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#### FROM THE EDITOR

The printing of this volume was deferred to allow the publication of the papers of the Conference: 'Dairy Ingredients - Production & Utilisation', held in Melbourne in March this year. These were presented in a double issue, listed as Volume 51, Nos. 1 & 2.

K.C. Richardson

### Rapid Methods for the Detection of Microbial & Viral Contamination\*

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#### Introduction

A bewildering array of rapid methods for food microbiology is described in the scientific literature. These methods are based on a variety of technologies, from conventional cultural methods that have been modified in various ways, to genuinely innovative techniques for the rapid detection of microbial cells, metabolic activity or macromolecules. The methods commonly referred to as 'rapid methods' fall into two categories. The first includes those that are aimed primarily at reducing the amount of labour required. It includes instruments that automate conventional analyses (e.g. the Spiral Plater™, automated colony counters), instruments and miniaturised kits that identify bacteria (e.g. API<sup>™</sup> kits, Vitek<sup>™</sup> instruments), and devices that reduce media preparation (e.g. Petrifilm<sup>™</sup>). These products improve labour efficiency, reduce the range of media and reagents that laboratories must stock and expand the range of tests that a laboratory can perform reliably. However, in most cases they do not substantially reduce the time that elapses before results are obtained.

The second category, the subject of this discussion, includes tests that are designed primarily to substantially reduce the time taken to obtain results. In keeping with the theme of the meeting, 'The Challenges of the 90's', I have concentrated on developments that might revolutionise the ways in which we detect small numbers of foodborne pathogens by the end of the decade.

Conventional methods for detecting pathogens in foods have substantial deficiencies, particularly the time that elapses before results are obtained (Figure 1). Microbiological

\* Reprinted with minor amendments from the Proceedings of the First Asian Conference on Food Safety; Kuala Lumpur, Malaysia; September 2-7, 1990. analysis may allow a recall of a contaminated batch, punitive action by regulatory authorities, or it may permit a food processor to prevent a problem from recurring in future batches. It often fails to allow us to prevent a contaminated product from reaching the market, or to keep contaminated raw materials out of a processed product. When it is possible to store foods until days or weeks of testing is complete, the holding costs are large. Conventional methods that employ selective media may not allow growth of stressed cells of bacteria and they cannot detect the viable, non-culturable forms of enteric pathogens found naturally in aquatic environments or induced in laboratory ecosystems.

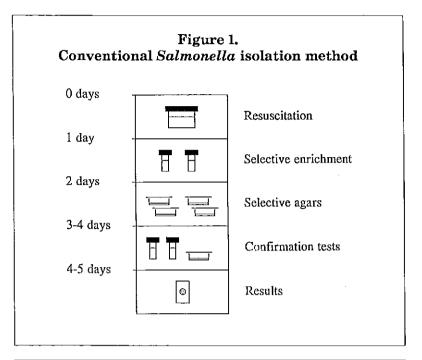
Food microbiology has been in need of a technological revolution for a long time. The pioneers of the discipline would require little retraining to be able to perform many tests used in modern food microbiology laboratories. For example, techniques that are the basis for the slow and expensive *Escherichia coli* counts that we perform now were familiar to microbiologists decades ago. At present, routine microbiological analyses of food have received relatively few benefits from the spectacular advances that molecular biologists and biotechnologists have made in the past decade.

The overdue revolution may be beginning. The new techniques that I will discuss enable us to detect pathogens in mixed cultures after selective enrichment, reducing the need for lengthy plating and confirmation procedures. These techniques also permit relatively rapid detection of toxins produced by microorganisms and they are beginning to make possible rapid detection of foodborne viruses.

#### Detection of Bacterial Nucleic Acids

Gene probes are the basis for a developing technology that has many potential advantages for the detection of foodborne viral and bacterial pathogens (Hill, 1988). The application of gene probes to routine food analysis so far has been very limited; the technology is still in its infancy. There are many developments taking place that will substantially increase the sensitivity, simplicity and value of gene probes for the detection of pathogens and indicator organisms in foods.

Briefly, a gene probe is a labelled DNA molecule with a sequence complementary to DNA that is present in the pathogen that we wish to detect, the target gene. DNA is normally double-stranded. If the two strands of DNA containing the target gene are



#### Table 1. Variable Specificity of Gene Probe & Polymerase Chain Reaction Methods

Target gene	Group detected	Reference
lacZ	Coliforms, Shigella	Bej <i>et al</i> . (1990)
lamB	E. coli, Shigella, Salmonella	Bej <i>et al</i> . (1990)
LT, ST	Enterotoxigenic E. coli	Cravioto et al. (1988)
Invasive	Enteroinvasive E. coli, Shigella	Lampel <i>et al.</i> (1990)

separated by heat and then allowed to recombine in the presence of the probe, the probe will bind to the complementary sequence in the target gene. The label on the probe allows the double stranded DNA containing the probe, and therefore the target gene, to be detected. Radioisotopes and biotin are common labels. Other labels that can be detected by a colour reaction or by chemiluminescence have been devised (Walker and Dougan, 1989).

DNA is not the only nucleic acid in the bacterial cell. Probes can also detect RNA sequences. Ribosomes, the cellular structures in which protein synthesis takes place, contain RNA as part of their structure. Ribosomal RNA differs from species to species and the differences can be exploited to allow the development of probes that detect a single species or a broader taxonomic

group. Unlike DNA, thousands of copies of ribosomal RNA are present in each bacterial cell, so probes for ribosomal RNA are inherently very sensitive. Ribosomal RNA probes have been developed for foodborne pathogens, including *Listeria*.

Gene probes can be tailored to specific needs. Depending on the nature of the target gene, they can detect one species, a broader group such as the coliforms, or strains with a particular characteristic within a species (Hill, 1989). Table 1 illustrates this capability of gene probes. The rapid detection of characteristics associated with virulence can be particularly useful because different strains of a species may vary in their pathogenicity. E. coli is a good example. Therefore information on virulence may be essential to the correct interpretation of the results of microbiological analyses of foods. Many conventional virulence tests, such as those that use animals or cell cultures, are slow to complete and they are impossible to perform in food laboratories that lack the necessary specialised expertise and facilities.

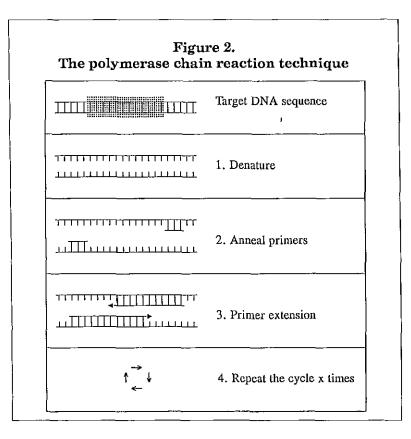
Gene probes have been developed for many foodborne pathogens (Hill, 1988; Hill, 1989) and there are commercial kits for the detection of Salmonella, Listeria, and E. coli. Comparisons with standard methods have shown that the probes can be reliable. The time that the current kits save is relatively modest because the techniques are not sufficiently sensitive for direct detection of the small numbers of cells of enteric pathogens usually present in food samples. Cultural enrichment is necessary before the probes are used.

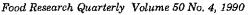
Another potential advantage of probes is that the chosen gene is always detectable, regardless of whether or not it is being expressed. Therefore, the successful use of gene probes does not depend on the production of antigens or enzymes, which may be suppressed by environmental influences. An example is provided by Clostridium perfringens. C. perfringens produces its enterotoxin during sporulation, but the organism sporulates poorly in laboratory media, so probes for the enterotoxin gene have been developed to allow rapid identification of toxigenic strains (Van Damme-Jongsten et al., 1990).

This feature of gene probes might also be a disadvantage sometimes. The presence of the target sequence might not be significant if the organism that carries it is incapable of producing the product that the gene codes for, for a variety of possible reasons. Gene probes might also give false reactions by detecting nucleic acids in dead bacteria in food samples. On the other hand, they may detect the viable, non-culturable forms of enteric bacteria that have been found in some environments.

#### The Polymerase Chain Reaction

The polymerase chain reaction, or PCR, is an important innovation that is enhancing the value of gene probes. The PCR can amplify a chosen DNA sequence very specifically. It





permits the detection of a single DNA molecule containing the target sequence and, therefore, a single bacterial cell or virus particle.

PCR is based on the ability of DNA polymerase enzymes to synthesise the complementary strand of a DNA sequence if a short priming region of double stranded DNA is present (Figure 2). The steps that follow extraction of DNA from bacteria in the sample are as follows:

- 1. The sample is heated to about 95°C to separate the two strands of DNA.
- 2. The temperature is reduced to a level at which primers anneal (bind) to the DNA strands if the target sequence is present (e.g. 40-55°C). These primers are short, synthetic DNA sequences. They are complementary to sequences at each end of the DNA that is to be amplified. They can be constructed readily if the sequence of the target DNA is known or if it can be deduced from the amino acid sequence of the protein for which the DNA codes. The primers are often about 20-25 bases long.
- 3. The sample is heated, usually to 70°C, and DNA polymerase extends from each primer that has bound to its target, creating a new strand.
- 4. The cycle is repeated as often as is necessary to produce the desired amount of DNA. Automated apparatus completes each cycle in a few minutes.

Following the PCR, target DNA can be detected by a gene probe. Alternatively, detection of amplified DNA by gel electrophoresis or another technique may be sufficient evidence for the presence of the target sequence. Many novel detection systems for gene probes used in conjunction with PCR are being investigated, with the objective of producing practical diagnostic kits (Kemp, 1989).

Some potential modifications of this technique are quite exciting. PCR techniques can detect a number of pathogens simultaneously if several different primers are used. The increases in speed that are probably possible might allow in-process monitoring of food production. Several authors have shown that results can be obtained in less than one day. A kit that provides results in three hours is technically feasible at present (Atlas, 1990), and further time reductions may be possible. Methods that will detect foodborne pathogens and indicator organisms are being developed. For example, PCR has been applied to the detection of invasive Shigella in food (Lampel et al., 1990), and a gene probe-PCR technique that is capable of detecting 1-5 viable coliforms in 100ml of water has been reported (Bej et al., 1988).

The simple outline above ignores many technical problems that must be overcome in developing practical, reliable PCR techniques. These include finding a DNA sequence that is specific to the group or species of microorganisms that is to be detected, identifying a DNA sequence that has appropriate properties for the PCR tech-

nique, preventing non-specific amplification of non-target DNA, and eliminating components of the test sample that will interfere with the reaction.

Most research on the detection of pathogens by PCR has been oriented towards clinical applications. Food samples present special problems. The major problem is that the target DNA must be extracted from the food into the relatively small volume of sample that the PCR system can accommodate. Foods vary widely in their composition and structure, and different protocols will be required for different foods.

#### Detection of Viral Nucleic Acids

Gene probes and the PCR technique might be particularly valuable for the detection of enteric viruses. The detection in food of the viruses that are most significant in foodborne disease, i.e. hepatitis A virus and the Norwalk-like gastroenteritis viruses, is impossible in most food laboratories. Immunological assays have been developed, but their sensitivity is inadequate for many applications. Detection of viruses in a living host (i.e. a cell culture or laboratory animal) is usually a more sensitive technique, but laboratory hosts for these viruses either do not exist, or they are unsuitable for the direct detection of viruses in foods. Cell culture techniques that are available for the detection of some enteroviruses in food are slow and expensive. Consequently, research on foodborne viral disease has been severely hampered.

Gene probes have been constructed for most of the major groups of enteric viruses, including hepatitis A virus, and used successfully to detect viruses in shellfish and water samples (Gerba et al., 1989). Probes containing a radioactive label can be used in such a way that the sensitivity of the assays is similar to that of cell cultures. The sensitivity of nonradioactive probes is significantly lower at present. The material and labour costs for the tests are not particularly high. Disadvantages of the probes include their inability to differentiate between infectious and non-infectious virus particles.

PCR techniques will improve the sensitivity of gene probes for enteric viruses. Gene probe-PCR methods that can detect either numerous enterovirus types or specific enteroviruses have been reported recently (Chapman et al., 1990). The nucleic acid of the important foodborne viruses is RNA, whereas the usual starting material for PCR techniques is DNA. DNA produced from viral RNA by enzymic methods can be used as the starting material for PCR.

As with tests for bacteria, there are many problems to be overcome before these techniques are suitable for routine use in food laboratories. In particular, it will be necessary to separate viral nucleic acid from relatively large samples of food as a preliminary step in gene probe assays. Fortunately, research on cell culture assays for some viruses in foods has provided food virologists with considerable expertise in extracting viruses from foods. It seems likely that the detect-

ion and investigation of food and waterborne viral illness will be enhanced by gene probes and PCR techniques.

Techniques Based on Genetic Manipulation Molecular biologists are investigating a range of other genetic techniques that may lead to rapid, simple detection methods. One such technique is based on bacteriophages (viruses that infect bacteria). The bacteriophages are genetically modified to contain genes that code for readily-detectable products. When the bacteriophages infect a susceptible bacterium, the introduced genes give the bacteria characteristics that can be detected by sensitive, rapid assays. The technique is potentially very specific, because each bacteriophage can infect only a defined range of bacteria.

Methods based on bacteriophages containing genes that code for bioluminescence are being developed (Stewart, 1990). Bacterial cells that are infected by the bacteriophages acquire the bioluminescence genes and express them, thereby becoming capable of producing light. A luminometer can detect the light emitted by 100 bioluminescent cells per ml, so only a short enrichment time is required when the technique is used to detect small numbers of pathogens in foods. A related technique employs genes that code for ice nucleating proteins. Pathogens infected by the modified bacteriophage are detected by a simple test for the ability to initiate ice formation.

#### Immunological Assays Immunological assays are

available for the detection of a variety of bacterial cell components or products against which antibodies can be prepared, including toxins, cell wall components and flagellae (Notermans, 1989). Of the techniques available, enzyme immunoassays are the most widely used by food microbiologists at present. They are the basis for successful commercial kits that detect foodborne pathogens and toxins, including Salmonella, Listeria, staphylococcal enterotoxins and aflatoxins. The range of organisms and toxins that can be detected in this way will expand in the near future.

Various independent trials have shown that the kits are reliable. Table 2 shows the results of collaborative studies that have shown good agreement between enzyme immunoassay kits and conventional cultural methods, with very low levels of false negative results.

Like gene probes, enzyme immunoassays detect pathogens in mixed cultures and they provide results within a few hours if the concentration of the pathogen is above the limit of sensitivity of the test. The assays cannot detect small numbers of pathogens directly in food samples (Table 3), so enrichment cultures must be used to increase the number of cells and to promote development of the antigens to be detected. This cultural process usually takes about two days at present, limiting the time savings.

Immunological assays are superior to genetic techniques for toxin detection. Gene probes can determine only whether genes for toxin production are present, they cannot determine whether toxins have been, or can be, produced. A potential disadvantage of immunological assays is that they may detect toxins that have been inactivated (Notermans, 1989), or conversely, they may fail to detect toxins that have been denatured to some extent by heat treatment but retained their toxicity.

Enzyme immunoassays for toxins are relatively sensitive in comparison with many other immunological assays, therefore lengthy procedures to extract and concentrate toxins may be unnecessary when this technique is used, so results can be obtained within a few hours. Some kits, such as the Tecra kits from Australia, do not require expensive equipment. Positive results are detected visually.

Latex agglutination tests have advantages in some applications and latex agglutination kits that detect toxins produced by foodborne pathogens are available. Immunoassays, whether using enzymes or latex agglutination as detection systems, make assays for microbial toxins possible in food laboratories that would not otherwise have this ability.

Immunological tests are still being improved, with the development of faster enrichment procedures, more sensitive systems for detecting positive reactions, better antibody preparations, and better kit designs (Lee *et al.*, 1990). Monoclonal antibodies may have advantages in some circumstances. Monoclonal antibodies are more specific than antibodies prepared using laboratory animals in the conventional manner, and they can be

#### Table 2. Results of Comparisons Between Enzyme Immunoassay Kits and Standard Culture Methods for the Detection of *Salmonella* in Foods (Data from Flowers *et al.* 1988, 1989, Curiale *et al.* 1990).

Kit	Overall	False neg	ative (%)
	agreement (%)	Immunoass	ay Culture
TECRA	96.7	1.4	1.6
Q-TROL	98.5	0.8	1.1
Salmonella-Tek	99.1	0	1.1

#### Table 3. Sensitivity of a Commercial Enzyme Immunoassay Kit (Data from Curiale *et al.* 1990).

Salmonella concentration (cells/ml enrichment broth)	Samples positive (%)
108	97
107	97
1.06	89
10 <sup>5</sup>	37

directed against a particular site on an antigen (e.g. the active site of a toxin). Monoclonal antibodies can reduce the possibility that inactivated toxins will be detected (Notermans, 1989).

#### Instrumental Methods

Several sophisticated instruments rapidly detect and enumerate microorganisms by detecting microbial metabolites or various broad indicators of microbial activity or contamination (e.g. measurements of microbial ATP, automated direct cell counts by epifluorescence, turbidity measurements). Instruments that measure impedance or conductance have been the most widely accepted for the examination of food.

Microbial growth leads to changes in the chemical composition of the growth medium. Many of these changes can be detected electronically by measurements of the imped-

ance or conductance of the medium (Firstenberg-Eden and Eden, 1984). Impedimetric or conductimetric microbiological growth analysers, such as the Bactometer<sup>™</sup> or Malthus<sup>™</sup>, automatically monitor one or more of these parameters in test cells incubated under precise temperature control. The data are stored and interpreted by a computer. These instruments are becoming widely accepted for the rapid measurement of microbial contamination of food products. Their principal use initially was for tests that replace total plate counts and related procedures (e.g. shelf life prediction).

Methods are now being developed that allow these instruments to be used for the rapid detection of bacterial pathogens and indicator organisms in food. Most emphasis has been on the detection of Salmonella. Selective media in which the growth of Salmonella causes readily detectable changes in conductance or impedance have been devised. Several strategies have been used to allow other indicators of the growth of Salmonella (e.g. infection with bacteriophages, colour changes caused by the utilisation of specific substrates) to be observed in parallel with conductance changes in order to increase the specificity of the test (Bullock and Frodsham, 1989). Salmonellae can be recovered from the Bactometer or Malthus cells for identification by conventional procedures.

Several studies have shown that these instruments are useful in screening foods and related samples for Salmonella (Bullock and Frodsham

1989; Smith *et al.*, 1989), and some food processors are using them routinely for this purpose. Conductance or impedance tests offer worthwhile savings in time and may reduce the cost of Salmonella testing. The ability to use these instruments for pathogen tests substantially increases their value in an industrial food microbiology laboratory, an important consideration given their cost.

#### Conclusion

The acceptance of the techniques discussed earlier has varied in different parts of the world. Several factors may have delayed their acceptance in some places. These include cost. Some of the techniques are quite expensive in terms of both capital cost and running costs and the time savings that they achieve are often not sufficient to justify the additional expense. Some kits have not been designed well for laboratories that do not perform a large number of tests routinely, and the reagents used in some kits have a limited shelflife after reconstitution. However, these new techniques will become cheaper and easier to use as they are developed further and used more widely. It is important to remember that these methods are at various stages in a complex process of development and acceptance that might take a decade or two.

All new techniques must be validated adequately, both by regulatory organisations and individual industrial laboratories. The performance of some existing kits varies with the type of sample analysed and other circumstances. Industry labs using kits approved by regulatory bodies have encountered excessive numbers of false reactions in particular applications. Most laboratories using gene probe or immunoassay kits to detect *Salmonella* or *Listeria confirm* their positive results by cultural methods. However, we must remember in this context that cultural methods can also yield incorrect results.

Food microbiologists and regulatory bodies must ensure that adequate mechanisms exist for evaluating and accepting new techniques, and that excessive conservatism does not cause the benefits of new technology to be ignored. In some countries, including Australia, difficulties in obtaining regulatory acceptance of new analytical technologies have been perceived to be a major factor inhibiting their introduction in industry.

#### References

- Atlas, R.M. (1990) Personal communication.
- Bej, A.K., Steffan, R.J., Dicesare, J., Haff, L. and Atlas, R.M. (1990) Detection of coliform bacteria in water by polymerase chain reaction and gene probes. *Applied and Environmental Microbiology*, 56: 307-14.
- Bullock, R.D. and Frodsham, D. (1989) Rapid impedance detection of salmonellas in confectionery using modified LICNR broth. Journal of Applied Bacteriology, 66: 385-91.

- Chapman, N.M., Tracy, S., Gaunt, C.J. and Fortmueller, U. (1990) Molecular detection and identification of enteroviruses using enzymatic amplification and nucleic acid hybridisation. Journal of Clinical Microbiology, **28**: 843-50.
- Cravioto, A., Trujillo, F., Beltran, P. and Hill, W.E. (1988) DNA hybridisation with oligonucleotide probes for identifying enterotoxinproducing Escherichia coli. Molecular and Cellular Probes, **2:** 125-30.
- Curiale, M.S., Klatt, M.J., Robison, B.J. and Beck, L.T. (1990) Comparison of colorimetric monoclonal enzyme immunoassay screening methods for detection of *Salmonella* in foods. *Journal of the Association of Official Analytical Chemists*, **73:** 43-50.
- Firstenberg-Eden, F. and Eden, G. (1984) Impedance Microbiology. (John Wiley & Sons, New York).
- Flowers, R.S., Klatt, M.J. and Keelan, S.L. (1988) Visual immunoassay for detection of Salmonella in foods: collaborative study.Journal of the Association of Official Analytical Chemists, **71**: 973-80.
- Flowers, R.S., Klatt, M.J., Keelan, S.L., Swaminathan, B., Gehle, W.D. and Chan-

donnet, H.E. (1989) Fluorescent enzyme immunoassay for rapid screening of *Salmonella* in foods: collaborative study. *Journal of the Association of Official Analytical Chemists*, **72**: 318-27.

- Gerba, C.P., Margolin, A.B. and Hewlett M.J. (1989) Application of gene probes to virus detection in water. *Water Science and Technology*, **21**: 147-54.
- Hill, W.E. (1988) Detection of bacteria in foods using DNA hybridisation. In 'DNA Probes for Infectious Diseases'. (Ed. F.C. Tenover). (CRC Press, Boca Raton).
- Hill, W.E. (1989) Detection of pathogens by newer methods. In 'Modern Microbiological Methods for Dairy Products'. (International Dairy Federation, Brussels).
- Kemp, D. (1989) Blue genes: colourimetric detection of PCR products for diagnostics. *Today's Life Sciences*, 1: 64-73.
- Lampel, K.A., Jagow, J.A., Trucksess, M. and Hill, W.E. (1990) Polymerase chain reaction for detection of invasive Shigella flexneri in food. Applied and Environmental Microbiology, 56: 1536-40.
- Lee, H.A., Wyatt, G.M., Bramham, S. and Morgan, M.R.A.

(1990) Enzyme-linked immunosorbent assay for Salmonella typhimurium in food: feasibility of l-day Salmonella detection. Applied and Environmental Microbiology, **56:** 1541-6.

- Notermans, S. (1989) Detection of toxinogenic microorganisms and their toxins by newer methods. In 'Modern Microbiological Methods for Dairy Products'. (International Dairy Federation, Brussels).
- Smith, P.J., Boardman, A. and Shutt, P.C. (1989) Detection of salmonellas in animal feeds by electrical conductance. Journal of Applied Bacteriology, **66:** 575-88.
- Stewart, G.S.A.B. (1990) In vivo bioluminescence: new potentials for microbiology. *Letters in Applied Microbiology*, **10:** 1-8.
- Van Damme-Jongsten, M., Rodhouse, J., Gilbert, R.J. and Notermans, S. (1990) Synthetic DNA probes for detection of enterotoxigenic *Clostridium perfringens* strains isolated from outbreaks of food poisoning. *Journal of Clinical Microbiology*, 28: 131-3.
- Walker, J. and Dougan, G. (1989) DNA probes: a new role in diagnostic microbiology. Journal of Applied Bacteriology, 67: 229-38.

### **Recombined Kiwifruit Products**

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The chloroplast fraction and aroma have been separated from pulped kiwifruit prior to processing the pulp into concentrate. Kiwifruit products may be made by recombining the chloroplast fraction, aroma extract and reconstituted juice. Seeds and fibre may also be added as needed. The recombined products, such as sorbet, ice-cream topping, juice and ice cream have the green colour of kiwifruit and are indistinguishable in sensory attributes from similar products freshly prepared from fresh kiwifruit pulp and which therefore have the colour and aroma characteristic of the fresh fruit.

#### Introduction

The Australian kiwifruit industry is expanding rapidly; annual production of kiwifruit (Actinidia deliciosa var deliciosa) is forecast to grow from 11,000 tonnes in 1988 to more than 20,000 tonnes in 1995 (NSW Kiwifruit Growers Association, 1988). Continued growth of the kiwifruit industry will be aided by developing markets for processed products to complement the market for fresh fruit.

Fresh kiwifruit has an attractive green flesh, due to chlorophyll, and a distinctive, pleasant aroma. These sensory attributes are crucial to the market success of fresh kiwifruit: however, it is difficult to retain the desirable sensory attributes of fresh kiwifruit in processed products. Kiwifruit prod-

ucts are naturally acidic and in acid environments chlorophyll is converted into pheophytins (Lodge & Robertson, 1990), a process which is accelerated by the elevated temperatures of pasteurisation and evaporative concentration. The aroma of kiwifruit is also sensitive to elevated temperatures (Anon... 1981; Wilson, Burns and Hogg, 1982), being subject to damage during pasteurisation and evaporation. Thus, processed kiwifruit products do not retain the attractive colour and distinctive aroma of the fresh fruit, but have an olive to grey colour due to pheophytins and the aroma develops a 'cooked' quality. Also, it is reported (Venning et al., 1989) that kiwifruit products contain a factor which causes an irritation in the mouth and throat. the sensation being described as 'catch in throat' or 'fish-hook effect'; the factor responsible has been identified as crystalline calcium oxalate (Perera *et al.*, 1990).

Thus, the expansion of markets for processed kiwifruit products will depend on the development of methods for conserving the sensory attributes of the fresh fruit into the products. One way of accomplishing this objective is to avoid heat treatments. For example, kiwifruit may be pulped and frozen immediately to -18°C. This product does have the attractive green colour and the pleasant aroma of kiwifruit and it is an item of commerce. However, acid degradation of chlorophyll continues even at -18°C and the green colour is lost between two

(Robertson, 1985) and twelve months (Venning *et al.*, 1989). The factor responsible for the irritant effect and active enzymes, including the proteolytic enzyme, actinin, remain present. Because evaporation is not feasible, the product is bulky and expensive to store and transport.

A concept for conserving the colour is now suggested: chlorophyll is separated immediately after pulping the fruit, the aroma is extracted and juice concentrate made by conventional methods without damage to the separated chlorophyll and aroma. After storage and transport, the chlorophyll, aroma extract and reconstituted juice are recombined into products.

Organic solvents usually used for extraction of chlorophyll should be avoided. Ideally the separation should be mechanical and, indeed, mechanical separation of chloroplasts, the chlorophyll-containing entities of plants, from plant material is an established laboratory technique (Spencer and Wildman, 1962).

The chloroplasts of kiwifruit are contained within the cells of the fruit (Possingham *et al.*, 1980). Pulping kiwifruit disrupts the fruit cells and releases chloroplasts (Lodge and Robertson, 1990); it follows that kiwifruit chloroplasts should be recoverable from the pulp

To test this concept, AUS-TRADE, the New South Wales Kiwifruit Growers Association and CSIRO initiated a joint project with the aim of developing a process for isolating the chloroplast fraction of kiwifruit. The granting agency, AUSTRADE, laid out guide-

lines for the conduct of the project. To ascertain that products which retain the characteristics of fresh fruit are indeed attractive, it was required that sensory evaluations of products freshly prepared from fruit be held at the start of the project. Due to the methods of preparation, these fresh products retained the colour and aroma characteristic of the fresh fruit. AUSTRADE also specified that, six months later, products prepared by combining kiwifruit chloroplasts with kiwifruit juice be evaluated at a workshop to demonstrate the recombined products. The process for isolating chloroplasts from kiwifruit was to be developed in the interval between the sensory evaluations.

This paper describes the process of partitioning kiwifruit into chloroplast, aroma and juice fractions and reports the results of the sensory evaluations.

#### Materials & Methods Fruit

All fruit were of the Hayward variety.

#### Analytical

Chlorophylls were assayed by a spectrophotometric method (White et al., 1963; Food and Agriculture Organisation, 1988). The concentrations of aldehyde constituents in aroma extracts were estimated by the hydroxylamine hydrochloride method (AOAC, 1980). Total soluble solids (TSS), expressed as degrees Brix (°B), were estimated by refractometry and titratable acid, calculated as g citric acid per 100 g product, was found by titration with 0.1 M sodium hydroxide to the phenolphthalein end-point.

#### **Chloroplast isolation**

In a typical separation, 25 kg of kiwifruit were coarsely milled in a Fitz Mill (Model M) fitted with a screen with 12 mm openings. The chloroplast-containing juice was removed from the fruit fragments and seeds by repeatedly passing the pulp over a 'Kason' gyratory screen with 50 micron openings and washing with a total of 16 kg water. The fragment-free diluted juice was passed through a centrifuge, Alfa-Laval Type B 1324, at a relative centrifugal force (RCF) of 200 to separate the chloroplast fraction. After neutralisation by suspension in dilute sodium bicarbonate solution and recovery by centrifugation, the chloroplast fraction was washed by suspension in water and recovered by centrifugation. The neutral, wet fraction (190 g) was freeze-dried to obtain a chloroplast fraction (17 g), the chlorophyll contents of which, in g per 100 g, were: chlorophyll a, 0.14; chlorophyll b, 0.065; pheophytin a, 0.02; pheophytin b, 0.14. The time from pulping the fruit to neutralising the chloroplast fraction was about 70 minutes.

#### Aroma extraction

After removal of chloroplasts, 100 kg of diluted juice, equivalent to 42 kg single strength juice, was passed through a spinning cone column (Casimir and Huntington, 1978) at 30 kg feed per hour. The column operated at atmospheric pressure and the strip rate was 6%. The volume of condensate, i.e. aqueous aroma extract, was 3 litres. The aqueous extract was passed through a 100 ml bed of XAD-16 adsorbent (Rohm and Haas Australia Pty Ltd, Cam-

berwell, Vic.) and the adsorbed aroma constituents eluted with ethanol to yield 50 ml of alcoholic aroma extract, aldehyde content (calculated as hexenal) 0.8 g/100 g.

#### Fresh Pulp

The fresh products were made from a pulp prepared from peeled kiwifruit. The TSS and titratable acid contents of the pulp were 14.0°B and 1.4 g per 100 g respectively.

#### **Fresh juice**

Fresh juice was prepared by lightly centrifuging pulp which separated coarse pulp but left the green chloroplasts suspended in the upper juice layer. After removing the juice, the coarse pulp was centrifuged at an RCF of 6000 which separated an opalescent, pale green liquid which was combined with the juice containing the suspended chloroplasts. The overall yield of fresh juice was 1.1 kg from an initial 1.5 kg of pulp. The TSS and titratable acid contents were 14.0°B and 1.40 g per 100 g respectively.

#### **Processed** juice

Kiwifruit juice which had been freed of chloroplasts was pasteurised, 60°C for 30 seconds, a pectic enzyme preparation added at the rate of 0.2 ml per kg ('Ultrazyme SP', Novo), and then held at 4°C overnight. The precipitated protein was removed by centrifugation at an RCF of 200 to obtain the processed juice. The TSS and titratable acid contents of the juice were 14°B and 1.2 g/100 g respectively.

#### Preparation of products

Fresh sorbet was prepared from the fresh pulp by adding

sucrose to raise the TSS content to the required level, 500 mg potassium metabisulphite per kg to prevent browning, a whipping agent (Versawhip 520) 2.5 g per kg pulp, and a gum (Supercol G.F.) 1.25 g per kg pulp to give the sorbet a creamy mouthfeel. The mixture was frozen in a scraped surface Taylor ice confection maker and the product stored at -20°C.

Recombined sorbet was prepared in the same way as fresh sorbet, except that processed juice was used in place of fresh pulp. Prior to freezing, kiwifruit components were added in amounts per kg juice: dried chloroplast powder, 2 g; aqueous aroma extract, 60 ml; and seeds, 7 g.

The fresh ice-cream topping and the recombined icecream topping were prepared in a similar manner to the fresh and recombined sorbets. However, the whipping agent and gum were not added and the products were chilled to 4°C in a conventional refrigerator until required (about 4 h).

The fresh juice drinks were prepared by diluting the fresh juice to lower the acidity and adding sucrose to raise the TSS contents of the products. The recombined juice drink, 6 L, was prepared from a processed juice in a similar manner to the fresh juice drinks, but chloroplast powder (12 g) and dried pulp (60 g) were also dispersed into the product.

The recombined kiwifruit ice-cream was made by mixing the seeds (70 g) with chloroplast powder (20 g) and aqueous aroma extract (600 ml) and mixing evenly into 10 litres of a tempered ice-cream base. The mixture was quickly frozen in

a scraped surface Taylor ice confection maker.

#### Sensory evaluations

The evaluation of fresh products used a panel consisting of 30 experienced judges, while the evaluation of recombined products was made by a 25 member panel of workshop participants. In each evaluation, the products were assessed for appearance, flavour and general acceptability on a nine point hedonic scale arranged so that scores of one, three, five, seven and nine were respectively 'extremely poor', 'poor', 'satisfactory', 'good' and 'extremely good'.

#### Results

#### Chloroplast fractions

An average of 1.0 g of freezedried chloroplasts was obtained per kg of fruit extracted. Typical chlorophyll and pheophytin compositions of extracts are summarised in Table 1. The intervals between pulping and neutralisation of the chloroplast fractions were about 40 to 70 minutes and partial conversion of chlorophylls to pheophytins would have occurred during the exposure of the chloroplasts to the acidic juice.

### Effect of time of exposure to acid on chlorophyll

The length of time that the chloroplasts are exposed to the acid environments of the pulp and juice before neutralisation is a factor in the extent of conversion of chlorophylls to pheophytins. To demonstrate this effect, fruit was pulped, the pulp passed through a brush finisher and the green juice divided into two portions. The first juice portion had pH of 3.4 and the chloroplasts were ex-

tracted from the juice at this pH, with the time from pulping to neutralisation of the extracted chloroplasts being more than seven hours. About 10 minutes after pulping the second portion was neutralised to pH 7 with dilute sodium hydroxide and the chloroplasts extracted from the neutral juice. The compositions of the two dried chloroplast fractions are recorded in Table 1 as extracts 1A and 1B for the fractions taken from the acid and neutral juice portions, respectively. The proportion of chlorophyll a, 50%, in the high pH extract was greater than the proportion of chlorophylla, 21%, in the low pH extract. However, the proportion of chlorophyll b in the high pH extract, 28%, was only slightly greater than the proportion of chlorophyll b, 20%, in the low pH extract. The proportions of pheophytins a and b, 15 and 7%, respectively, in the high pH extract were much less than their proportions, 48 and 11% respectively, in the low pH extract.

### Removal of cause of the irritant effect

The irritant effect or 'catch in throat', though present in fresh pulp, was greatly reduced in recombined products derived from pulp. The calcium oxalate crystals responsible for the effect deposited with a starchy paste in the distributor tube of the centrifuge during chloroplast separation. The paste had a gritty texture and caused an excruciatingly painful sensation in the mouth and throat when tasted. Dried paste had an oxalate content of about 0.8 to 1 g/100 g dry paste calculated as oxalic acid.

<b>Fable</b>	1	

Chlorophyll and pheophytin contents of freeze dried chloroplast extracts

		Relative pro	oportion (%) o	of		
Extraction No.	Chlor	Chlorophyll Pheophytin		hytin	Total <sup>E</sup>	
	a <sup>A</sup>	þв	a <sup>c</sup>	bp	Concentration	
1A <sup>r</sup>	21	20	48	11	0.80	
1B <sup>F</sup>	50	28	15	7	0.60	
2	54	26	12	6	0.60	
3	62	24	1	13	0.45	
4	50	24	17	9	0.41	
5	59	27	8	6	0.24	
6	47	25	22	6	0.32	
7	46	20	25	11	0.15	

- Chlorophyll a
- B Chlorophyll b

A

- C Pheophytin a
- D Pheophytin b
- E Total chlorophyll plus total pheophytin contents. Units: g per 100 g freeze-dried extract.
- F Extracts 1A and 1B were obtained by dividing a chloroplastcontaining juice sample in two. The chloroplasts were extracted from the first portion at its natural pH of 3.4; the time elapsed from pulping to neutralisation of chloroplast extract (1A) was over seven hours. The pH of the second portion was adjusted to 7 within 10 minutes of pulping, and the chloroplasts extracted (1B) from the neutral juice.

#### Sensory evaluations

Prior to the evaluation of the fresh products, a pilot study, utilising ten experienced sensory judges, established the optimum contents of the TSS and titratable acid contents of kiwifruit products. Of three levels of TSS contents, 14, 24 and 30°B, the intermediate level, 24°B, was most acceptable for both sorbet and topping and this value was selected for the TSS contents of the sorbets and toppings presented for the two sensory evaluations. The titratable acid contents of kiwifruit juices, generally around 1.2 g per 100 g, were too high for good acceptability, so the juices were presented as drinks in which the titratable acid contents had been lowered to 1 g per 100 g by

dilution and the TSS contents raised to 14.0°B by addition of sucrose.

Table 2 contains the results of the sensory evaluations of the fresh and recombined products. The two evaluations were conducted six months apart but the results allow the sensory attributes of the recombined products to be compared to those of the fresh products.

The sensory attributes of the recombined sorbet were consistently scored as being equal to or better than those of the fresh sorbet. The appearance of the recombined sorbet was essentially equal to that of the fresh sorbet: the mean scores of both were not significantly different and were equivalent to 'good'. The flavour score of the recombined sorbet corresponded to 'good' and was significantly better than that of the fresh sorbet, the score of which corresponded to 'satisfactory' to 'good'. Overall, the acceptability of the recombined sorbet was better than that of the fresh sorbet, the score of the recombined sorbet corresponded to 'good', and was significantly greater than that of the fresh sorbet which corresponded to 'satisfactory' to 'good'.

The sensory attributes of the recombined topping were equal to those of the fresh topping. The two toppings scored equally for appearance, both being considered to be 'good'. The flavour score of the recombined topping was not significantly lower than that of the fresh topping which corresponded to 'good'. The two products were equally acceptable, both being scored as better than 'satisfactory'.

The sensory attributes of

Sen	sory	v scores	for ki	wifruit	proc	lucts	
	Mean Panel Scores <sup>A</sup>					·	
	Sor	·bet	Top	ping	Ju	lice	Ice Cream
Appearance							
Fresh <sup>B</sup>	6,9	(1.6)	6.9	(1.4)	6.5	(1.8)	-
Recombined <sup>c</sup>	7.0	(0.9)	7.0	(1.2)	5.9	(1.4)	7.4 (1.3)
FLAVOUR							
Fresh <sup>B</sup>	6.0ª	(1.8)	6.9	(1.8)	6.1	(2.0)	-
Recombined <sup>c</sup>	<b>7.2</b> ⁴	(0.3)	6.6	(1.4)	6.6	(1.7)	6.9 (1.7)
ACCEPTABILITY							
Fresh <sup>B</sup>	6.2 <sup>ь</sup>	(1.9)	6.7	(1.7)	5.9	(2.0)	-
$Recombined^c$	7.0⁵	(1.2)	6.6	(1.2)	6.1	(1.4)	7.2 (1.5)
	scores						mean atly different
B Fresh p	roduc	ts.					
C Recomb	ined	Products					

the recombined juice drink were comparable to those of the fresh juice drink. Though the appearance of the recombined juice drink was scored as more than 'satisfactory' and that of the fresh juice drink as slightly less than 'good', the difference was not significant. The flavour of the recombined juice drink was scored as being slightly less than 'good' and that of the fresh juice drink as being 'satisfactory' to 'good' however, the difference was not significant. In overall acceptability the two juice drinks were scored equally between 'satisfactory' and 'good'.

The recombined ice-cream could not be compared to an equivalent fresh product. Nevertheless this product was well-liked by the judges, its appearance being scored better than 'good' and its flavour as 'good'. In overall acceptability this product was scored as being slightly better than 'good'.

#### Discussion

The process developed by this project enables separation of the chloroplast fraction from pulp prior to processing into juice. The chloroplast fractions obtained by this process have chlorophyll and pheophytin contents, the sums of which range from 0.15 to 0.8 g per 100 g(Table 1), somewhat less than the range of 4 to 8g total chlorophylls per 100g reported by

Menke (1966) and Hall (1976) as being typical of chloroplasts in general.

The sum of the chlorophyll plus pheophytin contents of the chloroplast fractions declined with the length of time fruit spent in postharvest storage before removal of the chloroplast fraction. For example, Table 1 shows that chloroplast fractions 1A to 4, which were derived from fruit stored for up to three months, had chlorophyll plus pheophytin contents 1.6 to 5 times greater than those of fractions 5 to 7 which were derived from fruit stored four to six months.

A good quality chloroplast extract should have greater proportions of chlorophylls a and b, as percentages of total chlorophylls and pheophytins, than pheophytins a and b. To ensure good quality, conversion of chlorophylls a and b to pheophytins a and b should be minimised during extraction.

Extraction of chloroplasts from juice neutralised to pH 7 within 10 minutes of pulping resulted in a better quality extract than extraction from the same juice at pH 3.4 with several hours between pulping and neutralisation of the extract.

The evaluation of the fresh products showed that kiwifruit products which retain the essential character of fresh kiwifruit, i.e. the colour and aroma, are attractive. The evaluation of recombined products showed that these processed products are also attractive, and in terms of the attributes of appearance, flavour and acceptability, are not inferior to the fresh products.

The shelf lives (as defined by the time for unacceptable degradation of the chlorophyll contents to occur) of the recombined products have not been investigated. It could be assumed that the shelf lives of frozen recombined products, i.e. sorbet and ice cream, would be similar to that of frozen kiwifruit pulp. The shelf lives of the recombined products which are not normally frozen, i.e. topping and juice, would of course be much shorter. Indeed, the technique of recombination is ideal for producing attractive frozen kiwifruit products apart from sorbet and icecream, for example frozen pulp, fruit bars and fruit yogurts.

Compared to unprocessed frozen kiwifruit pulp, the recombined products offer outstanding advantages. The factor responsible for the irritating sensation, the 'catch in throat', is reduced during separation of the chloroplast fraction — enzyme activity in the juice fraction is destroyed by pasteurisation, an operation which precipitates the proteolytic enzyme actinin. The juice fraction can be concentrated. so reducing bulk for ease of transport and storage. During storage, the chloroplast fraction is not in contact with acid juice, so acid degradation of chlorophyllisnot possible. Acid degradation of chlorophyll commences only when the chloroplast fraction is recombined with juice to give the final product.

We have not determined the storage stability of the freezedried chloroplast powders. It is possible that chloroplast extracts osmotically dried by sugars will be as acceptable as freeze-dried extracts. Research into these matters is needed.

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#### References

- Anon. (1981). Fruit nectars could give NZ a multimillion dollar industry. Orchardist NZ S4, No.l.
- AOAC (1980). Official methods of analysis of the Association of Official Analytical Chemists. 14th Edition, (AOAC Washington),
- Casimir, D.J. and Huntington, J.N. (1978). A spinning cone distillation column for essence recovery. Internationale Fruchtsaftunion Symposium. **15:** 115-124.
- FAO (1988). FAO Food & Nutrition Paper. **38:** 69-72,
- Hall, C.O. (1976). the coupling of photophosphorylation to electron transport in isolated chloroplasts. In *The Intact Chloroplast.* (Barber, J. ed). Elsevier. p. 135-170.
- Lodge, N. & Robertson, G.L. (1990). Processing of kiwifruit. In Kiwifruit: Science and Management. (Warrington, I.J. and Weston, G.C. eds.) New Zealand Soc. for Horticultural Science: Auckland, pp. 460-484.
- Menke, W. (1966). The structure of chloroplasts. In

Biochemistry of chloroplasts. Vol. 1 (Goodwin, T.W. ed.) (Academic Press: London) pp 3-18.

- NSW Kiwifruit Growers Association. (1988). *Private Communication*.
- Perera, C.O., Hallett, I.C., Nguyen, T.T. and Charles, J.C. (1990). Calcium oxalate crystals: the irritant factor in kiwifruit. Journal of Food Science, **55:** 1066-1069, 1080.
- Possingham, J.V., Coote, M. and Hawker, J.S. (1980). The plastids and pigments of fresh and dried Chinese gooseberries (Actinidia chinensis). Annals of Botany. **45:** 529-533.
- Robertson, G.L. (1985). Changes in the chlorophyll and pheophytin concentrations of kiwifruit during processing and storage. Food Chemistry, 17: 25-32.

- Spencer, D. and Wildman, S.G. (1962). Observations on the structure of grana-containing chloroplasts and a proposed model of chloroplast structure. Australian Journal of Biological Sciences, 15: 599-610.
- Venning, J.A., Burns, D.J.W., Hoskin, K.M., Nguyen, T. and Stec, M.G.H. (1989). Factors influencing the stability of frozen kiwifruit pulp. *Journal of Food Science*, **54**: 396-400, 404.
- White, R.C., Jones, I.D. and Gibbs, E. (1963). Determination of chlorophylls, chlorophyllides, pheophytins, and pheophorbides in plant material. *Journal of Food Science*, **28**: 431-436.
- Wilson, E., Burns, D. and Hogg, M. (1982). Breakthrough in juice research'. Food Technology in New Zealand, June: 17,18.

### Bacterial Spoilage of Specialty Pasteurised Milk Products

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The bacterial spoilage at 6°C was determined over a range of specialty pasteurised milk products containing varying levels of fat and protein and manufactured by three Melbourne processors. Bacterial spoilage was independent of the initial microbial load in all of the different types of milk, as determined by total microbial count. In 80% of the samples, spoilage was dependent upon the outgrowth of an initially small number of pseudomonads, presumably present as postpasteurisation contaminants. These results are in agreement with those for plain milk, i.e. that pseudomonads are the major spoilage microorganisms. At 6°C, spoilage (as defined by a bacterial concentration of 3x107 cfu/mL) occurred in 6 to 12 days and was independent of the type of specialty milk (i.e. level of fat or protein).

#### Introduction

Spoilage of pasteurised whole milk at refrigeration temperatures (46°C) has been shown to be due mainly to the growth of psychrotrophic bacteria predominantly pseudomonads (Chandler and McMeekin, 1985; Griffiths et al., 1988). These organisms are killed by pasteurisation and gain access to the milk later as contamination during further processing and packaging. In recent years a number of new specialty pasteurised milk products with altered levels of fat (mostly reduced) and protein (mostly elevated) have been introduced to the market. No studies have been reported on the shelf life or bacterial spoilage of these new products.

In this study we have compared the bacterial spoilage of four new products with altered levels of fat and protein.

#### Materials & Methods Milk Products

A range of pasteurised and homogenised white milk products were obtained directly from three Melbourne milk processing plants over a threeweek period in December-January, 1988-1989. Details of the types of homogenised, pasteurised milk samples used are presented in Table 1. In total, 32 one litre cartons were collec-

<sup>1</sup>Current address: Royal Melbourne Institute of Technology, GPO Box 2476V, Melbourne, Victoria, 3001.

<sup>2</sup> Recipient of a CSIRO Vacation Studentship Award. ted from the production lines of the processors and transported on ice to the laboratory where they were incubated in a 6°C water bath. This variation from the recommended storage temperature of 4°C was chosen to reduce the duration of experiments and to take account of the higher temperatures in summer sometimes experienced by retail containers of milk.

#### Enumeration of Spoilage Bacteria

Duplicate milk samples were removed aseptically from each carton daily. Ten-fold serial dilutions of the milk samples were made in 0.9% NaCl prior to enumeration by spreading 0.1mL aliquots onto petri dishes containing Plate Count Agar (Oxoid CM325) (PCA) and

Pseudomonas Agar Base (Oxoid CM559) containing 10ml/L Cetrimide, 10mg/L Fucidin and 50mg/L Cephaloridine (Oxoid SR103) (PsA) (Mead and Adams, 1977). The resultant plates were incubated for four days at 30°C prior to enumerating total colony forming units (cfu). Generation times were calculated from data obtained during the period of exponential increase in total and pseudomonad counts in the milk products and were the times taken for one doubling of bacterial numbers.

#### **Bacterial Spoilage**

Products were assumed as spoiled on achieving a total bacterial count of  $3 \times 10^7$  cfu/mL (log 7.5) (Griffiths, *et al.*, 1984).

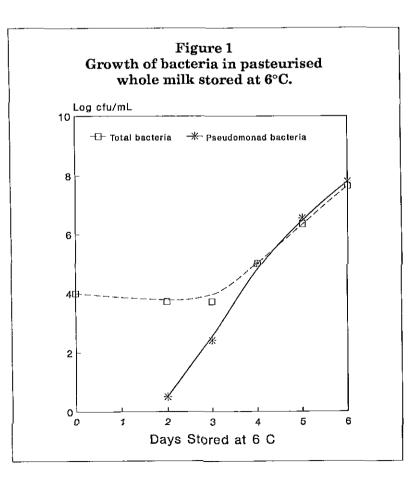
#### **Identification of Isolates**

Ten bacterial isolates were picked at random from each of the total and pseudomonad plates of 10 milk samples when their microbial levels were greater than  $3 \times 10^7$  cfu/mL. Presumptive identification of these isolates was made using the diagnostic tests prescribed by Shewan, *et al.*, (1960).

#### **Results & Discussion**

The growth at 6°C of pseudomonad and total bacteria was measured in each of 32 one litre cartons of commerciallyproduced plain and specialty pasteurised milks. All samples exhibited an initial total plate count in the range  $2.9-9.9 \times 10^3$ cfu/mL which remained relatively constant or decreased slightly during storage until such a time as the pseudomonad count was similar. Further storage showed both total and pseudomonad counts to

		l compo		analyses of arket milks	Ĉ
Milk Sample <sup>1</sup> L	Fat (%)	Protein (%)	Lactose (%)	Calcium mg/100mL	Energy kJ/100m
x.fat	4.8	3.3	4.6	117	317
whole	3.8	3.3	4.6	118	278
l.fat	1.5	3.9	5.3	145	214
uf	1.4	5.2	4.9	205	219
v.l.fat	0.1	<b>3.4</b>	4.7	129	142
	พ ไ. บ	fat lo f ultrafilt	o additior w fat	ns or subtrac	tions



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increase at similar rates. A typical example is shown in Figure 1 for pasteurised whole milk. Bacterial spoilage was assumed to have occurred when the total bacterial count reached 3 x 10<sup>7</sup> cfu/mL. All milks reached this level within 14 days. This microbial growth outcome was typical for all of the five types of milk studied, i.e. independent of fat, protein or calcium levels in the milk and was observed in 80% of the samples studies. These results are in agreement with those observed for pasteurised plain milk by Chandler and McMeekin (1985) and Griffiths et al., (1988), that bacterial spoilage is mostly due to growth of pseudomonads.

The mean time taken to reach log(7.5) cfu/mL (bacterial spoilage), a level associated with organoleptic spoilage (Griffiths et al, 1988) falls within a relatively narrow range (7.2-8.0d) for all of the different milk types tested, excepting the ultrafiltered low fat milk (one sample only tested, 5.5d) (Table 2). However there was a large variation between individual samples, as has been recognised by Maxcy and Wallen (1983). They demonstrated large differences in the shelflife of refrigerated, cartoned milks, even when they were removed sequentially from the production line.

Generation times for spoilage bacteria varied widely from 0.14 to 0.40d, presumably due to generic or species differences. This compares favourably with the observations of Griffiths and Phillips (1988), who demonstrated that the distribution of generation times of the natural microbiota in past-

Mean time in days $\pm$ standard deviation								
Milk1	$n^2$	Time to 1	09(7.5)	Generation	Time			
Sample		PCA	PsA	PCA	PsA			
x.fat	5	7.2±2.4	$7.1\pm2.4$	0.30±0.14	0.25±0.12			
whole	8	7.7±3.3	$7.8 \pm 2.9$	$0.23 \pm 0.08$	0.20±0.08			
I.fat	5	7.7±3.0	$7.1 \pm 2.4$	$0.34 \pm 0.11$	0.28±0.08			
uf	1	5.5	5.6	0.14	0.14			
v.l.fat	5	$8.0 \pm 1.9$	7.9±1.7	0.40±0.29	$0.35 \pm 0.30$			
Total	24	7.6±2.6	7.4±2.3	0.30±0.17	0.26±0.16			

eurised milks stored at 6.4°C was in the range 0.08 to 0.38d.

All isolates from pseudomonad plates were characterised as pseudomonads. This confirms the selective nature of this medium. In addition, 70 of 100 colonies isolated from total plate counts showing (>3)x10<sup>7</sup> cfu/mL) were characterised as pseudomonads. This is in agreement with the results of Griffiths et al., (1988) for milk stored at 6°C. They found the spoilage microbiota to be dominated by pseudomonads, with minor contributions from bacilli and other Gram-positive microorganisms. The similarity in total and pseudomonad numbers at spoilage levels also supports the use of this medium for monitoring the spoilage microorganisms present in post-pasteurisation contaminated milk.

Chandler and McMeekin (1989) reported a correlation between storage temperature and the rate of deterioration of pasteurised milk which was based on the square root model of Ratkowski et al., (1982). This model predicts that increasing storage temperature from the recommended 4°C to 6°C will reduce the shelf-life from 10 to 7.7d. In this study, 63% of milk products stored at 6°C for 7.7 days, contained spoilage concentrations (>log 7.5 cfu per mL) of pseudomonad organisms. This implies that after storage for 10 days at 4°C only 37% of these products would remain unspoilt.

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#### References

Chandler, R.E. and McMeekin, T.A. (1985). Temperature function integration and its relationship to the spoilage of pasteurised, homogenised milk. *Australian Journal of Dairy Technology.* **40:** 37-40.

Chandler, R.E. and McMeekin, T.A. (1989). Temperature function integration as the basis of an accelerated method to predict shelf life of pasteurised, homogenised milk. *Food Microbiology.* **6**: 105-11.

Griffiths, M.W. and Phillips, J.D. (1988). Modelling the relation between bacterial growth and storage temperature in pasteurised milks of varying hygienic quality. Journal of the Society of Dairy Technology. 41: 96-102.

- Griffiths, M.W., Phillips, J.D. and Muir, D.D. (1984). Methods for rapid detection of post-pasteurisation contamination in cream. Journal of the Society of Dairy Technology. **37:** 22.
- Griffiths, M.W., Phillips, J.D., West, I.G. and Muir, D.D. (1988). The effect of extended low-temperature storage of raw milk on the quality of pasteurised and UHT milk. *Food Microbiology.* 5: 75-87.
- Maxcy, R.B. and Wallen, S.E. (1983). Heterogeneity of samples as a problem in shelf-life prediction. Journal of *Food Protection*. **46:** 542-4.

- Mead, G.C. and Adams, B.W. (1977). A selective medium for the rapid isolation of Pseudomonads associated with poultry meat spoilage. *British Poultry Science*. 18: 661-70.
- Ratkowsky, D.A., Olley, J., McMeekin, T.A. and Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. Journal of Bacteriology. 149: 1-5.
- Shewan, J.M., Hobbs, G. and Hodgkiss, W. (1960). A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the Pseudomonaceae. Journal of *Applied Bacteriology*. **23:** 379-90.

### The New National Food Authority: What Role Will It Play?\*

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The efforts by many people to change the present food regulatory system will be wasted unless the new arrangements, centred on the National Food Authority (NFA), lead to a more efficient and competitive food industry able to deliver safe and nutritious food, employ more Australians and add greater value to Australia's agricultural raw materials.

Before answering the central question about the role of the NFA in future development of the industry, we must consider several other questions:-

- The food industry and food regulation what is the present position?
- The National Food Authority:-
  - Where has it come from?
  - What is it?
  - How will it operate? and finally,
- What is the future role for industry?

#### The Food Industry and Food Regulation - What is the Present Position?

The food industry in Australia is an efficient industry which accounts for 20% of Australian manufacturing industry. It is a major domestic user of agricultural commodities and packaging materials and of other services such as transport and advertising. It supplies the bulk of the Australian market but exports only a small share of its output of processed food products. Imports to the Australian market are growing, fuelled by the efforts of retailers to seek out cheap generic imports, often from centrally planned or subsidising economies.

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† The views expressed in this paper are those of the author and the Association he represents.

There is an increasing realisation amongst economic policy makers and commentators that the processed food industry must expand and export more if Australia is to resolve its balance of payments problems and to reverse the trend in imports. It can do so only if the total economic environment is conducive to new investment in plant and branded product exports. Obviously, factors such as interest rates, the exchange rate, inflation and micro-economic reform are vital elements of that environment, but food regulation is also an important element. The broader economic environment question has been taken up by the Grocery Manufacturers of Australia (and many other industry organisations) in submissions to the Commonwealth Government.

Food regulations encompass food standards, packaging and labelling regulations, Trade Practices and Fair Trading Acts, not to mention a host of other specific product regulations, health regulations and industrial legislation.

There have been improvements in some areas, including in some recently revised food standards. However, we still have a body of food regulation which has as some of its worst features:-

- Lack of clear objectives
- Excessively complex and prescriptive standards
- Slow and cumbersome procedures.
- Lack of consistency in decisions
- Lack of access to the process for interested companies, and
- Administrative inefficiencies.

That is not just my view but is also the view of regulatory review authorities and the Industries Assistance Commission as the following quotes confirm:-

> 'A great many food industry regulations tightly define product composition, labelling, use of additives and packaging. The Inquiry's view is that many such requirements reduce the scope of the industry to search out new ingredients and adapt to changing areas of demand. These interventions needlessly add costs to the food industry, raise prices and tend to direct rivalry towards traditional rather than innovatory products.

Non-uniformity of the Food Standards Code with other food industry regulations and differing administrative procedures and approaches add further unnecessary costs. Notwithstanding major moves towards greater uniformity in recent years, significant regulatory disparities persist.'

(Effect of Food Industry Regulations Report by the Commonwealth Business Regulation Review Unit and the Victorian Regulation Review Unit, November 1988.)

#### And again:

Regulations governing the composition and description of foods entail significant compliance costs. They also delay the introduction of new products and hamper innovation, disadvantaging both producers and consumers. There is scope for reducing the complexity and prescriptiveness of these regulations to enhance the competitiveness and efficiency of the industries.

Food regulations can be reformed in ways which do not detract from their essential role of protecting the public health and safety of consumers, and which increase the amount of information available to consumers.'

(Report on the Food Processing and Beverages Industries Recommendations - Industries Assistance Commission (IAC), 15 December 1989.)

Despite attempts to overhaul the food standards system in 1987, it was portrayed by the IAC in Table 1.

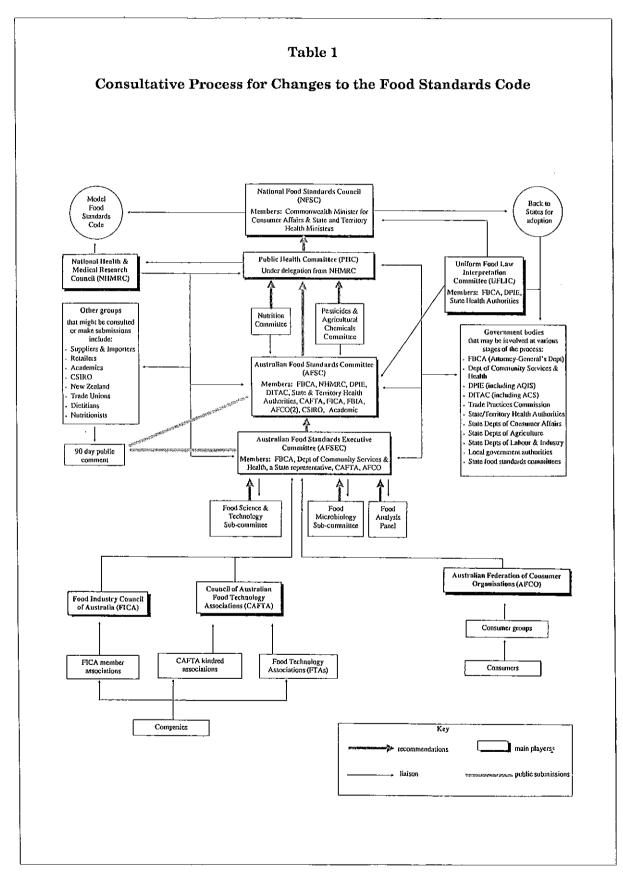
#### The National Food Authority - Where Has It come From?

Those reports from the Industries Assistance Commission and the Federal and Victorian Regulatory Review Units were part of a steadily increasing stream of criticism of the food regulatory system which, as Table 1 so clearly demonstrated, was certainly open to criticism. The Food Industry Council of Australia, the Grocery Manufacturers of Australia (GMA) and the Steering Committee for Reform of the Food Regulatory System and individual food companies all called for fundamental changes to the system.

The industry views were encapsulated in the following paragraph from a GMA letter to Health Ministers:-

> 'Excessive delays in establishing standards, inconsistencies and inadequate consultation with affected companies or industries are well-documented. The food industry believes that a complete overhaul of the system is needed to achieve the improvements which will enable an efficient, competitive industry to deliver new and better products more quickly to consumers in Australia and overseas.'

Debate ensued within the Commonwealth and State bureaucracies and the National Health and Medical Research Council, much of which was concerned with defending the existing system from its



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detractors and retaining as large a part as possible of the present system.

The food industry's proposal for change contained the following elements:-

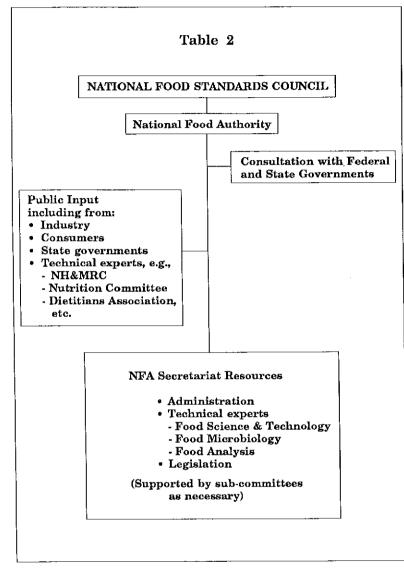
- Clearly stated objectives which identify public health and safety as the prime focus
- Uniform national standards
- Closer alignment with international standards
- An independent food commission supported by its own adequately resourced secretariat
- An open inquiry process with specified response times; and
- A timely and effective appeal mechanism

The much simpler system proposed by industry could be as set out in Table 2.

Industry put this position vigorously to government in the run up to the September 1990 Premiers' Conference.

A favourable decision was made by the Premiers in Brisbane when they agreed to the establishment of the National Food Authority with the following words in the communique:-

"... there is agreement that uniform national food standards should apply across the nation and be regulated by a National Food Authority. This should lead to substantial increases in the efficiency of food



production and distribution. In developing new standards, the National Food Authority will seek to promote as far as possible consistency between domestic and international food standards, in recognition of the benefits to be gained from increased international harmonisation.'

#### The National Food Authority - What is it?

Since the Premiers' Conference work within government has concentrated on specific details of the NFA and the preparation of legislation to bring it into being. That legislation is, I understand, still being drafted

for submission to the Autumn Session of Federal Parliament.

The substance of the Premiers' decision was set out in a November 1990 paper prepared by the Commonwealth Department of Community Services and Health which was provided to members of the Australian Food Standards Committee. Key features of the new food regulatory system include:-

 More specific objectives for domestic food standards:-

(a) to protect public health and safety;

(b) to provide sufficient information on food ingredients to enable consumers to make informed choices;

(c) to promote fair trading practices at the national level.

In developing standards, the NFA is intended to recognise the need to promote domestic uniformity and alignment with international requirements to promote trade and commerce in the food industry.

- Decisions will be taken by State and Federal Ministers, serving as the National Food Standards Council, on a majority basis and will be implemented by reference and without variation.
- The establishment of the National Food Authority:-
  - with a full-time Chairman and four part-time Members appointed on the basis of their individual backgrounds, skills and expertise;

- with the power to establish necessary advisory committees and hold public hearings as necessary;
- to be supported by appropriate technical and administrative staff dedicated to the work of the Authority with a set time period within which to reach decisions (although not set out in the paper, it is understood a time period of 6-10 months has been proposed);
- to undertake a variety of functions including:-

a) Development and review of domestic food standards

b) Co-ordination of domestic food surveillance

c) Development of food safety initiatives

d) Co-ordination of food recalls, and

e) Co-ordination of imported foods assessment policy.

While the enabling legislation is being drafted, the position of Interim Chairman has been advertised and applications are being processed and administrative details (such as the location of the Authority) are being determined.

An organisation chart for thenew system would look very similar to the industry proposal, but with two notable variations (Table 3). These are the formalised National Food Authority Advisory Committee and the NH&MRC Food Committee, on which I comment later.

A quick look at the major elements of the new system in tabular form confirms that we have been very successful in achieving a system which is much closer to industry requirements than that which exists at present. However, the food industry will need to maintain its vigilance during the period up to and including the tabling of the legislation to ensure that an open, independent and qualified system eventuates. Appointments to the Authority and its secretariat will be crucial for its credibility. Its location is also an important issue - the NFA's accessibility to industry, consumers and the necessary food experts would be enhanced if it were located in Sydney or Melbourne, rather than Canberra (the government preference).

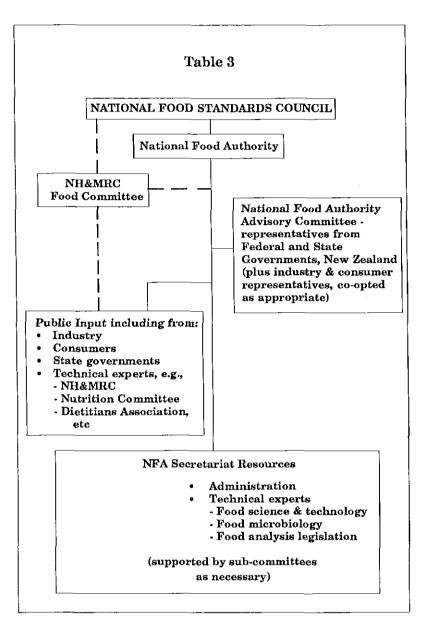
#### The National Food Authority - What Role Will it Play?

The answer to this question will be determined largely by the legislation, by the appointments of Authority members and staff, by their willingness to adopt a fresh new approach to food regulation and by the support they are given by the National Food Standards Council. The opportunity remains all too real for the narrow perspective of the past to be reinstated in the process. Ideally the National Food Authority will:-

 Consider applications quickly, providing ample opportunity for industry (and the public) to be involved. I believe this could be achieved in most instances

through a relatively small meeting of parties such as has been used in anti-dumping investigations. The time limits I have suggested should also avoid the totally unacceptable delays which have been encountered in setting standards for, to take an example, artificial sweeteners, table spreads and reduced fat ice cream.

- Make decisions which are consistent with its objectives and with international practice. The first and overriding objective must be the protection of public health and safety. It is essential also that the NFA take heed of the direction to promote domestic uniformity and alignment with international requirements and to promote trade and commerce in the food industry. That will require that generally no Australian standard should be out of line with those in competing nations. The past proposal for the world's toughest restrictions on caffeine in kola drinks and the present proposal for controls on vitamins and minerals which run ahead of international convention are examples of issues to which this directive is particularly relevant. The AFSC's current preoccupation with the regulation of negative claims is another example which has absolutely no public health and safety implications but which could cost industry massive amounts of money in relabelling and promotion expenditure.
- Move away from the present set of complex and prescrip-



tive standards which require amendment to permit every new product or product variant and which give rise to demands for local variations, such as for milk in Western Australia.

• Ensure greater uniformity and more timely adoption of standards for all states. In a press statement following the Premiers' Conference, the Federal Minister for Health referred to 44 variations to the Food Standards Code which existed at that time — and to additional standards which existed in NSW, WA, SA and Tasmania.

• Co-ordinate related regulatory and food safety initiatives, such as food recalls, imported food assessment, export standards, and uniform national interpretation of regulations.

More actively defend Australian food supplies through the wider dissemination of the results of the Market Basket Survey and through the publication and promotion of booklets such as 'Food Additives - Food for Thought'. There is a great deal of information collected by governments which is not used effectively to allay concerns about Australian food products.

How many are aware that the Federal Bureau of Consumer Affairs spent months developing the 'Food for Thought' booklet and that industry contributed both time and money to ensure that a balanced document was produced and widely available?

How many are aware that the document was released not by Minister Tate but by his Department on 11 February, accompanied by a bland four paragraph press statement?

At that time the 'Choice' article on additives was in every newspaper and many talk-back radio programs yet not one media outlet referred to the 'Food for Thought' booklet. If they try, the National Food Authority must be able to do better than that!

 Review existing regulations and simplify them according to the new philosophy and objectives. Although not spelt out in the Department of Community Services and Health paper the Authority will be asked to complete within 18 months a review of the policy underpinning food standards and to establish a detailed timetable for a review of individual standards. If it is to be completed quickly, such a review will be resourceintensive and may require additional staff from within government, supported by the provision of relevant industry personnel on secondment specifically for this purpose.

In his statement after the Premiers' Conference, the Minister described the decision to set up the National Food Authority as 'a significant piece of micro-economic reform, which would cut costs to producers and therefore consumers, and remove barriers to interstate trade.' If it operates as I outlined earlier, it will do much more than that. It will, for example:-

٠ Encourage innovation in the food industry by enabling new products to be developed and approved more quickly and through a more certain process. Less prescriptive standards will remove the need for the standard to be amended every time there is a change in the composition of a product. Furthermore, by removing at least one layer in the decision-making process (namely, the Public Health Committee) and by focussing responsibility for standards much more specifically on the Authority and its secretariat, there will be less opportunity for others

outside the system to frustrate and delay the standard approval process.

- By aligning Australian standards more closely with international standards, facilitate production in Australia on a scale which will contribute to greater international competitiveness for the Australian industry. Competitiveness will also be encouraged, of course, by innovation and by the reduced costs of compliance referred to by the Minister.
- Have more incentive to counter the often ill-founded and malicious criticism of the Australian food industry. The Authority and its dedicated secretariat will be solely responsible for food regulation and monitoring. Problems of divided responsibility which exist between various Commonwealth agencies and state governments in the present system should be very largely removed. Provided adequate resources are made available, it will be much easier for the Authority to carry out a food monitoring program such as the Market Basket Survey and to promulgate the results in a way which is better understood by the general public. The access to adequate resources is a big proviso, however. as the Market Basket Survey has consistently suffered from this problem, while the latest FBCA booklet. 'Food Additives - Food for Thought' required funding through GMA members to ensure a reasonable print run.

Reduce inconsistencies between food regulations. Responsibility will lie within the Authority for food product and hygiene standards, food recalls, emergency public health situations, imported food assessment and uniform interpretation of legislation. Again, a move to simpler and less prescriptive regulations will remove the possibility of inconsistent treatment of related or competing food products.

I have given a very positive assessment of what might be achieved under a national food authority. However, the seeds of doubt about aspects of the new system have been sown and some may already be germinating. I understand, for example, that:-

- At least some states are seeking to back away from the commitment to eliminate all variations from the current Food Standards Code and to require the automatic adoption of all new standards.
- The independence of the NFA may be compromised by the administrative influences of the Department of Community Services and Health which is, at present, having a major say about issues such as location and staffing of the secretariat.
- The expertise available in the new secretariat may also be compromised by excessive reliance on the officers of the Federal Health and Consumer Affairs departments which are at present involved in food standards administration. Too

little effort seems to be taken to identify and engage appropriately qualified people with a new contribution to make.

- A possible Canberra location is one of the constraints

   and bureaucratic rigidities within the Commonwealth Public Service is another.
- Potential candidates for the Chairman's job and for senior administrative positions are a further cause of worry as the Canberra rumour mill is churning out the names of people who are very much involved in the present system and/or who have little affinity with the food industry.

Mechanisms are also being established to oversee or 'second guess' the Authority, including a formal advisory committee, the National Food Authority Advisory Committee. While the premiers' decision specifically says that the advisory committee will 'not routinely be part of the standards development process' and is intended as 'a vehicle for coordination and consultation with the states and territories', it has the potential to frustrate the Authority's activities. This would be particularly so if its membership were to be expanded to include industry and consumer representatives, whereby it would become a virtual re-creation of the present Food Standards Committee. The NHMRC is seeking to retain its influence on food issues and also, by implication, on the Authority by establishing a Food and Health Committee which will report to the Public Health Committee. This need not create problems if the input to the Authority is made during the Authority's open public consultation phase. However, if that NHMRC Food and Health Committee seeks to exert its influence directly on the ministers who comprise the National Food Standards Council, or through contradictory publications, it could be a most unhelpful element in the new food regulatory system.

## What is the Role for Industry?

The final details of the new food regulatory system and the operation of the National Food Authority will obviously determine the role which industry is able to play in the new system. The extent of industry involvement will also depend, however, on the attitude which is adopted by food industry representatives towards the new system. They can either:-

- Accept the comfort of a predictable, if less than ideal, situation which is provided by the present set of regulations and which, as I have said, has an inhibiting effect on innovation and competition, or
- Seek to correct the problems of the existing system by aggressively pursuing *less* prescriptive regulations and relying on their own competitive abilities to increase their market share in Australia and overseas.

Those of us who have worked towards achieving a new system have done so in the belief that the companies we rep-

resent have been supportive of a much freer regulatory environment and will follow the second course.

Taking that positive approach, industry can do much to maintain the pace of regulatory reform. It can:-

- Identify major constraints in existing standards and recognise new market opportunities which could be pursued in a changed regulatory environment
- Promote reviews of existing standards, starting with those which impose greatest constraint
- Consistently and strongly advocate the move to less restrictive and prescriptive standards
- Devote sufficient resources to food regulatory issues to ensure that industry itself does not provide an excuse for not meeting the prescribed time limits; and
- Present well-argued cases including, where possible, drafts of proposed standards to meet industry requirements. Expertise exists within companies and within organisations such as CAFTA to draft standards

in the most effective manner. There is greater knowledge about food manufacture and consumption within industry organisations and within companies (including within their overseas affiliates) than in government and those resources should be harnessed to achieve the best result from the new system.

Industry's credibility within the new arrangements will depend of course, on the accuracy of its submissions and on its responsibility in adhering to food standards. Industry is required to act responsibly at all times: it is particularly important that it does so at a time of significant change, such as we are experiencing at present. It is all too easy for the regulators to overreact to irresponsible behaviour by even the minority of the industry at a time when new procedures are being established.

In short, there are many steps which the food industry can take to set the agenda for food regulation reform. It need not be reactive only to government initiatives or to consumerist attacks on the industry. The National Food Authority has the potential to be very good for Australian industry and we in the food industry must do all we can to make that happen. At the end of the day, however, it will only happen if the commitment by state and federal governments which led to the very welcome food regulatory initiatives at the Premiers' Conference, is maintained as those initiatives are put in place and become operational. I have mentioned a series of doubts about how well that commitment will stand up to the inter- and intragovernmental activities associated with the establishment of the new arrangements. The way in which those doubts are handled will provide the first indication of how firm is the commitment to speedy and significant reform.

I said earlier that regulatory reform was only one, albeit important, element in a process of reform which is necessary to increase the competitiveness of the food industry. Events in the near future will be crucial to that process. We may have less influence over the broader process of economic reform but we must use every opportunity to remind the governments of Australia of their commitment to the reform of food regulation. The future development of the food industry depends on it.