VOL. 46 No. 4 December 1986

CSIRO Food Research Quarterly

Engineering wheat-grain proteins to suit processing requirements: Studies on wheat quality in the CSIRO Wheat Research Unit

By C.W. Wrigley

CSIRO Wheat Research Unit, North Ryde, New South Wales, 2113

The production of wheat grain that is of a quality type suited to the intended food process is critical to successful processing and also to maximizing returns on export sales to Australia. Research in the Wheat Research Unit has revealed the existence of major genes for quality by identifying quality-related gluten proteins.

The nutritional importance of cereal grains

What combination of foods provides the cheapest complete nutrition? One answer to this question can be obtained by applying a least-cost-rations computer program, commonly used in formulating animal feeds, but substituting supermarket prices and nutritional values for food products and including the daily requirements for an adult (energy, protein, vitamins and minerals). The best-value combination is a mix of wheat-based breakfast cereal and oatmeal (contributing half the energy and most of the protein, iron, thiamin, riboflavin and niacin), sugar (providing 40% of the energy), and smaller amounts of skim milk, liver and potatoes (mainly providing calcium, vitamins A and C, respectively).

This illustration of the nutritional value of cereal grains neglects their additional contribution of fibre to the diet, further justifying the recommendation of cereal products in the nutritional guidelines of Australia and other countries.

For these and other reasons, the cereal grains have occupied a very important position in world food supplies, particularly as sources of protein and energy. Current production levels for wheat, rice and maize (together) average about a kilogram of grain per person per day. In fact, it has been calculated that this level of production is sufficient to satisfy the world population projected for the end of the century (Pimental *et al.* 1975). Such calculations, of course, do not take into account the realities of transportation problems, inabilities of poorer nations to buy and the many non-food uses of cereals.

Competition in world grain trade

Australia is a significant contributor to world trade in cereal grains particularly for wheat because of a five- to ten-fold surplus of production over consumption (Wrigley 1986). However, to maintain a position of prominence in world trade and to maximize sale prices, cereal chemists must also come to grips with the further reality of consumer acceptance: is the grain produced suitable for processing into the desired products?

Recent years have brought major changes in the markets to which Australia exports grain, and consequent changes in the products made from it. Food chemists have thus had to study the grain types, dough properties and milling procedures best suited to making flat breads for Middle East countries, steamed bread as made in China, noodles of many types for Asian countries, in addition to the more familiar risen breads of western countries.

The Bread Research Institute has been prominent in establishing the technological requirements of wheat for this wider range of products. These studies have been complemented by a more basic examination in the CSIRO Wheat Research Unit of the types of proteins, lipids and carbohydrates best suited to these uses.

Objectives of the Wheat Research Unit

Study of the chemical basis of grain quality has been the primary objective of the unit since its establishment in 1957 with a grant from the then newly-formed Wheat Industry Research Council. It was located in the same building as the Bread Research Institute intentionally to foster close

CSIRO Food Res. Q. 1986, 46, 73-78

collaboration. This day-to-day interaction has ensured industry-relevance for the Unit's projects, becoming more valuable as the Bread Research Institute has become closely associated with the Australian Wheat Board and as its industry involvement has spread well beyond the original concept of 'bread' research. Consequently, the most recent restatement of the Unit's objective is that it should strive to develop a deeper understanding of the relationship between grain composition and quality, in order to assist in optimizing returns for Australian grain and grain products.

Early wheat-quality research

Australia made an important contribution to the infant science of cereal chemistry between 1891 and 1910 through the collaboration of William Farrer with Frederick Guthrie (Chief Chemist of the NSW Department of Agriculture). During this period, Guthrie pioneered small-scale procedures for test milling and test baking, as well as establishing basic concepts concerning gluten proteins (Wrigley and Rathjen 1981).

Subsequent research has shown the validity of Guthrie's accent on gluten (Tracey 1967) because of its central importance in determining dough properties and baking quality. This role for gluten involves both the quantity and the composition of the gluten in a wheat sample.

A realisation of this importance also prompted a focus on gluten composition in the research of the young Wheat Research Unit, under the leadership of Michael Tracey (later to become Chief of the CSIRO Division of Food Research). Further insight into the heterogeneity of gluten was provided by the (then) new techniques of gel electrophoresis and column chromatography (Lee and Wrigley 1963).

This knowledge led to a study of the possibility that the presence of specific gluten components might indicate quality type. No such relationship was revealed at that stage by a limited study of only a few wheats (Lee *et al.* 1963). This concept was further discounted when gel isoelectric focusing was combined with electrophoresis to show that the gliadin portion of gluten was even more complex than was previously realised (Wrigley and Shepherd 1974).

Protein composition and genotype identification

The loss of interest in searching for 'quality-related' protein components did not mean a disinterest in protein composition, since electrophoretic analysis of the gluten proteins was developed as an effective means of identifying cereal varieties. Electrophoresis in starch gel was used initially to check on segregation of premium wheats. Further simplification of the method was provided by the use of preformed gradient gels of polyacrylamide, thus providing a standard procedure (R.A.C.I. 1981) that became an essential part of the introduction of variety-controlling legislation for improving wheat quality (Wrigley 1980).

Re-evaluation of 'quality-proteins'

The resulting accumulation of information on gliadin and glutenin composition for many Australian wheat varieties produced the stimulus to again search for protein components that might statistically relate to specific aspects of grain quality. Such associations were found in this re-assessment (Wrigley *et al.* 1982), providing encouragement to continue the search.

Durum wheats

These studies coincided with successes in France (Damidaux *et al.* 1978) and Canada (Kosmolak *et al.* 1980) in the discovery that specific gliadin proteins were consistently present in durum wheats of superior (protein 45) or poor (protein 42) pasta-making quality. This conclusion was confirmed and extended in studies at the Wheat Research Unit of about 100 durum breeding lines, showing that two groups of gliadin proteins were involved in the associations (duCros *et al.* 1982). Furthermore, low-molecular-weight glutenin polypeptides showed similar associations (duCros 1986).

Attention has focused on durum gliadins 42, 45 and associated proteins, showing the two groups of proteins to be products of two allelic blocks of tightly linked genes on the short arm of chromosome 1B. N-terminal amino-acid sequencing of the purified proteins has so far revealed little difference between corresponding components of the two groups. Sequencing proved quite difficult after the first 20 amino acids because of long repeats of glutamine/glutamic-acid residues with frequent prolines. Resolution of this problem will probably require nucleotide sequencing.

The conclusions from these studies of durum wheats are now being applied in Australia's durum-wheat breeding program by Dr R.A. Hare at the Agricultural

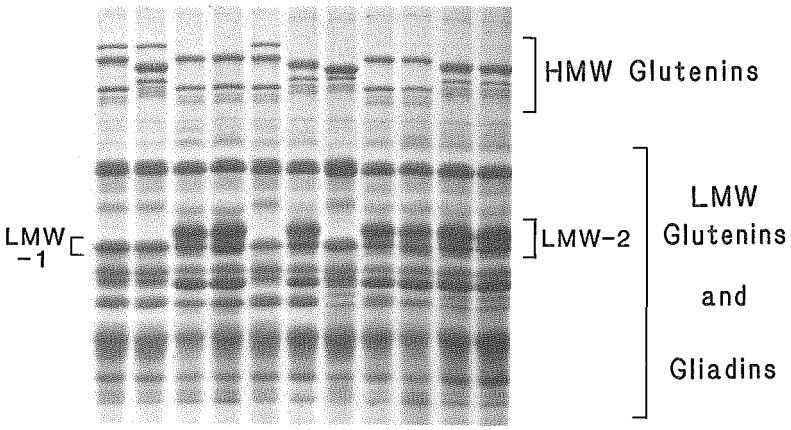

Fig. 1. SDS gel electrophoresis of gluten proteins from breeding lines of durum wheat showing variation in their high-(HMW) and low molecular weight (LMW) proteins. *Good pasta-making quality is commonly associated with the presence of the group of* glutenins marked LMW-2 and poor quality with those marked LMW-1.

Research Centre, Tamworth. In particular, a close association between glume (head) colour, gliadin 42/45 and pasta-making quality is proving a valuable selection tool (Hare and duCros 1986).

Bread wheats

These successful experiments with durum wheats provided encouragement to further examine protein composition in hexaploid (common) wheats. Payne and co-workers (1981), among others, had drawn attention to two pairs of high-molecular-weight (HMW) glutenin polypeptides; 5 and 10 were often present in good baking wheats whereas 2 and 12 were present in other wheats.

As it appeared that these conclusions might not apply universally, an ambitious project was initiated in collaboration between the Wheat Research Unit and the DSIR Wheat Research Institute (Christchurch) to examine one-kilogram samples of grain of two large sets of wheat, chosen to represent wide ranges of genotypes, pedigrees and quality types. Sufficient grain was available to permit full quality evaluation by conventional dough testing and by mechanical dough-development and baking. Protein evaluation included two types of gel electrophoresis to indicate the presence of up to 50 gliadin components and 21 HMW glutenins.

Some aspects of quality (e.g. dough extensibility) were not correlated with protein composition, but very highly significant correlations were observed with other aspects, particularly resistance to dough extension, work input to dough mixing and loaf volume. High values for these were related to the presence of HMW glutenins 5 and 10 (mentioned above) and certain gliadins (not 45 of durum wheats), low values with HMW glutenins 2 and 12, and other gliadin components.

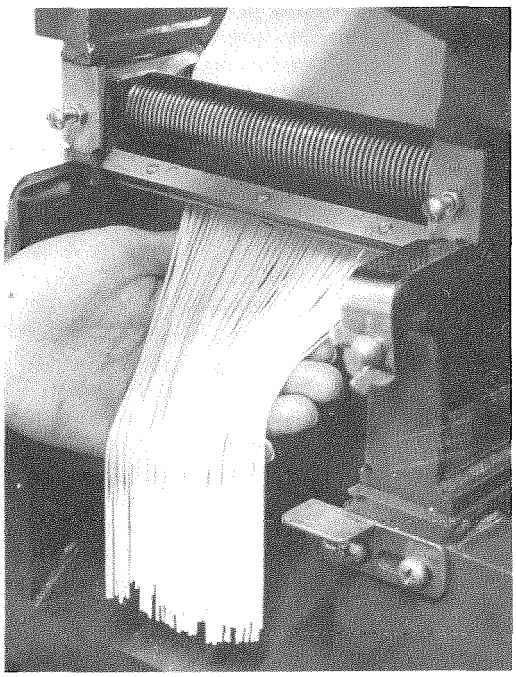

Fig. 2. Noodles being cut from a dough piece. Australian wheat is commonly used in SE Asia for noodle manufacture.

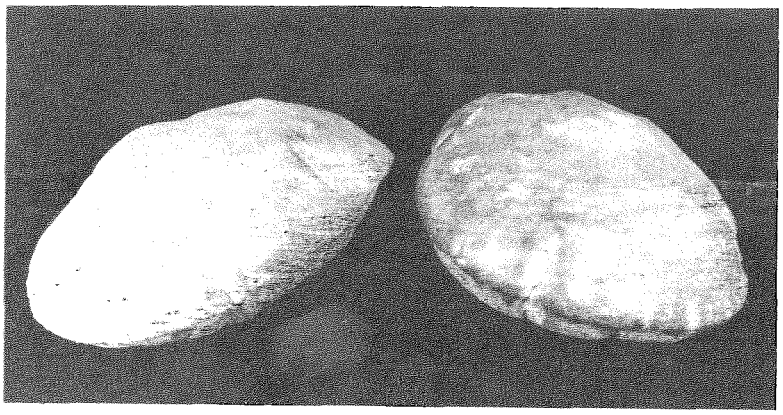

Fig. 3. Flat bread. Much of the wheat exported to the Middle East from Australia is used for the manufacture of this type of product.

New approaches to breeding

Further confirmation of the importance of some of these proteins has more recently come from quality testing of isogenic lines of wheat obtained by segregating, from Australian wheats, biotypes that differ in their HMW-glutenin composition (Lawrence 1986). Dough properties (particularly resistance to extension) were as predicted from the correlation studies.

The growing agreement between cereal chemists in many countries on the existence of key 'quality-related proteins' opens new approaches to quality segregation in wheat breeding. Such proteins can be taken as marking 'major genes' for quality. The use of gel electrophoresis to detect them in segregating lines has been given as one of the main reasons for the major improvements in quality for English wheats from the Plant Breeding Institute, Cambridge.

Novel breeding techniques

Furthermore, ready identification of the direct gene products makes quality-type directly amenable to genetic manipulation. The possibility of direct gene insertion becoming practicable for monocotyledonous plants provides strong motivation for cloning and characterization of these genes. In fact, partial nucleotide sequences from such studies are already available (Shewry and Miflin 1985). Collaborative investigations of copy DNA clones are progressing in the Wheat Research Unit and the CSIRO Division of Plant Industry (Canberra). In addition, somaclonal variation in the protein composition and quality of durum wheats is being studied.

Monoclonal antibodies

In such studies and more general investigation of these proteins, the availability of specific antibodies would be of immense value. Monoclonal antibodies offer the best possibility for producing such a degree of specificity, but attempts to select clones reactive with a single gluten polypeptide (especially a quality-related one) have so far been unsuccessful, presumably due to the very high degree of sequence homology between gluten proteins.

However, monoclonal antibodies have been obtained with quite high specificity (e.g. reactive only with omega-gliadins) and they are providing useful information about relationships between gluten proteins and between cereal genotypes (Skerrit *et al.* 1984). Furthermore, they have great potential as a basis for highly specific and sensitive food-analysis kits (Skerritt 1985). Prototype reagents for gluten analysis are already being tested as a result of commercial collaboration between the Wheat Research Unit and Biocon (Australia) Ltd.

The next critical step of producing monoclonal antibodies specific for distinct quality-related proteins (e.g. glutenins 5+10 vs 2+12) may require that the full amino-acid sequences of these proteins be determined to see what part of the sequence is distinctive for each. Synthetic peptides could then be made to reflect these differences and to produce monoclonal antibodies of the required specificity. A project to achieve this objective is currently being pursued in collaboration with the CSIRO Division of Protein Chemistry (Melbourne).

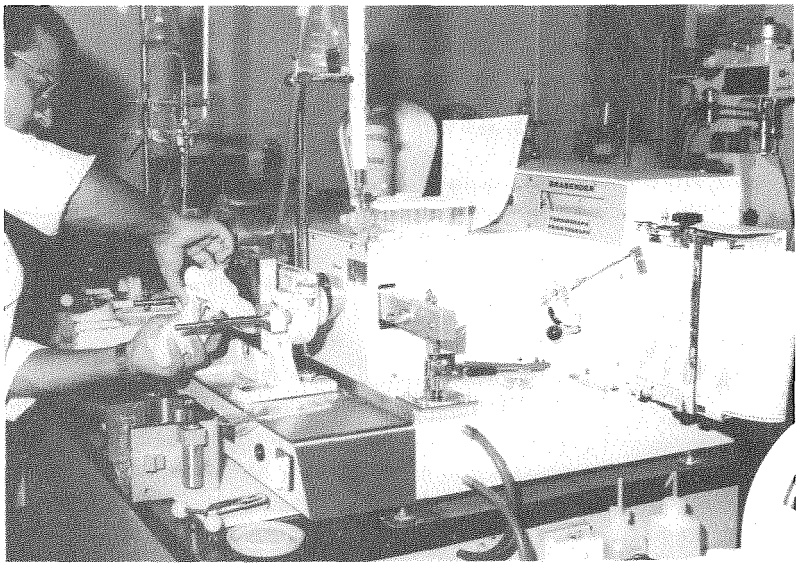

Fig. 4. The Farinograph, an instrument for measuring the mixing properties of wheat flour doughs. This instrument is one of several used in assessing the suitability of wheat for pan and flat bread manufacture.

Fractionation and reconstitution of flour components

It is still premature to say whether or not these quality-related proteins themselves confer the differences in dough properties that have been observed. For example, the 'major genes' may not actually code for the protein markers, but for some other critical component. Genetic manipulation of the protein-coding genes will provide a stringent test of the hypothesis.

In the meantime, an alternative approach is to purify the components and to add them to a basic dough to evaluate their ability to modify dough properties. This approach requires very careful attention to the details of purification to avoid damage to the proteins' functional properties. Guidelines for doing so have been established by MacRitchie (1985).

Modification of gluten

These studies have so far been primarily directed at identifying functional properties of cruder fractions than those resulting from gel electrophoresis. They have thus demonstrated the possibility of tailoring gluten fractions to give almost any desired combination of extensibility and resistance to stretching (MacRitchie 1986). Adaption of these simple gluten-extraction techniques thus provides the possibility of mass-producing gluten products with mechanical properties matched to specific requirements.

Treatment of gluten with alkali or enzymes offers another approach to manipulating the properties of gluten (Batey 1986). Current collaboration between the Wheat Research Unit, CSIRO Division of Food Research and Goodman-Fielders Pty Ltd is producing a series of modified gluten products suitable for a range of purposes in the food industry (as extenders, foaming agents, emulsifiers, dough-softening agents and other uses).

Thus, the engineering of the functional properties of wheat proteins can be achieved either through conventional breeding and, in time, through genetic engineering, or by the use of appropriate manufacturing processes which modify the protein directly. Knowledge being gained about the structure and inheritance of these proteins is assisting in these approaches to optimize protein properties for particular end products.

References

Batey, I.L. (1986). Enzymic solubilization of wheat gluten. *Journal of Applied Biochemistry* **7**, 423-9.

Damidaux, R., Autran, J.C., Grignac, P., and Feillet, P. (1978). Mise en evidence de relations applicables en selection entre l'electrophoregramme des gliadins et les proprietes viscoelastiques du gluten de *Triticum durum* Desf. *CR Academie Science Serie D* **287**, 701-4.

duCros, D.L. (1986). Glutenin proteins and gluten strength in durum wheat. *Journal of Cereal Science* (in press).

duCros, D.L., Wrigley, C.W., and Hare, R.A. (1982). Prediction of durum-wheat quality from gliadin-protein composition. *Australian Journal of Agricultural Research* **33**, 429-42.

Hare, R.A., and duCros, D.L. (1986). Genetic linkage of glume colour and certain gliadin proteins in durum wheat. *Crop Science* **26**, 831-3.

Kosmolak, F.G., Dexter, J.E., Matsuo, R.R., Leisle, D., and Marchylo, B.A. (1980). A relationship between durum wheat quality and gliadin electrophoregrams. *Canadian Journal of Plant Science* **60**, 427-32.

Lawrence, G.J. (1986). The high-molecular-weight subunit composition of Australian wheat cultivars. *Australian Journal of Agricultural Research* **37**, 125-33.

Lee, J.W., and Wrigley, C.W. (1963). The protein composition of gluten extracted from different wheats. *Australian Journal of Experimental Agriculture and Animal Husbandry* **3**, 85-8.

Lee, J.W., Tracey, M.V., Winzor, D.J., Wrigley, C.W., and Zentner, H. (1963). Properties of gluten fractions prepared by ion-exchange chromatography on carboxymethyl-cellulose. *Australian Journal of Biological Science* **16**, 717-27.

MacRitchie, F. (1985). Studies of the methodology for fractionation and reconstitution of wheat flours. *Journal of Cereal Chemistry* **3**, 221-30.

MacRitchie, F. (1986). Physiochemical processes in mixing. In 'Chemistry and Physics of Baking' (J.M.V. Blanshard, P.J. Frazier, and T. Galliard, eds). Royal Society of Chemistry, London. pp.132-56.

Payne, P.I., Corfield, K.G., Holt, L.M., and Blackman, J.A. (1981). Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. *Journal of the Science of Food and Agriculture* **32**, 51-60.

Pimental, D., Dritschilow, W., Krummel, J., and Kutzmann, J. (1975). Energy and land constraints in food protein production. *Science* **190**, 754-61.

Royal Australian Chemical Institute (1981). Electrophoretic identification of cereal varieties. Official Testing Method E2. Cereal Chemistry Division (RACI): Melbourne.

Shewry, P.R., and Miflin, B.J. (1985). Seed storage proteins of economically important cereals. *Advances in Cereal Science and Technology* **7**, 1-83.

Skerritt, J.H., Smith, R.A., Wrigley, C.W., and Underwood, P.A. (1984). Monoclonal antibodies to gliadin proteins used to examine cereal grain protein homologies. *Journal of Cereal Science* **2**, 215-24.

Skerritt, J.H. (1985). A sensitive monoclonal-antibody-based test for gluten detection. 3. Quantitative immunoassay. *Journal of the Science of Food and Agriculture* **36**, 987-94.

Tracey, M.V. (1967). Gluten: new light on an old protein. *Cereal Science Today* **12**, 193-7, 214.

Wrigley, C.W., and Shepherd, K.W. (1974). Electrofocusing of grain proteins from wheat genotypes. *Annals of the New York Academy of Science* **209**, 154-62.

Wrigley, C.W. (1980). Improving Australian wheat quality by varietal management: Identification of varieties. *Food Technology in Australia* **32**, 508-11.

Wrigley, C.W. (1986). Improving the market value and utilization of Australia's cereal crop through research. *Food Technology in Australia* (in press).

Wrigley, C.W., and Rathjen, A.J. (1981). Wheat breeding in Australia. In 'Plants and Man in Australia'. (D.J. Carr and S.G.M. Carr, eds). Academic Press Inc., New York, pp.96-135.

Wrigley, C.W., Lawrence, G.J., and Shepherd, K.W. (1982). The association of glutenin subunits with gliadin composition and grain quality in wheat. *Australian Journal of Plant Physiology* **9**, 15-30.

Humidity: measurement and control during the storage and transport of fruits and vegetables

By A.K. Sharp

CSIRO Division of Food Research, North Ryde, New South Wales, 2113

During long-term refrigerated storage the quality of horticultural produce depends on both temperature and humidity. The humidity is sometimes too low, but is so difficult to measure and control that, in most cases, permanent humidity monitoring or control instrumentation is not recommended. Instead, it is suggested that humidity should be checked from time to time, using portable equipment, and if it is persistently too low the cooling system should be modified.

What is humidity?

The term 'humidity' describes the amount of water vapour in the air. There are many ways of expressing humidity, each useful for particular purposes. Expressions of humidity used in horticulture include:

(i) absolute humidity or moisture content (g water/kg dry air)
(ii) water vapour pressure (Pa)
(iii) relative humidity (ratio of the absolute humidity to the absolute humidity of air, at the same temperature, saturated with water vapour)
(iv) dew-point (the temperature at which dew would begin to form if the air were cooled).

The maximum amount of water vapour that air can hold depends on the air temperature. Air that is holding the maximum amount of water vapour at a given temperature is said to be saturated, and has a relative humidity (R.H.) of 100%. Air at the same temperature, but holding half that amount of water vapour, therefore, has a relative humidity of 50%. As the temperature of air is increased, the amount of water vapour required to saturate it increases, approximately doubling for each 10 deg C rise in temperature.

When saturated air is warmed, its relative humidity will fall; although the amount of water vapour in it remains the same, at the higher temperature the air is capable of holding more water vapour. This is because saturated air at 0 °C contains 3.8 g water per kg dry air, but at 2 °C it could hold 4.4 g per kg. If air saturated at 0 °C is warmed to 2 °C, therefore, its relative humidity will fall to 86% (i.e. 100% x 3.8/4.4).

When saturated air is cooled, it must lose water because at the lower temperature it cannot hold as much water vapour. When cooled by passing over a cold surface, the excess water vapour from saturated air will produce condensate or ice, and when cooled by mixing with colder air, the excess water vapour forms fog. (If air saturated at 2 °C is cooled to 0 °C by passing over cooling coils, its water vapour content will fall from 4.4 g/kg to 3.8 g/kg, depositing 0.6 g of ice for each kg of air passing the coils.)

When moist, but unsaturated, air is cooled, the temperature at which it just becomes saturated, and the excess water vapour first begins to condense, is called the dew-point temperature. The dew-point depends only on the water vapour content of the air, and so is an alternative way of expressing humidity.

When unsaturated air passes over a wet surface that is neither heated nor cooled, the surface will be cooled by evaporation of water. Provided that the air speed is high, the extent of cooling depends mainly on the temperature and R.H. of the air. If a temperature sensor is covered with a wet wick, the temperature reached by the sensor is called the wet-bulb temperature. This value, together with the air (dry-bulb) temperature, can be used to determine the humidity of the air. Alternatively, the humidity can be expressed as the dry-bulb temperature and the wet-bulb depression (the difference between the dry-bulb and wet-bulb temperatures).

The relationships between temperature and

CSIRO Food Res. Q. 1986, 46, 79-85

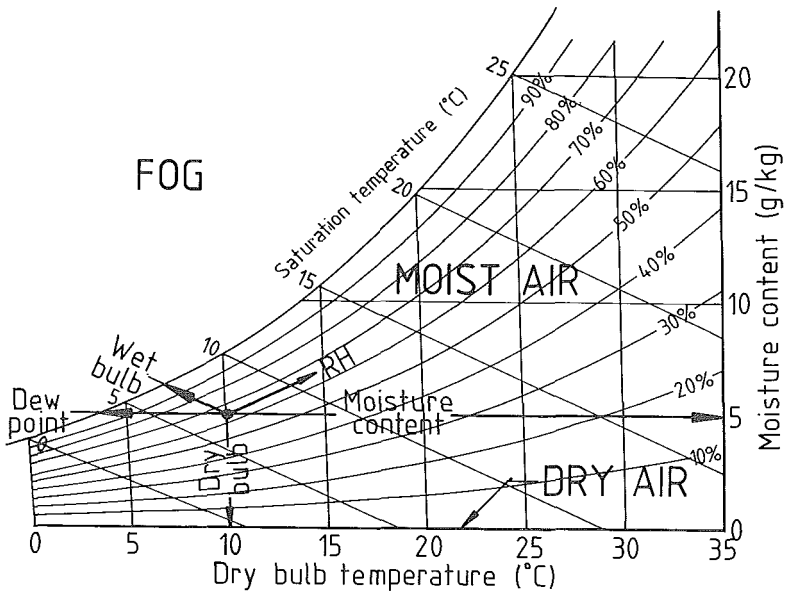

Fig. 1. Psychrometric chart, relating humidity to wet- and dry-bulb temperatures. The point marked is at 10 °C dry-bulb, 7 °C wet-bulb. The chart shows that this is equivalent to a moisture content of 5 g water vapour/kg dry air, a dew-point of 3.7 °C and a R.H. of 65%.

the various measures of humidity are often presented in the form of two families of graphs called a psychrometric chart (see Fig. 1). The dry-bulb temperature is the common horizontal axis: the vertical lines represent constant dry-bulb temperatures, the horizontal lines represent constant moisture contents (and dew-points), the inclined straight lines represent constant wet-bulb temperatures, and each curved line represents air at a constant relative humidity. The use of the psychrometric chart is explained in the ASHRAE Handbook of Fundamentals (1972), in most text books on drying or air-conditioning, and in the paper by Gaffney (1978).

Effect of humidity on produce, and vice versa

All foods contain water, and if they are placed in a sealed enclosure, they will gain or lose water until the humidity inside the enclosure reaches a value that is characteristic of that food at that temperature. This value is called the equilibrium relative humidity (E.R.H.). Most fruits and vegetables have an E.R.H. of 97-98%, and will lose water if allowed to stand in air that has a lower relative humidity (R.H.). The rate of water loss depends on the difference between the water vapour pressure exerted by the produce and the water vapour pressure in the air, known as the water vapour deficit (W.V.D.), and also on the air speed past the produce. The loss of as little as 5% of their weight causes fruit and vegetables to shrivel or wilt. To minimize water loss most fruits and vegetables should be stored at 97-98% R.H., but this is impracticable for reasons that will be explained later in this paper, and even if it were possible, at such high relative humidities fibreboard cartons lose their strength, and spoilage organisms grow rapidly. In practice, therefore, coolrooms usually are operated at a humidity a little below the E.R.H. of the produce, so the rate of moisture loss is not too great, but spoilage is retarded. For short-term storage 90% R.H. is adequate, but for long-term storage 95% R.H. is preferable, used in conjunction with protective chemical treatments to limit the rate of spoilage.

Of course, changing the humidity of the room is not the only way to alter the humidity of air in contact with the produce. Many types of packaging limit moisture movement and so create a microclimate within the pack that differs from the climate of the room. An extreme example of this effect is the film-wrapping of oranges described by Tugwell and Gillespie (1981) which halved the weight loss.

Other products, such as nuts and seeds, are dried before storage until the E.R.H. is sufficiently low to prevent the growth of

moulds (i.e. to an E.R.H. below 70%), so these products must be stored at a similar humidity to prevent the reabsorption of water.

When is humidity important?

The amount of water lost from an object in contact with air depends on the time of exposure, and also on the exposed surface area, and the water vapour deficit.

i.e. Amount of water loss
= a constant x time x area x W.V.D.

This equation shows that for any particular cooling situation the water loss depends on the time and the water vapour deficit. During long-term storage, therefore, when time is large, the water loss will become large unless the W.V.D. is kept small. This means holding the R.H. of the air close to the E.R.H. of the food. Cooling warm produce, however, takes only a small time, so the total amount of water lost will be small even if the W.V.D. is large; the humidity of the air therefore is of minor importance during cooling. In fact a low humidity actually aids cooling because evaporation of water removes heat as the latent heat of vaporization, so increasing the cooling rate. Evaporation increases the rate of cooling of fruits only slightly, but can be used to provide very rapid cooling of leafy produce such as lettuce, which has a large surface area. In a vacuum cooler the total pressure is reduced, reducing the water vapour pressure in the air, and so increasing the W.V.D. and hence the rate of evaporation. Vacuum coolers are used widely in the USA and in Europe to cool leafy produce.

High humidity, therefore, is desirable during long-term storage, but, except for produce that is extremely sensitive to moisture loss, is of little benefit during cooling because of the short times involved.

Measurement of humidity

Devices used to measure humidity are called hygrometers. They can respond to any mechanical, electrical, chemical or physical characteristic that changes with humidity. Hair, for example, absorbs water and stretches when the relative humidity increases. In the familiar hair hygrometer this change in length is used to move the pointer across the scale. Other materials change their electrical properties, and can be used to produce changes in an electronic circuit. Electronic hygrometers are used commonly in the laboratory, and in air-conditioning systems.

The accuracy of mechanical and electronic hygrometers depends on how well they are calibrated. While the manufacturer may adjust hygrometers to read correctly before despatch, they are liable to drift from calibration. All indirect hygrometers, therefore, should be checked regularly. Indirect hygrometers also have other disadvantages; the reading obtained is likely to be influenced by variables other than humidity; a temperature change of 10 °C changes the length of hair, for example, by the same amount as a 4% R.H. change, and some electronic sensors respond not only to water vapour, but also to other gases and vapours, including sulphur compounds. Both mechanical and electronic hygrometers are affected to some extent by their recent history, so the reading obtained depends on whether the humidity is rising or falling (hysteresis) and by how long they have been exposed to the current humidity (drift). Generally hysteresis is more of a problem at medium humidities, and drift is more of a problem at high humidities. However, the main problems with the use of these devices in coolrooms is that at relative humidities above about 85% they are not sufficiently accurate or stable to be useful. For example, the manufacturer of one portable hand-held electronic hygrometer claims an accuracy of ±2% between 10 and 80% R.H., ±4% R.H. between 80 and 90% R.H., and does not specify accuracy above 90% R.H.

A more reliable way to measure high humidities is to measure the cooling effect of air moving over a wet temperature sensor. Instruments based on this principle are called psychrometers. Psychrometers have the great advantage over indirect hygrometers in that they need not be calibrated against a humidity standard, because only temperatures are measured, and temperature sensors should be accurate and stable. Provided that air is flowing over the sensors and that the wick is kept wet and clean, the humidity measurement will be reliable. The simplest such instrument is the sling (or whirling) psychrometer, which consists of two thermometers, one dry and one wet, mounted in a frame that swings around a handle (see Fig. 2). An automated version of this, the aspirated psychrometer, is an instrument incorporating an electric fan to move air over wet and dry temperature sensors. This can be a large, permanently-installed instrument (see Fig. 3), or a small hand-held instrument. Alternatively, the wet and dry temperature sensors can be mounted in an existing air stream.

The R.H. or other expression of humidity is obtained from the wet- and dry-bulb temperatures by using a psychrometric chart,

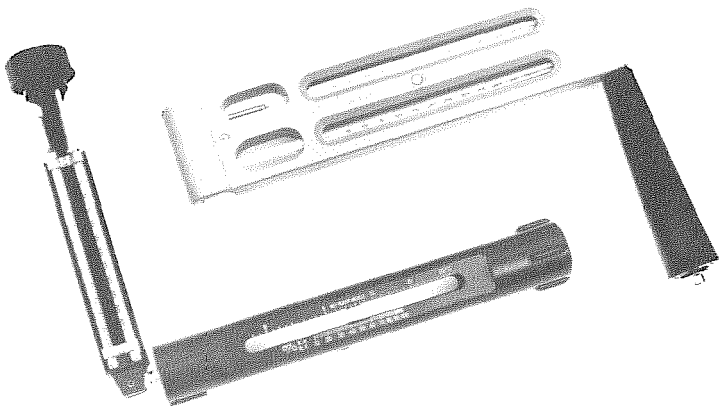

Fig. 2. Commercial sling psychrometers.

tables, a special slide rule (often provided by psychrometer manufacturers), or by solving a set of psychrometric equations. Weiss (1977) gives a set of equations suitable for use with a desk calculator or microcomputer, and some psychrometers, which use electronic temperature sensors, automatically calculate and display the relative humidity and/or dew-point.

Humidity can also be measured with automatic dew-point meters that use a light beam directed onto an electronically-cooled mirror exposed to the air, to detect the first formation of dew. Like aspirated psychrometers they do not need to be calibrated against a humidity standard because they measure the dew-point temperature directly. However, they are expensive, and prone to error if dirt is allowed to accumulate on the mirror.

How accurately can humidity be measured by psychrometry?

The accuracy of measurement of humidity by psychrometry depends on how accurately the temperatures can be measured. For this reason it is extremely difficult to measure humidity more accurately than about ±3% in fruit and vegetable stores and transport systems, i.e. it is just possible to distinguish between 90 and 93%.

The main problem is that the wet bulb depression is small at coolroom temperatures, and therefore difficult to measure accurately under commercial conditions. At 5 °C and 90% R.H. the wet bulb temperature is 4.3 °C, so a humidity difference of 10% is measured as a temperature difference of only 0.7 °C. A change from, say, 90% to 93% R.H., therefore cools the wet bulb by only 0.2 °C. Great care is necessary to measure temperatures to this degree of accuracy, and high-precision platinum resistance elements are usually specified. To illustrate the degree of precision required, standard industrial high accuracy platinum resistance elements (BS1094: Class A) are accurate to ±0.16 °C at 0 °C. If two of these are used to measure wet and dry bulb temperatures, the error in the temperature differences could be as much as ±0.32 °C, producing an error in the derived R.H. of ±4.5%. In some aspirated psychrometers pairs of sensors are carefully matched so that although the absolute accuracy is not improved, the error in the measured temperature difference is reduced. Typically, psychrometer manufacturers claim sensing errors of no more than ±2% R.H., and perhaps as low as ±1%, implying error due to temperature sensors of less than ± −0.15 °C and ±0.075 °C respectively. The total error in R.H. of the installed, operating measuring system will always be greater, however, because of errors in measuring the sensors, and because of contamination of the wick.

The wick used to cover the wet bulb must be kept very clean; it must not be touched, even with clean fingers; only distilled water should be used to wet it, and it should be

replaced when it becomes contaminated with airborne dirt. The risk of contamination is minimized by ventilating the sensors only when required; one commercial device incorporates a circuit that switches on the fan only 2 min before readings are taken.

Inside coolrooms and containers

A coolroom or refrigerated container is an insulated enclosure with a door, an air cooler, a temperature controller and a fan to circulate the air past the cooler.

Heat enters refrigerated spaces by conduction through the walls, and air leakage. Additional heat comes from produce as it cools and as it respires, and as the heat equivalent of the electrical energy supplied to the fan. A coolroom or container is kept below the temperature of its surroundings by the air stream which warms as it circulates, collecting this heat and carrying it to the air cooler, where it is transformed to the refrigeration system (see Fig. 4). If the air stream neither gains nor loses water as it circulates around the room, becoming warmer, its moisture content remains constant, but its relative humidity will fall, purely because of the temperature rise. On a hot day more heat will penetrate the walls of the coolroom or container, the temperature of the air stream will rise more, and consequently its relative humidity will fall further than on a cool day. Air leaving the cooler at 0 °C and 100% R.H., will fall to 86% R.H. if the temperature rises by 2 °C before returning to the air cooler, but it will fall to 70% R.H. if the air warms by 5 °C. If the circulating air is picking up moisture as well as heat, the fall in R.H. will be reduced, and if there is a great deal of

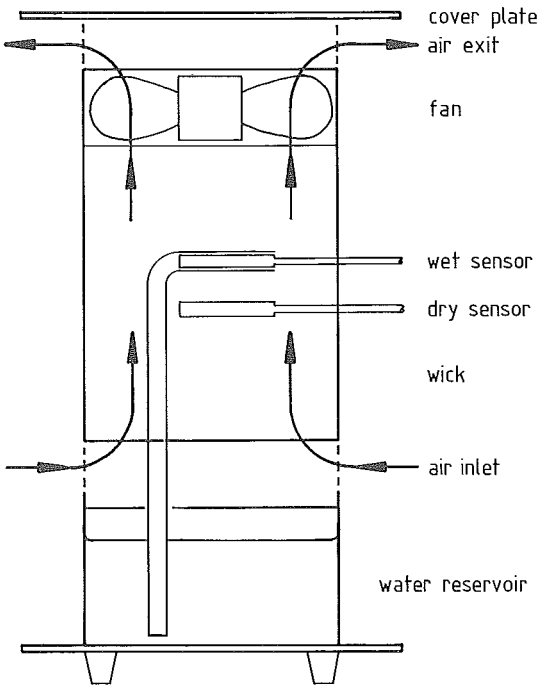

Fig. 3. Commercial aspirated psychrometer.

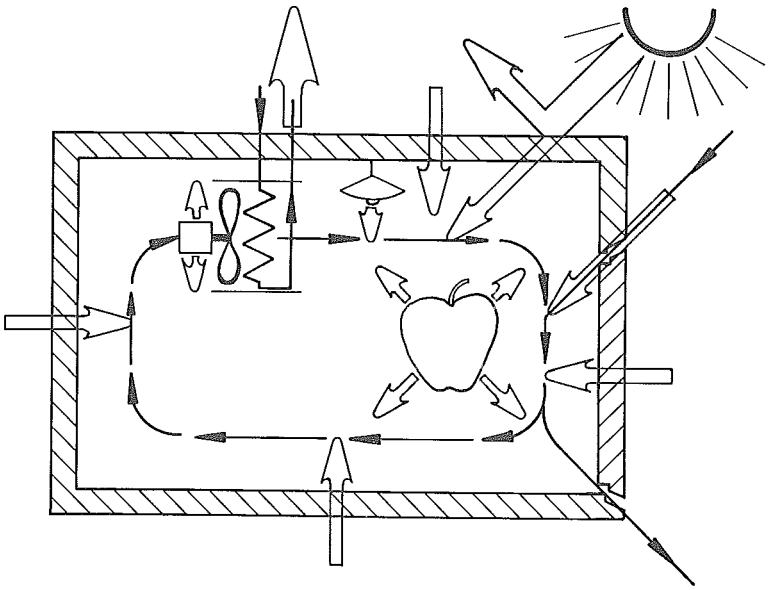

Fig. 4. Sources of heat and moisture in a coolroom.

warm produce in the room, or the door is left open on a warm, humid day, the air returning to the cooler could be at 100% R.H., and might even contain free water as fog. The refrigeration system removes this additional water, forming frost on the coils, so decreasing their efficiency, and also causing the refrigeration system to shut down more frequently to defrost. Van Nieuwenhuizen (1985) showed that by spraying water into the load space of a container it was possible to increase the return air humidity to 85% R.H., but at the expense of decreasing the effective output of the refrigeration system by 30%.

The R.H. of the air in contact with produce can be increased by designing the store such that the circulating air is split into two streams, one that circulates around the walls, through a 'jacket', intercepting the wall heat leakage, and a separate, parallel stream that circulates through the produce. In such a 'jacketed' store the air in contact with the produce rises in temperature very little, and therefore the R.H. remains high.

Conventional air coolers are usually banks of finned coils through which a cold refrigerant is circulated, and can operate at any desired temperature. An alternative type, used when a very high humidity is required, circulates the room air through a curtain or shower of chilled water, and therefore cools the air stream no lower than 0 °C.

Air leaves a conventional cooler with its dew-point close to the temperature of the coils. The temperature of the coils is regulated to give the desired air temperature, and the lower the temperature of the coils, the lower the humidity of the air leaving them will be. If the air cooler has a large surface area, its temperature need not be much colder than the required air delivery temperature, and the air stream leaving it will have a humidity close to 100% R.H. If the air cooler is small, however, it must operate at a lower temperature in order to transfer the same quantity of heat, and therefore the humidity will be lower. It is quite practicable to build a room that will operate at a high R.H. provided that an appropriate cooler is installed. Good design requires a realistic estimate of the refrigeration capacity needed to cool the room and its contents, selection of a cooler of adequate size, and correct matching of the cooler to the rest of the refrigeration system. For long-term storage it is also important to insulate the room well so as to reduce the heat load which directly decreases the R.H.

A water-shower cooler will deliver air at a temperature close to that of the water, and at a humidity close to 100% R.H. Variations in humidity throughout the room will be no less in rooms fitted with water-shower coolers than in rooms equipped with conventional air coolers, since these variations are caused by temperature change, not by the type of air cooler used.

Relative humidity depends on the inside and outside temperatures and also on the position within the coolroom or container. We must consider carefully, therefore, just what we mean when we ask 'At what R.H. is this room operating?' It is important to specify where the measurement is made (e.g. air delivery from cooler), the internal and external temperatures, what is in the room, whether produce is being cooled, and whether the room is being worked.

Control of humidity in coolrooms

Control of humidity requires some way to measure humidity, plus some means of removing water from the air (to reduce the humidity) and/or of adding water (to increase the humidity). It follows that humidity control can never be better than the precision of measurement or of addition or removal of water.

The temperature of a refrigerated space is set by adjusting the thermostat of the refrigeration system, but there is no comparable simple way to adjust the humidity. Indeed, for most applications, active control of humidity is unnecessary, because the operating humidity is determined by the design of the refrigeration unit, especially by the size and operating temperature of the cooler. The operating relative humidity can be altered to some extent by adjusting the evaporator pressure regulating valve, to change the coil temperature. However, increasing the R.H. by increasing the coil temperature also decreases the refrigeration capacity, so this technique is limited to periods of operation when the full capacity of the refrigeration plant is not required, such as for the long-term storage of apples, after the fruit has been cooled to storage temperature.

The alternative way to increase the humidity is to leave the refrigeration system operating normally, but to add water directly to the air in the room. This method is simple, and may be adequate for occasional use, but it will degrade the refrigeration performance by increasing the rate of frosting of the air cooler, it can cause water damage to produce and fibreboard cartons, and it will permit more rapid growth of most spoilage organisms.

Generally, it is sufficient to check the humidity from time to time using a portable

device such as a sling psychrometer. If only occasionally the humidity is found to be too low, and a high humidity is necessary, simple measures can be employed, such as spraying water over the produce or the floor. If the humidity is too low persistently, this probably indicates that the cooler is operating at too low a temperature, and should be replaced with a larger one.

For some applications, such as for rooms handling a variety of produce, it may be desirable to regulate the humidity by automatic control of water atomizers or steam boilers. Electronic signal converters and controllers are available which monitor wet- and dry-bulb sensors, calculate and display the R.H., and provide an electrical output suitable to operate the water-injection device, but such devices are expensive. Over a small range of temperatures the relative humidity is approximately proportional to the wet-bulb depression, so for coolrooms operating at a set temperature the cost of instrumentation can be reduced with little loss of accuracy by using this temperature difference, in place of the R.H., to drive the controller.

Occasionally, it is desirable to reduce the relative humidity of a coolroom. The only practical way is to lower the operating temperature of the cooler, by adjusting the evaporator pressure regulating valve, and to reduce the size of the cooler, to maintain the same cooling capacity.

Control of humidity in containers

Freight containers currently in use are not designed to allow humidity to be controlled. Generally, they have a small air cooler and provided the interior of the container is below ambient temperature, the coils will be cooler than the delivery air stream, and the R.H. will be low. There is no simple way of reducing the R.H. further, but it can be increased. A technique sometimes used is to seal the defrost and floor drains, allowing the defrost water to accumulate on the floor of the container. The disadvantages of wetting the floor are the risk of water damage to the cargo, the increased cost of cartons made from water-resistant fibreboard, and the risk of more rapid spoilage. Generally, it is better to control the microclimate within the package or carton, and allow the container to operate normally.

Equipment

The author can supply a list of equipment suitable for measuring and controlling humidity, with the Australian agents and approximate prices.

References

American Society of Heating, Refrigeration and Air-Conditioning Engineers; (1972). 'Handbook of fundamentals'. (ASHRAE: New York).

Gaffney, J.J. (1978). Humidity: basic principles and measurement techniques. *HortScience* **13**, 551-5.

Tugwell, B.L., and Gillespie, K. (1981). Australian experience with citrus fruit wrapped in high density polyethylene film. *Proceedings of the International Society of Citriculture* **2**, 710-4.

Van Nieuwenhuizen, G.H. (1985). Transport refrigeration units working under humid conditions. Technology Advances in Refrigerated Storage and Transport, Meeting International Institute of Refrigeration, Orlando, Florida, Nov. 1985. Preprints, 163-72.

Weiss, A. (1977). Algorithms for the calculation of moist air properties on a hand calculator. *Transactions of the American Society of Agricultural Engineers* **20**, 1133-6.

A pilot scale preheating system for the treatment of milk and whey

By P.T. Clarke and F.G. Kieseker

CSIRO Division of Food Research, Highett, Victoria, 3190

Introduction

The preheating of milk plays an important role in the manufacture of milk powder. During preheating, changes are induced in milk constituents, in particular the whey proteins and minerals. While the basic reasons for these changes are not fully understood they result in the development of specific functional characteristics in milk powders which are essential to the successful manufacture of a number of dairy and allied products. These characteristics include heat stability, viscosity, rennetability, and behaviour in bread making.

In an endeavour to quantify these changes the American Dry Milk Institute (1971) established a heat classification system for non-fat milk powders based on the interaction of whey proteins with casein during the preheating stage. As the level of heat treatment is intensified either by raising the temperature or extending the holding time, the extent of complexing between these proteins is increased so that the concentration of undenatured whey proteins is reduced. This allowed an arbitrary division of milk powder into low-, medium-, and high-heat classes as determined by the amount of undenatured whey protein nitrogen in mg/g of powder after a specific heat treatment.

Recently the International Organization for Standardization issued a standard for the classification of non-fat milk powders, ISO 6735-1985(E), which is also dependent on the denaturation of the whey proteins.

While these methods are useful in that they provide a guide as to the level and constancy of heat treatment applied, they do not provide information relating to the development of specific functional properties which must be confirmed by analysis of the powders. These properties are established by the application of controlled heat treatment to the milk. However, the level of heat treatment may have to be adjusted from time to time to account for variations in the composition of milk.

The equipment used in commercial operations for preheating may vary from tubes for short holding times e.g. 72.2 °C/15 s for milk pasteurization and 120 °C for 1–2 min, or vats and holding tanks for longer times such as 85 °C/30 min. It is important that the specified time-temperature relationships be achieved for all product processed. Under-treatment may result in failure to achieve the required functional characteristics whereas over-treatment may result in damage to the product. In any system the level of under- and over-treatment must be kept to a minimum.

In commercial operations, failure to achieve the required level of heat treatment can arise from faulty instrumentation, radiation losses, failure to maintain set levels in tanks or vats, channelling or by-passing of product through the system.

In view of the importance to many projects of controlling heat treatment, a pilot-scale preheating system has been developed which minimizes these problems. The unit is currently being used in the preheating of milk for the manufacture of specialized milk powders, heat treatment of permeate from the ultrafiltration of milk or whey and the treatment of whey protein concentrates.

Plant description

The unit, constructed of stainless steel, has three major components: a heating system, holding tubes and a cooling section.

The heating system consists of two tubular heat exchangers both of which are heated by water circulated from separate electronically controlled balance tanks. The water heating medium is steam and/or electricity. The first stage raises the temperature of refrigerated milk to approximately 60 °C. The second stage is capable of heating the product to any predetermined temperature up to 95 °C using the return water from the holding tubes. A probe senses the temperature of the product leaving the second heat exchanger and the

CSIRO Food Res. Q. 1986, **46**, 86-89

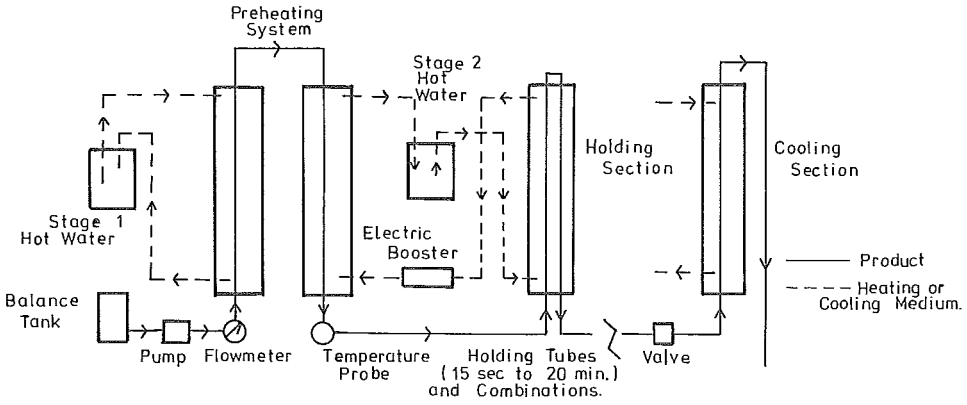

Fig. 1. Diagrammatic layout of pilot scale preheating system.

incoming water is electrically boosted to obtain the required temperature (Fig. 1).

The holding section comprises eight jacketed tubes with capacities equivalent to 0.25, 0.5, 1, 2, 2, 5, 10 and 20 min at a flow-rate of 100 l/h. The tubes may be interconnected as required to meet variable time requirements. The product is held within 1°C of the required temperature by circulation of water at high velocity. The product flows upward through the tubes and returns via small diameter pipes which are enclosed in the water jacket. A relief valve is provided at the top of each tube to allow the removal of air during start up and a small valve is positioned at the exit of the last holding tube to create a slight back pressure and avoid siphoning.

The cooling section consists of a tubular heat exchanger serviced by chilled water at 4°C thereby efficiently terminating heat treatment after holding, reducing the by-product to ambient temperature.

Holding efficiency

The inverse relationship between the speed of the fastest particle and the theoretical average speed of the product through the tube is known as the holding efficiency and is expressed as a percentage:

$$\text{Holding efficiency} = \frac{T_1}{T_2} \times 100\%$$

T_1 = actual time for first detectable particle to pass through holding tube.

T_2 = theoretical average time for milk to pass through holding tube calculated from capacity of tube and rate of flow of the product.

The holding efficiency for a tube in which the flow is laminar is about 50% but where the flow is turbulent the efficiency may be as high as 80% (Green 1983).

Several methods have been used for determining the holding efficiency of plants. These include:

- Dye injection
- Colour method of Dummett and Mongar (1944)
- Conductivity

Dummett and Mongar state that the sensitivity of the dye injection method corresponds to a dilution of about 1 in 5000. For their own method it is 1 in 100 000 and for conductivity 1 in 2000. Because of its sensitivity and the ease of application the method of Dummett and Mongar was used in principle allowing for the pilot-scale nature of the plant and the range of holding times involved.

Using this method the holding efficiency of each individual tube was determined as shown in Table 1.

The holding efficiency of each tube up to and including 5 min hold was equal to or exceeded the theoretically obtainable values.

TABLE 1

The holding efficiency of tubes of increasing holding times calculated on a flow rate of 100 l/hour.

Tube (min)	Holding efficiency (%)
0.25	88
0.5	79
1	81
2	82
5	83
10	72
20	65

The larger capacity tubes with increased volume and diameter provide greater potential for channelling and have demonstrated a decreased holding efficiency. Typical holding efficiency values for combinations of holding tubes to achieve specified times are shown in Table 2.

TABLE 2

The holding efficiency of tubes used in combination

Time (min)	Tubes	Holding efficiency (%)
30	20 + 10 min	70
10	5 + 2 + 2 + 1 min	79
0.75	30 + 15s	83

Adjustment of flow rates may be necessary in the case of the larger tubes to improve holding efficiencies. However, this may not always be compatible with the capacity of drying equipment.

Residence time distribution

The time taken for the fastest particle to travel through a holding system is of particular concern for processes such as pasteurization. However, it is also essential to have an overview of the residence time distribution to establish the extent of under- or over-holding of the product being processed.

A conductivity method was used to determine the residence time distribution of the product in the system. This involved the use of an 18% sucrose solution (viscosity at 20 °C, 1.786 cp) to simulate skim milk (viscosity at 20 °C, 1.79 cp) using the addition of 0.01N KCl as indicator. The preheating system was stabilized using the 18% sucrose solution which was replaced with the KCl/sucrose solution by turning a valve at the commencement of the holding time determination. Samples were taken at the outlet at specified intervals and the conductivity determined at 20 °C using a digital conductivity meter.

The weight of KCl eluted per unit of time was established from a prepared graph relating concentration of KCl to conductivity over the range 0 to 0.01N KCl. This weight per unit of time was expressed as a percentage of the total weight of KCl eluted from that first detected until reaching a concentration of 0.01 N.

The results of two trials for the 5 and 20 min tubes are shown in Fig. 2. The residence time distribution for the 20 min tube is obviously greater than that of the 5 min tube. The introduction of baffles and a product mixer to the 20 min tube had the effect of reducing the residence time by approximately 4 min as compared with the control. Raw skim milk to which 0.03 N KCl was added and processed with an in-line mixer demonstrated a similar time pattern to the sucrose/KCl solution.

Using the same technique the 5 min tube which had given a holding efficiency of 83% as compared with 65% for the 20 min tube demonstrated a similar time distribution pattern for skim milk with mixer to that of the control.

The overall performance of this unit is in marked contrast to the baffled holding tank system described by McDonald (1975). In trials designed for a 21 min holding time the sodium chloride used as an indicator was detected at the outlet within 5 min. The residence time in the system extended to approximately 50 min. The use of a tubular system was recommended.

Radiation losses

Heat losses from preheated milk held in uninsulated systems such as tubes and vats can be significant and may influence the properties of the resultant powders.

Changes in inlet and outlet temperatures of preheated milk held in uninsulated tubes in this system are shown in Table 3. Heat losses from milk held over a similar range of

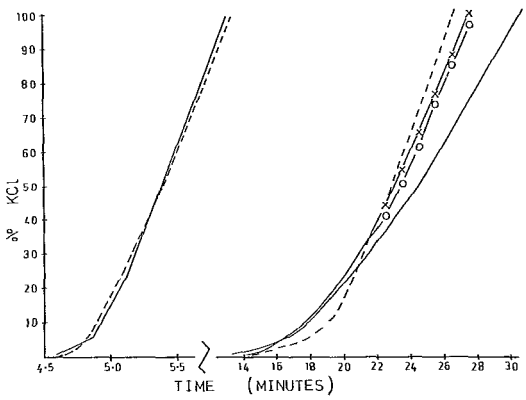

Fig. 2. The residence time distribution for the 5 and 20 minute holding tubes.
18% sucrose solution (control) —
18% sucrose solution plus mixer —x—x—
18% sucrose solution plus mixer and baffles —o—o—
Skim milk plus mixer – – – –

TABLE 3

Temperature losses from preheated milk held in uninsulated holding tubes

Tube (min)	Temperature (°C) In	Out
0.25	75	69
	71	67
1	62	59
5	83	79
	64	62
10	82	75
	72	69
20	86	80
	73	68
30	85	76

temperatures in the tubes, protected by the circulation of water at high velocity at the same temperature, were negligible.

Conclusion

The use of the pilot scale preheating system has provided a positive basis for the study of changes occurring in a number of dairy products during heating. For holding times in excess of 5 min some adjustment of flow rates may be required to achieve a specific heat treatment.

The residence time within the larger tubes could be improved by the installation of baffles and appropriate mixing devices. Radiation losses are eliminated by the installation of water jackets which contribute to the uniformity of heat treatment.

The unit has been used successfully in the heat treatment of whey for the isolation of specific fractions, preparation of milk for ultrafiltration, studies relating to changes in the mineral balance of milk resulting from the application of various preheating regimes and the preparation of specialized milk powders.

References

American Dry Milk Institute (1971). Standards for grades of dry milks including methods of analysis. *Bulletin of the American Dry Milk Institute* (916), 53pp.

Dummett, G.A., and Mongar, J.L. (1944). A new method for checking holding time in short-time pasteurizing plants. *Dairy Industry* **9**, 265.

Green, E. (ed.) (1983). Pasteurizing plant manual. 3rd ed., Society of Dairy Technology, London, 104pp.

McDonald, G. (1975). Holding systems used for preheating. *Dairy Technology* **6**, 13.

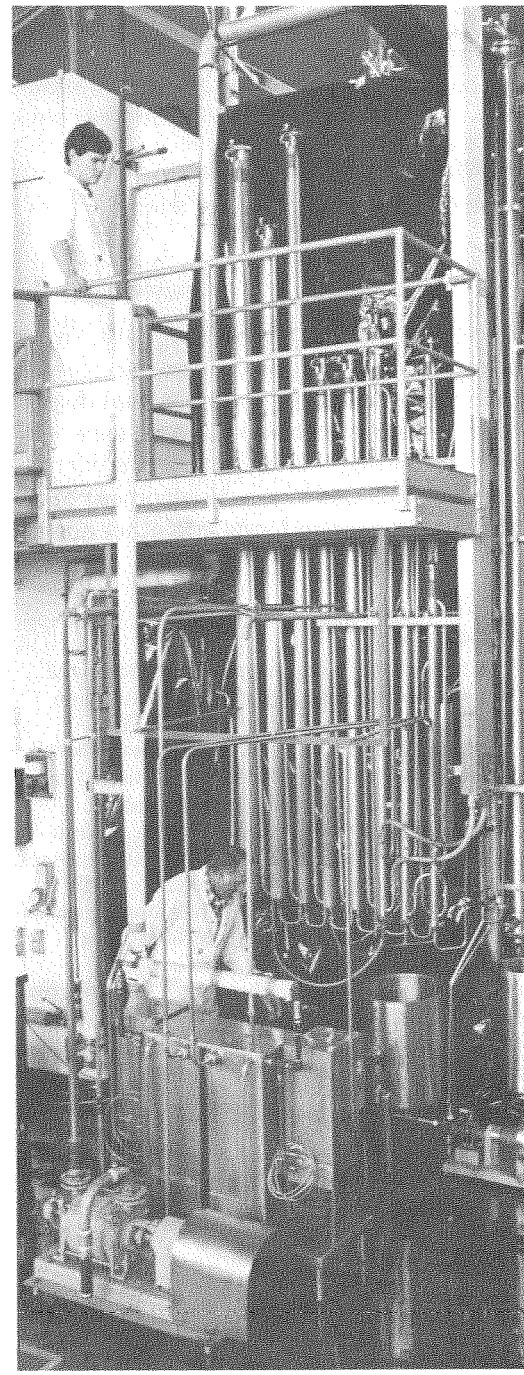

Fig. 3. Pilot scale preheating system at Dairy Research Laboratory.

Rapid chromatographic detection of microbial spoilage of commercially-sterile foods

By M.J. Eyles and R.F. Adams

CSIRO Division of Food Research, North Ryde, New South Wales, 2113

Traditional cultural methods of detecting microorganisms have many disadvantages and limitations. To overcome these problems, microbiologists are adopting instrumental techniques and other methods that do not involve the preparation of cultures. Analysis for microbial metabolites using a variety of chromatographic techniques permits rapid, and often inexpensive, detection and characterization of microbial activity in foods. Recent advances in the development of these techniques for use in investigations of spoilage of canned foods and other commercially-sterile products are discussed below.

Introduction

During growth, microorganisms modify the chemical composition of the substrate from which they obtain their nutrients. The concentrations of some components of the substrate are reduced as they are utilized by the microorganisms, while microbial metabolites that may not have been present initially are added. Chromatographic analyses can detect these changes and show rapidly whether or not microbial growth has occurred.

There are many different fermentation pathways by which microorganisms may obtain energy from a substrate. The spectrum of metabolic products produced varies with the pathways that are used. The biochemical characteristics of an organism, the composition of the substrate, and the growth conditions (e.g. pH and redox potential) determine which pathways are used. Thus, in addition to showing whether or not microbial growth has occurred, chromatographic analysis of metabolic products may provide information about the identity of microorganisms. Table 1 lists some of the many different classes of metabolic products that bacteria may produce in food or culture media, and that may be readily detected by chromatographic techniques.

Chromatographic detection of chemical changes produced by microbial activity has a number of potentially useful applications in the food industry. This paper discusses the use of several chromatographic techniques in detecting microbial spoilage and determining the cause of spoilage of canned foods and related commercially-sterile products. The indicator compounds that have been most widely used for these purposes are the short-chain organic acids and alcohols.

TABLE 1

Some microbial fermentation products

Class of product	Examples
Acidic	
Monocarboxylic acids	Acetic
Hydroxy acids	Lactic
Dibasic acids	Succinic
Keto-acids	Pyruvic
Aldehydo-acids	Glyoxylic
Miscellaneous	Malic
Basic	
Amines	Histamine
Neutral	
Gases	Carbon dioxide
Alcohols - primary	Ethanol
secondary	2-propanol
tertiary	2-methyl-2-propanol
Glycols	Butanediol
Aldehydes	Acetaldehyde
Ketones	Acetone
Acyloins	Acetoin
Diketones	Diacetyl
Trihydric alcohols	Glycerol

CSIRO Food Res. Q. 1986, **46**, 90-94

Conventional microbiological investigations

Spoilage of canned foods and related products may involve one or more of a range of microbiological, chemical or physical defects. For example, swelling of cans may be due to microbial growth, electrochemical reactions, overfilling, or other problems. The defects leading to microbial spoilage may be broadly classified as due to under-processing, post-processing contamination or pre-processing microbial growth. In each of these cases, microbial survival and/or growth may occur as a result of a variety of deficiencies. For example, under-processing may occur because of the use of raw materials of inappropriate microbiological quality, an improper heat process, incorrect product pH, or other problems.

Microbiological examination of a spoiled product has two principal aims: (1) to determine whether the spoilage was caused by microorganisms and, if so, (2) to obtain information about the nature of the spoilage organisms that will help to determine why spoilage occurred. For example, spoilage of a low-acid canned food by a single type of sporeforming bacterium usually indicates under-processing, while the presence of a mixture of non-sporeforming microorganisms normally indicates post-processing contamination. The steps in a conventional microbiological examination are summarised in Fig. 1.

The microbiological analyses outlined in Fig. 1 are only part of an investigation of the cause of spoilage (Hersom and Hulland 1980). The microbiological data must be considered in the context of a body of other information, including the history of the samples, details of the process, the condition of the containers, the pattern of spoilage and other information.

Conventional microbiological investigations are labour-intensive and may be lengthy. When defects in commercially-sterile products are detected, it is often necessary that the nature and cause of the defect be determined quickly so that industry or regulatory authorities can take appropriate action. Analyses involving microbiological cultures take days or weeks to complete, whereas many chromatographic techniques for detecting metabolic activity are complete in less than an hour.

Conventional procedures may not always differentiate unequivocally between microbial and non-microbial spoilage, since each indicator of microbial growth may give a negative result in foods that have suffered microbial spoilage. The pH may not change significantly, especially in acid foods; microscopic examination can fail to reveal significant numbers of microorganisms; cultural examination may also yield negative results, since spoilage organisms in canned foods may die before an investigation commences (auto-sterilization). In contrast, chemical changes caused by microbial activity remain detectable irrespective of whether the microorganisms are viable.

Gas chromatography

Gas chromatography (g.c.) has become important in many microbiology laboratories for the semi-quantitative determination of the end-products of microbial fermentations. For example, g.c. analyses of metabolic products are used in clinical laboratories for the identification of some groups of anaerobic

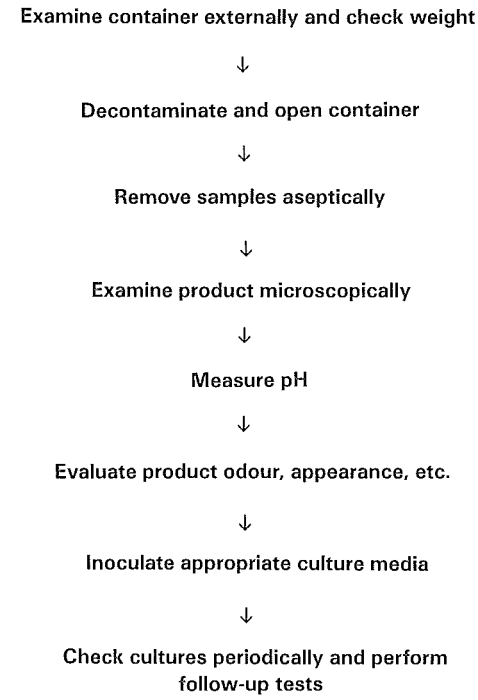

Fig. 1. Steps in conventional microbiological examination of spoiled canned foods.

bacteria. G.c. was first used to detect bacterial metabolites in foods some years ago (Guarino and Kramer 1969; Mayhew and Gorbach 1975) and has been used more recently to detect and diagnose microbial spoilage of commercially-sterile products (Schafer *et al.* 1982; Eyles and Adams 1986).

Studies at the CSIRO Food Research Laboratory have concentrated on analysis of spoiled canned foods for volatile fatty acids (VFA), i.e. the C_2–C_8 aliphatic acids, and alcohols. These compounds are markers of microbial growth that g.c. analyses can detect quickly and, if an instrument is available, inexpensively. The sample preparation procedures used for these analyses are minimal. Many samples, for example fruit juice products or brines from vegetable packs, require only acidification before injection into the instrument (Table 2). In our experience, the injection of relatively crude preparations of this kind has not had any substantial adverse effects on the g.c. column.

TABLE 2

Preparation of foods for analysis of volatile fatty acids by g.c.

Juices, brines, other liquids

1. Add 100µl 50% sulphuric acid/mL sample
2. Centrifuge 8000 g/10 min (optional)
3. Inject 2–10µl into g.c.

Solids, semi-solids

1. Prepare a 20% suspension in water
2. Treat as above
3. Extract with equal volume of diethyl ether (optional)
4. Inject 2–10µl of sample or ether extracts into g.c.

The technique can show within about 30 min of collection of the sample whether microbial spoilage has occurred. The amount of labour required is comparable to that involved in preparation and microscopic examination of a smear. It is possible to extend the analyses to non-volatile acids (lactic, dicarboxylic, keto acids) at the expense of extra sample manipulation.

We have subjected a wide range of foods that have become spoiled as a result of defects in commercial food processing operations to both g.c. and conventional microbiological examinations. Samples of canned foods that have been artificially inoculated with spoilage organisms have also been studied. The foods examined have included vegetable, fruit, meat, fish and dairy products. Conventional canned foods, aseptically-packed products in flexible packages and chemically preserved products have been included. Aerobic and anaerobic sporeformers (thermophiles and mesophiles), various non-sporeforming bacilli and cocci, and yeasts have been among the spoilage organisms studied. Some of these analyses have been described in detail elsewhere (Eyles and Adams 1986).

Spoilage is detected by comparing the VFA profiles obtained by g.c. analysis of unspoiled and spoiled samples. Substantial differences in the VFA profiles have always been observed between sound and microbially-spoiled products. As expected, the VFA profiles of spoiled and unspoiled samples did not differ in a limited range of products that had suffered non-microbial spoilage (e.g. swelling due to defective filling). Analysis for alcohols can provide useful additional information.

The g.c. profiles of unspoiled products have always contained several peaks and are often quite complex (Fig. 2). Many foods contain naturally-occurring or added VFA. Since minimal sample preparation procedures are used, compounds other than VFA also appear in the profiles. Peaks produced by other components are usually identifiable, being excessively broad, asymmetric or having retention times different to those of the VFA. These other components do not detract from the value of the analyses and provide additional information in many cases. Clearly, correct interpretation of g.c. data depends on the availability of good control data. It is necessary to know the profile of unspoiled samples of the product and the extent of likely variation between samples.

Microbial spoilage may cause one or both of two types of change in the VFA profile. Peaks are increased in size or added to the profile as a result of production of metabolites (Figs. 3, 4). Less frequently, peaks may be eliminated or reduced in size as a result of microbial utilization of food components (Fig. 3). The number of peaks affected varies with the type of spoilage. Where spoilage has been caused by a single microorganism and it has been possible to culture the organism, the metabolites detected in cultures have been similar to those detected in the food. Our experience indicates that there may be less correlation between culture profiles and product profiles when a mixture of organisms is present, presumably because of the metabolic interactions that occur between organisms.

Analysis for metabolites by g.c. has advantages other than the ability to show rapidly whether microbial spoilage has occurred. The g.c. profiles of spoiled products may also yield information about the nature of

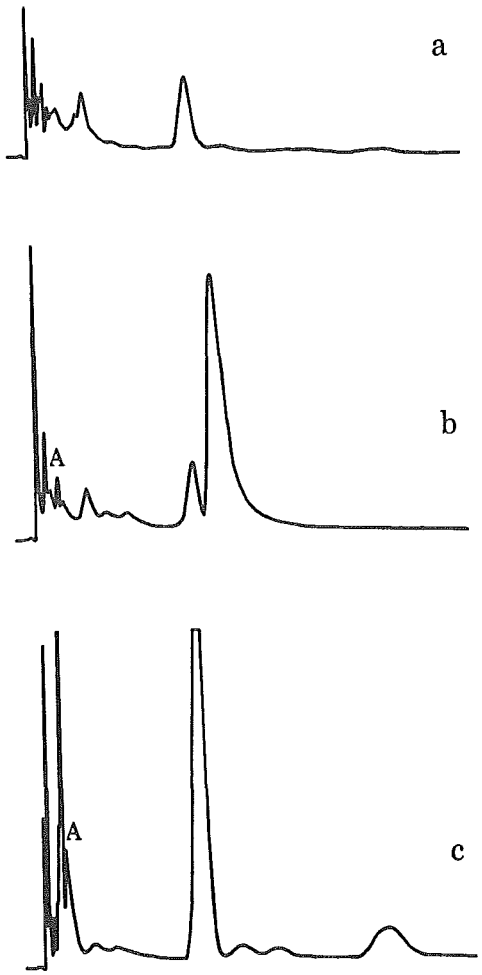

Fig. 2. G.c. profiles of unspoiled canned foods; (a) mushrooms, (b) tuna, (c) tomato. Key to Figs. 2–4: A, acetic acid; P, propionic acid; B, n-butyric acid; iV, iso-valeric acid; nV, n-valeric acid; C, iso-caproic acid. Chromatographic conditions: Perkin-Elmer Sigma 3B gas chromatograph with a flame ionization detector; glass column (2 m, 2mm internal diameter) packed with Chromosorb 101; the column was heated at 180 °C and the flow rate of the carrier gas (nitrogen) was 40 mL/min.

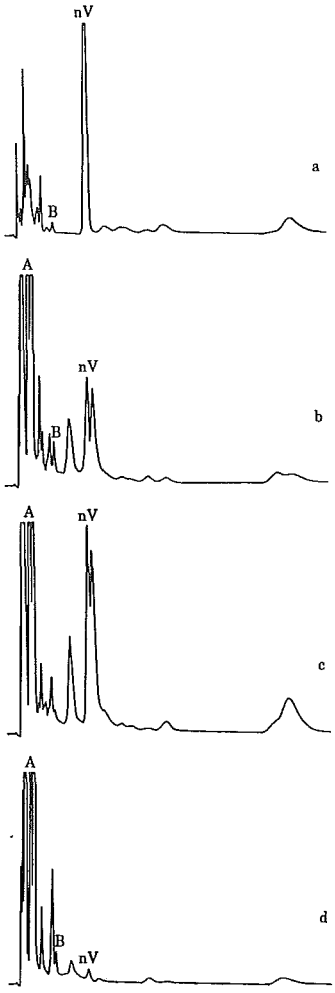

Fig. 3. G.c. profiles of UHT cream; (a) unspoiled, (b,c,d) samples from different containers from the same batch spoiled by a mixture of microorganisms. Key: see Fig. 2.

the spoilage organisms that can be useful in several ways. Such information may be particularly valuable if the organisms are no longer viable and thus cannot be characterized using cultural procedures.

In some circumstances, g.c. analyses may provide strong evidence that spoilage has been caused by a particular group of micro-organisms. For example, Gram positive bacilli causing spoilage of certain fruit products are likely to be lactobacilli or butyric anaerobes (*Clostridium butyricum* and similar organisms). Lactobacilli do not produce butyric acid, unlike the butyric anaerobes. G.c. analysis of the product can therefore be used to differentiate between these two groups.

Because different microorganisms produce different spectra of metabolic products, g.c. can indicate whether spoilage has been caused by a single type of organism or more than one. If a mixture of organisms has caused spoilage, different containers from the same spoiled batch frequently yield different g.c. profiles. Fig. 3 shows profiles obtained with a product that had become contaminated with a mixture of organisms as a result of container defects. Fig. 4 shows profiles of a product spoiled by a single *Clostridium* species.

A g.c. procedure that detects certain metabolites in spoiled low-acid canned foods

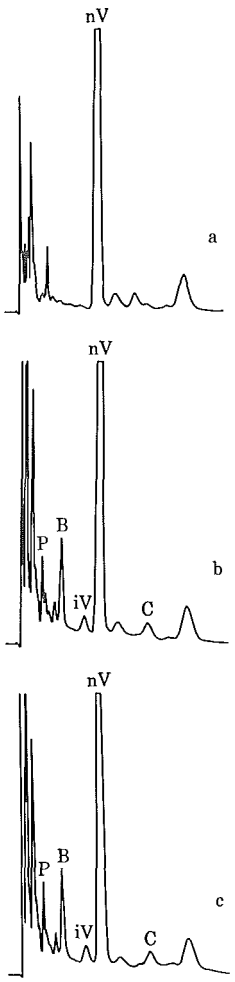

Fig. 4. G.c. profiles of canned corn; (a) unspoiled, (b,c) spoiled by a single *Clostridium* species. Key: see Fig. 2.

can differentiate spoilage caused by sporeforming bacteria (i.e. heat resistant bacteria) from spoilage due to non-sporeformers (i.e. heat labile) (Schafer *et al.* 1982; Schafer *et al.* 1985). Differentiation between these two groups is based on the detection of D-(-)-2,3-butanediol and butyric acid, which are produced by sporeformers but not by non-sporeformers. This information helps to distinguish underprocessed cans from cans that have suffered post-processing contamination. The method is intended for use with cans in which auto-sterilization has occurred, preventing the use of the usual cultural procedures. It has been adopted by the Association of Official Analytical Chemists (AOAC) (Anonymous 1985).

Several metabolites, including volatile acids, alcohols and amines, have been proposed as indicators of pre-processing spoilage of heat-processed foods, especially canned fish (Jay 1986). Several studies have indicated that the ethanol content of canned tuna or salmon can be a useful index of the degree of spoilage that has taken place in the raw fish. A method for determination of ethanol in canned salmon by g.c. has been adopted recently by the AOAC (Anonymous 1986). Histamine and other amines have been used frequently as indicators of pre-processing spoilage of canned fish, especially tuna and other scombroid fish. G.c. analyses have been developed for their detection (Jay 1986).

Thin-layer chromatography

Ackland *et al.* (1981) developed a thin-layer chromatography (t.l.c.) method for the detection of several short-chain organic acids in spoiled canned vegetables. Their aim was to develop a rapid, reliable, inexpensive test that could be used to screen for microbial growth in cans that showed no external evidence of spoilage (e.g. swelling). The t.l.c. method fulfilled these requirements, detecting organic acids in all of the microbially-spoiled cans examined. Results were obtained within about 2 h of sampling.

Lactic acid was detected in all spoiled cans during the study described above, therefore a rapid (5 min) spot test for lactic acid was developed for use as a screening method for suspect cans (Ackland and Reeder 1984). The test was found to be reliable in trials with a large number of containers and a range of products, although it was not suitable for foods that have a naturally high content of lactic acid. These two tests for short-chain acids were able to detect microbial spoilage in some instances where one or all of the conventional tests failed to do so.

High performance liquid chromatography

The type of information obtainable from g.c. analyses of spoiled products can also be obtained from metabolite profiles generated using high performance liquid chromatography (h.p.l.c.). An advantage of h.p.l.c. over g.c. is that a broader spectrum of acid metabolites can be detected in a single h.p.l.c. analysis. The non-volatile acids require derivatization and subsequent extraction of the derivatives before analysis by g.c. but not by h.p.l.c. Therefore, different classes of organic acids can be detected simultaneously using h.p.l.c. Adams *et al.* (1984) demonstrated that a wide range of short-chain organic acids

can be detected quantitatively in food by h.p.l.c. Although many microbial metabolites in foods may be detected by h.p.l.c., the technique has been applied to commercially-sterile foods only occasionally. For example, h.p.l.c. analyses for a range of amines have been used to indicate the quality of canned fish (Jay 1986).

Conclusion

The preceding discussion has indicated some of the potential uses of chromatographic techniques for the detection of microbial metabolites in canned foods and related products. Although the capabilities of these procedures have not been fully established, several studies have shown that chromatography can be a valuable adjunct to conventional microbiological investigations of spoilage of these products. Rapid chromatographic analyses can determine whether microbial spoilage has taken place and differentiate between microbial and non-microbial spoilage. Metabolite profiles may provide several kinds of information about the nature of the spoilage flora and thereby assist in diagnosing the cause of spoilage. Chromatography may be particularly useful for characterizing spoilage in situations where conventional techniques yield equivocal answers. In some circumstances, the rapidity of chromatography may enable a diagnosis to be made much more quickly than would be possible otherwise.

References

Ackland, M.R., and Reeder, J.E. (1984). A rapid chemical spot test for the detection of lactic acid as an indicator of microbial spoilage in preserved foods. *Journal of Applied Bacteriology* **56**, 415-9.

Ackland, M.R., Trewhella, E.R., Reeder, J., and Bean, P.G. (1981). The detection of microbial spoilage in canned foods using thin-layer chromatography. *Journal of Applied Bacteriology* **51**, 277-81.

Adams, R.F., Jones, R.L., and Conway, P.L. (1984). High performance liquid chromatography of microbial acid metabolites. *Journal of Chromatography* **336**, 125-37.

Anonymous (1985). Sporeformers in low-acid canned foods. Gas chromatographic method. *Journal of the Association of Official Analytical Chemists* **68**, 404-5.

Anonymous (1986). Ethanol in canned salmon. Headspace gas chromatographic method. *Journal of the Association of Official Analytical Chemists* **69**, 358-9.

Eyles, M.J., and Adams, R.F. (1986). Detection of microbial metabolites by gas chromatography in the examination of spoiled canned foods and related products. *International Journal of Food Microbiology*, **3**, 21-30.

Guarino, P.A., and Kramer, A. (1969). Gas chromatographic analysis of head-space vapors to identify micro-organisms in food. *Journal of Food Science* **34**, 31-7.

Hersom, A.C., and Hulland, E.D. (1980). 'Canned Foods — Thermal Processing and Microbiology'. (Churchill Livingstone: Edinburgh).

Jay, J.M. (1986). Microbial spoilage indicators and metabolites. In 'Foodborne Microorganisms and Their Toxins: Developing Methodology', (Eds M.D. Pierson and N.J. Stern) (Marcel Dekker, New York).

Mayhew, J.W., and Gorbach, S.L. (1975). Rapid gas chromatographic technique for presumptive detection of *Clostridium botulinum* in contaminated food. *Applied Microbiology* **29**, 297-9.

Schafer, M.L., Peeler, J.T., Bradshaw, J.G., Hamilton, C.H., and Carver, R.B. (1982). A rapid gas chromatographic method for the identification of sporeformers and non-sporeformers in swollen cans of low-acid foods. *Journal of Food Science* **47**, 2033-7.

Schafer, M.L., Peeler, J.T., Bradshaw, J.G., Hamilton, C.H., and Carver, R.B. (1985). Gas chromatographic detection of D-(−)-2,3-butanediol and butyric acid produced by sporeformers in cream-style corn and canned beef noodle soup: collaborative study. *Journal of the Association of Official Analytical Chemists* **68**, 626-9.

News from the Division

Deaths

Dr David Bishop

Dr David Bishop, Senior Principal Research Scientist at the Division of Food Research died recently at a result of an accident while involved in his hobby, scuba diving. Dr Bishop had been a member of the Plant Physiology Unit of the Food Research Laboratory for over 18 years, initially at Sydney University, and more recently at Macquarie University. He was an outstanding scientist with an international reputation in the field of lipid biochemistry, particularly on the relationship between chloroplast membrane structure, composition, and biological activity.

At one time Dr Bishop was particularly interested in lipid metabolism in photosynthetic organisms that live in symbiotic association with clams and corals on the Great Barrier Reef and he made several significant discoveries in this field. He was able to use his expertise in scuba diving in this work.

One of Dr Bishop's most valuable attributes was his ability to initiate research and collaborate with colleagues within CSIRO and in other organizations including those overseas. These collaborative studies have involved not only fundamental studies in the area of plant lipid biochemistry, but also more practical problems including membrane lipid changes in bananas as a controlling factor in ripening, the lipid composition of ripening cherries, the structure of lipopolysaccharides in oral bacteria, the lipid composition of wool fibre in relation to wearing quality, lipids as a food chain marker in tuna and mutton birds and the role of lipids in the chilling injury of plants. At the time of his death, Dr Bishop was evaluating the role of molecular species of phospholipids in the chilling tolerance of crop plants. The study was a collaborative one with Japanese scientists and aimed to identify biosynthetic enzymes critical to the development of chilling tolerance.

Dr Bishop's international reputation resulted in invitations to attend major overseas conferences and to work in overseas laboratories. This was also reflected in the number of visiting scientists working with Dr Bishop in Sydney. He was an excellent and lucid speaker who could present his ideas clearly and concisely to audiences who were not specialists in his field.

David Bishop will be sorely missed by his friends, colleagues and the international scientific community.

Mrs Phyllis Moy

Mrs Phyl Moy's retirement from CSIRO in July 1984 was recorded in this column in the September 1984 issue of the Quarterly (Vol. 44, No. 3).

Not long after her retirement Mrs Moy became ill and after being hospitalized on several occasions over the last few years she died on 15 September 1986. Despite the burden of her illness Mrs Moy maintained her interest and support for Community Aid Abroad, the aborigines, the Australian Historical Society and for her family and friends. Whenever possible she visited the laboratories at North Ryde where she was loved and respected by so many of her colleagues as a very fine lady. She shall be sadly missed by all who knew her.

Dr Judith Howard

We also note, with sadness, the death on 24 September 1986, of Dr Judith Howard (nee Waltho), after a long illness. Dr Howard was a microbiologist at FRL until her resignation in 1976. More recently she worked as Scientific Assistant to the Chief of CSIRO's Division of Molecular Biology at North Ryde.

New Chief for the Division of Food Research

The CSIRO Executive has announced the appointment of Dr D.J. Walker, currently Officer-in-Charge of the Meat Research Laboratory at Cannon Hill, Queensland, as the Chief of the Division of Food Research for a term of six years. He succeeds Dr J.H.B. Christian, whose term as Chief ends on 31 December 1986.

Fuller particulars will appear in the March 1987 (Vol. 47, No. 1) issue of the Quarterly.