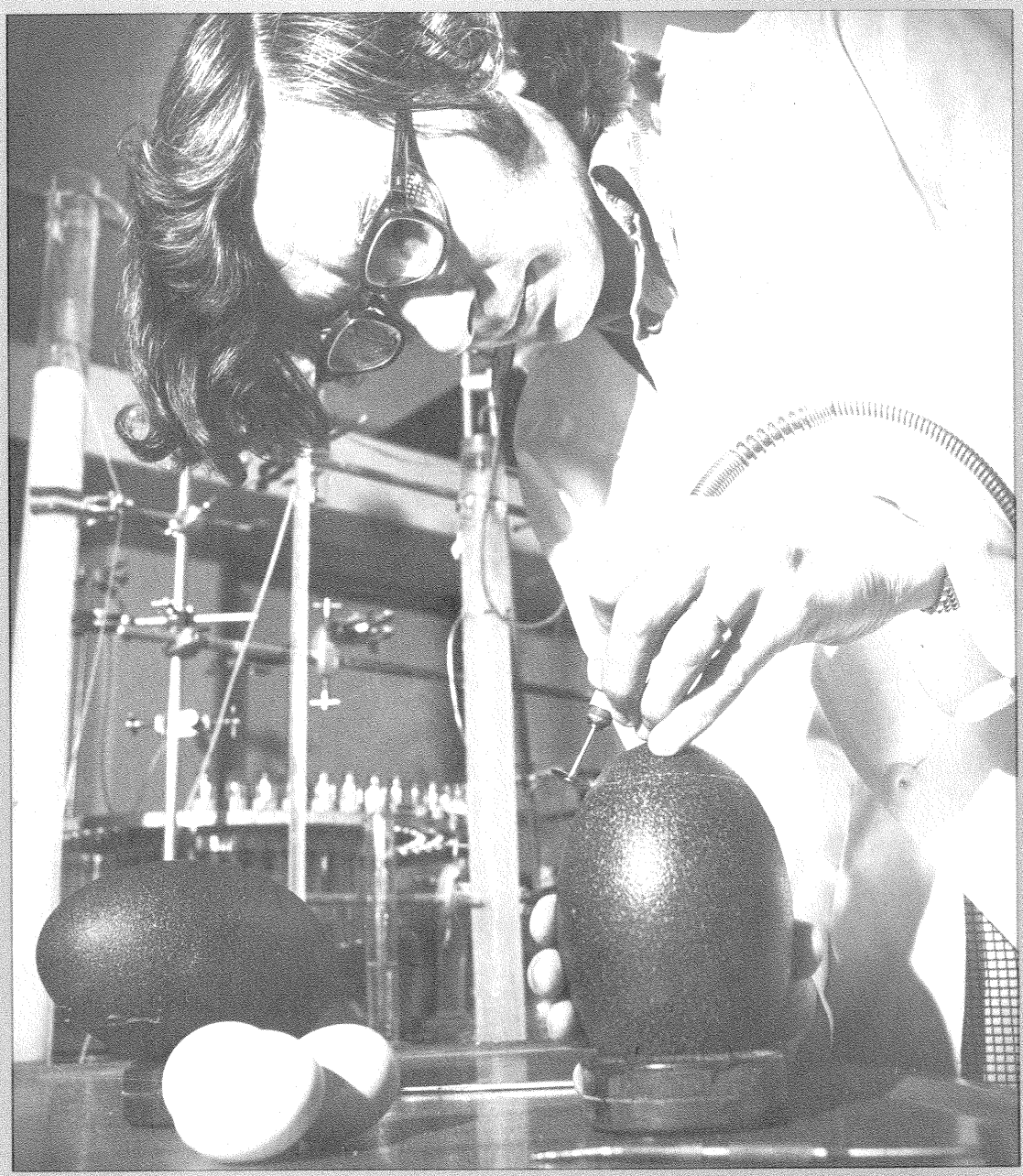
Vol. 35 No.1 March 1975

Mr Rooney

CSIRO Food Research Quarterly



Recent advances in the chemistry of egg yolk

By R. W. Burley

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How a study of emus' eggs is helping to unravel the secrets of hens' eggs

Eggs have a long history as a human food and now form an important part of the diet of nearly everyone in Australia. It is, therefore, surprising how little we really know about the chemistry of the contents of an egg. Our ignorance is especially profound when we consider the yolk. Not only are we uncertain exactly how many chemical substances are present in yolk, but the nature of the interactions between the major constituents (lipids, proteins and water) is largely obscure.

For these reasons practical questions about the behaviour of egg yolk during processes such as baking, and about the source of the emulsifying and other properties of yolk, cannot be answered satisfactorily, nor can we adequately explain the alterations that occur in yolk during freezing and storing. In fact it has been stated by Forsythe in the *Baker's Digest* (1970) that: '*Egg yolk is undoubtedly the least understood lipid-containing ingredient used in the mix and snack food industry*'.

Lack of knowledge of the chemistry of lipid–protein interactions is also a disadvantage in branches of the food industry that do not use egg yolk as well as in biology and medicine where the functioning of lipid–protein membranes is now a major subject of research.

This article attempts to summarize what is known about some broad aspects of the chemistry of egg yolk and discusses some recent studies in the Division on egg yolk and lipid–protein interactions in general.

Biologically, avian egg yolk is part of the food supply of the embryonic bird. It provides all the embryo's lipid, i.e. oil plus fat, much of its protein, all its iron and most of its phosphorus and calcium. The yolk also contains *c.* 50% water. These substances, some of which are highly reactive chemically, are packed in a form that remains stable until they are needed, perhaps months after the egg was laid.

Exactly why the yolk remains stable for so long we do not know, nor do we know much about the state of the yolk before the egg is opened. We do know that yolk is synthesized as a series of concentric layers, and we suspect that these may be stabilized in the intact egg by a fragile membranous honeycomb (Burley 1969). There are few techniques capable of telling us much about the intact egg. After the yolk has been removed from the shell and separated from the white it is easier to study, which is fortunate because this is the form that is of particular interest to food technology.

When viewed under a microscope, egg yolk is both complicated and fascinating (Fig. 1). It appears to consist of a series of globular particles in a background of minute granules, both floating in a clear yellow fluid. The composition of the globules is still unknown as they have never been isolated. There is no doubt, though, that most of the yolk solids are in solution in the liquid phase.

Chemically hens' egg yolk has the approximate composition given in Fig. 2; more precise values may be found in a reference such as Shenstone (1968).

The macromolecular constituents of yolk may be separated by high speed centrifuging, and this was the way the chemical nature of yolk was originally worked out by W. H. Cook and coworkers in Ottawa in the 1950s (e.g. Vandegaer *et al.* 1956; Cook and Martin 1969). After dilution with isotonic saline solution and centrifuging, yolk appears as shown in Fig. 3 (see inside front cover). The

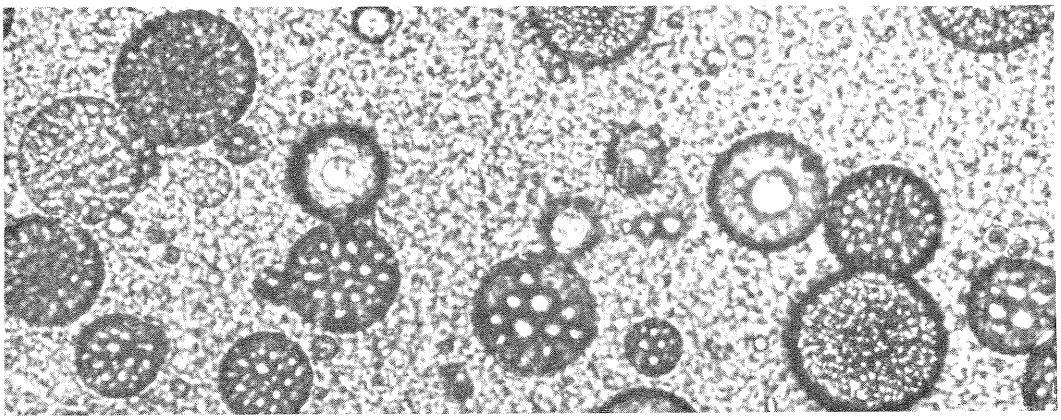

Fig. 1. Egg yolk as it appears under the microscope (low power). Photo: Joan M. Bain.

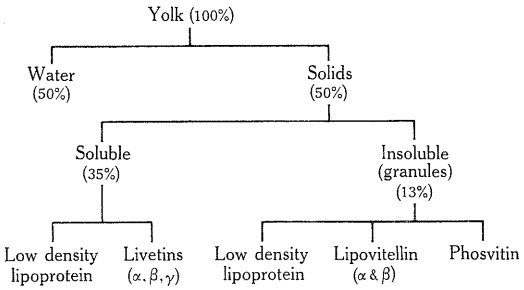

Fig. 2. Approximate composition of hens' egg yolk. Only macromolecular constituents and water are indicated.

major constituent of hens' egg yolk is a lipoprotein (Band A) that contains more than 60% of the dry weight of the yolk. This material was first isolated by Cook and coworkers who determined its composition. Unlike the other constituents of yolk, which are named after the Latin word for yolk, 'vitellus', this lipoprotein has never been given a specific name but has been referred to as the yolk 'rising fraction', 'floating fraction', 'low density lipoprotein', 'very low density lipoprotein' or 'high lipid lipoprotein'.

Lipoproteins are complexes of neutral lipid, phospholipid and protein with properties that differ from those of their constituents but are not stabilized by covalent bonds. An important result of Cook's work was the recognition that the lipid in yolk is not free, but is all bound in lipoprotein particles in some way at present obscure. It is, therefore, incorrect to refer to 'free lipid' in yolk. Part of the lipid of yolk is easily extracted with ether, for example; but this is probably because organic solvents readily disrupt the lipoprotein particles. Typical lipoprotein particles from the yolk of hens' eggs are shown as they appear in the electron microscope in Fig. 4. Most appear nearly spherical but some appear faceted or partly flattened. The electron microscope is not sufficiently powerful to reveal the detailed arrangement of the protein and lipid in these particles but, as discussed below, the Division is tackling this problem of structure.

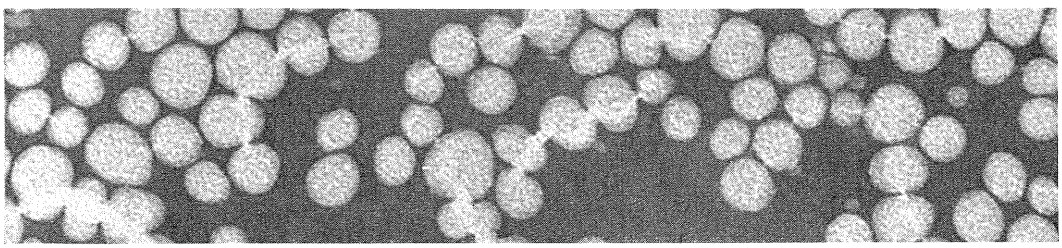

Fig. 4. Electron micrograph of low density lipoprotein of hens' egg yolk (×28 000). Photo: D. W. Gove.

The yolk's intermediate layer (B in Fig. 3) contains the soluble proteins, the livetins, three of which have been recognized (α, β and γ) although their function is still unknown. One suggestion, not supported by conclusive evidence, is that they are analogous to the globulins of blood serum.

The lower layer (C in Fig. 3) consists of the so-called 'yolk granules'. The contents of the other layers (A and B) are soluble in the aqueous phase of yolk, which is essentially a 30% solution of lipoprotein and a 12% solution of protein, but the granules are insoluble in the yolk fluid and form the background of irregular particles seen in yolk under the light microscope at low magnification (Fig. 1). The granules are composed of high density lipoproteins (α and β lipovitellin), a phosphoprotein (phosvitin) and calcium. They may be dissolved in concentrated salt solution, when the macromolecular constituents are liberated. The granules also contain a small amount of a low density lipoprotein which differs from the lipoprotein in the upper layer (A). Little is known about the organization of the yolk granules, and their function is unknown. They contain all the iron in the yolk and much of the calcium (Burley and Cook 1961).

Because of its high concentration, the low density lipoprotein (A, Fig. 3) would be expected to determine most of the important physical and chemical properties of yolk. Evidence of this has recently been provided by Kamat and Lawrence (1973), who studied the baking of cakes in which whole yolk was replaced by the various yolk fractions. They found that the low density lipoprotein was essential for the production of a satisfactory cake.

The composition of the low density lipoprotein of hens' egg yolk is given in Fig. 5, which shows that it contains a variety

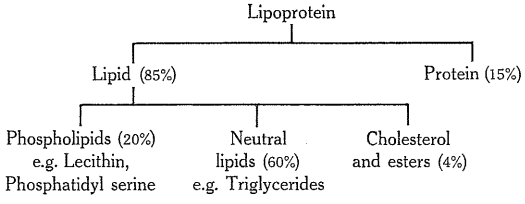

Fig. 5. Composition of low density lipoprotein of hens' egg yolk.

of neutral and polar lipids in addition to protein. Previous studies at this laboratory were especially concerned with the protein and the chemical properties of the lipids, although now more attention is being given to the physical properties of the lipids. The function of the protein is not likely to be elucidated until more is known about its chemical properties, and before these can be studied the protein has to be isolated and characterized. This presented considerable difficulty largely because it was not possible to dissolve the isolated protein without the use of degradative solvents or other drastic chemical treatments. However, we have now solved this problem and are able to isolate the total protein fraction from the lipoprotein of hens' egg yolk in a form that is soluble and amenable to study by the conventional methods of protein chemistry.

This achievement came as a result of studying the yolks of the eggs of other species. It was argued that properties of importance in determining the ease of isolation and solubility of the yolk proteins are unlikely to be of survival value to the organism and so would be expected to vary in a more or less random way from one species to another. Thus, assuming a reasonably rapid rate of mutation consistent with the probable non-enzymic nature of the yolk proteins, we should expect to find other birds' eggs with more amenable proteins.

After examining the eggs of several other kinds of birds we found, as we had hoped, that not all are as difficult to deal with as those of the hen. We were fortunate in finding that the low density lipoprotein of the emu's egg yolk has proteins that are especially suitable for study. A female emu lays about 20 very large eggs (Fig. 6) in a season, each being equivalent to almost 12 hens' eggs. In spite of its larger size, the yolk from an egg of an emu which has been eating the same sort of food as hens is indistinguishable in appearance and general characteristics from that of a hen's egg. Eggs of the wild emu have a slightly different lipid composition depending on the bird's diet.

Chemically the constituents of the egg yolk from both birds are the same (Fig. 2) but the proportions are somewhat different. Nevertheless, the main constituent of emus' egg yolk is a low density lipoprotein which is very similar to that of hens' egg yolk, and cakes made from it are similar.

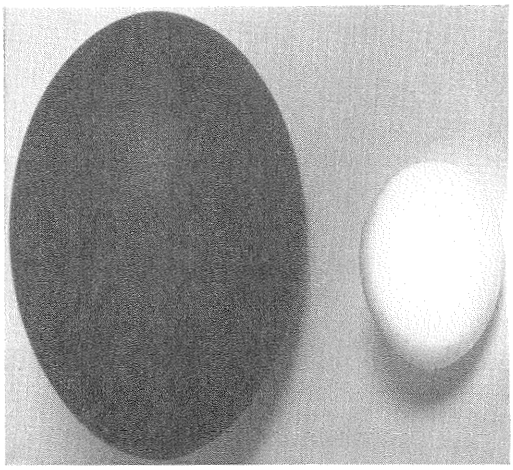

Fig. 6. Emu's egg and hen's egg.

From the emu's lipoprotein we have been able to isolate the major protein in a pure state. It has been named 'emu's apovitellenin I'. Its amino acid composition is unusual

Lys-Ser-Ile-Phe-Glu-Arg-Asp-Asn-Arg-Arg-Asp-Trp-Leu-Val-Ile-Pro-Asp-Ala-Val-Ala-Ala-Tyr-Val-Tyr-Glu-Thr-Val-Asn-Lys-Met-Phe-Pro-Lys-Val-Gly-Gln-Phe-Leu-Ala-Asp-Ala-Ala-Gln-Ile-Pro-Val-Ile-Val-Gly-Thr-Arg-Asn-Phe-Leu-Ile-Arg-Glu-Thr-Ser-Lys-Leu-Ser-Ile-Leu-Ala-Glu-Gln-Met-Met-Glu-Lys-Val-Lys-Thr-Leu-Trp-Asn-Thr-Lys-Val-Leu-Gly-Tyr-Tyr.

Fig. 7. Amino acid sequence of emu's apovitellenin I.

and the molecular weight, *c.* 10 000 (Burley 1973), is very low. At the CSIRO Division of Protein Chemistry, Dr Theo Dopheide and Dr Adam Inglis have recently worked out its amino acid sequence (Dopheide and Inglis 1974). The sequence is one of the

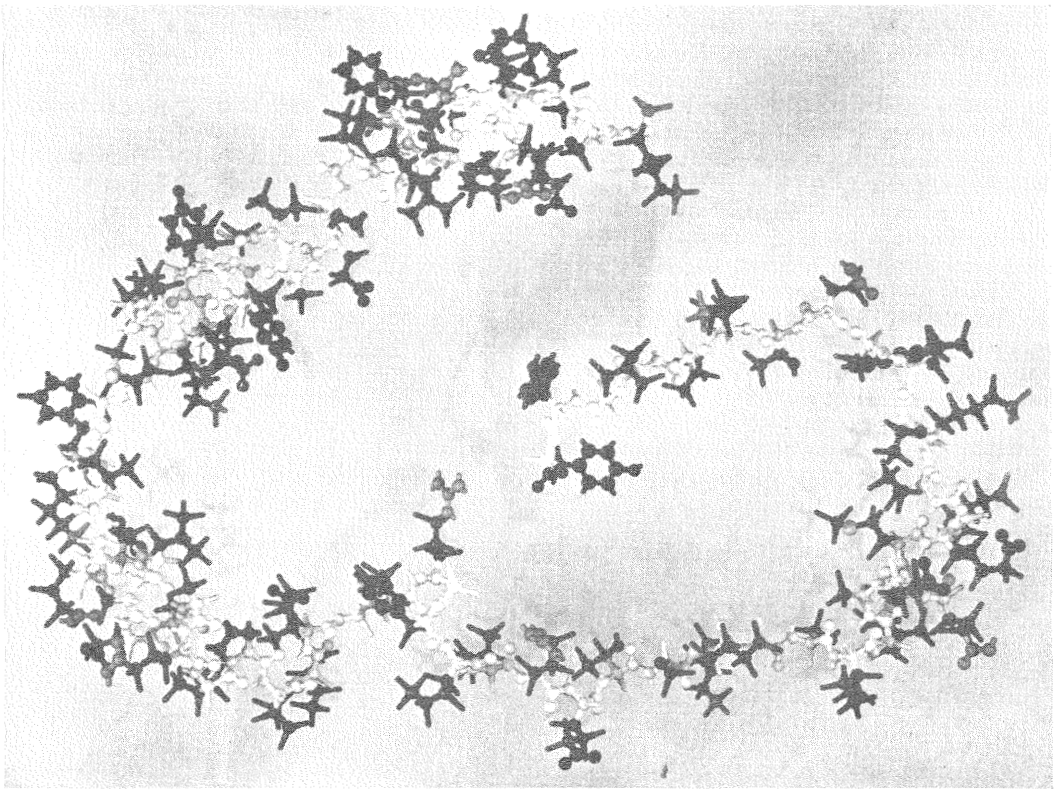

Fig. 8. Atomic model of the possible structure of emu's apovitellenin I.

most important properties of a protein and this is the first time that a protein from a yolk lipoprotein has been sequenced. It is also one of the first lipid-associated proteins to be sequenced.

The sequence is shown in Fig. 7 and a large-scale molecular model is shown in Fig. 8. On this scale the original egg would be about the size of Australia, and a lipoprotein particle would have a diameter of several metres. We do not yet know the structure of the molecule, i.e. whether it is globular, linear or circular, although from its physical properties we postulate that in the lipoprotein it is present in an extended and aggregated state. Fig. 9 is a highly speculative diagram of how we imagine the protein might exist in the lipoprotein. It is based on the 'lipid core' model recently elaborated by Schneider *et al.* (1973) in which the lipoprotein particle is essentially a globule of neutral lipid (shown in Fig. 9 by removing a segment of the particle) surrounded by phospholipid and protein. According to our model the protein occurs as an aggregated network around rafts of phospholipid that float on the lipid core.

By studying the properties of protein in emus' egg yolk we have gained a better understanding of the proteins associated with lipid in the egg yolks of other species. In particular, the investigations involving emus' eggs have enabled us to devise methods of solubilizing and isolating the proteins not only of the low density lipoprotein of hens' egg yolk, but also of the yolks of other birds' eggs as well. However, we have found that the proteins in the hen's egg are more chemically reactive and more complicated than those in the emu's egg. Possibly because the emu's egg has to wait longer before hatching, an evolutionary adaptation has led to the elimination of some of the more reactive chemical groups. We have isolated a pure protein from hens' eggs that is analogous to emu's apovitellenin I and we are at present trying to ascertain its properties as a knowledge of these could help solve practical problems encountered when egg yolk is used in food processing.

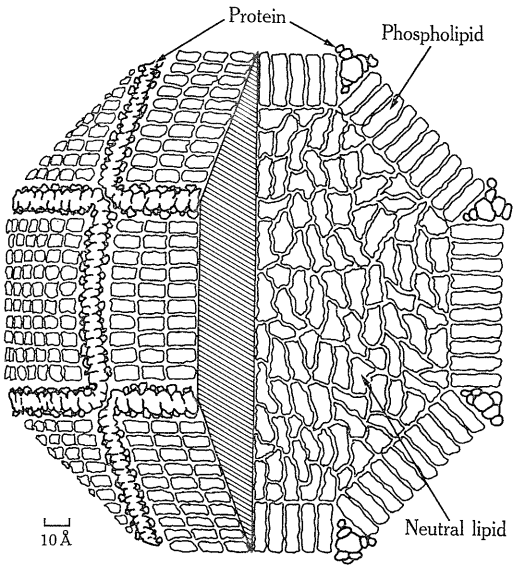

Fig. 9. Part of a lipoprotein particle showing possible arrangement of neutral lipid, phospholipid and protein.

References

Burley, R. W. (1969). Lipid–protein interactions in the liquid phase of egg yolk. Proc. Australas. Poultry Sci. Conv. pp. 141–7.

Burley, R. W. (1973). Isolation and properties of a low molecular weight protein (apovitellenin I) from the high-lipid lipoprotein of emu egg yolk. *Biochemistry* **12,** 1464–70.

Burley, R. W., and Cook, W. H. (1961). Isolation and composition of avian egg yolk granules and their constituent α- and β-lipovitellins. *Can. J. Biochem. Physiol.* **39,** 1295–1307.

Cook, W. H., and Martin, W. G. (1969). *In* 'Structural and Functional Aspects of Lipoproteins in Living Systems', eds E. Tria and A. M. Scanu. p. 579. (Academic Press: New York.)

Dopheide, T. A. A., and Inglis, A. S. (1974). The amino acid sequence of a protein (apovitellenin I) from the low-density lipoprotein of emu egg yolk. *Aust. J. Biol. Sci.* **27,** 15–21.

Forsythe, R. H. (1970). Eggs and egg products as functional ingredients. *Baker's Dig.* **44,** 40–6.

Kamat, U. B., and Lawrence, G. A. (1973). Contribution of egg yolk lipoproteins to cake structure. *J. Sci. Food Agric.* **24,** 77–88.

Schneider, H., Morrod, R. S., Colvin, J. R., and Tattrie, N. H. (1973). The lipid core model of lipoproteins. *Chem. Phys. Lipids* **10,** 328–53.

Shenstone, F. S. (1968). *In* 'Egg Quality: a Study of the Hen's Egg', ed. T. C. Carter. p. 26. (Oliver and Boyd: Edinburgh.)

Vandegaer, J. E., Reichmann, M. E., and Cook, W. H. (1956). Preparation and molecular weight of lipovitellin from egg yolk. *Arch. Biochem. Biophys.* **62,** 328–37.

Microbiological quality control in foods*

By K. C. Richardson

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A successful program depends on close supervision of raw materials, preparation and processing, and only secondarily on inspection of the finished product

The most quoted words encountered in recent years whenever microbiological standards, whether internal factory standards or legislatively enforceable standards, are discussed are those spoken by Sir Graham Wilson (1970) in his concluding remarks at a symposium on the subject in England:

'*Bacteriologists are better employed in devising means to prevent or overcome contamination than in examining more and more samples. Their chief contribution to the supervision of our food supply should be to ensure that the preparation and processing are properly carried out. This means, of course, paying attention to the quality of the raw supply and to the treatment of the food all along the line. Control of processing, using this term in a broad sense, is of far greater importance than examination of the finished article. Processing, that is to say, should be controlled primarily by technique and only secondarily by results.*'

All food microbiologists would agree with this statement. The concept that it contains forms the basis of this article and should be the basis of any quality control program.

Principles of microbiological control

Examination of finished products is a process of inspection, not of control, and it is economically desirable to reduce to a minimum inspection at the finished stage. Microbiological examination is relatively expensive in terms of time and labour and rarely gives immediate results. Besides, the results, far too often, put somebody in the invidious position of having to decide whether a product should be released. He has to judge, usually on the basis of a very limited sample, whether the product is a potential or actual health hazard, whether it will have a useful storage life during commercial distribution or whether it should be dumped.

It is far better to identify and isolate the source of trouble early in the processing chain so that remedial action can be taken to minimize the quantity of product that will be defective. In practice it is seldom possible to guarantee complete control over raw materials and processing conditions. Consequently, some finished-product inspection will always be necessary. Nevertheless, the control principle is achievable and should be aimed at. It is difficult to justify to management the expense of such rigorous process checks, particularly at the raw-material stage, but experience, notably in the canning industry, has demonstrated that they provide the cheapest long-term form of both quality control and quality assurance.

There is also the practical advantage that some raw materials can and should be held in storage until time has elapsed for a clearance to be advised from the laboratory. This is frequently not possible with the finished product. When the raw materials have been shown to be satisfactory, the microbiologist should concentrate his efforts on providing the production staff with firm guidelines on desirable processing technique from his point of view. He must first convince senior management that his point of view is more important than anybody else's, or at least equally important, and he must be responsible only to senior management.

The guidelines should consist of recommended temperatures for handling a

*From a lecture in the course on 'Food-borne microorganisms of public health significance' arranged by CSIRO, AIFST and the University of New South Wales, and given at the University of New South Wales in July 1974.

given foodstuff, the time it can be held at this temperature without undue deterioration, and procedures for handling different foods to eliminate cross contamination and contamination by personnel. Contamination from equipment can be kept to a minimum by procedures defined by the plant microbiologist. He should check the efficiency of any cleaning and sanitizing procedures, and on the basis of his figures he should recommend how often the steps should be carried out and any necessary modifications.

All this can be done only if the microbiologist has a thorough understanding of the processes he is monitoring in order that he can carry out the right kind of investigations and obtain significant answers. If he plans his job properly, a thermometer and a wall clock become his main instruments in controlling the bacteriological quality of the final product.

Setting internal standards

Quality control is often considered under the three aspects—control of raw material, process control and inspection of the finished product (Hawthorn 1967). The factory microbiologist should also play an integral role whenever a new product is being developed or an existing one modified. If new plant is involved, experience of suitable cleaning must be gained so that the type of cleaning program and materials to be used can be written into the factory schedule. The new product must be critically examined from two aspects.

- The spoilage pattern of the food should be ascertained at different temperatures of storage.
- The fate of any contaminating, food-poisoning organisms must be determined beyond reasonable doubt.

These are not necessarily easy questions to answer and for this reason qualified microbiologists should be employed by food manufacturers. By answering these questions during developmental work, the microbiologist draws up internal standards which become the basis for controlling the quality of the product. Commercial experience may cause initial standards to be modified, but this in no way diminishes the need to establish them.

To elaborate on this point it is convenient to divide foods into three categories: non-sterile foods fully cooked before consumption, non-sterile foods not cooked before consumption and commercially sterile foods.

Non-sterile foods fully cooked before consumption

Fresh poultry has been chosen as an example of a major group in this class—fresh flesh foods.

Spoilage pattern

Evidence of off odours can be detected in fresh poultry when the standard plate count is in the range 10^6–10^8 organisms per cm^2 (Green 1974). Assuming an initial population as low as might reasonably be attained, the quality control microbiologist must determine how long it will be before spoilage levels are reached at different temperatures. If he does not have reliable figures to work from he must obtain them by doing his own plate counts.

The usual bacterial population of chickens is 95% *Pseudomonas* spp. (Ayres 1960) and the accepted storage temperature for chilled poultry is 2–5°C. In this temperature range the mean generation time for *Pseudomonas* spp. is between 7 and 14 h (Tompkin 1973). Thus, if poultry is kept for 72 h at 2–5°C, a maximum of 10 generations could be attained by the initial contaminants. Therefore, unless the product has an initial count of less than 10 000 organisms per cm^2 it is unlikely to remain wholesome during a reasonable period in retail or domestic storage. Products with a count between 1000 and 10 000 may be considered satisfactory, but if a high proportion of the samples examined is at this level, action is necessary. It is not the function of the microbiologist to put things right himself; he should inform production through appropriate channels that a potentially serious situation is developing which must be rectified.

Chilled poultry is an extreme case, but the same basic reasoning can be applied to all products in the category of fresh flesh foods and, in fact, must be applied if a basis for quality control is to be achieved.

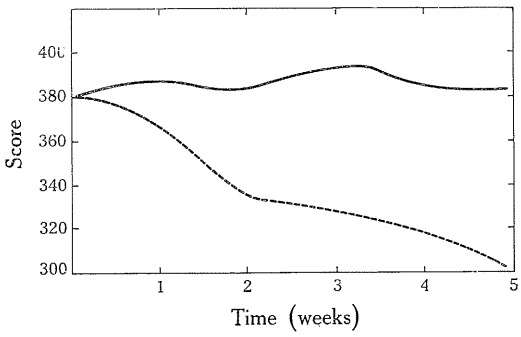
The concept used in this discussion is described in detail by Dyett (1970) who used smallgoods as an example. He described a system of internal grading for pork sausages which is based on a standard plate count. There are four grades—A, B, C and D, although the system can be varied, of course, to suit a particular

situation. There seems no good reason to extend the number of grades beyond four and in many cases three will be sufficient. The simplest system involves only two grades—satisfactory and unsatisfactory, and this is the one most widely used. However, this system must be quite arbitrary and it is not realistic in many industrial situations.

Boundaries between the different grades described by Dyett are so arranged that under normal circumstances the bulk of production falls into grade A (< 10 000/g). A few results will fall into grade B (10 000/g–50 000/g) with an occasional result in grade C (50 000/g–100 000/g). When the system is feeding back information to the production supervisors, no results should come into grade D (> 100 000/g).

If each grade is given a numerical value, for instance grade A = 4, B = 3, C = 2 and D = 1, the bacteriological status of a food over a period, as determined either by sampling the end product or by sampling at a selected point during production, can be represented graphically. If 100 samples are taken over the given period, the maximum score possible is 400. Two possible results are shown in the figure. The solid line indicates that a high quality product is leaving the factory and that raw material and process controls are operating satisfactorily. The broken line indicates a rapidly deteriorating situation which should have been arrested in the factory after a week or so.

When setting internal standards, the bacteriologist must use common sense. Reducing the plate count of a product generally costs money. If a reduction in the count has no practical effect on commercial performance then the microbiology laboratory falls into disrepute and its future advice may be poorly received or even ignored. On the other hand, if what is normal is not regarded by the bacteriologist as satisfactory, it would be foolish to let 'normal' become synonymous with 'satisfactory'. If this is allowed to happen the situation could backfire both on the company and on the microbiologist. Such a position could become particularly serious when the quality control program concerns bacteria that could cause food poisoning.



Quality control scoresheet.

The standard plate count is not, of course, the only test that can be used as an index of quality. However, keeping quality or storage life will always be a major concern of any control program, and one should never lose sight of this fact among the plethora of tests available to microbiologists. Bacteriological cleanliness, as indicated by low plate count recordings, will ensure a reasonable storage life for a product; it will not necessarily ensure that the food is free from the risk of food poisoning contaminants.

Food poisoning organisms

What happens to contaminating organisms depends, first of all, on what happens to the food they are in. In the previous example it is reasonable to expect that the chicken will be cooked, either fried, roasted or stewed, before being eaten. It may then be eaten hot or consumed at a later time after cooking.

Accepting our standard for storage life and assuming reasonably fast distribution, the chicken can be a cause of food poisoning only if it is mishandled in the kitchen after cooking. The manufacturer and his microbiologist cannot set standards to avoid this, but they do have an obligation to see that the product does not contain more than a certain number of food poisoning bacteria. Given the present state of technology in this country, we must accept that a significant proportion of birds will enter the factory contaminated with *Salmonella* and *Staphylococcus aureus*, and probably all will be contaminated with *Clostridium perfringens*. It then becomes the business of the microbiologist to ensure that

- as many of these contaminants as possible are removed or destroyed during processing,

- their growth is not permitted, and
- contamination from bird to bird and from equipment to bird is minimized.

With this product microbiological examination can be used only to supplement process inspection and to pinpoint possible sites of contamination. When these sites are ascertained, modification to the process line may be indicated.

Non-sterile foods not cooked before consumption

This class of food enables an illustration of the basic thesis concerning food poisoning organisms. In the example previously given, the standard plate count was suggested as being the essential tool for microbiological quality control. With this class of food which will include many dried, frozen and cured products, we must extend the range of organisms for which testing becomes necessary. From this extremely wide range of products, a frozen dessert item, chocolate mousse, may be selected to demonstrate the kind of thinking involved in drawing up an appropriate control program.

The food is frozen and will be consumed in this state, so we must assume that the microbial population of the product as it is eaten will be the same as when it leaves the factory. The factory microbiologist must determine how many and what types of organism are likely to occur as a result of the company's manufacturing practices. He must then decide whether new techniques should be introduced in order that the product can be marketed safely within the specifications considered essential.

After he has studied the ingredients, the microbiologist may decide that his specification for the finished product should include a plate count to alert him to the possible cause of off flavours detected by other branches of the quality control program. He may decide upon a specification for salmonella and a coagulase-positive staphylococcus in order to protect the consumer's health and his company's reputation. He may select other parameters such as coliforms, *E. coli* or staphylococcal enterotoxin. It is his job to decide what will really tell him something about the product and what he can cope with in the laboratory.

Nevertheless, internal standards are more important than this final inspection and should be the ones used in the factory to ensure a suitable shelf life and freedom from public health hazard. Incidentally, such standards should only rarely be used to ascertain process efficiency which is better measured by physical means and visual inspection.

Plant hygiene needs to be evaluated on the basis of microbiological standards, as physically clean surfaces may not be free of microorganisms. It may be decided to use a standard plate count (SPC) for salmonella and staphylococcus determinations, and that the limits should be set as:

SPC $\neq$ 100 000/g;
Salmonella absent in 50 g;
Coagulase-positive staphylococci $\neq$ 100/g.

If we take up the aspects of quality control emphasized by Hawthorn, it should be clear that priority of attention must be given to the more important raw materials in planning a quality control program. Hawthorn gives three very practical rules.

- The dominant raw materials are selected for priority attention. These are not necessarily the raw materials used in largest amount, but may, for instance, be ones that have a record of association with food poisoning organisms.
- The selected materials are tested in relation to their contribution to product quality.
- The materials tested are released from store only after the test results have been properly assessed and recorded.

These are general rules which should be applied to all quality control procedures.

In our example, chocolate mousse, raw materials may be chocolate, shortening, flavouring essence, dried or frozen whole egg, emulsifier, whole milk or at times dried milk and water. The selected materials to undergo rigorous microbiological inspection would be the chocolate (salmonella, staphylococcus), the processed egg (salmonella) and the dried milk (SPC, staphylococcus). In this case all three could be considered dominant raw materials.

The microbiologist then determines what specification he will put on each raw material, taking into account his specification for the final product and the proportion of each raw material in the product. In general it is unwise to rely on any processing step to eliminate food poisoning organisms or their toxins; in this

particular instance it is impossible.

If results obtained on raw materials show that the proposed specification for the final product cannot be met, the bacteriologist must advise management that higher quality raw materials are essential. He should not take this step, however, until convinced that his specifications are realistic and related to a real health hazard or risk to quality.

Having established the principles on which raw materials may be cleared for production, the microbiologist should next turn his attention to the process. Hawthorn has two further general rules.

- Process control must relate the processing results to the raw materials tests.
- The critical points in the process must be defined and subjected to concentrated attention.

The order could perhaps be reversed, but the commonsense in these rules is indisputable.

It is difficult to discuss or set specifications for raw material control until we know what increase or decrease in microbiological and pathogenic load may occur during processing. Equally, one cannot stipulate process control with certainty, without assuming that proper control of raw materials is in operation.

In planning a scheme for microbiological process control it is advisable to study a flow diagram, taking care to show alternative processing steps where these exist to accommodate changes in raw material, as in our example where the alternatives are whole or dried milk. The flow diagram should be drawn up after an inspection of the processing line in company with the plant engineer. With chocolate mousse, process control will consist of strict temperature control during production, even during breakdowns, and care of hygiene for plant and personnel. This is a general rule, except that with some products temperature control will consist of keeping the material above the maximum growth temperature of pathogenic and most other microorganisms, rather than below the minimum growth temperature, as in this instance. A series of temperature checks, which should be recorded, will tell the microbiologist just as much as bacteriological sampling.

Further control, beyond that provided by temperature checks, is needed for plant and personnel hygiene. However, it should be emphasized that contamination of a foodstuff by factory sources is usually not a problem until contaminating microorganisms have been given a subsequent opportunity to proliferate in the food.

Plant hygiene

During line inspection the potential points of bacterial build up can be ascertained. The bacteriologist should then bring these points to the attention of the process and engineering staff and try to have them eliminated. If this is not possible the points should be marked on the flow sheet for special attention during cleaning and disinfection. They should also be monitored by microbiological checks which could be applied either to the work surfaces or to the product after it has passed over them. The SPC provides a good measure of general cleanliness and there are other techniques that may be used (Brownlie 1971). Pathogenic organisms known to be present in the raw material, or likely to have been transmitted by operatives, may be checked with differential selective media. The purpose of the check for organisms known to be in the raw material is to ensure that no multiplication is occurring. It should not be necessary if proper process control is operating. By the same token, where the risk of product contamination by personnel exists, the microbiologist should advise on what handling precautions are necessary, rather than conduct counts to determine what is happening. Such counts need only be done as occasional checks unless examination of the final product gives cause for concern.

With proper planning, the testing of surfaces for undue microbial contamination can also be minimized. The microbiologist should participate in designing the cleaning program and he should monitor the program when it is introduced. Techniques for the microbiological examination of work surfaces were discussed in last September's issue of the *Quarterly*, and also by Favero *et al.* (1968), and in greater detail by Brownlie (1971) who gives guidelines for the level of contamination which may be tolerated. If proper control is exercised over the disinfecting program for the factory, such checks should not be necessary more frequently than once a week. Physical inspection is less satisfactory in

plant hygiene control than in process control, and the frequency of checks may be determined by experience and by the degree of confidence one has in the operatives.

It is important, when carrying out such tests, to select the growth medium which yields the most informative results—with a high-salt product, the plate count should be carried out in a suitably adjusted medium. Similarly with an acid product, one should select a medium with a pH which will screen out non-acid-tolerant organisms.

The standard of water used in processing and cleaning is also better checked by chemical means rather than bacteriologically. For day to day quality control bacteriological checks are not as useful as measuring free chlorine and contact time. Nevertheless, one must always take into account the possibility of human error and equipment malfunction, and checks should be maintained at selected intervals because only they give direct information.

Testing the final product

When checks on raw material have been carried out in detail and process control is properly designed and effectively practised, inspection of the finished product merely serves a confirmatory function. Since resources in any control laboratory are limited, one has a choice of examining many samples by comparatively simple tests, or of subjecting a smaller number of samples to many different or more complicated tests.

I consider the first procedure has more general application than the second. The need for detailed tests should be obviated by earlier quality control procedures. Returning to the example of chocolate mousse, the SPC and salmonella check on selected samples off the line would be adequate. This routine should, however, be varied from time to time. As an alternative to checking for salmonella when the raw materials have been cleared, checks for *E. coli* or coagulase-positive staphylococci may be substituted.

If these product checks indicate that a problem situation is approaching, this means, of course, that the process checks have failed to give a warning, and in-line checks and work surface counts will need to be carried out to pinpoint the source of trouble. Such checks would be taken at points already defined on the flow sheet as possible problem areas which should have received strict process inspection. The list of these points may have to be extended to cover other areas when the result of an investigation is known.

The group of non-sterile foods not cooked before consumption, and the processes involved, are so diverse that one example cannot adequately cover all points. For instance, in a dried food-mix containing vegetables or meat, it may be appropriate to have an internal standard for *Clostridium perfringens*, an organism which is more likely to be a real hazard than a heat-labile pathogenic organism. Process control may be used to eliminate growth of such an organism if it is present in acceptable raw material.

Commercially sterile foods

Canned foods form a peculiar group and some special points need to be made within our basic framework of raw material control, process control and finished product inspection.

The lethality of any given process for a low-acid food is based on an initial spore population. When the initial spore load is exceeded, the process may become inadequate—dangerously so in some circumstances.

Canners have a tendency to restrict their microbiological checks on raw materials to the standard materials of starch and sugar for which they may have purchase specifications. In many instances, they may completely ignore even these specifications. This is particularly true of the meat canning industry where shortages sometimes cause lower grades of raw material to be used. These include not only minor, but also major ingredients which could be a source of excessive spore loads. An examination of the history of the ingredients should tell the microbiologist whether a standard must be met before the ingredient is released for further processing.

The importance of process control cannot be over emphasized as a means of avoiding spoilage through under processing or leakage of seams, with the attendant risk, in both instances, of food poisoning. A particularly important aspect here is the handling of the cans after they have left what we will assume was correctly

chlorinated water. The risk of entry of bacteria into the can through leaks continues as long as the seams of the can remain wet. Hence, if food poisoning bacteria are to be eliminated from sites of infection, special attention must be paid to disinfecting the can races and to the hygiene of operatives working in this area.

Conclusion

When a finished product is faulty in microbiological terms usually nothing can be done short of rejection. Recovery is rarely possible and never to be recommended. Therefore, inspection of the finished product has a relatively minor part to play in a good system of quality control. Its function is to confirm that earlier controls have operated properly and to disclose any weaknesses which may exist in these controls. For this reason it is legitimate, periodically, to analyse the final product for food poisoning organisms which received little or no attention in tests at earlier stages. The frequency of sampling will diminish progressively as confidence in the earlier controls increases, but a minimum level of sampling will always be required.

The problem is to define this level. Some would employ statistical calculations to estimate the chances of a given sample size reducing the risk of inadequate sampling to 1 in 10^3, or 1 in 10^6, or to any other level considered desirable. None of these calculations will eliminate the element of risk, unless 100% sampling is used. Statistics enable us to assess the chances of error, but judgment must be exercised to decide the level of acceptable risk.

While this is a legitimate procedure and may well be essential when the microbiologist is called upon to examine a consignment of food which was not produced under his direct control, e.g. an ingredient obtained from another company and perhaps in another country, it is a dubious exercise in in-plant control of the final product.

Controls of raw materials and processes should be designed to do the real work. Quality control is a continuous and evolving concept in which the collection of figures on the final product is only a small part.

References

Ayres, J. C. (1960). The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. *J. Appl. Bacteriol.* **23,** 471–86.

Brownlie, L. E. (1971). Microbiological testing of work surfaces. *In* 'Quality Control Techniques School Notes'. Pap. 4. (CSIRO Div. Food Res., Meat Res. Lab., Cannon Hill, Qld.)

Dyett, E. J. (1970). Microbiological standards applicable in the food factory. *Chem. Ind.* (*Lond.*) 189–92.

Favero, M. S., McDade, J. J., Robertsen, J. A., Hoffman, R. K., and Edwards, R. W. (1968). Microbiological sampling of surfaces. *J. Appl. Bacteriol.* **31,** 336–43.

Green, S. (1974). Microbiology in the poultry processing industry. *Process Biochem.* **9,** 27–8.

Hawthorn, J. (1967). The organization of quality control. *In* 'Quality Control in the Food Industry', ed. S. M. Herschdoerfer. Vol. 1, pp. 1–31. (Academic Press: London.)

Tompkin, R. B. (1973). Refrigeration temperature as an environmental factor influencing the microbial quality of food. *Food Technol.* **27,** 54–8.

Wilson, G. (1970). Symposium on microbiological standards for foods: concluding remarks. *Chem. Ind.* (*Lond.*) 273–4.

Cheese flavour: what is it?

By J. F. Horwood

CSIRO Division of Food Research, Highett, Vic.

An account of recent research into the basis of cheese flavour. From a talk given in June 1974 at the Conference of the Australian Institute of Dairy Factory Managers and Secretaries, Queensland

Cheese is a complex food consisting primarily of protein, fat and water. The components which contribute to cheese flavour are distributed in the fat and water phases and at a much lower concentration in the protein. Just as the flavour components arise from several sources, so the flavour response is composed of several elements. Flavour is sometimes inaccurately called taste; this is incorrect because, as well as the taste sensations of salt, sour (or acid), bitter and sweet in the mouth, flavour also includes the response of the person tasting to the aroma from vapours entering the nasal cavity as the food enters the mouth or from the vapours rising from the back of the throat as the food is swallowed. Other regions of the mouth and nose respond to pain and temperature, and to tactile sensations. The interpretative processes of the brain dealing with the signals transmitted from the taste and smell centres are not yet understood.

The importance to flavour of both taste and aroma is illusrated in this example from an issue of the *Givaudan Flavorist* (1973): 'Taste buds differentiate only between what is sour, sweet, salty or bitter. When smell is hampered taste suffers. This fact is vividly illustrated in an old parlor game where someone is blindfolded and given two different types of "apples" to eat and distinguish between them while holding his nose. Unable to do so, he opens his eyes to see that one of the "apples" is an onion'. This anecdote also points up the important contribution to flavour of the tactile sensations—texture, body and mouthfeel. Such considerations are taken into account by the cheese grader who, in making an assessment, gives points for colour, texture and body as well as flavour.

In the following discussion the flavour of Cheddar cheese will be regarded as the basic type of cheese flavour as most work has been done on this variety. In addition, we will assume that the water content, fat content, texture and body, and temperature are correct for the type of cheese and do not interfere with the perception of flavour.

The taste of cheese

Saltiness and acidity

We well know that cheese tastes salty and acidic (or sour). Bitter taste in cheese is recognized as a defect which has presented some problems recently. Apart from mentioning that the bitterness is caused by an accumulation of bitter peptides, i.e. products arising from protein breakdown (proteolysis), this aspect will not be discussed further as bitterness is not a component of standard cheese flavour.

The salt level required to produce the desirable taste and to control microflora is well established. There is not the same agreement or certainty about the levels of components such as free fatty acids which contribute to the acidic taste. Although the level of free fatty acids rises during maturation, so far no significant correlation has been established between the level and typical Cheddar flavour.

Of the fatty acids derived from the action of lipolytic enzymes on the fat of the cheese, the ones mainly responsible for the acid taste are those containing four to ten carbon atoms. However, it appears that the contribution of these fatty acids to flavour may come more from their aroma than from their taste (Baldwin *et al.* 1973). There is also an important contribution

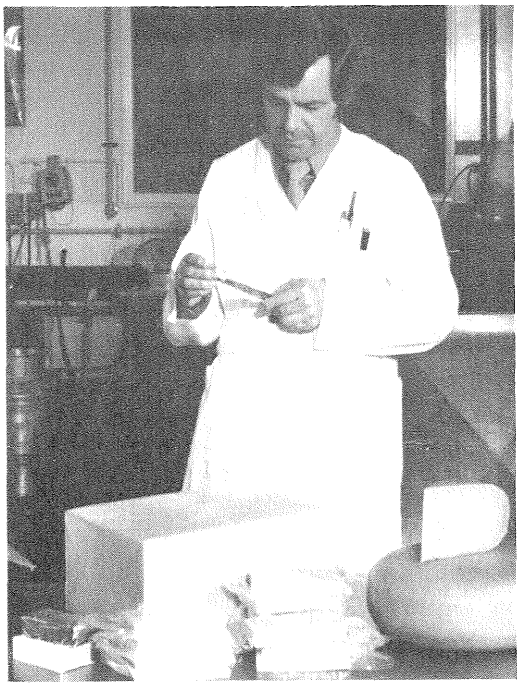
from acetic acid to cheese flavour, but this compound is derived from the enzymatic breakdown of lactose or citrates and not from the fat. If the level of acetic, butyric and hexanoic acids is too low the cheese is mild and lacking in flavour. From our own recent work (Czulak *et al.* 1974) we have found that when the acetic acid level is low and the butyric acid level is high Australian Government graders rate the cheese flavour 'unclean'. At this level butyric acid vapour is being released from the cheese, and can be detected in the aroma by gas chromatography. At higher levels of butyric and hexanoic acids the cheese is said to be rancid.

Influence of bacteria in milk

Recent work in the Netherlands (Stadhouders and Veringa 1973) aimed at determining the influence on cheese flavour of bacteria in the milk. Milk drawn aseptically from cows had a bacterial count of 2–6 per 100 ml! The cheese prepared aseptically from the milk had a very low level of free fatty acids.

It was also shown that enzymes from the starter bacteria were not able to split the milk fat to produce free fatty acids. Control cheese made from the same milk, normally drawn, contained normal levels of free fatty acids. Other workers had observed that if the milk initially contained high levels of bacteria the levels of free fatty acids were higher. However, the Dutch workers also showed that starter bacteria were able to produce free fatty acids from mono- and diglycerides present in fat which already had been partially split.

At present, then, it appears that the presence of some bacteria in raw milk is desirable provided the level is not too high. If the level is too low the combined action of these enzymes from the bacteria in the milk and the starter culture is insufficient to yield enough free fatty acids, and the flavour of the cheese is not Cheddar-like. On the other hand, as cheesemakers can tell us, heat treatment of milk of bacteriologically poor quality does not ensure that unclean and rancid flavours will not develop in the cheese. This is because the fat-splitting enzymes (lipases), which survive the heat treatment, produce too much butyric and hexanoic acids and result in off-flavours. Reiter and Sharpe (1971) have pointed out that if the bacterial quality of cheesemilk further improves, and if some form of continuous cheesemaking process has been adopted which reduces the incidence of contamination from equipment, a method of innoculating the milk with the required flora before or after heat treatment may be necessary.



Evaluating quality characteristics of experimental cheeses at the Dairy Research Laboratory.

Other acidic components

A number of other acidic compounds are formed in cheese. α-keto acids derived from lactose, citrate or protein are strong acids but tend to be unstable. In isolation they undergo condensation reactions to produce compounds with spicy, caramel or curry-like odours. The α-keto acids are intermediates in the enzymic production in cheese of other well recognized and important flavour compounds and they would be expected to remain at relatively low concentrations. A range of α-keto acid has been found in Cheddar cheese. Their contribution to cheese flavour is not certain although they have been included in an interesting formulation for synthetic cheese flavour, which occurs in a recent Unilever patent. At Highett we are in the process of developing a quantitative method for the

determination of α-keto acids in rapidly maturing cheese-curd slurries.

Certain amino acids are present in cheese as breakdown products of protein. They themselves have a broth-like flavour and impart an important background to the flavour of cheese, but do not contribute to the acid taste.

Sweetness

Sweetness is the remaining aspect of taste to be discussed. Small amounts of sugars, such as glucose and galactose, occur in cheese but normally the term 'sweet flavour' as applied to cheese implies lack of acidity. The response may refer to the presence in cheese of small amounts of compounds called esters which have a fruity aroma.

Aroma

Ketones

One group of interesting compounds are the methyl ketones. A study of the formation of methyl ketones during cheese maturation has recently been reported by Walker and Keen (1974) in New Zealand. They concluded that the C3 to C15 odd-numbered ketones, once formed, are not reduced to alcohols or other compounds, as was formerly thought. The levels in Cheddar cheese, even up to 12 months of age, are low and represent only 15–20% of the levels attainable from the β-keto esters present, which are precursors to the ketones. Apparently the β-keto esters are unstable even at the low temperature of the maturing process and some methyl ketones are generated. Alternatively, the conversion of β-keto acids to methyl ketones may be enhanced by the activity of lipolytic enzymes present in the cheese. The C4 methyl ketone reached levels higher than expected and this was thought to be due to the action of bacteria.

From these studies it was concluded that although none of the C3 to C15 odd-numbered ketones imparted a Cheddar-like flavour it was likely that the C7 ketone (heptan-2-one) contributed directly to the flavour and that the C5 (pentan-2-one) and the C9 (nonan-2-one) might also contribute. It should be pointed out that high levels of these ketones are found in mould-ripened cheese varieties such as blue vein cheese and that they are considered to contribute to the characteristic flavour. The ketones in these products have been demonstrated to arise from the oxidation of higher fatty acids by enzymes (oxidases) from the mould.

Fruitiness

Small amounts of the fruity esters have been found in Cheddar cheese. Farrer and Weeks (1970) have found ethyl butyrate (the ester of butyric acid) to be most beneficial to the flavour of Cheddar cheese. At Highett we have detected ethyl butyrate in the vapour from Cheddar cheese of good quality.

If the concentrations of ethyl butyrate and ethyl hexanoate rise above a certain value the cheese becomes unpleasantly fruity. Ethyl alcohol normally occurs in Cheddar cheese and is readily detected in its vapour. The ethyl alcohol can be made to react with the butyric and hexanoic acids also present in the cheese to form esters in sufficient amounts to give the fruity flavour defect. Several factors appear to be responsible: the presence of enzymes from particular bacteria such as *Pseudomonas fragi*, the presence of lipases and insufficient heat treatment of the milk from which the cheese is to be made. Several years ago the laboratories of the Queensland Department of Primary Industries showed that the activity of *Pseudomonas fragi* is not easily stopped by heating. American workers have found that this ubiquitous species survives in dairy factories and that it can esterify butyric and hexanoic acids with ethyl alcohol in milk cultures. Lipase in the cheese vat could provide higher amounts of butyric and hexanoic acids. One might expect that a slow-acting vat would especially favour the growth of *Pseudomonas fragi* and the production of cheese with 'unclean' and 'fruity' flavours.

Other compounds

Compounds considered to be interrelated through the action of enzymes produced by the starter bacteria are diacetyl, acetoin, 2, 3-butyleneglycol, 2-butanone and 2-butanol. Of these, it has been established that diacetyl contributes to the flavour of Cheddar cheese. Diacetyl is produced by fermentation of citrate in the milk from which the cheese is made. Acetoin is produced by the reduction of diacetyl or directly from excess of pyruvate produced by bacteria. In recent work carried out in New Zealand (Keen and Walker 1974) it

was shown that with two strains of starter acetoin was reduced to 2, 3-butyleneglycol, whereas a third strain seemed to lack the enzyme necessary. In the same investigation it was concluded that 2-butanone and 2-butanol arise from bacterial action. However, 2-butanone has not been detected in some cheese with good Cheddar flavour, so its contribution to flavour is in doubt. Lactones derived from fat (hydroxy-acid triglycerides) have been found in a variety of cheeses, but because of their low levels they have not been generally associated with cheese flavour. However, it is interesting to note that the C10 and C12 δ-lactones have been included in the latest Unilever patent mentioned earlier.

Sulphur compounds

Manning and Robinson (1973) of the National Institute for Research in Dairying, in England, reported on the analysis of Cheddar aroma. The vapour of one fraction, when isolated, gave an odour closely resembling that of Cheddar cheese. They considered that, of the compounds identified, hydrogen sulphide, methane thiol, dimethyl sulphide and diacetyl were the most important. Many readers will remember from their school days that hydrogen sulphide is 'rotten-egg gas'. The contribution of this compound to good flavour in Cheddar cheese illustrates the importance of the level of concentration. The other compounds, with the exception of diacetyl, are also evil-smelling in anything but very low concentration.

Since then, Manning (1974) has shown that only hydrogen sulphide and dimethyl sulphide are found in cheese without true Cheddar flavour such as that made without starter or from skim milk. However, methane thiol was included in the mixture in the case of conventional Cheddar cheese of good flavour. Kristoffersen (1973) has recently reaffirmed that there exists an optimum ratio for free fatty acids and hydrogen sulphide for balanced Cheddar cheese flavour. A low ratio indicated a 'sulphide' flavour characteristic and a high ratio a 'fermented' flavour characteristic. The importance of sulphur compounds thus seems to have been established. Over the last decade or so, a number of workers have measured the levels of these sulphur compounds in cheese. Sulphur compounds are important, too, in the flavour of beer, and some years ago a German brewing company set up a quality control system based on a commercially available apparatus. In this unit the level of sulphur compounds was automatically measured in the headspace of partially emptied bottles of beer. Will similar quality control procedures ever be used for cheese?

Conclusions

I hope that I have managed to convey how delicate is the balance of flavour compounds which gives typical, good Cheddar cheese flavour. Many factors contribute and quite a few of these are under the control of the cheesemaker. He can, for example, control saltiness although he may be less able to control the action of the enzymes which split the fat and give either a pleasant acid bite or unclean and rancid tastes.

The cheesemaker can influence the level of bacteria in the milk. Some bacteria are apparently needed to help produce the free fatty acids, but he certainly does not want the types which can lead to fruity flavours if conditions during manufacture are not suitable. There is evidence that the particular starter used can influence final flavour. This is certainly true for bitterness and applies to such compounds as diacetyl.

Just how he can best achieve the optimum ratio of free fatty acids and sulphur compounds is not entirely clear, but it obviously depends on the activities of enzymes from bacteria, starter and rennet. These in turn can be controlled to a large extent by conditions during cheese manufacture.

The continuing joint efforts of the microbiologist, chemist and cheesemaker should ensure that in the not too distant future complete long-term control of the flavour of cheese will be attained.

References

Anon. (1973). *Givaudan Flavorist* (1), 3–4, 8.

Baldwin, R. E., Cloninger, M. R., and Lindsay, R. C. (1973). *J. Food Sci.* **38,** 528–30.

Czulak, J., Hammond, L. A., and Horwood, J. F. (1974). *Aust. J. Dairy Technol.* **29,** 128–31.

Farrer, K. T. H., and Weeks, K. J. (1970). *Food Technol. Aust.* **22,** 620–3.

Jago, G. R. (1974). *Aust. J. Dairy Technol.* **29,** 94–6.

Keen, A. R., and Walker, N. J. (1974). *J. Dairy Res.* **41,** 65–71.

Kristoffersen, T. (1973). *J. Agric. Food Chem.* **21,** 573–5.
Manning, D. J., and Robinson, H. M. (1973). *J. Dairy Res.* **40,** 63–75.
Manning, D. J. (1974). *J. Dairy Res.* **41,** 81–7.
Ney, K. H., Wirotama, I. P. G., and Freytag, W. G. (Unilever N. V.) (1972). Neths Pat. 7204792.
Reiter, B., and Sharpe, M. Elizabeth (1971). *J. Appl. Bacteriol.* **34,** 63–80.
Stadhouders, J., and Veringa, H. A. (1973). *Neth. Milk Dairy J.* **27,** 77–91.
Walker, N. J., and Keen, A. R. (1974). *J. Dairy Res.* **41,** 73–80.

New Australian Standard

The Division has helped to frame a new Australian Standard for Frozen-food Retail Cabinets, replacing AS B220.

Experiments have shown that the greatest hazard to temperature control in cabinets is the large amount of thermal radiation from the shop ceiling onto the top layer of food. To keep the average temperature of this layer below $-15°C$, the air blown over the top of the frozen food must be delivered at $-29°$, and this makes necessary a particularly efficient cabinet design.

Manufacturers have given assurances that they can build cabinets capable of meeting the new standard, and trial cabinets have done so satisfactorily.

The committee responsible has concluded its work and the standard has now been published as AS 1731. The National Health and Medical Research Council will incorporate the new standard into its proposed frozen food regulations.

Further reading

Middlehurst, J., Richardson, K. C., and Edwards, R. A. (1973). Berechnung der Wärmeübertragung durch Strahlung in oben offene Tiefkühltruhen für Lebensmittel. *Klima & Kälte Ing.*, **1**(12), 27–30.
Middlehurst, J. (1974). Radiation shielding of frozen food cabinets. *Aust. Refrig. Air Condit. Heat.* **28**(8), 19–21.

Codex Alimentarius

Food temperature survey

Because of difficulties in getting agreement on a standard carrying temperature for frozen foods, the nations who are signatories to Codex Alimentarius were asked to determine the actual temperatures of such foods during transport and display.

On behalf of the Australian Government, the Division of Food Research carried out a survey of temperatures in refrigerated vehicles; other recent work provided data on temperatures in commercial frozen-food cabinets. The cities chosen for the survey were Townsville, Brisbane, Wagga Wagga and Melbourne. Measurements were taken during 70 commercial delivery runs over a period of 7 weeks; the temperatures of the foods were measured at loading into the vans, each time the doors were opened and at the conclusion of the run. All the data were collated in a form ready for computer analysis and sent to the Codex contact point in Britain.

Our measurements were taken in winter to coincide with tests in the northern hemisphere and therefore the Division repeated some of the tests in Sydney in the Australian summer. Results of the whole survey will be published soon.

Research in dairy hygiene

By D. G. Dunsmore

Dairy Research Centre, Richmond, N.S.W. 2753

Testing centre

A laboratory known as the Commonwealth Advisory Laboratory on Dairy Detergents and Sanitizers has been established at Hawkesbury Agricultural College, N.S.W., to provide a single Australian testing centre for these products. The function of the laboratory is to provide the dairy industry with information on the performance of the detergents and disinfectants (sanitizers) that are used in its cleaning programs.

'Performance' is determined by testing the cleaning material according to specifications published by the Standards Association of Australia (SAA).

All sections of the dairy and detergent industries may submit samples which are then tested by the methods described in the Australian standard for that type of product. The results are notified in detail to the organization which submitted the sample and to the manufacturer of the product.

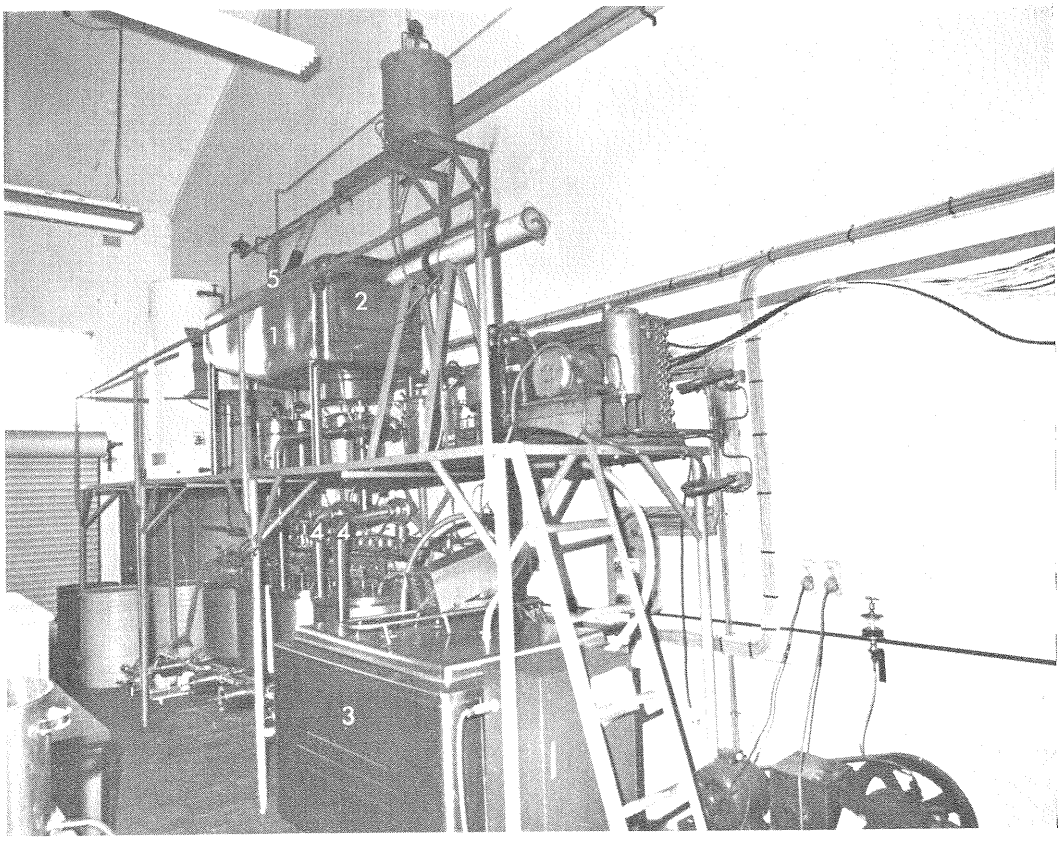


Fig. 1. Bulk tank simulator to aid in assessing CIP systems for bulk handling equipment. 1, Test tank; 2, stainless steel test panel (rear view); 3, milk storage vat; 4, CIP piping; 5, position or detergent-spraying equipment.

A quarterly bulletin is published listing those products that have proved satisfactory.

The laboratory also investigates the public health aspects of detergents and disinfectants, concentrating particularly on detergent residues in milk.

A further function of the laboratory is the development, on behalf of the SAA, of new physical, chemical and microbiological testing methods for cleaning materials. Several have been incorporated into published standards and a test procedure for general purpose disinfectants is currently under development. The laboratory is expanding the scope of its work and will shortly begin developing standards for the meat industry, including abattoirs, and for fish- and chicken-flesh products. However, it will be several years before any such standards can be fully defined and published.

In addition to the testing centre, the Dairy Hygiene Research Group at Richmond is conducting the following projects.

Cleaning bulk tanks

Developments in the bulk handling of milk, both on the farm and in the factory, have created a need for methods of 'cleaning-in-place' (CIP) that will be economic and effective for such equipment as refrigerated farm vats, road tankers, factory vats and silo tanks. Because of the size of these items, manual cleaning is often impossible and CIP techniques already in use for other equipment in the dairy industry have not proved successful. Aspects needing to be improved include the the design of the tanks and the cleaning apparatus, the properties of the cleaning and disinfecting products, and the nature of the system in which they will be used. In the current project the physical aspects of applying cleaning solutions and the chemical cleaning systems to be applied are being investigated. Two novel pieces of cleaning-assessment equipment have been developed for this task—an impact rig, which is used for examining the cleaning effect of solutions sprayed onto surfaces under pressure, and a bulk-tank simulator (Fig. 1) which simulates under controlled laboratory conditions the soiling and cleaning that take place on dairy farms and in factories. A stainless steel panel in the test tank is soiled with milk and then washed with the rinses, detergents and disinfectants of the cleaning system. This process is repeated a number of times before an assessment of the soil remaining on the test plate is made. The simulator is able to perform the equivalent of one month's operations in one day, thereby greatly speeding up the research program.

Results obtained in this project will also be applicable in other industries using bulk handling equipment.

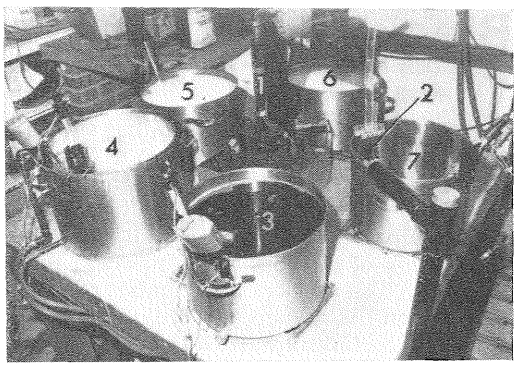


Fig. 2. Circular simulators used in assessing methods of cleaning milking machines. 1, Rotating central arm; 2, test panels; 3, pre-milk rinse; 4, milk-soiling tank; 5, rinse tank; 6, detergent-wash tank; 7, post-detergent rinse tank.

Cleaning milking machines

The current method of cleaning milking machines, which is used throughout Australia, was developed at the Dairy Research Centre in 1965. Although the method ensures adequate cleaning, it is complex and expensive. To overcome these deficiencies a project is underway to develop a new system which will be equally effective but simpler and more economical.

The cleaning simulator illustrated (Fig. 2) has been developed to evaluate the efficiency of experimental cleaning systems. The machine operates by immersing test panels of stainless steel like that used in the construction of milking machines, into milk in order to soil them, and then into the rinsing and detergent solutions. This simulates the cleaning operation on the dairy farm.

Good progress has been made and improved cleaning systems are now being evaluated by extensive field trials before being promoted in the dairy industry.

Black spot in prawns

Recent research in the Division has shown that the enzymatic defect known as 'black spot' can be better controlled by dipping raw prawns in a dilute solution of sodium metabisulphite than by the haphazard application of metabisulphite powder; the treatment also reduces the number of bacteria on the prawns. In order to promote the use of metabisulphite in this way, a dipping machine has been built at the FRL workshops. It can handle 5000 kg prawns per h and is intended for use on large prawning trawlers and in processing factories and unloading depots.

The prototype dipper is composed of a circular drum with four compartments, each holding *c.* 45 kg prawns in a perforated plastic lug box (Fig. 1). The drum revolves in a tank containing the metabisulphite solution (3000 ppm SO_2). The revolving action was chosen because it helps to displace small air bubbles that might otherwise cause some areas of the prawn to go untreated. Boxes of prawns are loaded and unloaded manually on plywood slides fitted

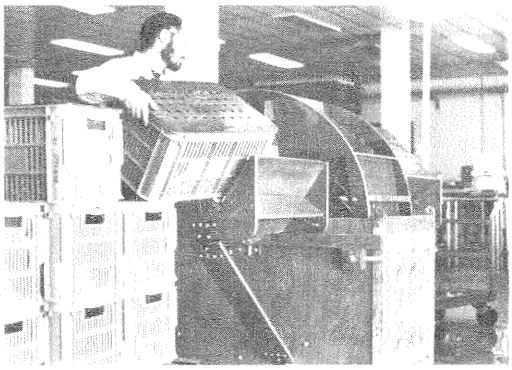


Fig. 2. Dr Sharp loading the machine on the factory floor.

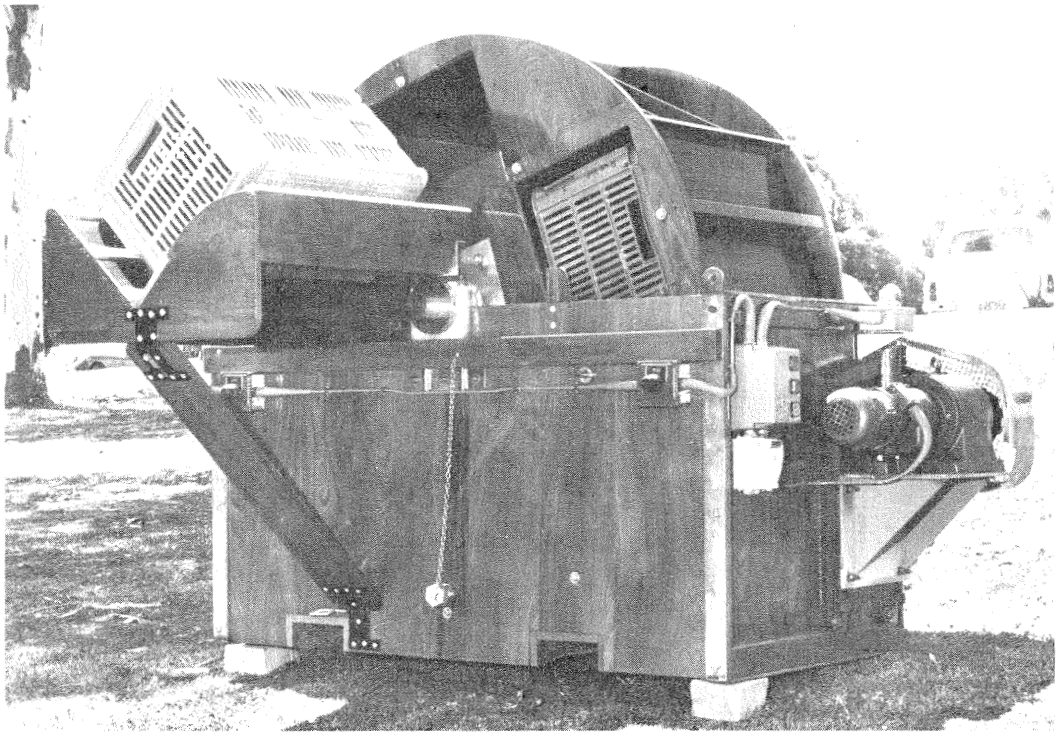


Fig. 1. Prawn dipping machine.

with nylon runners. As the drum rotates, the prawns are immersed for 30 s and then drain for 30 s.

Slots and lifting eyes have been provided so that the dipper may be moved easily with a forklift; the loading slides may be detached and the drum may be removed from the tank whenever necessary for transporting or thorough cleaning.

Dipping trials under commercial conditions (Fig. 2) were carried out at the Queensland Fish Board's Processing Division at Colmslie during December 1974 by Mrs Judith Ruello, Dr Alister Sharp and Miss Vivien Beilby of FRL. More than 4·5 t raw banana prawns were dipped. Results of the trials confirmed the good results obtained under laboratory conditions. The Division is indebted to the Queensland Fish Board for the facilities and assistance provided during the trials.

Fishermen and processors may inspect the prawn dipping machine at MRL, Cannon Hill, or alternatively a film showing its operation is available from the the Technical Secretary, FRL.

News from the Division

Retirements

Barbara E. Johnston

An effective library service is essential to modern scientific research and Miss Johnston has done her utmost to provide this during her long association with this Division. She joined C.S.I.R. in 1939, spending the first four months in training at the Head Office Library in Melbourne. On returning to her home city of Sydney in 1940, she assumed responsibility for the libraries at both the McMaster and the Food Preservation Laboratories, and in 1945 became full-time librarian at Homebush.

Before qualifying for membership of the Australian Institute of Librarians, Barbara Johnston had graduated in Science from the University of Sydney, majoring in Botany and Zoology. She also studied German and some Russian. Some of her colleagues may be surprised to know that she took the course in Industrial Microbiology at the Sydney Technical College. All this made a firm base on which to build a career as a scientific librarian, and this she did with enthusiasm. Several overseas visits, both official and private, kept her well informed on the international library scene. Her report on 'Special Library Practice', written in 1949 after visits to libraries in England, France, Canada and the U.S.A., became well known in its field and for many years was used as a set book for library examinations.

Her influence on the development of scientific and technical libraries was profound. Much of it was exerted through her membership, from its inception in 1944, of the Special Libraries Committee of the N.S.W. Branch of the Australian Institute of Librarians; when the Institute became the Library Association of Australia she was on the first elected committee of its Special Libraries Section and held all its major offices during the early years. With her wide experience it was natural that she should undertake extensive teaching of specialized library courses.

She wrote informatively on many aspects of special libraries, and drew on her wide experience to produce in 1969 the authoritative pamphlet 'Libraries of Australian Government Departments and Agencies'.

Throughout much of her career Miss Johnston carried heavy responsibilities in supervising the libraries of other CSIRO Divisions in N.S.W., particularly those of Fisheries and Fuel Research, on behalf of the Chief Librarian. She was regarded as the senior CSIRO consultant in library matters in N.S.W., and it was this Division's great good fortune that her close family ties in Sydney made her unwilling to leave this city.

Research in food science and technology embraces many disciplines in the physical and biological sciences and in engineering. For library services to cater adequately for this broad spectrum is difficult indeed. From primitive beginnings, a table and no typewriter, Barbara Johnston built up one of the finest libraries in this field, intended to serve not only this Division, but the related industries as well. It was part of her philosophy that important books in this field, if unlikely to be purchased by other libraries in Australia, should be on her shelves and available to anyone who might need them.

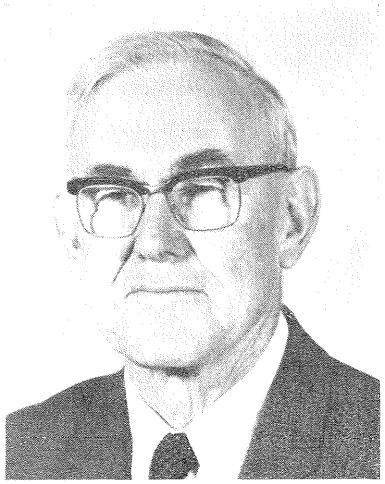
Miss Johnston was deeply involved in the production of the *Quarterly* and served on the Editorial Committee from its inception. By answering thousands of industry inquiries, she saved the scientific and technical staff countless hours for research. But the acid test of a librarian must be the service the library gives to its everyday users. This has always been excellent, and Barbara Johnston's helpfulness, experience and thoroughness were appreciated by all. She has left us with a well-stocked and well-run library, most appropriate to the needs of the Food Research Division and the industry which it serves.

J.H.B.C.

Robert S. Mitchell

Mr Robert Mitchell, 'Mitch' to his colleagues and many friends, retired on 24 July 1974 after two periods of service with the Organization dating from 1934.

Mr Mitchell holds the Queensland Diploma in Agriculture and the degrees of



B.Sc.Agr. and M.Sc.Agr. awarded by the University of Queensland in 1934 and 1937. He first joined what was then the C.S.I.R. Section of Food Preservation and Transport in 1934 and worked on the storage disorders of bananas, 'black end' and 'squirter', in association with the Queensland Department of Agriculture. From 1937 until 1942 Mr Mitchell carried out research on soils with the University of Queensland and later with the Soil Conservation service in N.S.W.

In 1942 he rejoined the Division of Food Preservation and Transport and started a long period of collaborative work with Mr L. J. Lynch who was a close friend and a fellow student at the University of Queensland. Mr Mitchell's main research contributions concerned the measurement of food texture, the measurement and prediction of crop maturity, and the development of new equipment, processes and products. He made major contributions to the development of the Maturometer and its use for predicting the optimum harvest time of pea crops for processing. This prediction technique and another for sweet corn are widely used in the Australian industry and overseas. In 1953 he received a Fullbright award and a Smith-Mundt grant to work on pea processing at Geneva, N.Y., U.S.A. His work has resulted in 6 patents and about 30 technical publications.

Mr Mitchell has been involved with the food technology course at Hawkesbury Agricultural College since its planning stage and was a part-time lecturer and examiner. He was also a Colombo Plan expert in

Indonesia in 1961 and Western Samoa in 1967.

A readiness to help his colleagues and people in industry with a wide variety of problems characterized Mitch's attitude during his working career. His quiet friendly personality, his practical approach to problems and his extensive knowledge of food technology are sadly missed.

P.W.B.

Appointments

Following Dr J. R. Yates' transfer to CSIRO Head Office in Canberra, Dr V. H. Powell has been appointed Leader of the Industry Section at MRL. Dr Powell graduated from the University of Queensland and held post-doctoral positions at the Universities of Rochester, U.S.A., and Manchester, England. From 1967 to 1970 he was a Research Fellow in the Research School of Chemistry at the Australian National University, after which he became Research Manager of the Ampol Research and Development Laboratory in Brisbane.

Miss Betty Baker, whose appointment to the staff was announced in the June 1974 issue of the *Quarterly*, has succeeded Miss Barbara Johnston as the Librarian at FRL. Miss Baker has also taken Miss Johnston's place on the Editorial Committee of the *Quarterly*.

Mrs L. Piip has joined FRL as a Librarian. Previously, she was in charge of the library at the Bermuda Biological Station for Marine Research for some 12 years.

Transfer

Mr H. A. Bremner, Experimental Officer at MRL, has transferred to the Division's Tasmanian Food Research Unit where he will work on fish products.

Visiting workers

Dr F. F. Busta, Professor in the Departments of Microbiology and of Food Science and Nutrition at the University of Minnesota, U.S.A., commenced a year's sabbatical attachment to the Division in October 1974. He is working on heat-induced modification of bacterial spores.

Dr Henryk Melcer from Massey University, N.Z., worked in the Environmental Biology Section at MRL as a Visiting Scientist during January and February. Dr Melcer's particular field of research is effluent treatment systems.

Professor E. K. Akamine of the College of Tropical Agriculture at the University of Hawaii will work with PPU at North Ryde for about six months. His main interest is in tropical fruits and he has already established contact with growers and producer organizations in tropical and subtropical areas in Queensland and northern N.S.W.

General

Mr M. V. Tracey, Chief of the Division, has become a member of a Working Party on the Thiamine Status of Australians, established by the National Health and Medical Research Council.

Congresses

The Fourth International Congress of Food Science and Technology was held in Madrid in September 1974, with the theme of 'Food Science for Health and Peace'. The Division was represented by Mr M. V. Tracey, while Mr J. F. Kefford and Dr June Olley attended in a private capacity. Mr Tracey gave a paper on behalf of himself and Dr A. R. Johnson on 'Polyunsaturated ruminant food products'. The Congress was followed by a meeting of the General Assembly of the International Union of Food Science and Technology, and the Australian delegate, Mr J. F. Kefford, was re-elected as a member of the IUFoST Executive for a further four-year term and appointed Chairman of the Committee on Education and Training.

The Nineteenth International Dairy Congress took place in New Delhi early in December 1974 and was followed by the annual meeting of the International Dairy

Federation (I.D.F.). Among the Australian participants were Drs Harrap, Jago and Lloyd and Messrs Muller and Kieseker of DRL and Dr Sidhu of FRL. Dr Harrap was the Australian Government's representative at the meetings of the I.D.F.

Work overseas

While on a two-year secondment to the Australian Department of Foreign Affairs, Dr R. A. Buchanan of DRL is stationed at the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand, as a Colombo Plan Expert. His main task there is to provide Australian expertise and assistance in the production of high-protein low-cost foods. A new weaning food has already been formulated.

Mr D. W. Roberts, the MRL Industry Section's Extension Officer in Perth, has gone to Teheran for eight weeks on behalf of the Australian Meat Board, to introduce and provide advice on freezing, bulk storage and defrosting procedures in cold stores operated by the Iran Meat Authority.

Earlier, Mr Roberts supervised a trial shipment of vacuum-packaged chilled sheep meats despatched to Iran and Kuwait by Westralian Farmers' Cooperative Ltd. Our export industry may benefit from shipment of chilled meat to the Middle Eastern market where frozen meat is not yet readily accepted. At the same time, at the request of the Iranian Minister of Agriculture, the Australian Meat Board is developing a program of trade and consumer education in the area.

While he was overseas, Mr Roberts joined officers of the Western Australian Government in a technical study of meat industries and meat marketing in Singapore, Bahrein, Dubai, Oman, Qatar and Saudi Arabia.

Dr R. W. Shorthose of MRL's Meat Science and Technology Section is spending one year at the Howard Florey Institute of Experimental Physiology and Medicine at the University of Melbourne, studying methods of assaying hormones.

Specialist courses

As part of its continuing series, MRL held a two-day course in November entitled 'Advances in meat technology: meat chilling and handling'.

New Division

The Australian Minister for Science, the Hon. W. L. Morrison, has announced the formation of a new CSIRO Division of Human Nutrition, following a revision by the CSIRO Executive of its previous policy on research with likely application to health or medicine. The Division of Food Research welcomes the establishment of the new Division and looks forward to fruitful cooperation with it in the future. The Division of Human Nutrition is based in Adelaide in the premises of the Division of Nutritional Biochemistry which now ceases to exist; its Chief, Dr A. T. Dick, is Interim Chief of the new Division. It is expected that the new Chief will have been appointed by the end of the year.