M. Rooney

Vol. 38 No. 2 June 1978

# CSIRO Food Research Quarterly

# Prevention of condensation on canned foods in ISO* containers

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## Condensation damage

During the transport and distribution of canned foods, condensation of water often results in damage to cans, labels and cartons.

When a can is wet for more than a few days it rusts; the rust appears first at points where the base-plate steel is exposed, e.g. around the seams, but eventually rusting may become general over the tinplate surface.

Wetting of the printed paper labels on cans causes wrinkling, fading of the inks, and the appearance of a whitish bloom in the varnish; eventually, mould growth on the label and rusting under the label may cause severe discoloration.

The first consequence of wetting a carton is loosening of the flaps; then when the carton is lifted the bottom opens and the contents are discharged. The carton board is also softened by wetting, and tears and breaks readily. When a wet carton dries out again there is usually discoloration in the form of 'bleached' areas and 'high-water marks' because the dyes in the board are water-soluble and tend to migrate. Mould growth may also discolour damp cartons, while on stapled cartons the staples may rust and cause further discoloration. In addition, when damp cartons rub together in a ship, the surface layer of the board may be substantially disintegrated, leading to the condition known as 'chafing'.

Usually, the contents of water-damaged cans remain sound and wholesome, but carton and label damage and rusting make the packages unmerchantable, so that the importer is faced with the costs of repacking cartons and cleaning and relabelling cans.

The aims of this paper are to review the causes of condensation and the nature of the condensation associated with each cause, to analyse remedies that have been tried or proposed, and to suggest procedures that will help to minimize condensation. Although the emphasis is on the prevention of condensation in ISO general cargo shipping containers, which are essentially closed steel boxes of dimensions 7 by 2·5 by 2·5 m, the principles discussed can be applied in any other situation where condensation occurs.

There is no magical cure for condensation, although understanding its origin and taking precautionary measures can reduce it. Under certain climatic conditions complete prevention can be extremely expensive.

## Water vapour and air

Ordinary atmospheric air consists principally of three gases: nitrogen, oxygen and water vapour. The ratio of oxygen to nitrogen is constant, but the amount of vapour is variable. Unlike the situation with oxygen and nitrogen, there is a maximum amount of water vapour that can occur in air at any particular temperature. When air contains the maximum amount of water vapour that it can hold it is said to be saturated.

In Fig. 1 the line for RH = 100% shows, for a range of temperatures, the maximum mass of water vapour (in kg) that can be held in air, per kg dry air. At 30°C, air can hold a maximum of 0·0273 kg/kg whereas at 20°C it can hold only 0·0148 kg/kg. This means that if air, saturated with water vapour at 30°C, is cooled to 20°C, 0·0125 kg of water vapour condenses out as liquid water for each kg dry air. The ability of warm air to hold more water

*International Standards Organization

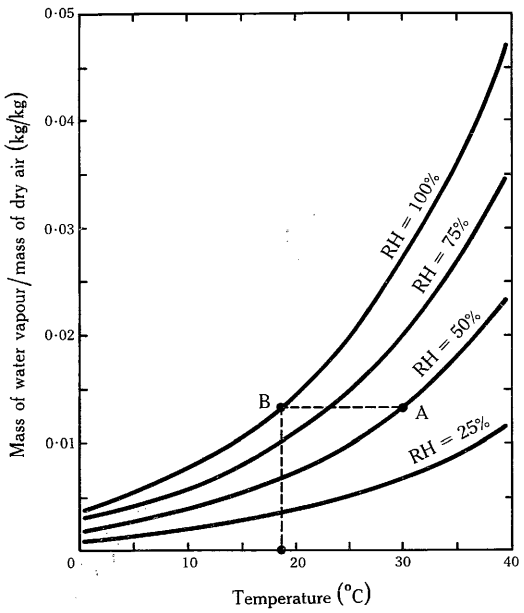



**Fig. 1.** Mass of water vapour/mass of dry air (kg/kg), over a range of temperatures, for moist air at selected values of RH.

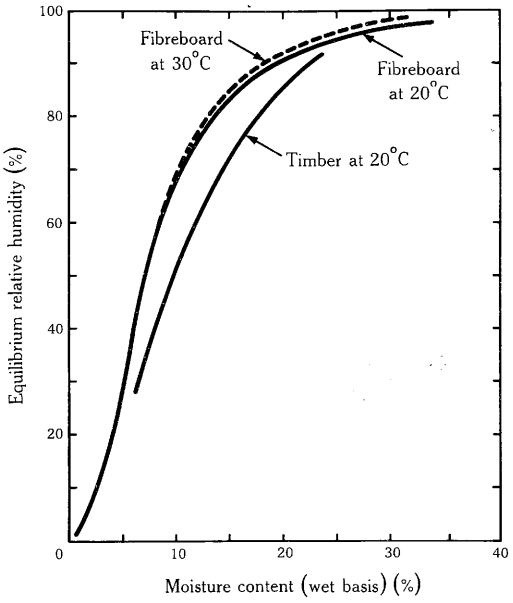



**Fig. 2.** ERH of typical timber at 20°C, and typical corrugated fibreboard at 20°C and 30°C, as a function of moisture content.

vapour than cold air is the basic cause of the occurrence of condensation.

*Relative humidity*

Air that is saturated with water vapour has a relative humidity (RH) of 100%. Air that is not saturated has an RH of less than 100%. The RH of moist air is related to the ratio of the number of molecules of water vapour to the number of molecules of dry air associated with it. The water vapour content of air at various values of RH is shown in Fig. 1.

*Equilibrium relative humidity*

Air can be saturated with water vapour by keeping it in contact with liquid water. Let us consider what happens when we hold air in contact, not with liquid water, but with an absorbent material containing water, e.g. damp timber, cloth, or fibreboard. The water in these materials is bound to them to a greater or lesser extent depending on their moisture content (MC). In general, the lower the MC of a material, the more tightly is the remaining water bound to it. Therefore, when dry air is placed in contact with a material containing water, although the water in the material is not able to saturate the air, some water vapour is transferred to the air, producing an RH of less than 100%. The RH finally attained by air in contact with a material, is called the equilibrium relative humidity (ERH) of the material. The ERH depends on the degree to which the water is bound to the material and hence on both the nature of the material and its MC. Figure 2 shows the ERH of a typical timber at 20°C, and of a typical fibreboard at 20°C and 30°C, as a function of MC. Note that relatively large changes in ERH are produced by small changes in MC but only small changes in ERH are produced by large changes in temperature.

Curves such as those shown in Fig. 2 are called sorption isotherms. For some materials the sorption isotherm taken with increasing values of MC can be different from that obtained with decreasing values of MC.

*Interaction of moisture-containing materials*

When two materials containing moisture are placed in a sealed enclosure, water vapour migrates from the one having the higher ERH to that having the lower ERH until the ERH of the two materials is the

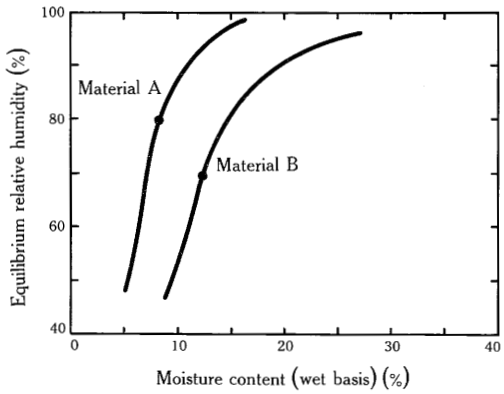



**Fig. 3.** Sorption isotherms of two materials in a sealed enclosure. Water vapour moves from A (ERH = 80%) to B (ERH = 70%), even though the MC of B is > the MC of A.

same. Figure 3 shows the sorption isotherms of two different materials; suppose material A has initially an MC of 8% (ERH of 80%) and B has an MC of 12% (ERH of 70%). Water vapour migrates from A to B and the final ERH of both is somewhere between 70% and 80%, depending on the relative amounts of each material. For these two materials with these initial conditions the water vapour migrates from the one having the lower MC to that with the higher MC. It is not possible to tell from the MC of the two materials which way the water vapour will flow unless their sorption isotherms are known.

*Dewpoint*

If air at 30°C and 50% RH (point A on Fig. 1) is cooled without changing the amount of water vapour in the air, eventually a temperature is reached at which the amount of water vapour in the air is the maximum that the air can hold, i.e. it becomes saturated. The temperature at which this occurs is known as the dewpoint (DP), and it is represented by the temperature corresponding to point B on Fig. 2. Thus, air at 30°C and 50% RH has a DP of 18·6°C.

## Condensation experiment

Consider a metal enclosure containing fibreboard and having the sorption isotherm shown in Fig. 2. Let us suppose that the material is in equilibrium with the air in the enclosure and is producing an ERH of 75% (MC = 11·5%) and that initially the entire enclosure and material is at 30°C. Cool the walls of the enclosure to 20°C. This is below the DP of the air in the enclosure (from Fig. 1, 30°C, ERH 75%, DP = 25°C). The result is a complicated interaction of water movement and temperature change.

- As the temperature of the enclosure is below the DP of the air it contains, water condenses on its inside surface
- The water condensing on the surface tends to raise the surface temperature towards the DP of the air. If the temperature of the enclosure is not maintained at 20°C, the surface temperature rapidly reaches the DP and condensation stops
- If the enclosure is maintained at 20°C, its temperature remains below the DP, so water continues to condense on its inside surface
- This loss of water vapour from the air tends to lower the RH in the enclosure
- As soon as the RH of the air in the vicinity of the material falls below the ERH of the material, water evaporates from it to try to maintain the RH equal to the ERH of the material
- The condensed water on the surfaces of the enclosure is rapidly cooled to about 20°C. If sufficient accumulates, it runs down the walls and drips off the roof onto the outer layers of the material and saturates them. The outer layers are cooled towards 20°C by this water.
- The part of the material that remains at about 30°C still tries to maintain a DP of 25°C, so water vapour from it condenses on any surface having a temperature below 25°C. This means that water now condenses not only on the surface of the enclosure but also directly onto the cool outer layers of the material in the enclosure
- This process continues until the temperature of all the material falls sufficiently. Condensation stops when the DP of the air is equal to the temperature of the enclosure (20°C)
- At this stage, neither the temperature nor the MC is uniform throughout the material. As the temperature of the contents continues to fall to 20°C, both

the temperature and the MC tend to become uniform

- Because no water has been lost from or gained by the enclosure, the MC of the material returns to its initial value of 11·5%
- Ultimately the enclosure and its contents are at 20°C and the MC of the material is 11·5% throughout
- The whole process described above may take many weeks depending on the size of the enclosure, the initial temperatures involved, the nature and quantity of the material containing moisture and the volume of the air space.

## Requirements for condensation

It can be seen from this description that there are three conditions which must exist *simultaneously* before condensation can take place in an enclosure. There must be:

- a source of water vapour
- a temperature difference
- a pathway that enables the water vapour to move from the source to the cold surface.

These requirements apply to any circumstances where condensation can occur, e.g. in warehouses, ships or containers. However, in this review subsequent discussion is directed specifically at condensation on canned foods in ISO containers.

The cause of specific cases of condensation can often be determined by a close examination of the exact pattern of the damage. Some of the more common types of damage are listed in the Table. Owing to the serious financial losses caused by condensation damage, much effort has been expended to reduce it, if not prevent it. Methods of combatting one or more of the three causes of condensation listed have been studied.

### *Sources of water*

The principal sources of water are:

Nature of incidence of condensation and diagnosis of its cause

| Nature of damage | Probable cause |
|---|---|
| Wet, rusty or mouldy cans, top layer of stow | Roof of container has been allowed to get too cold, probably left in the open on a clear night |
| Top and bottom layers and pallets wet | As case 1 but worse. Water has run down walls to pallets |
| Rusty cans at bottom, saturated pallets | As case 2 but condensation has drained to floor level rather than dripping from the roof |
| Rusty cans in top and bottom layers but MC of cartons same as at loading | As case 2 but there has been time for the water to redistribute itself. The cans will be cooler than at loading |
| Outer layers good but central pallet loads damp, mouldy and possibly rusty | Container walls have been too hot, probably caused by standing in hot ambient conditions or in sun. This has heated outer cartons, and water has migrated to the cool inner ones |
| Damage down one side of container only | *a* Many air leaks in that side of container<br>*b* Opposite side of container has been hot—probably stowed near heat source<br>*c* Damaged side stowed near refrigerated space or left in a shaded position |
| Damage near doors observed when doors are opened | *a* Leaking door seals<br>*b* Door end kept cooler than rest of container |
| Water on cartons soon after opening doors | Ambient DP higher than temperature of cans |
| Spotty damage throughout stow | *a* Loose stow and container temperature has varied fairly rapidly and often<br>*b* Free access of ambient air to loose stow |

- liquid water, e.g. water left after cleaning a container
- ambient air
- fibreboard cartons
- paper labels
- water-based glues
- wooden pallets
- timber flooring and wall linings
- punctured cans.

It is interesting to note that all the solid materials that are sources of water vapour are derived from cellulose. There are two ways of attacking the problem arising from having sources of water vapour in the container:

- all materials that contain or release water should be excluded
- the container and contents should be dried to a sufficiently low moisture content before or after loading.

Several special types of packaging materials have been tested in attempts to reduce the amount of water in a loaded container. Such materials, e.g. 'Shrink-wrap', waxed cartons, and plastic carton board are more expensive than normal fibreboard. Each of these has been used in experiments to replace the 760 kg approx. of fibreboard that is commonly used in a container load of cartons of canned goods. However, the fact that condensation can still occur unless *all* the sources of water listed above are simultaneously eliminated, has been neglected and this has led to some conflicting reports on the virtues of particular packaging materials. For example, the cans in a container load of waxed cartons may suffer extensive condensation damage if the pallets, unknown to the shipper, have a high moisture content. Cans in standard cartons in an adjacent container used for comparison may be much less affected if their pallets have a low moisture content. Clearly the greater damage cannot be attributed to the use of waxed cartons! The ideal arrangement would probably be to use unlabelled cans tied with steel or plastic binding straps to metal or plastic pallets in unlined metal or plastic containers. However, the industry is not yet ready for this.

Condensation can be considerably reduced by lowering the moisture content of the various materials that are sources of water vapour, and the amount of water that must be removed can be quite surprising.

Suppose, for example, that we have an ISO container with 150 kg plywood in the walls and floor and that the protective epoxy finish has worn off. Let the MC of this plywood be 15%. Suppose, also, that the container is loaded with 12 wooden pallets (135 kg at 20% MC) of canned foods in cartons, and that the total load of fibreboard is 760 kg at 12% MC. If the temperature of the pallets and their load is 20°C and the expected minimum temperature is 10°C, we have the following values: from Fig. 1, for a DP of 10°C and a temperature of 20°C the RH in the container must not be above 53%; from Fig. 2, if the ERH of the timber of the pallets and container is 53%, they must have an MC not greater than 10·5%; similarly from Fig. 2, the MC of the fibreboard must not exceed 7·5%. So the amount of water to be removed is

$$\left(\frac{15{\cdot}0}{100} - \frac{10{\cdot}5}{100}\right) \times 150 \text{ kg from the plywood,}$$

$$\left(\frac{20{\cdot}0}{100} - \frac{10{\cdot}5}{100}\right) \times 135 \text{ kg from the pallets,}$$

and

$$\left(\frac{12{\cdot}0}{100} - \frac{7{\cdot}5}{100}\right) \times 760 \text{ kg from the fibreboard,}$$

making a total of *c.* 54 kg water. This is by no means an extreme case and gives some idea of the magnitude of the problem.

Clearly it is better (i.e. less expensive) to prevent the water from getting into the materials in the first place rather than to dry them out afterwards. Fibreboard can be stored at a low RH, as can fully made up and packed cartons. Pallets can be stored out of the rain, and the container walls and floor can be dried immediately after washing rather than allowing water to stand on them, thereby saturating the plywood.

Once the materials have been loaded into the container there are several ways of lowering their MC; (i) dehydrated warm air can be blown into the container, (ii) desiccants (e.g. silica gel) can be distributed throughout the load (usually during loading), and (iii) a refrigerated 'cold finger' or 'cold plate' can be installed in the container.

Air driers with a rotating drum of

desiccant are available. The desiccant first dries ambient air and then is rotated to a position where it is heated to drive off the moisture.

The drier is operated by opening the doors of the container and placing a false door across the opening. This false door has holes to allow the dry air to enter and the moist air to escape. An internal pipe may also be provided to distribute the air within the container. Driers are available in a large range of sizes. It has been found that a typical model, rated at $0{\cdot}24\,m^3\,s^{-1}$ (500 cfm), can in fact supply $0{\cdot}04\,m^3\,s^{-1}$ (80 cfm) of air at 45°C and 5% RH to each of two containers. From an expanded version of Fig. 1, air at 45°C and 5% RH holds 0·0030 kg water per kg dry air. When a container at 22°C was supplied with this air, the air at the exit had a temperature of 25°C and an RH of 48% during the first hour. Air under these conditions holds 0·0097 kg/kg, so the take-up of moisture is 0·0067 kg/kg dry air. At $0{\cdot}04\,m^3\,s^{-1}$ this gives a maximum rate of 1·2 kg water removed per hour. It is clear that either a very much larger airflow is required, or that drying must be continued over a long time to remove sufficient water.

One kilogram of dried silica gel can absorb 0·3–0·35 kg water before the ERH it generates rises to 60%. Consequently silica gel can be used to dry the outer layers of the cargo. Normally about 25 kg silica gel can absorb *c.* 8 kg water. If the MC of the load in the container is fairly low, silica gel can be used as an insurance against the chance occurrence of condensation caused by unexpected temperature gradients. When the MC of the load is high, silica gel is too expensive because it rapidly becomes saturated unless large quantities are used.

Any material that generates a low ERH can remove water from a material at a higher ERH, as explained in the paragraph on interaction of moisture-containing materials (p. 26). Thus fibreboard at very low MC and consequently a low ERH, absorbs moisture from, say, damp pallets having a high ERH. The change in MC of fibreboard is small for a large change in ERH, so dry fibreboard is nowhere near as efficient a desiccant as silica gel.

As explained earlier, water vapour condenses on any surface that is below the DP of the air surrounding it. If the condensed water is drained to the outside of the container, any moisture-containing material therein is slowly dehydrated. There have often been suggestions that this could be done by a mechanically refrigerated 'cold finger', but the difficulties of providing continuous refrigeration would have to be overcome first. A large cold surface would be needed to remove the quantities of water involved and the water would then have to be prevented from freezing.

*Temperature differences*

Temperature differences can occur at any time from the initial loading of the cartons until their final discharge. The following is a list of the various forms and causes of temperature differences.

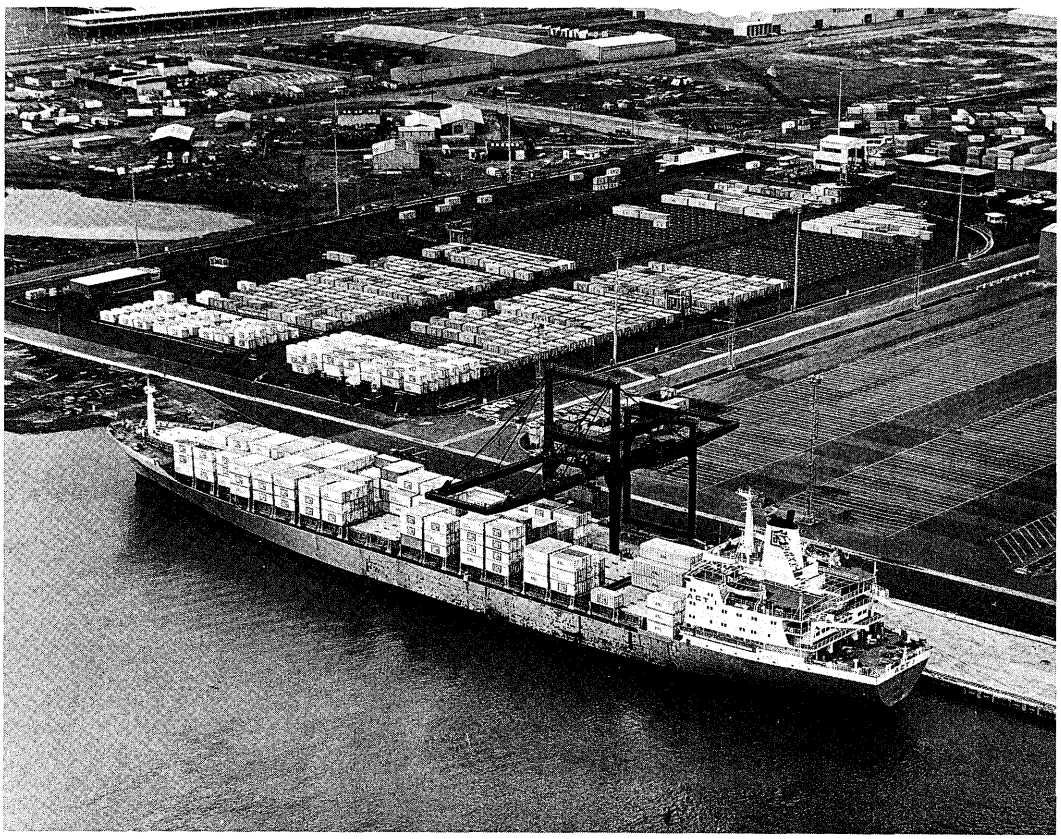
- Temperature differences between individual cartons before they are loaded.
- Temperature differences between cartons and the container during loading.
- Changes in the temperature distribution within the container caused by changes in the temperature of the ambient air.
- Fluctuations in the temperature of the container walls caused by
  - (a) exposure to the sun
  - (b) exposure to a clear night sky
  - (c) formation of dew or frost on the outside of the container
  - (d) stowing the container near a source of heat
  - (e) stowing the container near a refrigerated space.
- Temperature differences between the cartons and the ambient air at time of discharge.

Little attention has been paid to reducing these temperature differences that may occur in containers. Temperature differences may be minimized by: insulating containers, or by partially insulating them; shading containers from the sun or a clear night sky: placing containers below deck on ship; emptying containers rapidly; shipping warmed cans.

If cartons, initially all at the same temperature, are loaded quickly into an insulated ISO container it is almost impossible to induce condensation on them while they are in the container. Unfortunately, this solution is not feasible owing to the high costs involved.

As a step towards the insulated container, insulation may be applied to the roof only. It must be emphasized that the insulation must not itself introduce water; some trials have failed for this reason. Insulation of the roof reduces the range of temperatures of the inner surfaces of the container and reduces condensation on the roof. In an uninsulated container the rate of condensation on the inside of the roof on a clear night depends only on the rate at which the water vapour can migrate to the surface. In contrast to this, with a cargo at 20°C and a metal surface temperature of 0°C, a layer of polyurethane, 25 mm thick, on the inside of the roof of an ISO container 7 m long, limits the condensation rate to 0·4 kg $h^{-1}$. This insulation has no effect on the condensation on the walls and consequent run off to the floor; it only reduces condensation on the roof.

Temperature differences can be reduced by simply shading the container from direct sunlight or by protecting it from direct exposure to a clear night sky. This is not easy during land transport by rail or road. Fortunately, while the vehicle is moving the temperature of the outer surface of the container remains close to that of the ambient air; but, in Australia the surface temperature of a stationary container can rise to 70°C when exposed to summer sunlight, and can fall to 0°C on a clear winter night. When the top of the container is hot, the upper layers of cartons slowly heat up causing condensation on the cooler cans lower down. On the other hand, a cold roof on a container causes condensation as soon as its temperature falls below the DP of the air in the container. This can occur within minutes of the container becoming stationary if it is not suitably covered. Probably the best solution is to avoid land transport during winter nights.



A container ship berthed at Swanson Dock, Melbourne

Current practice in ships is to place uninsulated containers of canned food below deck. This has led to a reduction in the incidence of condensation.

At outturn, if the temperature of a load in a container is 20°C and the container doors are opened where the ambient conditions are, say, 25°C and 80% RH, the temperature of the cans is below the DP (21·3°C) and condensation forms on them. If they are left in pallet loads it takes a long time for their temperature to rise above the DP and until then the cartons cannot dry out. In this time the cans rust and mould grows. If the pallet loads can be broken down rapidly to individual cartons, condensation stops sooner and the cartons dry out more quickly.

In conventional ships, condensation is considerably reduced by shipping cans that have been kept warm (above 20°C), since this prevents the condensation of water vapour from humid sea air onto the cans. However, conditions are quite different in containers. Cartons loaded into a cold container can produce condensation on its roof even before the doors are closed. The cooler the cans are, the less likely is this to occur. Containers cool rapidly if, on a clear night, they are left standing at any stage during their transport from cannery to shipping port. Cool cans also reduce condensation in this instance. However, as already mentioned, cans at a low temperature can cause condensation when the doors of the container are opened at outturn.

*Pathways for vapour flow*

Water vapour can flow from warm regions to cold surfaces in a number of ways —through airgaps that allow water vapour to migrate within the container, e.g. between cans and cartons, between cartons, between cartons and container surfaces, under pallets. In addition moist ambient air can enter the container through airgaps in the container itself or at the door seals.

Unless specially treated, all cellulose materials allow relatively free passage of water vapour through them.

Plastic films in various forms have been used in attempts to block the flow of water vapour from the source to the cold surface. Fibreboard with plastic film laminated on one side was unsatisfactory because the plastic was on the outside of the carton; it can only be effective in preventing rust on the cans if it is laminated onto the inside of the carton. If plastic-faced fibreboard (which is more expensive than the normal type) were used routinely attention would have to be paid to any breaks in the carton, e.g. at the junction of the flaps.

In another experiment plastic 'snoods' —large inverted plastic bags—have been placed over each pallet load in a container. They remove the problem of condensation falling from the roof onto the top layer of cartons and they prevent the migration of water vapour from one pallet load to another. But, unless a plastic sheet is placed between the pallet and the cartons and unless this plastic sheet is effectively sealed to the snood, water vapour can flow from the pallet to the cans on it. Furthermore, temperature gradients can lead to condensation on the inside of the plastic snood. Although the amount of water is usually not large, it can produce extensive damage on the outer surfaces of the cartons within the snood.

Perhaps best included in this category is a special type of paint. It is not designed to prevent condensation but to prevent the condensed water from dripping from the surface on which it forms. The paint contains fibres that produce two sizes of capillary pores in the paint. Water vapour moves freely down the large pores and condenses on the cold metal surface of the container. The liquid water flows up the fine pores that act as wicks and this water then evaporates from the outer ends of the fine pores, i.e. from the surface of the paint. This system has been found to work well in abattoirs but extensive experiments have not yet been carried out in containers. This paint will not prevent condensation damage caused by temperature differences between different parts of the load.

## Design of experiments on condensation

Numerous experiments on condensation have been carried out in many countries. Almost all of them have produced results that are, at best, dubious, and more often than not completely misleading. The basic reason for this is the difficulty of accurately replicating all the conditions that affect condensation in a container.

For example, consider an experiment to study the effect of snoods on condensation.

It seems relatively easy to send two containers, one with and one without a snood, from cannery to consignee and to observe the different amounts of condensation in the two containers. Unfortunately, such a simple experiment is almost certainly doomed to failure. It will be realized from this review that it is essential to ensure that:

- the containers are identical in construction, air leakage, door seal leakage, MC of timber and temperature at loading
- the two loads are identical in every respect except for the snoods, i.e. identical in weight, stacking pattern, the quantity and MC of labels, fibreboard, and pallets, and also identical in temperature
- all containers experience the same temperature changes during transport, i.e. the land transport does not stop for a few hours in a position where one container is somewhat more shaded than the other, the containers are stowed in positions in the ship that are at the same temperature, and the two containers are everywhere treated as a pair and not stacked, for example, in different parts of the container terminal
- the containers are not opened under ambient conditions that can cause condensation.

Unless it can be shown that the containers undergo identical treatment through *all* these phases, any difference in the condensation experienced in the two containers cannot be interpreted as an indication for or against snoods. The same reasoning applies whether one is testing snoods, silica gel, hot air drying or any other method proposed for reducing condensation.

The type of experiment discussed above emphasizes the inherent difficulty of designing and carrying out tests involving condensation. By their nature they require extremely close observation throughout the entire duration of the test. This usually means that extensive instrumentation is essential.

This inherent difficulty of experiments on condensation has led to the many views that exist on 'how to stop condensation'. When the experiments that have 'proved' that a particular method works are examined, it is rare to find that even a few of the necessary precautions have been taken. So the apparent success of the method was a matter of luck and not necessarily related to any intrinsic merit in the method under test.

## Conclusions

Condensation problems in containers are accentuated by the small size of the load; this permits large temperature gradients with a consequent rapid movement of water vapour. Packaging and handling materials based on cellulose aggravate the problem still further. Despite this, many thousands of tonnes of cargo escape condensation trouble. However, if it is necessary to ensure that a particular cargo is not subject to damage one cannot rely solely upon the chance of getting good weather.

Since the guaranteed methods of ensuring that condensation cannot occur are:

- to remove *all* sources of water, or
- to remove *all* temperature differences, or
- to prevent *any* flow of water vapour,

it is possible, in principle, to use any one of these to achieve success. However, we cannot usually do any one of these things completely. So we can reduce the risk of condensation but not prevent it. Suggestions for operating procedures to reduce condensation are:

1. Do not put anything damp into containers and
   - ensure that containers are dried immediately after cleaning
   - store pallets in a covered dry area
   - store fibreboard in a dry area
   - use only sufficient water spray on the fibreboard to permit folding without cracking
   - use the minimum amount of glue on cartons
   - store loaded pallets in a dry area.
2. Avoid temperature differences in the container and
   - use insulated or partly insulated containers
   - load containers in a covered area
   - avoid temperature differences between different pallet loads, i.e. loading from widely different parts of a warehouse stack into the one container
   - avoid land transport on winter nights

unless the weather is cloudy, overcast or rainy

- ▶store loaded containers under cover
- ▶on ship, stow loaded containers below deck away from heated or refrigerated areas.

3. Use packaging materials that minimise transport of water vapour. In addition it is desirable to empty containers rapidly:
   - ▶break down pallet loads immediately after they are removed from container. If this is not possible, warm the cartons by blowing warm air on them. Some water will condense at first but this will dry out once the temperature of the can is higher than the DP of the ambient air.


## Acknowledgment

Much of the material in this review is taken from unpublished reports of work undertaken by the authors at the Division of Food Research CSIRO. We are grateful for permission to use other unpublished material from various shipping companies. We would also like to thank the organizations and the many people who willingly gave their assistance and provided containers, suitable loads, equipment, and transport facilities without which the tests forming the basis of this review could not have been undertaken.


## Further reading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## The Nutrition Society of Australia

The Society was formed at the ANZAAS Congress in Canberra on 23 January 1975 and incorporated several regional groups which were already in existence. The first President was Dr F. W. Clements, then of the School of Public Health and Tropical Medicine, University of Sydney and he was succeeded in 1976 by Dr I. W. McDonald, formerly Chief of the CSIRO Division of Animal Physiology at Prospect, N.S.W.

The objective of the Society is 'to advance the scientific study of nutrition and its applications to man and animals'. Thus the membership includes both human and animal nutritionists and a wide range of professions covering research in medicine, dietetics, agriculture, veterinary science, social work, public health, food technology and analytical standards.

The first annual scientific meeting was held in Melbourne in 1976 and the second, in 1977, in Sydney. Proceedings of the meetings are published, including general symposia and research communications. At the Sydney meeting in August, 1977, there were two symposia: 'Nutrition and the Australian Aborigine' and 'Early Post-natal Nutrition and Obesity'. There were also 25 research papers on a wide range of topics whereas there were only 8 at the first meeting in Melbourne. These figures indicate the rate of growth of the Society in its first two years. Membership has also grown rapidly and now stands at approximately 450.

There are regional groups in Armidale, Brisbane, Canberra, Melbourne, Perth and Sydney. Regular meetings are held and reported in a newsletter which acts also as a vehicle for comment and debate on a wide range of issues concerning nutrition.

A recent interesting activity of the Society has been to explore the possibility of establishing a Nutrition Foundation in Australia, along the lines of similar organizations in the U.K. and the U.S.A., mainly for education and the dissemination of information relating to sound nutrition.

Membership of the Nutrition Society of Australia is open to suitably qualified people and there are special financial arrangements for students. A number of companies in the food industry are sustaining members and provide welcome financial support as well as identifying themselves with the aims and objectives of the Society. Further information concerning membership can be obtained from the Honorary Secretary, Dr J. L. Black, c/o CSIRO, P.O. Box 239, Blacktown, N.S.W. 2148, telephone (02) 631 8022.

J.B.D.

# Producing heat stable milk powder

By F. G. Kieseker and R. J. Pearce

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In many developing countries the successful operation of a number of dairy plants that produce recombined dairy products is based on regular supplies of non-fat milk powder from Australia and New Zealand. The recombined products manufactured that have a requirement for heat stable powder are in-can sterilized milks (12–13% total solids) and evaporated milk (26–31% total solids). The recombined evaporated milk is prepared by dispersing the milk powder in water, adding either anhydrous milk fat or locally produced vegetable oils, homogenizing the blend and finally sterilizing the canned product at 120°C for 12 min. The manufacture of an acceptable product is highly dependent on the use of a non-fat milk powder that, on reconstitution, is sufficiently heat-stable not to coagulate during heat sterilization.

During the normal conditions of sterilization, recombined evaporated milk, made from heat stable milk powder, will increase slightly in viscosity to give a product with a desirable consistency. In commercial practice the required viscosity may be achieved by the addition of stablizing salts such as phosphate or citrate and by slight adjustment of sterilizing conditions. On the other hand, milk made from milk powder that is not heat stable will thicken excessively or coagulate.

Coagulation is a two-stage process consisting of an induction period of variable length, during which the milk remains fluid but increases in viscosity, followed by a rapid coagulation stage. The coagulum may be smooth or grainy and may be accompanied by whey separation. Obviously an induction period longer than the sterilization time is a minimum prerequisite for satisfactory processing.

The heat stability of milk may be defined as the time taken for the milk to thicken excessively or to coagulate when heated under strictly controlled conditions.

Heat coagulation of evaporated milk was described by Sommer and Hart in 1926 and has been under scrutiny ever since, although the problem of heat stable powder manufacture for recombined milk products is one more recently encountered. Empirical methods have been established to manufacture skim milk powder with a satisfactory level of heat stability but, for reasons that are not fully appreciated, they are not always successful. The physico–chemical changes that occur during heat coagulation are complex and not well understood.

## Preheating, evaporation and spray drying

At present the key to manufacturing skim milk powder with specific functional properties is the extent of the heat treatment given to the milk before evaporation, viz. the preheating or forewarming process. Heat stable powder suitable for the manufacture of recombined evaporated milk is produced from milk that has been extensively preheated, for at least 30 min at 85°C. This temperature is sufficient to denature the whey proteins and to allow them to interact and aggregate with themselves and the casein. Such a milk powder is referred to as high heat powder.

In some cases the heat stability of high heat powders may be increased by longer preheating and/or higher preheating temperatures. However, there appears to be an upper limit in the time/temperature relationship beyond which heat stability no longer increases and may actually decrease. At certain periods of the year no amount of preheating will yield a satisfactory heat stable product.

Marked variations in the heat stability of milk, that are reflected also in powder, seem to be a worldwide problem in spite of differences in milk production patterns. In Australia calving occurs predominantly in the late winter to early spring resulting in a flush of milk in spring and early summer, while in the intensive dairying areas of north-west Europe calving and

milk production are evenly distributed throughout the year. The heat stability problem appears, therefore, to be associated closely with seasonal 'environmental' conditions in addition to the stage of lactation.

During early lactation when the levels of non-casein protein and minerals are high, heat stability is low; the stability gradually rises and levels off during mid-lactation, and declines towards the close of lactation when milk composition again tends to change. Many attempts have been made to relate different factors in the composition of milk to the heat stability of evaporated milk or milk powder for recombined evaporated milk, however, positive correlations have not been established. A correlation between the urea level and the heat stability of skim milk is described below.

Small changes in the pH of milk (as little as 0·05 pH units) can also have a marked effect on the heat stability of the powder. During the lactation period there is often a slow change in the natural pH of the milk; this pH may not necessarily coincide with the pH at which maximum heat stability occurs.

In addition to the broad seasonal variation in heat stability of skim-milk powder, day to day fluctuations in the heat stability of skim milk are observed and these may be reflected in the powder. The plane of nutrition of the cow, which depends partly on prevailing weather conditions, strongly influences heat stability. During drought or prolonged dry periods heat stability may fall; a similar result may be noted during periods of prolonged wet weather when temperatures are low and

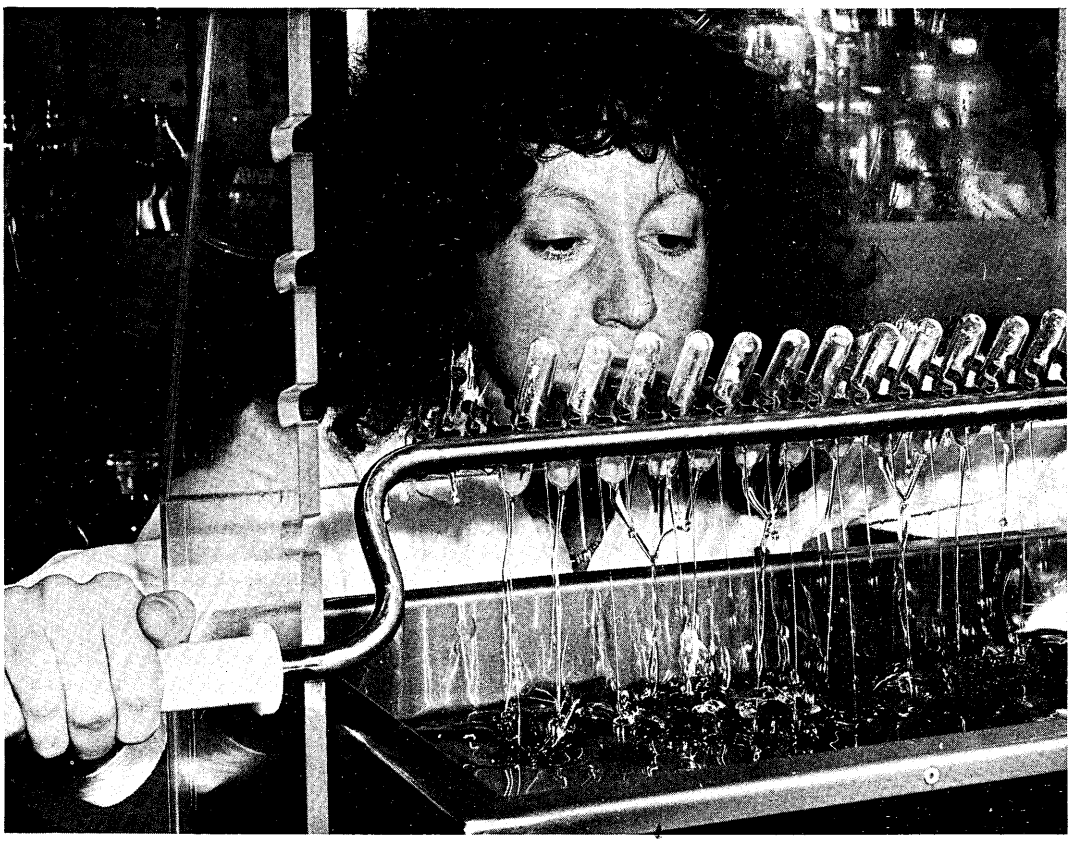

**Fig. 1.** Testing samples of milk for heat stability as a function of pH.

pastures become waterlogged. The health of the cow may also be a factor influencing heat stability, in particular, subclinical mastitis is known to decrease the heat stability of the milk.

In Australia at present, the period during which heat stable powders can be manufactured extends from October to late February. In irrigation areas this period may extend to late April.

Trials are currently being carried out, by the Dairy Research Laboratory, CSIRO in collaboration with the Gilbert Chandler Institute of Dairy Technology, to determine the preheating conditions required to extend the period during which heat stable powders can be manufactured. Differences in heat stability resulting from the type of preheating applied and different time/temperature combinations have been examined. Low temperature, long time preheating, e.g. 85°C for 30 min, and high temperature, short time preheating, e.g. 120°C for up to 180 s, when compared at the beginning of the season, gave powders that showed little difference in heat stability. However, high temperature, short time preheating as established by Newstead and coworkers at the New Zealand Diary Research Institute has been advantageous in giving improved heat stability to the powder at the end of the season. Increasing the holding period at 120°C from 60 to 180 s has, in some instances, also been beneficial. The reasons for these differences are not known and are being investigated further.

Since the heat stability is so sensitive to pH, adjustment of the pH of raw milk to the point of maximum heat stability would be expected to improve the heat stability of the powder. To date, only marginal improvement has been achieved. A problem has been that during preheating there is a decrease in the pH of milk, the extent being dependent on the level of preheating applied. It would appear that pH adjustment after preheating could be advantageous; however, results of a number of trials have been variable.

In order to attain maximum economy in manufacture and to establish the desired physical characteristics of the powder, such as high bulk density and good dispersibility, the milk is concentrated to a maximum of 42% total solids before spray drying. This is achieved by evaporation under vacuum at relatively low temperature. Under these conditions no further denaturation of the labile whey protein occurs. Preheating the concentrate before drying does not appear to influence the heat stability of the powder although it can affect other physical characteristics.

Spray drying yields a satisfactory product of good flavour and solubility. Other forms of drying have either a detrimental effect on product quality or are not economically competitive.

### Heat stability testing

Heat stability is normally measured as a function of pH to give a pH/heat stability curve. Milk powders are tested by reconstituting with water to the required level of total solids; the pH of the samples is adjusted with either $Na_2HPO_4/NaH_2PO_4$ or NaOH/HCl to give values within *c.* $\pm 0{\cdot}4$ units of the natural pH of the reconstituted milk. Aliquots are then sealed in glass tubes, or larger aliquots placed in bottles or cans. These are heated in an oil bath at 120°C for concentrated milk, or 140°C for milk of normal solids level (non-concentrated milk), as shown in Fig. 1. The samples are examined at regular intervals for the first signs of coagulation which may be only a general thickening, small isolated clots, or a complete coagulation.

Coagulation times are recorded and plotted against pH. A typical heat stability versus pH curve for non-concentrated milk is shown in *a*, Fig. 2. Commercial heat stability standards for milk powder have been set on the basis of this test (see Appendix). In order for a powder to be satisfactory, the heat stable period must be greater than the time needed for sterilizing the product, with due allowances for heating and cooling it.

Occasionally heat stable, high heat powders meeting the standard specifications do not perform satisfactorily when recombined with fat.

At the Dairy Research Laboratory milk powders have been tested for heat stability both as 20% reconstituted milks and as recombined milks containing 18% non-fat solids and 8% milk fat. The latter are homogenized with a laboratory scale homogenizer and are equivalent in composition to commercial recombined evaporated milk. It has been shown that the heat stability of the powder measured

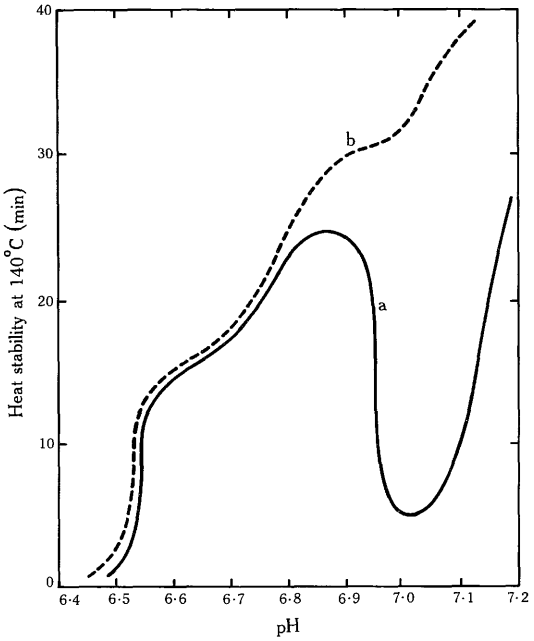


**Fig. 2.** Heat stability versus pH relationship for (a) non-concentrated skim milk, (b) the basic model system in the absence of whey protein.

as the recombined product is less than in the absence of fat. Heat stability is also dependent on the homogenizing pressures used. These results indicate that it would be advisable in commercial practice to test the suitability of milk powders for recombining as homogenized milks.

## Physico–chemical basis of heat coagulation

Despite many previous investigations there is still no clear understanding of the coagulation process although the involvement of several of the components of milk has been suggested. At the Dairy Research Laboratory investigations are being carried out on heat coagulation using simplified milk systems. As a preliminary to detailed study, several parameters were investigated to establish a relevant model system. Heating temperature, solids level, protein concentration, casein concentration, whey protein to casein ratio, calcium, magnesium, citrate and phosphate levels all substantially influenced the heat stability of non-concentrated milk. From these observations it was clear that the temperature needed to be accurately controlled and the protein or casein levels had to be standardized, as did the salt environment.

Similar general procedures have been followed in several previous studies but owing to failure to standardize protein or solids levels, the conclusions drawn from these studies are dubious.

A basic system has been adopted in which casein micelles are separated from milk by ultracentrifugation and resuspended in milk ultrafiltrate at a standard protein concentration. If whey proteins are added back to this model system, normal heat stability behaviour comparable to that in the original milk can be restored, indicating that the results obtained from the simplified system may be significantly related to those from skim milk.

## Protein interactions

The complex nature of the heat stability/pH curve of milk, shown in *a*, Fig. 2, is attributable in part to casein–β-lactoglobulin interactions. The basic model system in the absence of whey proteins shows a simpler behaviour, being almost a smooth curve of increasing stability with increasing pH as shown in *b*, Fig. 2. A sharp rise in heat stability of variable magnitude is always observed at *c.* pH 6·5 and a small shoulder may be apparent at *c.* pH 6·9 due to residual β-lactoglobulin carried over with the casein micelles. Addition of purified β-lactoglobulin to the model system results in a trough at this pH and this is accentuated with increasing β-lactoglobulin concentration. A small increase in stability at *c.* pH 6·8 is also induced, the final result being an apparently normal heat stability curve (*a*, Fig. 2) despite the lack of other whey proteins. The depth of the trough may be reduced by the addition of κ-casein either to milk or to the model system described above. A conclusion from this is that a β-lactoglobulin/κ-casein interaction is responsible for the heat coagulation in non-concentrated milk and previous studies have also attempted to establish the validity of this conclusion. However, seasonal variations in the level of β-lactoglobulin or in the β-lactoglobulin/κ-casein ratio do not correlate with variation in heat stability in non-concentrated milk.

Recently, it has been noted that at times of the year when the heat stability of concentrated milk is low, the opposite effect is observed in non-concentrated milk.

Moreover, heat stable powder for concentrated milks requires high heat treatment whereas for non-concentrated milk preheating decreases the heat stability so that low heat powder (pasteurized only, 72°C for 15 s) is necessary. Clearly, results of studies on non-concentrated milk may not be applicable to concentrated milk and *vice versa*. Consequently in all recent studies at the Dairy Research Laboratory both non-concentrated milk and concentrated milk derived from it have been examined.

### Effect of β-lactoglobulin

Seasonal measurements of the change of β-lactoglobulin levels over the season have not correlated with heat stability. However, since most of these studies have been performed on non-concentrated milk (*c.* 10% total solids) and as the effect of β-lactoglobulin is on the trough region of the pH-heat stability curve in non-concentrated milk as described in the previous section, the lack of correlation between natural or maximum heat stability and β-lactoglobulin level is not surprising. The effect of β-lactoglobulin on the heat stability of concentrated milk (*c.* 20% total solids) has received little attention. Recent reports from New Zealand have suggested that β-lactoglobulin levels are not important in determining the heat stability of concentrated milks. However, recent studies at the Dairy Research Laboratory indicate that this is not the case and that the concentration of β-lactoglobulin plays an important role in the heat stability of concentrated milks. In simplified systems it was found that without preheating, the casein coagulation was highly sensitized by the presence of β-lactoglobulin. If the milk was preheated the coagulation was de-sensitized. This has been confirmed in both pilot scale and semi-commercial powder manufacture.

The lack of correlation between heat stability and β-lactoglobulin levels, which have been observed previously, may also have been due to the standard analytical methods employed for the estimation of β-lactoglobulin. Recently it has been shown that the 'β-lactoglobulin' fraction, as normally isolated, also contains other protein components not previously recognized. A discriminating assay for β-lactoglobulin is required. This is currently under investigation.

### Effect of urea

Compositional analyses of milk have increased in complexity as more components have been identified. Attempts at correlating such analyses with heat stability and season proved fruitless until recently, when the levels of the various components constituting the non-protein nitrogen containing fraction were reported from th Hannah Research Institute in Scotland. A positive correlation was found between maximum heat stability and the level of urea in non-concentrated milk. Following this report the effect on urea concentration was tested at the Dairy Research Laboratory using the model system describe above. The results corroborated the seasonal data. The predominant effect was on the magnitude of the sharp rise in coagulation time at pH 6·5 (*a*, Fig. 2), and indicated that urea affected the casein coagulation markedly but not the interaction between casein and whey protein. In concentrated milk, however, no such influence of urea was observed. To confirm this, high heat powders were manufactured with and without urea and with urea added before and after preheating, but no differences in heat stability could be observed in concentrated milks.

### Conclusion

The production of milk powder with satisfactory heat stability characteristics is still an uncertain process. Work in progress is aimed at extending the period over which these powders can be made and at the same time at gaining a better understanding of the physico–chemical basis of heat stability so that processing conditions can be established on a rational basis.

## Appendix

### Heat stability test for skim milk powder

*Apparatus*

- A balance of approximately 500 g capacity, accurate to 0·05 g
- Heller laboratory stirrer or equivalent variable speed agitator
- McCartney bottles of 1 oz capacity
- Boiling water bath
- Glycerol bath fitted with thermostatically controlled immersion heater
- Safety shield

*Reagents*

Monosodium phosphate 7½% solution ($NaH_2PO_4.2H_2O$)
Disodium phosphate 7½% solution ($Na_2HPO_4.2H_2O$)
Distilled water

*Method*

1. Weigh 292·5 g of water (43°C) into a 600 ml beaker
2. With stirrer adjusted so as to create a vortex, add slowly 75 g skim milk powder. Continue to stir for 3 min, then let stand for 30 min
3. Set up McCartney bottles as follows:

| | | |
|---|---|---|
| A | 0·25 ml $H_2O$ | 0·25 ml $NaH_2PO_4$ |
| B | 0·375 ml $H_2O$ | 0·125 ml $NaH_2PO_4$ |
| C | 0·05 ml $H_2O$ | — |
| D | 0·25 ml $H_2O$ | 0·25 ml $Na_2HPO_4$ |
| E | — | 0·5 ml $Na_2HPO_4$ |

4. Weigh 25 g of the reconstituted milk into each of the McCartney bottles
   Screw the lids down *tightly* and mix well by inverting the bottles
5. Place bottles in boiling water bath for 5 min
6. Transfer bottles to glycerol bath controlled at 120°C
7. Check bottles after 3 min and then each succeeding 3 min for coagulation
8. Record time of first thickening or coagulation from time of entry into glycerol bath

*Note:* Carry out observations from behind a safety shield.

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# The 'settling phenomenon' in egg yolk. What do bacteria do to eggs?


By D. V. Vadehra* and R. W. Burley

CSIRO Division of Food Research, North Ryde, N.S.W.


## Introduction

Egg yolk is a surprising substance. In spite of its daily availability, details of its chemical nature are still elusive. The recent discovery at Cornell University, U.S.A., of unusual behaviour in yolk following the action of certain micro-organisms or chemicals presents the possibility that eventually we shall be able to solve some of the outstanding problems about the structure of yolk. The original aim of this research, which was partly successful, was quite different. It was to make egg yolk products with modified properties by allowing selected bacteria to grow in yolk. The beneficial effect of the growth of the right sort of microorganism in foods has long been familiar in the milk industry. Products such as cheese and yoghurt result from microbial action on milk under the right conditions. A particular aim of the work at Cornell University was to produce yolk products with a lower proportion of cholesterol than usual—a highly desirable aim at a time when low cholesterol foods are often prescribed medically.

*Since 1973 Professor D. V. Vadehra has been head of the Microbiology Department, Panjab University, Chandigarh, India. In 1972 the 'settling phenomenon' in egg yolk was discovered in his laboratory while he was Associate Professor in the Poultry Science Department, Cornell University. Professor Vadehra has since studied the phenomenon with his students in India. In 1976 he was awarded a CSIRO Senior Research Fellowship which he spent working on the 'settling phenomenon' and other topics at the Food Research Laboratory, North Ryde.

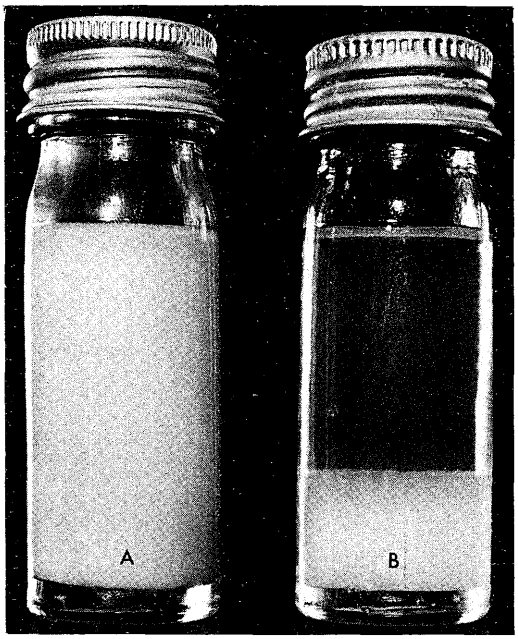


**Fig. 1.** The settling phenomenon. Tube B contained yolk from Australorp hen's eggs. This yolk had been diluted with 10% by volume of 1 M ammonium chloride and left for 24 h at 37° C under sterile conditions. Tube A, the control, contained yolk from the same sample that had been diluted with 10% sterile water. It was also left for 24 h at 37°C.

An unexpected result of this work was the discovery of the phenomenon illustrated in Fig. 1 (Sravani *et al.* 1972). Tube A, the control, contained hen's egg yolk diluted slightly with water. If it is properly sterile, such yolk will have the same appearance for months at temperatures below about 40°C; that is, there is no tendency for the various solid particles in yolk to settle or float. By contrast, if the yolk had been diluted with certain salts, such as ammonium chloride, or if certain bacteria had been added, within a few hours at 37°C (or longer at lower temperatures) the yolk started to become clear. The solid particles settled, and when settling was complete the appearance of the yolk was as shown in Tube B. A list of salts, bacteria, and other agents that cause this phenomenon, and an indication of their effectiveness, is given in the table. Substances that do not cause settling are also given.

*The significance of the settling phenomenon*

The new phenomenon is significant for two reasons:

(a) It reveals a new and unexpected way in which certain bacteria are able to alter yolk. That other kinds of bacteria (e.g. *C. perfringens*, *B. cereus*, (Kushner 1957)) release fats from combination with protein in yolk and degrade it in other ways has long been known. After the action of such bacteria the fats float to the surface of the yolk to form an oily layer. This process, known as the 'egg yolk reaction' is used as a diagnostic test for certain pathogenic bacteria. The mechanism of this reaction is reasonably well understood. It is a result of the action of lipases (specific enzymes), released by the bacteria, on the yolk lipoproteins (Burley and Kushner 1963). The settling phenomenon is quite different. Lipids are not released and visible damage to the yolk is minimal.

(b) Yolk settling raises the question of what keeps the solid particles in yolk in suspension. It is well known that eggs stored for years under sterile conditions show no signs that the solid particles have settled. The reasons for this are not known. They are possibly related to unsuspected interactions amongst the yolk constituents. The settling phenomenon offers a means of exploring this property of yolk.

## The nature of yolk

Avian egg yolk consists of a collection of particles of various sizes floating in a very concentrated solution (Burley 1975). Only about half the weight of yolk is water, which is unusually low for a biological material. The solution contains most of the proteins and fats of yolk, the fats all being combined as 'lipoprotein' (Cook and Martin 1969). Some of the proteins, known as the yolk 'livetins' are uncombined and are apparently free in solution. Of the solid particles in yolk, only the so-called 'yolk granules', *c.* 1–6 $\mu$m in diameter, that make up 12% of the yolk (Burley and Cook 1961), have a sufficiently high density ($1 \cdot 3\ g\,ml^{-1}$) to sink in yolk. Little is known about the other particles, although very recently a new variety, the 'insoluble yolk globules', has been recognized and isolated in this laboratory (Vadehra, Bain and Burley 1977). These particles have a relatively low density ($1 \cdot 02\ g\,ml^{-1}$). The relationships between the solid particles and the substances in solution have not yet been worked out.

Agents that show the settling phenomenon in hen's egg yolk. For comparison they were all tested under the conditions used for Fig. 1, i.e. the yolk was diluted with water plus the agent.

| These agents caused settling (in order of decreasing effectiveness) | | | These agents did not cause settling | | |
|---|---|---|---|---|---|
| Salts | Bacteria | Enzymes | Chemicals | Bacteria | Enzymes |
| Ammonium chloride | *Streptococcus faecalis* | Thermolysin | Ammonium thiocyanate | *Bacillus* species | Trypsin |
| Sodium chloride | *Serratia marcescens* | Bromelin | Calcium chloride | *Clostridium perfringens* | Chymotrypsin |
| Lithium chloride | *Vibrio parahaemolyticus* | Pepsin | Zinc sulphate | | Phospholipase C |
| Ammonium sulphate | | Subtilisin | Copper chloride | | Phospholipase D |
| Potassium chloride | | | Potassium bromide | | |
| Sodium sulphate | | | Potassium iodide | | |
| Ammonium acetate | | | Urea | | |
| | | | Sucrose | | |

## Experiments at North Ryde

*Experiments on the yolk granules*

We have studied the properties of the yolk granules because it is the sedimentation of these particles that causes the clearing seen in Fig. 1. So far no important differences have been found between granules that settled under gravity after the action of bacteria or chemicals (see the table) and those sedimented by high-speed centrifuging. We have, however, made the unexpected observation that the isolated granules contain a small but definite proportion of the yolk livetins, even after thorough washing. This was true for both 'settled' and 'centrifuged' granules.

Figure 2 shows the process of the washing of granules with 0·16 M sodium chloride (isotonic saline). For this purpose the granules were mixed well with a known volume of solution, centrifuged at 10 000 rpm for 30 min and the supernatant solution analysed for total protein; the individual proteins being detected and identified by gel electrophoresis. Initially the washings contained lipoprotein and livetins not firmly attached. Then more livetins were removed. These were evidently held quite firmly. They could not be removed with water alone, although water easily removed the unbound lipoprotein and livetins. The well-washed granules appeared to be more sticky and less opaque than those that were unwashed.

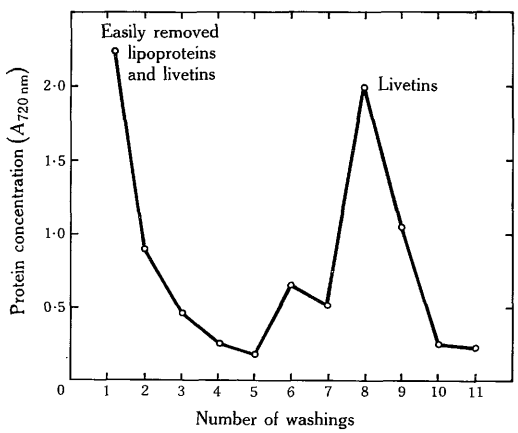


**Fig. 2.** Effect of washing with isotonic saline on egg-yolk granules from Australorp eggs. The granules were isolated by the usual procedure (Burley and Cook 1961) and mixed with 10 volumes of 0·16 M sodium chloride. They were then homogenized and centrifuged for 30 min at 10 000 rpm ($3 \times 10^4$ g) at 20°C. The supernatant solution was analysed for total protein by the Folin-biuret method using samples of 100 $\mu$l measured, after treatment, at 760 nm. The distribution of proteins was determined by polyacrylamide gel electrophoresis.

*Action of thermolysin on yolk*

We have examined the action of enzymes on yolk because it is most likely that bacteria affect yolk (see the table) by releasing specialized enzymes into the medium. We found that thermolysin, a reasonably specific proteolytic enzyme made by certain bacteria, causes settling very

effectively. Unlike the bacteria and salts in the table, this enzyme caused rapid settling when added to undiluted yolk as a solid. It is the only agent we have found that will affect undiluted yolk in this way. Another unusual property of thermolysin is that it enhances the formation, or visibility, of membranous structures in the yolk (Fig. 3). The reasons for this are not clear. We have tested the action of thermolysin on isolated constituents of yolk. It readily hydrolyses lipovitellin—one of the substances in the granules—and also $\gamma$-livetin both at pH 5 to 6. With isolated substances no signs of membranes have been seen after the action of thermolysin.

*Action of calcium chloride on yolk*

In studying the action of different salts (see the table) we have found that calcium chloride is unable to cause settling; nevertheless it has a large effect on the granules which become sticky and eventually dissolve. Although calcium chloride is quite different chemically from thermolysin it also gives rise to unusual membranous structures. These did not settle but could be centrifuged down. No clear explanation for the action of calcium chloride has been found.

*Separation of the yolk livetins*

As we found no evidence that changes in the properties of the granules are responsible for yolk settling, we studied the soluble proteins of yolk—the livetins. These could, in theory, maintain some kind of inner structure in yolk by forming entangling filaments or sheets, thus restricting the movement of the granules and other particles. Accordingly we have re-examined the livetins of hen's eggs by gel filtration column chromatography (Vadehra and

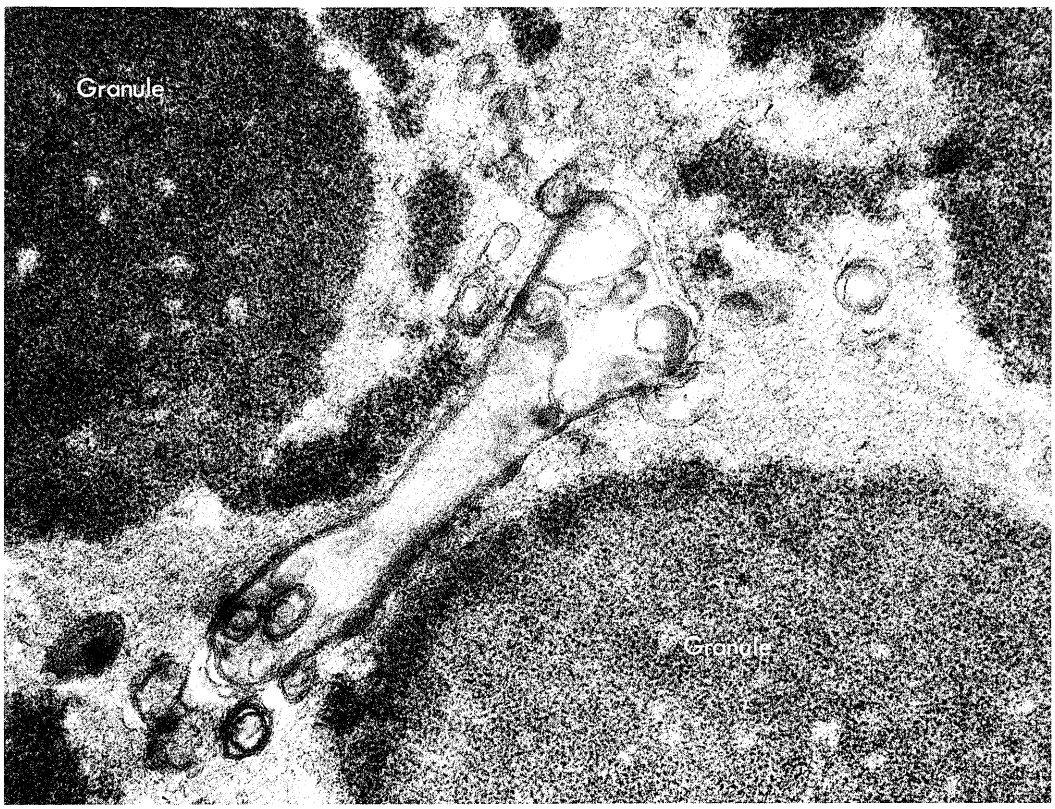


**Fig. 3.** Membranous structures that settled with the granules as a result of the action of the proteolytic enzyme thermolysin on the yolk of Australorp eggs. This is an electron-microscope photograph of a section after embedding and fixing. Mag. $\times 60\,000$. Photo by G. Bennett and J. M. Bain.

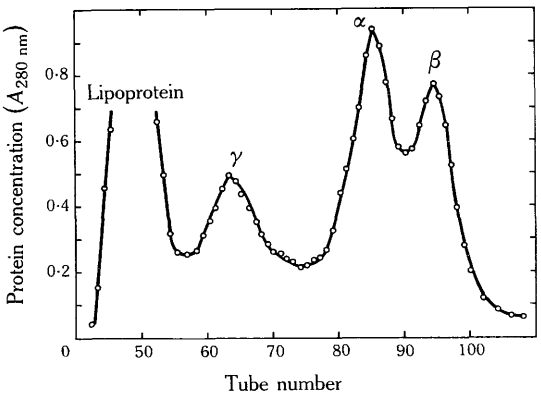


**Fig. 4.** Chromatographic pattern of the soluble proteins in egg yolk determined using a column (90 × 2·5 cm) of Sephacryl S–200. The granules and most of the low-density lipoprotein were first removed from the yolk by centrifuging. Protein was detected by measurement of optical density at 280 nm and identified by gel electrophoresis. $\alpha$, $\beta$, $\gamma$ refer to the three livetins.

Burley 1977). Figure 4, for example, shows the separation in M sodium chloride of the total livetin mixture from hen's egg yolk. The positions of the three major livetins ($\alpha$, $\beta$, and $\gamma$) are indicated.

We have also examined livetins from the egg yolk of other birds, including ducks, turkeys, and emus. Although the general pattern was the same, there were substantial differences in detail amongst the different species.

*Viscosity of yolk*

We have measured the viscosity changes during the settling of yolk with a coaxial-cylinder viscometer and a falling weight viscometer. We found an *increase* in viscosity during settling, an observation that suggested that one or more proteins in yolk had taken on a more elongated structure.

*Settling phenomenon in yolk of other eggs*

Although most of our experiments have been on hen's eggs because of their economic importance, we have also studied the settling phenomenon in egg yolk from eggs of other birds. The yolk of duck's and turkey's eggs was similar to that of hen's eggs, but emu's egg yolk was notably different in that settling was extremely slow. A difference was also observed between the two breeds of hen we have examined. Yolk from White Leghorn eggs settled faster than that from Australorp hens.

## Conclusion

In our efforts to explain the settling phenomenon we have examined many of the constituents of yolk in detail. We have found new constituents (the 'insoluble yolk globules', Vadehra *et al.* 1977) and new properties of yolk (e.g. the membranous structures, Fig. 3). However, we are not yet able to explain all our observations in chemical terms. At present the most likely explanation for the 'settling phenomenon' is that the active agent, bacterium, salt, or enzyme (see the table), acts by disrupting some sort of 'inner cohesion' in the yolk. The nature of this is not known. We suggest that some yolk proteins take part in weak intermolecular interactions that act as a barrier to settling by joining the granules, the low density lipoprotein, and the livetins. Such weak protein interactions could be sensitive to certain salts and would be disrupted by proteolytic enzymes. In our studies of the soluble proteins of yolk, however, we have not yet found a protein with the right characteristics to act as a weak intermolecular cement, although the presence of a small amount of livetins in the granules suggests that they are not confined to the aqueous phase. As with much work on yolk, a major disadvantage is the lack of information on the properties of the proteins. Few of the soluble proteins of yolk have been isolated in a pure state and their properties determined. This is a problem we are now examining. Some of the soluble proteins of hen's egg yolk are immunologically similar to those of hen's blood. This does not help much because information on the proteins of hen's blood is also sparse. Even less information is available about egg yolk of other birds. It is possible that the differences amongst different species in the settling phenomenon are related to differences in their soluble proteins.

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# Fitting thermocouples into cans

By P. W. Board and V. Stekly

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Ecklund (1949) was probably the first to describe a thermocouple assembly designed specifically for measuring temperatures in canned foods during the evaluation of heat sterilization processes. The hot junction and thermocouple wires are supported in the can by a plastic rod which enters the can through a threaded receptacle that is sealed into the can with a nut and rubber gasket. The Ecklund thermocouple is fitted to the empty can before filling and, since the external surface of the thermocouple assembly is flush with the can, the can may be closed on conventional closers. After closing the can, the lead wires are plugged into the thermocouple and measurements may be started.

Since Ecklund's assembly was developed there have been many alternative designs of thermocouples (see, for example,

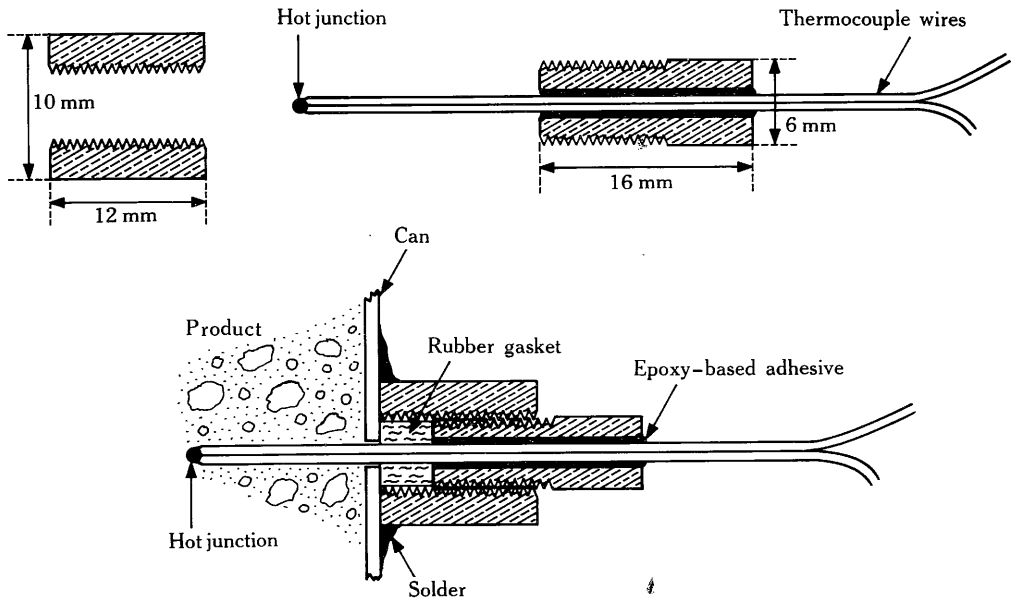


**Fig. 1.** Cross-section of thermocouple assembly before and after attachment to the can.

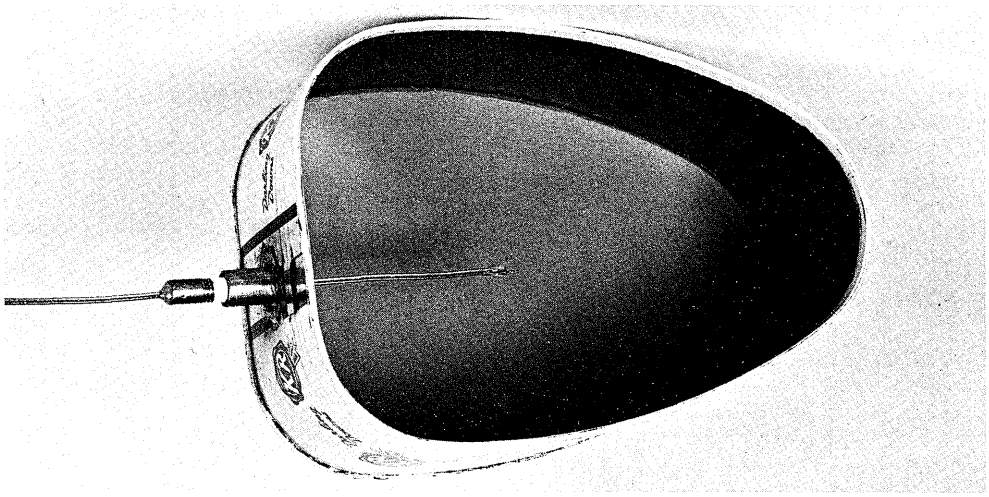

**Fig 2.** Assembled thermocouple attached to a can.

Beverloo and Weldring 1969) and of methods for fitting them into cans. For most of the heat penetration measurements made at these laboratories the simple thermocouple assembly described by Board (1977) is used. The thermocouple wires are sealed into small holes in the can with a commonly available epoxy-based adhesive. If there is a danger that the hot junction may move from the desired measuring point during filling, closing or processing, the separate wires of the thermocouple are introduced through holes at opposite points in the can.

One problem with cans that are fitted with thermocouples before filling is that the commercial filling operation may have to be modified or replaced by another method to avoid damaging the thermocouple. Recently there was a need to measure temperatures in cans containing a re-formed meat product that was normally filled mechanically; it was considered that hand filling might alter the structure and heat transfer characteristics of this product. This problem was overcome by designing a simple new thermocouple assembly which allowed the hot junction to be placed at the desired measuring point after the can was filled and closed; this assembly is described in this note.

The assembly (Fig. 1) consists of two brass tubes which are threaded so that they may be screwed together. The thermocouple is sealed into the narrow tube with epoxy-based adhesive, with enough wire extending from the threaded end of the tube to reach from the can to the selected measuring point in the product. Nylon insulated copper-constantan wires of light gauge (*c.* 0·3-mm diameter) are satisfactory for thermocouples for evaluating canning processes, but if conduction errors are likely to be important (Cowell *et al.* 1959) finer wires or wires of lower conductivity should be used. Fine wires that need mechanical support may be made more rigid by coating the length that enters the product with the epoxy-based adhesive.

The assembly is fitted to the filled, closed can by first soldering the wider threaded tube to the can wall. A hole *c.* 2-mm diameter is then made in the can inside this tube using a pointed steel skewer and the skewer is pushed further into the product to make a tunnel for the thermocouple. The thermocouple is then inserted into the hole and the assembly is screwed together.

If the threads on the brass tubes are carefully made, a satisfactory seal will usually be formed when the narrow tube is screwed into place. However, if there is a danger of leakage, a perfect seal may be obtained by placing a small rubber gasket between the leading face of the narrow tube and the can wall. The assembled thermocouple is shown in Figs 1 and 2.

In practice these thermocouples have usually performed satisfactorily but occasionally they may bend during insertion

into the can and the hot junction may be displaced. As with any type of thermocouple the test cans should be opened and inspected after the measurements have been taken and results from hot junctions found to be displaced must be discarded. The thermocouples described here are simple to make and they provide means for taking measurements in products that are not amenable to other techniques.

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# News from the Division

### Appointments

Mr Peter M. Husband, Scientific Services Officer, has been appointed Extension Officer (MRL) in Perth, W.A., following the resignation, early in January 1978, of Mr Denis Roberts. Mr Roberts has accepted a position in the Division of Dairying and Food Technology of the W.A. Department of Agriculture.

Mr Husband, who is a Bachelor of Applied Science, was formerly in the Industry Section at Cannon Hill.

### Honours and awards

The Chief of the Division, Mr M. V. Tracey, was named an Officer of the Order of Australia in the 1978 Australia Day Honours List, for services to science.

In the 1977 Academic Year the following were awarded degrees:
Dr D. J. Walker, Officer-in-Charge, MRL, D.Sc. (Sheffield)
Mr D. S. MacArthur (MRL) Ph.D. (Cambridge)
Mr M. A. Brown (FRL) M.Sc. (Macquarie)
Mr. A. Graham (MRL) M.Sc. (Griffith)
Mr D. Barnett (FRL) B.A. (Macquarie)

The Panjab Agricultural University named its Animal Nutrition Centre *The Dr G. S. Sidhu Animal Nutrition Centre* in recognition of the contribution made by Dr Sidhu (FRL) in initiating and developing this work at the University.

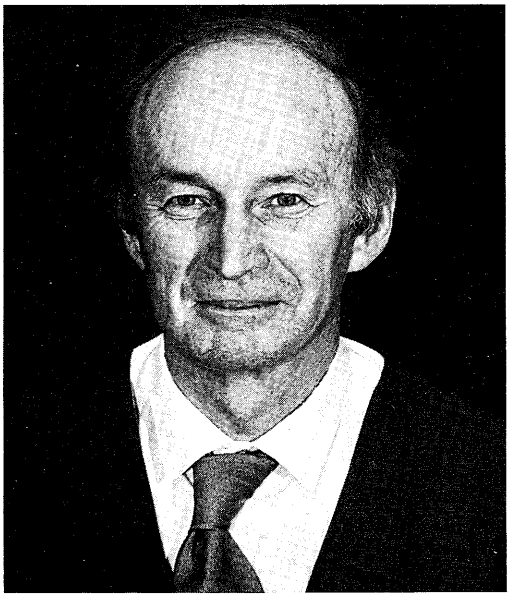
Dr A. R. Johnson (Officer-in-Charge, FRL) was elected a Fellow of the Australian Institute of Food Science and Technology (FAIFST).

## Obituary

Malcolm Franklin died of Hodgkin's disease on 17 March, aged 31. His untimely death cut short a most promising career. Malcolm joined the Division on 4 July 1966 as a Technical Assistant to Dr W. B. McGlasson. He undertook part-time technical college courses in biology which he passed with distinction. He became expert in the maintenance and construction of electronic laboratory equipment. During his short career his efforts in developing analytical methods and his active interest in research problems contributed a vital part to the Division's research program on ripening and physiological disorders in fruit. In recognition of his ability he was recently promoted to Senior Technical Officer.

Significantly, Malcolm's greatest contributions were made during the last five years. He underwent major surgery in 1974, following which he made a remarkable recovery and resumed his studies and also moved from the post of Chairman of the N.S.W. Branch of the CSIRO Technical Association to that of Federal President. He was serving a fourth year as President at the time of his death. His work with the CSIROTA brought him in contact with a wide spectrum of people, both in CSIRO and in other Public Service organizations. Malcolm was a quiet person but no one who came to know him failed to like and respect him. He will be greatly missed by all of his colleagues throughout CSIRO.

## Retirement



Mr Ronald Hill, Senior Principal Research Scientist and Second-in-charge of DRL, retired from CSIRO in March. Mr Hill began work with the Victorian Education Department, but at the outbreak of the Second World War he joined the Air Force and worked on radar for five years. After the War he returned to the Education Department for a year, then taught mathematics and statistics at RMIT until 1950, when be began his career with CSIRO.

From 1950–1959 Mr Hill was with the Division of Building Research, studying methods of manufacturing lightweight aggregates for concrete and plaster, together with the physical chemistry of ceramics. In 1960 he left that Division to set up a protein group at the Section of Dairy Research (now DRL). For the past 19 years Mr Hill has investigated a number of problems: during his early years with DRL he studied the physical structure of the casein micelle, and under his leadership the first electron micrographs were obtained that clearly showed the structure of the casein micelle. This led him, in 1964, to put forward a theory on the structure of the micelle which is now widely accepted. He was also the first research worker to elucidate the action of rennin on $\kappa$-casein. Mr Hill did this by making a well-characterized artificial peptide substrate for rennin. During his study of the coagulation and syneresis of casein the importance of a positively charged cluster of amino acids in coagulation was also demonstrated.

He also found that there were two different mechanisms and two different types of free radicals responsible for initiating oxidation in milk fat and showed that both of these processes could be inhibited by heating milk to 80°C for 15 s.

Mr Hill and his wife hope to settle in Queensland. We wish him good luck and a happy retirement.

### Membership of committees

Mr M. V. Tracey is a member of the International Mycotoxin Check Sample Advisory Group (of the WHO International Agency for Research on Cancer) for two years.

Drs J. R. Vickery (Convenor) and J. H. B. Christian and Messrs M. V. Tracey and D. McBean (FRL) are members of a Study Group on Food Technology for an Australian Academy of Technological Sciences working party to assess Australia's contribution to food production in developing countries.

Mr K. C. Richardson (FRL) is a member of a working party established by the NHMRC Food Microbiology Subcommittee, to draw up a General Code of Hygiene Practice for the Preparation, Handling, Storage and Sale of Foods for Consumption without Further Processing.

Drs A. D. Warth and A. G. Lane (both FRL) have become assessors for the NATA Biological Registration Advisory Committee.

Mr E. G. Davis and Dr F. B. Whitfield of FRL are members of a new committee of the Standards Association of Australia to develop a standard parallel to BS 3755: 1964, Methods of test for the assessment of odour from packaging materials used for foodstuffs.

### Visiting workers

Associate Professor E. C. Tigchelaar, Department of Horticulture, Purdue University, Lafayette, Indiana, U.S.A., returned to FRL in February to continue the tomato breeding program begun in 1977 with Dr W. B. McGlasson and the N.S.W. Department of Agriculture. The work is supported by the Rural Credits Development Fund.

Mr G. L. Robertson, Senior Lecturer in Food Processing in the Department of Food Technology, Massey University, Palmerston North, New Zealand, recently spent an 11-month sabbatical leave working with Dr B. V. Chandler at FRL. His main interest was the effect of pectic enzymes on citrus juice quality, with particular reference to bitterness.

### Overseas aid

Several members of the Division worked on overseas aid projects for some time during the period under review:

Dr W. B. McGlasson and Mr M. J. Franklin of FRL were released to the Australian Development Assistance Bureau in October/November 1977 and assisted in setting up a Postharvest Fruit and Vegetable Training and Research Centre at the University of the Philippines, Los Baños.

Dr R. A. Buchanan (DRL), Australian Liaison Officer for ASEAN Projects, stationed in Kuala Lumpur, has had his secondment extended for another two years, to August 1979.

Mr J. F. Kefford, Assistant Chief, External Relations, visited Malaysia from 16 October to 12 November 1977 at the invitation of the Malaysian Agricultural Research and Development Institute in Serdang, to conduct a review and appraisal of current research programs in food technology. Dr D. J. Walker, Officer-in-Charge, MRL, has since examined the meat research programs at the Institute and given a keynote address at the Malaysian Institute of Food Technology in Kuala Lumpur.

### Study visits overseas

Dr D. G. Laing (FRL) has commenced a year's official visit to the U.S.A., U.K. and Europe. He will work for nine months at the Monell Chemical Senses Center, University of Pennsylvania and for three months at the Swedish Food Institute.

Dr A. K. Sharp (FRL), is spending some months at the Sprenger Institute, Wageningen, Holland, as well as visiting research centres en route in South-east Asia, Europe and the U.S.A.

Dr J. K. Raison (FRL) is on a long-term visit to the U.S.A. to undertake collaborative studies on the effect of temperature on the structure and function of membrane-associated proteins at the Carnegie Institution of Washington, Stanford, California.

Mr F. G. Kieseker (DRL) is spending five months at the Netherlands Institute for Dairy Research, Ede, and will be visiting research centres in U.K., Eire and other parts of Europe.

Mr B. Y. Johnson (MRL) is on a study trip to observe meat works techniques and practices as well as new research and development and extension work in U.K., Sweden, Denmark, Germany, Canada and the U.S.A.

Messrs A. Graham and H. Chua (MRL) were invited to present a paper at the Council of Australian Public Abattoir Authorities Conference at Invercargill, New Zealand and subsequently to visit the Meat Industry Research Institute of New Zealand and meat works in New Zealand.

*Note:* Some detail of the results of some of these overseas visits will appear in subsequent issues of *Food Research Quarterly*.

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