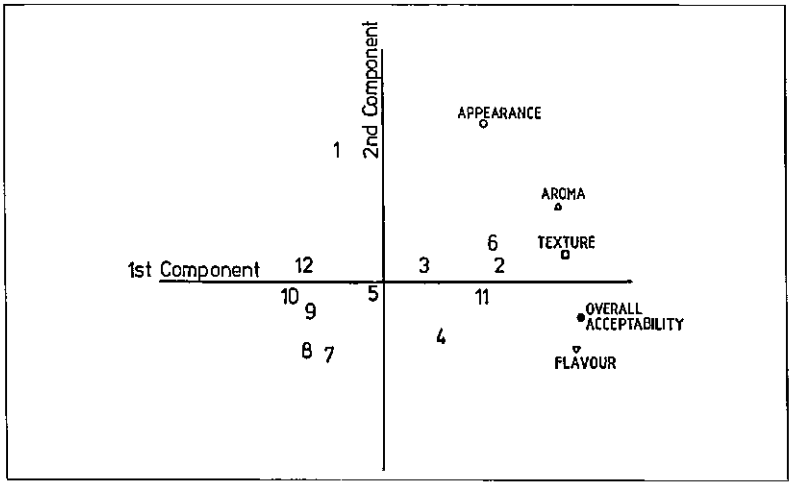


Fig. 1. Attributes of the sensory profile and the 12 salami types plotted relative to the first two components of the Principal Components analysis.

Fig. 2: Acceptability ratings of the sensory panel and the 12 salami types plotted relative to the first two components of the Principal Components analysis.



for 51.6% of the total variance and appeared to be associated with appearance attributes (colour and particle size), and textural attributes (initial bite) adhesion and chewiness. The second component accounted for 19.9% and was mainly associated with flavour attributes (spice, off flavour and, especially, smokiness) as well as with the textural attributes, but contrasting with greasiness. Smokiness contrasted with all other attributes. Off flavour contrasted with spice and, particularly, smokiness. The third component accounted for 9.6% and was predominantly associated with appearance (colour, particle size and fat content). The total variance accounted for, with just three components, was 81.1%.

The loadings of the attribute on the first two principal axes (71.5% of total variation) have been listed in Fig. 1. The coordinates for each attribute were determined from the magnitude of the correlations relative to the first and second axis. The positions of both the attributes and the samples relative to the first two axes have been plotted in Fig. 1. This allowed a direct comparison in terms of sample attributes and complemented the data presented in Table 3. As an example salami no. 2 was lower than salami no. 12 in off aroma and flavour but higher in spice and smokiness. Salami no. 12 was also greasier than no. 2. Salami no. 1 was quite different from the other salamis in all the appearance factors.

The result of the principal components analysis of the acceptability ratings has been shown in Table 7 and Fig. 2. The vector loadings in Table 7 indicate that the first principal axis accounted for 76.5% of the total variance and was strongly associated with aroma, texture and flavour acceptability and with overall acceptability rather than appearance. The second axis accounted for 16.0% and was primarily associated with appearance, with aroma and flavour contrasting with the other three. The third axis (4.2%) was associated with aroma and texture, with appearance and aroma contrasting with texture, flavour and overall acceptability. The results in Fig. 2 showed that the most acceptable salamis were nos. 2, 6 and 11. Liking was apparently unidimensional and was related principally to the texture, aroma and flavour of the salamis. Salami no. 1 was the exception largely because of its different appearance.

Objective measurements

The relationships between results obtained using the sensory attributes, and the various mechanical, chemical composition, pH and colour measurements carried out, have been shown in Table 8. None of the mechanical methods showed particularly high relationships with any of the textural attributes, although peak force values from Method II significantly ($P<0.01$) correlated with initial bite/adhesion and chewiness. These poor relationships were probably due to several reasons including that the salamis were all fresh (preliminary work showed that quite large physical changes occurred as the salamis dried out during hanging).

The percent moisture, fat and protein also showed few high correlations. High protein content related ($r=0.73$, $P<0.01$) with appearance factors such as colour and particle size and to textural attributes. Percent fat was inversely related to acid aroma.

Colour measurements had few significant correlations - most were with flavour attributes. The pH values were inversely related to smokiness.

The relationships between the acceptability ratings for appearance, aroma, texture, flavour and overall acceptability have been listed in Table 9. Percent fat content was inversely related to acceptability of appearance, i.e. the higher the fat content the less appealing in appearance. The colour measurements seemed to reflect the acceptability ratings. An increase in the amount of reflected red and yellow light (viz. higher 'a' and 'b' values respectively) related to whatever was increasing aroma, texture and flavour acceptability. An increase in L (greater L values equated with lighter or paler colour) led to a decrease in the acceptability of appearance. The chroma or colour saturation of the samples (a2 + b2) related well ($P<0.01$) with all the acceptability ratings except for appearance.

The pH values were poorly related to appearance, texture and overall acceptability but increasing pH tended to decrease aroma ($r = 0.56$, close to significance at $P<0.05$) and flavour ($r=-0.61$ $P<0.05$).

Using the sensory profile to assess different products

As an illustration of the prac-

tical application of the sensory profile developed for salamis, a direct comparison of Danish salamis from four different manufacturers or manufacturing methods was carried out. They were tasted in the same way as the other salamis (i.e. 3 mm thick slices) and under the same test conditions. Each salami was tasted four times at four separate sessions with the order of tasting randomised. The profile was modified by substituting 'saltiness' for 'smokiness' because the level of smoking was similar over the four salamis. The results have been listed in Table 10. Salami no. 1 was the leading brand and salami no. 2 was the next highest seller.

In appearance there was a) little difference in colour although no. 3 was significantly darker than no. 4 ($P<0.05$); b) salami no. 2 had finer particles than any of the others; and (c) no. 4 had the highest obvious fat content. Overall, no. 4 was rated the least acceptable in appearance.

In aroma, no. 2 had a significantly higher acid aroma than nos. 1 or 3 ($P<0.05$) but was not different from no. 4. In terms of 'off aroma', no. 4 had significantly higher values than no. 1 ($P<0.05$). Acceptability was significantly ($P<0.05$) higher for no. 1 than for nos. 3 and 4.

In texture, no. 4 was significantly lower in initial bite/adhesion and chewiness but significantly higher in greasiness. Salamis nos. 1 and 2 had significantly ($P<0.05$) more acceptable textures than nos. 3 or 4 and no. 3 was better than no. 4.

Acid flavour was lowest for no. 3, while spice flavour was lowest for no. 4, which also had the highest off flavour rating. Salami no. 1 was considered saltier than nos. 3 or 4. Flavour acceptability was significantly higher for salami no. 1 than for the others.

Overall acceptability followed the acceptability ratings for aroma, texture and flavour with no. 1 being ranked as significantly better than no. 2, which was, in turn, rated as significantly better ($P<0.05$) than nos. 3 or 4.

The results in Table 10 show how the salami profile can be used to compare a new product such as no. 3 with competitors in say nos. 1, 2 or 4. It was possible to say (from the comparisons in Table 10) that the aroma and flavour of no. 3 would need to be improved if it was to compete with the market leader, Salami no. 1.

Conclusions

A sensory profile for salamis has been developed using the technique of Free Choice profiling. However, rather than using Free Choice profiling per se, the panelists converted their individual sensory profiles into a consensus or Fixed Choice profile. This was achieved by each individual going through his/her profile and explaining what they meant by each descriptive term they used. After discussion, the entire panel then agreed on a profile which they believed reflected their individual profiles. This method for arriving at a complex profile has been used for salamis, but can be used for other meat products of varying kinds.

The mechanical measurements tried did not appear overly successful in discriminating between samples probably due to other factors being perceived as more important. Colour and chemical measurements such as pH, % protein, % fat and % moisture were useful for product specification. The mechanical measurements would be more suitable for looking at the effects of conditioning or other treatments where loss of moisture etc., would lead to an increase in hardness or toughness.

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TABLE 1
Percent moisture, fat, protein and pH in the 12 samples of salami used in sensory panels.

Sample No., type and manufacturer[a]			Moisture	Fat	Protein	pH
1	Copenhagen	A	41.8	36.6	15.0	4.80
2	Danish	B	44.5	31.2	18.0	4.84
3	Hungarian	B	39.6	32.4	20.4	5.23
4	Milano	B	41.8	26.9	22.0	5.23
5	Veneto	B	46.5	29.6	17.5	5.28
6	Csabai	B	36.6	35.8	21.4	5.01
7	Hungarian	C	47.5	23.9	21.0	5.34
8	Veneto	C	45.2	32.7	17.2	5.69
9	Cacciatori	C	34.9	33.5	25.2	5.88
10	Sopressa	C	45.2	28.7	22.0	5.23
11	Pizza	D	43.5	29.2	20.8	5.24
12	Veneto	E	34.7	33.9	23.7	5.21

[a] Manufacturers represented by A, B, C, D or E.

TABLE 2
Sensory Profile derived for salamis

Appearance	1. Colour; Light - Dark 2. Particle Size; Fine - Coarse 3. Fat content (%); Low - High 4. Acceptability of Appearance; Very Poor - Very Good
Aroma	1. Acid/Sour; Very Weak - Very Strong 2. Off-Aroma; Very Weak - Very Strong 3. Smoke; Very Weak - Very Strong 4. Acceptability of Aroma; Very Poor - Very Good
Texture	1. Initial Bite/Adhesion; Mush - Very Firm 2. Chewiness; Very Tender - Very Tough 3. Greasiness/Oiliness; Low - High 4. Acceptability of Texture; Very Poor - Very Good
Flavour	1. Acidic/Sour/Tang; Low - High 2. Hot/Spice; Low - High 3. Off-Flavour; Low - High 4. Smoke; Low - High 5. Acceptability of Flavour; Very Poor - Very Good
Overall Acceptability of Product	Very Poor - Very Good

TABLE 3
Values obtained for the appearance, aroma, texture and flavour attributes and ratings for all 12 salamis

Attribube	Salami identification number												
Appearance	1	2	3	4	5	6	7	8	9	10	11	12	LSD[a]
Colour	18.5	56.5	40.1	73.0	47.6	67.7	81.6	61.9	71.4	58.0	52.1	77.5	11.3
Particle size	12.0	36.0	37.7	58.1	60.2	81.9	34.2	55.1	77.9	82.4	36.8	82.3	7.3
Fat content	43.2	59.6	56.0	52.2	52.4	68.2	38.5	61.6	67.2	68.1	49.5	67.4	12.5
Acceptability	30.1	56.6	50.4	64.9	63.0	54.2	50.6	52.5	51.7	48.8	61.0	47.7	10.3
Aroma													
Acid/sour	40.7	42.3	46.8	56.0	43.0	38.0	50.7	41.4	44.8	54.0	45.5	48.4	7.1
Off	16.2	14.4	21.6	25.7	26.2	15.2	26.1	25.2	28.0	33.2	16.1	33.0	6.6
Acceptability	52.5	65.8	56.0	53.0	51.6	69.5	48.5	49.5	42.2	41.0	63.3	44.9	10.0
Texture Initial bite/adhesion	39.8	60.0	44.6	66.1	39.6	78.0	40.5	29.5	67.8	64.0	51.6	58.2	13.1
Chewiness	31.4	53.0	36.8	56.7	38.8	74.3	33.1	25.8	63.0	67.1	43.1	62.2	13.1
Greasiness/ oiliness	34.7	42.2	40.1	44.2	51.4	38.8	45.8	56.8	52.7	55.9	43.6	64.7	7.6
Acceptability	46.6	64.7	60.6	59.9	51.0	64.3	52.5	48.3	50.6	48.3	67.0	41.9	9.2
Flavour													
Acid/sour	44.2	54.0	62.2	60.7	47.4	54.7	49.1	48.2	42.2	65.1	49.5	47.3	8.1
Hot/spice	48.2	62.0	58.3	61.1	58.8	63.6	36.8	47.8	39.3	54.9	71.4	46.7	7.4
Off	17.2	12.3	21.8	22.6	25.9	13.5	26.9	24.8	27.0	32.6	15.0	28.7	7.6
Smoke	51.9	52.7	40.0	34.4	26.2	62.8	24.1	25.6	28.5	41.4	24.3	24.3	10.1
Acceptability	50.8	71.4	58.1	58.6	49.1	66.3	39.5	40.0	39.6	37.3	66.7	42.7	8.1
Overall Acceptability	44.9	69.1	56.8	59.8	50.6	64.0	40.1	40.5	40.7	37.2	68.0	37.2	9.1

[a] Least significant different at $P<0.05$ level

TABLE 4

Correlations between the various sensory profile attributes and acceptability ratings for appearance, aroma, texture, flavour and overall Sensory attribute number

Sensory attributes		1	2	3	4	5	6	7	8	9	10	11	12
Colour	1	1.00											
Part	2	0.60	1.00										
Fat	3	0.31	0.83	1.00									
Acid	4	0.40	0.18	-0.11	1.00								
Off	5	0.47	0.60	0.31	0.63	1.00							
In.bite	6	0.41	0.61	0.58	0.16	-0.01	1.00						
Chewiness	7	0.45	0.78	0.72	0.17	0.19	0.96	1.00					
Greasiness	8	0.52	0.66	0.51	0.30	0.84	-0.04	0.19	1.00				
Acid	9	0.03	0.17	0.20	0.53	0.09	0.32	0.30	-0.14	1.00			
Hot	10	-0.29	-0.05	0.08	-0.11	-0.52	0.27	0.20	-0.38	0.46	1.00		
Off	11	0.36	0.48	0.19	-0.59	0.97	-0.15	0.04	0.76	0.06	-0.60	1.00	
Smoke	12	-0.41	-0.31	-0.04	-0.52	-0.87	0.35	0.20	-0.80	0.16	0.54	-0.88	1.00
Appearance		0.66	0.13	-0.12	0.44	0.09	0.15	0.07	0.11	0.26	0.23	0.02	-0.26
Aroma		-0.21	-0.31	-0.11	-0.51	-0.89	0.20	0.04	-0.67	0.13	0.73	-0.93	0.83
Texture		-0.04	-0.21	-0.13	-0.06	-0.67	0.43	0.24	-0.66	0.38	0.73	-0.71	0.64
Flavour		-0.25	-0.31	0.09	-0.32	-0.82	0.31	0.14	-0.67	0.24	0.82	-0.90	0.80
Overall		-0.18	-0.28	-0.11	-0.25	-0.78	0.30	0.12	-0.64	0.27	0.84	-0.85	-0.71

[a] R = 0.58, P<0.05; R = 0.68, P<0.01; R = 0.89, P<0.001

TABLE 5
Correlations between appearance, aroma, texture, flavour and overall acceptability ratings

Acceptability rating factor		Factor identification[a] A	B	C	D	E
Appearance	A	1.0	-	-	-	-
Aroma	B	0.18	1.00	-	-	-
Texture	C	0.47	0.81	1.00	-	-
Flavour	D	0.20	0.93	0.87	1.00	-
Overall	E	0.36	0.91	0.92	0.98	1.00

[a] P<0.05 r = 0.58
P<0.01 r = 0.68
P<0.001 r = 0.80

TABLE 6
The vector loading values obtained for the first three principal components for all 12 sensory attributes

Attribute	Principal component 1	2	3
Colour	-0.387	-0.240	-0.757
Particle size	-0.653	-0.101	-0.384
Fat content	-0.221	-0.058	-0.410
Acid aroma	-0.061	-0.119	-0.037
Off aroma	-0.099	-0.253	0.094
Initial bite	-0.350	0.412	-0.194
Chewiness	-0.436	0.351	-0.066
Greasiness	-0.167	-0.284	0.184
Acid flavour	-0.061	0.057	0.077
Spice flavour	-0.016	0.295	0.034
Off flavour	-0.080	-0.275	0.126
Smoke flavour	0.102	0.559	0.050

TABLE 7
The vector loading values obtained for the first three principal components for the acceptability ratings.

Acceptability	Principal component 1	2	3
Appearance	-0.181	-0.917	0.274
Aroma	-0.406	0.235	0.781
Texture	-0.408	-0.166	-0.523
Flavour	-0.557	0.274	-0.086
Overall	-0.570	-0.025	-0.184

TABLE 8
Relationships between the various objective measurements and the attributes derived from the sensory profile

Measurement used	Seensory profile attributes [a,b] Appearance 1	2	3	Aroma 4	5	Texture 6	7	8	9	Flavour 10	11	12
Method I H	0.16	0.58	0.57	0.10	0.35	0.35	0.53	0.51	0.18	-0.21	0.18	-0.02
Ch	0.24	0.51	0.42	0.52	0.64	0.26	0.46	0.63	0.35	0.02	0.51	-0.32
Method II IY	-0.56	-0.31	-0.21	-0.37	-0.43	-0.06	-0.08	-0.33	-0.22	0.43	-0.48	0.45
IYD	-0.11	-0.58	-0.70	0.09	-0.16	-0.40	-0.48	-0.20	-0.33	-0.05	-0.16	-0.11
PF	-0.13	0.46	0.50	-0.13	-0.18	0.69	0.72	-0.13	0.34	0.64	-0.30	0.53
Method III	0.02	0.56	0.59	0.04	0.35	0.25	0.45	0.50	0.21	0.24	0.21	-0.04
Method IV IY	0.07	0.55	0.53	-0.04	0.25	0.34	0.52	0.31	0.22	0.27	0.27	0.13
PF	0.17	0.69	0.66	0.06	0.41	0.33	0.55	0.57	0.22	0.18	0.29	-0.08
% H_2O	-0.18	-0.47	-0.57	0.17	-0.07	-0.57	-0.58	-0.11	0.16	0.10	0.05	-0.16
% Fat	-0.43	0.10	0.45	-0.72	-0.31	0.31	0.16	-0.11	-0.32	0.01	-0.33	0.45
% Protein	0.73	0.62	0.42	0.43	0.63	0.63	0.37	0.04	0.04	-0.18	0.31	-0.29
L	-0.28	-0.25	0.01	-0.25	-0.12	-0.03	-0.03	0.06	-0.40	-0.42	-0.10	0.17
a	0.22	-0.07	-0.21	0.13	-0.41	0.27	0.16	-0.45	0.53	0.60	-0.46	0.49
b	-0.10	-0.04	0.02	-0.27	-0.59	0.35	0.21	-0.61	0.29	0.55	-0.62	0.70
Hue (a/b)	0.24	0.17	0.04	0.28	0.55	-0.22	-0.06	0.59	-0.10	-0.37	0.56	-0.59
Chroma ($a^2 + b^2$)	0.06	-0.03	-0.08	-0.10	-0.55	0.36	0.22	-0.58	0.44	0.62	-0.59	0.66
pH	0.46	0.41	0.26	0.16	0.56	-0.12	-0.07	0.57	-0.27	-0.48	0.58	-0.74

[a] Sensory attributes are listed in Table 4

[b] R = 0.58, P<0.05; R = 0.68, P<0.0l; R = P<0.001

TABLE 9

Relationships between the ratings for appearance, aroma, texture, flavour and overall acceptability and percent moisture, fat and protein contents as well as colour measurements and pH

			Acceptability rating[a]		
Measurement	Appearance	Aroma	Texture	Flavour	Overall
% Water	0.30	0.02	0.01	-0.06	0.03
% Fat	-0.78	0.18	-0.18	0.14	0.01
% Protein	0.41	-0.23	0.12	-0.14	-0.10
L	-0.61	-0.22	-0.39	-0.18	-0.29
a	0.52	0.68	0.68	0.60	0.64
b	0.03	0.71	0.63	0.63	0.61
Hue (a/b)	0.15	-0.53	-0.46	-0.51	-0.47
Chroma ($a^2 + b^2$)	0.29	0.77	0.71	0.68	0.68
pH	0.30	-0.56	-0.38	-0.61	-0.48

[a] Correlations >0.58 significant at P<0.05
>0.68 significant at P<0.01
>0.80 significant at P<0.001

TABLE 10

Comparison of salamis obtained from either different manufacturers or manufacture

	Product Identification				
Attribute	1	2	3	4	LSD
Colour app	59.8	59.9	65.4	55.8	7.1
Particle size	53.8	42.9	57.0	60.6	8.5
Fat content	55.1	49.2	55.5	64.6	6.8
Appearance acceptability	60.9	63.2	65.1	48.9	6.4
Acid aroma	44.9	53.8	43.8	50.8	7.7
Off aroma	20.2	25.6	23.8	29.9	6.3
Aroma acceptability	65.8	60.4	54.0	53.6	8.3
Initial bite	48.7	62.9	50.0	34.8	6.3
Chewiness	46.4	57.1	48.9	33.4	4.1
Greasiness	43.0	45.8	54.7	60.9	6.0
Texture acceptability	64.6	66.5	58.5	45.9	5.3
Acid flavour	54.0	55.6	43.6	49.4	6.0
Spice flavour	50.1	46.6	51.2	38.7	8.4
Off flavour	22.0	24.1	25.2	28.3	8.1
Salt flavour	57.7	51.2	44.7	48.6	8.3
Flavour acceptability	66.4	57.2	53.3	50.0	8.9
Overall acceptability	67.7	60.4	52.9	46.6	6.9

News From The Division

Death of Dr Alan David Warth

Alan David Warth died suddenly on the 21st June 1990 aged 55. Alan suffered a stroke in 1989 and underwent successful triple by-pass heart surgery in January this year. Unfortunately a blood clot necessitated another operation on the 18th June and a further one on the 20th. He died shortly after the latter.

Alan was born in Christchurch, New Zealand, and graduated from the University of Auckland in 1958 with an M.Sc (1960) in organic chemistry. He joined the CSIRO Division of Food Preservation in 1959 as an experimental officer to work with Dr W.G. Murrell on the chemistry of bacterial spores particularly in relation to their heat resistance properties. Bacterial spores are renowned for their resistance to heat and to many other drastic physical and chemical treatments. They are responsible for determining the process requirements for many foods and the sterilisation treatments for surgical, pharmaceutical and medical materials and equipment. Alan devoted over twenty years of his scientific life to bacterial spores and the mechanism of their heat resistance. The vital germ cell of the bacterial spore, containing all the heat labile DNA and enzymes, is packaged within six to seven layers. Very little was known about their chemical composition and structure when Alan started his research, which involved separation of these layers by physical and enzymic methods and analysing their chemical composition and structure.

His most important contribution to science is probably the elucidation of the structure of the cortex as a peptidoglycan polymer consisting of a backbone of amino-sugar units cross-linked by the tetrapeptide sidechains. One of the alternating amino-sugars was shown to be muramic acid, some moieties of which uniquely contained a muramic lactam ring which reduced the degree of crosslinking compared to the polymer in vegetative cell walls. Part of this work was done during the tenure of a CSIRO studentship with Professor Jack Strominger at the University of Wisconsin and during a post-doctorate year at Harvard University.

Other aspects of Alan's spore research concerned studies on the enzymes involved in the synthesis of spore peptidoglycan and development of two sensitive methods for assaying dipicolinic acid, a unique constituent of bacterial spores. His studies also showed that although the level of heat resistance of spores of the different species varied 100,000-fold the sporulation process itself in individual species increased the resistance over that of vegetative cell resistance by a similar extent in all the species tested.

He showed that the enhancement in heat resistance by the sporulation process could be explained by a reduction in internal a_w of the spore to about 0.73, a level needed to stabilise labile spore enzymes to the same degree as in the intact spore. His theory, 'the anisotopic swollen cortex', may prove to be involved in the reduction of the internal a_w and part of the basic mechanism of achieving heat resistance.

His work on bacterial spores led to his reclassification to Senior Research Scientist (1969) and eventually Principal Research Scientist (1974), to international recognition, several invitations to the International Spores Conferences in the USA and to the European Spore Conferences, and to invited reviews on spores in the Advances in Microbial Physiology Series. He was also an invited participant in the specialist scientific workshops on spores organised under the US-Australia Co-operative Science program.

During the last several years, as a result of changes in the direction of CSIRO research, Alan concentrated on the physiology of microbial cells growing in acid conditions and particularly the mechanism of the action of CO_2 and the very important organic acid preservatives (benzoic, sorbic, propionic). Alan carried out an in-depth analysis of the chemistry of their preservative action and the transport of such acids and ions into cells.

This resulted in the publication of about ten papers, some written since his first illness which largely forced him to give up laboratory bench work. The significance of his contribution in this area has received worldwide recognition.

Altogether Alan published some 40 papers, nearly all on original research findings.

Alan, a member of the Australian Society for Microbiology (MASM), served three years (1973-75) on the NSW Branch Committee as treasurer. He was also a committee member of the CSIRO Officer's Association and served the FRL as group representative in 1974 and 1975. For recreation he enjoyed bushwalking, skiing, gardening and canoeing, and was a member of the Wakehurst Touring Canoe Club, serving as a committee member for several years and as president on two occasions (1984, 1985).

Alan will be remembered for his keen, critical scientific mind and his death will be a loss to science and his many friends here and overseas. It is most unfortunate that his research career should end prematurely.

He is survived by Carol and their three daughters Francelle, Susan and Jeannette.

W G Murrell